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EFECTO DE LA TEMPERATURA, MATERIA ORGÁNICA Y pH SOBRE LA TOXICIDAD DE SPINOSAD Y SPINETORAM EN LARVAS DE *Aedes aegypti* L. (DIPTERA: CULICIDAE)

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RESUMEN

El mosquito *Aedes aegypti* L. (Diptera: Culicidae), se considera el animal más peligroso al ser humano debido a la severidad de las enfermedades que transmite. La eficacia del combate químico de esta plaga depende, en parte, del efecto que tienen las variables abióticas sobre la eficacia biológica de los insecticidas que se utilizan para su control. En la presente investigación, se estimó el impacto que tienen la temperatura, contenido de materia orgánica y pH, sobre la toxicidad de spinosad y spinetoram en larvas del cuarto instar de la raza susceptible, New Orleans de *Ae. Aegypti*. Se emplearon los bioensayos reconocidos por la Organización Mundial de la Salud. Inicialmente, se determinaron los rangos de supervivencia de las larvas, a los valores de las variables indicadas. Los resultados obtenidos demuestran que hay sobrevivencia sin que se presente efecto adverso significativo en las primeras 24 h a temperaturas de 10° C a 35° C. No se observaron, en las larvas, efectos adversos relacionados con el contenido de materia orgánica. Los rangos de pH en los que se observó supervivencia van de 4 a 12. Posteriormente, se incluyeron valores intermedios dentro de esos rangos para realizar los bioensayos completos de cada una de las variables bajo estudio. Temperatura: la CL_{50} varió de 0.041 (1.0×; 25 °C) a 0.053 mg L^{-1} (1.29×; 30 °C) y de 0.10 (0.83×; 30 °C) a 0.12 mg L^{-1} (1.0×25 °C) para spinosad y spinetoram, respectivamente. Materia orgánica (MO): la CL_{50} de spinosad fluctuó entre 0.064 (1.0×; 0% MO) y 0.22 mg L^{-1} (3.43×; 13% MO); en spinetoram, la menor toxicidad se observó con 0% de materia orgánica (CL_{50} =0.095 mg L^{-1}; CL_{95} =0.36 mg L^{-1}); los límites fiduciales al 95% se traslaparon

para el resto de concentraciones tanto a nivel de CL_{50} como de CL_{95}. pH: la mayor toxicidad del spinosad se observó a valores de 7, 8, 9 y 10; mientras que para spinetoram, la mayor toxicidad se observó a pH = 12 (CL_{50} = 0.040 mg L^{-1}; CL_{95} = 0.13 mg L^{-1}). Se estima que, a pesar de que en algunos casos se observaron diferencias estadísticas, es poco probable que se reflejen faltas de control en campo.

Palabras clave: Bioensayo, mosquitos, larvicida, control químico, spinosinas.

EFFECT OF TEMPERATURE, ORGANIC MATTER AND pH ON THE TOXICITY OF SPINOSAD AND SPINETORAM AGAINST LARVAE OF *Aedes aegypti* L. (DIPTERA: CULICIDAE)

Jaime García Severiano, M. en C.
Colegio de Postgraduados, 2019

ABSTRACT

The mosquito *Aedes aegypti* L. (Diptera: Culicidae), is considered the most dangerous animal to the human being due to the severity of the diseases it transmits. The effectiveness of chemical control of this pest depends, in part, on the effect that abiotic variables have on the biological effectiveness of the insecticides used for their control. In the present research, the impact of temperature, organic matter content and pH on the toxicity of spinosad and spinetoram in larvae of the fourth early instar of the susceptible strain, New Orleans of *Ae. Aegypti*. The bioassays recognized by the World Health Organization were used. Initially, the survival ranges of the larvae were determined, that is, the values of said variables. The results obtained show that there is no significant adverse effect in the first 24 hours at temperatures of 10 ° C to 35 ° C. Adverse effects related to the content of organic matter were not observed in the larvae. The pH ranges in which survival was observed range from 4 to 12. Subsequently, intermediate values within those ranges were included to perform the full bioassays of each of the variables under study. Temperature: the LC_{50} ranged from 0.041 (1.0×; 25 °C) to 0.053 mg L^{-1} (1.29×; 30 °C) and from 0.10 (0.83×; 30 °C) to 0.12 mg L^{-1} (1.0×; 25 °C) for spinosad and spinetoram, respectively. Organic matter (OM): the spinosad LC_{50} fluctuated between 0.064 (1.0×; 0% OM) and 0.22 mg L^{-1} (3.43×; 13% OM); in spinetoram, the lowest toxicity was observed at 0% organic (LC_{50} = 0.095 mg L^{-1}; LC_{95} =0.36 mg L^{-1}), the fiducial limits at 95% in the rest of concentrations overlapped at both LC_{50} and LC_{95}. pH: the highest toxicity of spinosad was observed at pH of 7, 8, 9 and 10, as the pH was lower than 7

or higher than 10, the LC_{50} values increased.; while for spinetoram, the highest toxicity was observed at pH =12 (LC_{50} = 0.040 mg L^{-1}; LC_{95} = 0.13 mg L^{-1}). It is estimated that even though, in some cases, statistical differences were observed, it is unlikely to reflect field control failures.

Keywords: Bioassay, mosquitoes, larvicide, chemical control, spinosyns.

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CONTENIDO

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PÁGINA

INTRODUCCIÓN GENERAL

Las enfermedades al ser humano transmitidas por insectos vectores representan más del 17 % de todas las enfermedades infecciosas, causando más de 700, 000 muertes al año (WHO, 2017). Dentro del orden Diptera, la familia Culicidae, representa un grupo de artrópodos de gran importancia médica. Este grupo incluye varias especies capaces de transmitir importantes enfermedades a los seres humanos como la malaria, filariasis linfática, encefalitis japonesa y la fiebre del oeste del rio Nilo (Jayapriya & Gricilda, 2015; WHO, 2015).

El género *Aedes* spp se caracteriza por ser un importante vector de arbovirus (WHO, 2015); entre estos se encuentran, chikungunya, fiebre amarilla, zika, virus Mayaro, dengue clásico y dengue hemorrágico, esta última transmitida principalmente por *Aedes aegypti* L. (Ringu-Perez *et al.*, 1997: WHO, 2009)

El dengue es la enfermedad viral humana más importante transmitida por insectos vectores. La incidencia y distribución geográfica del dengue se ha incrementado en los últimos años (Ringu-Perez *et al.,* 1997). Mundialmente, a causa del mosquito trasmisor del dengue *Ae. aegypti* más de 3.9 mil millones de personas en más de 128 países están en riesgo de contraer esta enfermedad. Anualmente se estiman 96 millones de casos de infección (Brady *et al.,* 2012; Bhatt *et al*., 2013). En México, durante el año 2018 se registraron 12,706 casos de infección y 45 muertes confirmadas por dengue, el 82 % de los casos confirmados corresponden a Chiapas, Veracruz, Jalisco, Nuevo León y San Luis Potosí. (SNVE, 2018)

La primera línea de control de vectores consiste en la implementación de estrategias de control como la reducción o eliminación física de sitios de reproducción (criaderos) de larvas (Darriet *et al.*, 2010). Estas medidas incluyen el cubrimiento y limpieza de recipientes o depósitos de agua en

dónde se desarrollan los instares acuáticos del mosquito. Cuando no es posible eliminar todos los sitios de reproducción larval se recomienda realizar tratamientos con insecticidas (larvicidas). Para la protección del hogar se recomienda también el uso de mosquiteros, repelentes y materiales impregnados con insecticidas; además de la aplicación de insecticidas en exteriores e interiores contra adultos (WHO, 2011; WHO, 2012).

Para el control biológico de *Ae. aegypti* se han empleado peces del género *Gambusia* spp. los cuales han demostrado ser eficientes depredadores (Griffin, 2014). También se han empleado crustáceos de las siguientes especies *Macrobrachium pantanalense, M. amazonicum, M. brasiliense* y *M. jelskii*, las cuales en condiciones de laboratorio han logrado 100 % de control de todos los instares larvales. En liberación en campo se observó una reducción considerable de adultos (Dourado, 2017).

El control mediante el uso de la Técnica de Insectos Estériles (TIE) es un método que se puede utilizar para el control de vectores de enfermedades. Se trata de control específico, basado en la cría masiva de la especie objetivo y el empleo de la radiación para esterilizar machos para su posterior liberación en campo. La cantidad de machos estériles liberados debe ser alta, lo que permite que machos estériles compitan con machos silvestres por el apareamiento. Si un macho estéril se aparea con una hembra silvestre, no se originará progenie, el resultado es una reducción drástica o en casos extremos, la erradicación de la especie objetivo (Bourtzis *et al.*, 2016; Benelli, 2015).

Un método reciente para el control de mosquitos se basa en enfoques transgénicos. Esta estrategia se puede usar para la supresión de la densidad poblacional y se basa en la liberación continua de machos que llevan un sistema genético que puede reprimirse durante la cría, pero que es letal para

la progenie sin represor (Thomas *et al*., 2000). Los experimentos basados en ensayos empleando estas tecnologías los ha realizado Oxitec® en Brasil, Islas Caimán, México y Malasia. (WHO, 2015). Recientemente, se evaluó una cepa autolimitada de *Ae. aegypti*, OX513A, en la etapa de evaluación de campo en Brasil se observó que liberaciones sostenidas de machos *Ae. aegypti* OX513A condujeron a una supresión de 80 a 96 % de la población silvestre (Carvalho *et al.*, 2015: WHO, 2015).

El uso de agentes de control microbiano como *Bacillus thuringiensis* var. *Israelensis* (Bti), se emplea como un eficiente larvicida, cuya acción insecticida se debe a un cristal paraesporal el cual se constituye por proteínas cristalinas (endotoxinas) las cuales se disuelven en el intestino medio de la larva del mosquito, dañando las células epiteliales y causando la muerte. Sin embargo, se ha demostrado que varias especies de insectos incluido el mosquito, manifestaron resistencia a las proteínas de *Bacillus thuringiensis* var. *israelensis* (Lacey, 2007; Naqqash *et al*., 2016)

El uso de insecticidas sintéticos para el control de *Ae. aegypti*, se ha convertido en un elemento importante y esencial para dicho propósito (Aponte *et al*., 2013). El control del mosquito en la etapa larvaria es necesario y eficiente ya que, durante la etapa inmadura, los mosquitos son relativamente inmóviles y permanecen más concentrados que en la etapa adulta. Sin embargo, algunos efectos colaterales del uso no adecuado de los insecticidas químicos resultaron en contaminación ambiental, efectos sobre organismos no blanco y en el desarrollo de resistencia fisiológica entre las especies de mosquitos vectores (Arivoli *et al*., 2015; Jayapriya & Gricilda, 2015).

Muchos de estos programas de manejo integrado para el control de vectores se basan principalmente en el uso de dos estrategias de control; temefos para el control de larvas e

insecticidas organofosforados y piretroides como adulticidas (Benelli, 2016). En México el uso de insecticidas sintéticos se ha adoptado como una de las principales estrategias de control, dándose el uso de temefos por más de 30 años y permetrina por más de 10 años (Flores *et al*., 2005). El uso de insecticidas piretroides, principalmente deltametrina y permetrina, los cuales han sido utilizados extensamente como adulticidas y representan una fuerte presión de selección en diferentes zonas del país originando poblaciones con desarrollo de resistencia múltiple que ponen en riesgo la eficacia de los piretroides y en consecuencia los programas de control del vector del dengue (Aponte *et al*., 2013). En México se han detectado casos de resistencia a insecticidas organofosforados, carbamatos y piretroides en poblaciones de *Aedes aegypti* (Flores *et al.,* 2006, 2013; Chino-Cantor *et al*., 2014; Flores, 2016). El uso de insecticidas de origen vegetal se ha incrementado en las últimas décadas debido a la baja cantidad de residuos persistentes en el ambiente. Los productos vegetales se consideran un enfoque alternativo potencial, ya que son poco contaminantes para el medio ambiente, específicos para el objetivo y biodegradables (Jayapriya & Gricilda, 2015). Los insecticidas botánicos en combinación con agentes microbiales de biocontrol y reguladores de crecimiento en insectos pueden ser una opción amigable para el ambiente, toxicológicamente segura y aceptable para su implementación en programas de control de vectores (Arivoli *et al*., 2015).

Para atenuar el desarrollo de poblaciones resistentes en campo se ha hecho énfasis en la importancia del manejo de fuentes de desarrollo larval y la influencia de los factores en la expresión de la resistencia hacia algunos insecticidas (Owusu *et al.,* 2017). Para este fin se han incorporado a través de trabajos de investigación nuevos grupos químicos de insecticidas para el control de instares acuáticos, que se caracterizan por ser principalmente reguladores de crecimiento como: metopreno, diflubenzuron y pyriproxyfen, este último ha demostrado que actúa

eficientemente contra pupas. Se ha comprobado que estas moléculas formuladas para su liberación lenta y aplicadas en combinación con *Bacillus thuringiensis* var. *Israelensis* o spinosad son altamente eficaces para el control de mosquitos (Darriet *et al.*, 2010; Alkenani, 2018). Las spinosinas y sus análogos producidos por la fermentación de *Saccharopolyspora spinosa* (Mertz & Yao, 1990), han dado lugar a insecticidas importantes como el spinosad y el spinetoram, mismos que constituyen una herramienta importante para control de larvas debido al bajo impacto ambiental, baja toxicidad a humanos y especies no blanco (Huang *et al.*, 2009). Por tanto, deben considerarse como una herramienta altamente útil para el manejo de vectores en áreas con antecedentes de resistencia a insecticidas.

Con base a la problemática abordada anteriormente, surge la necesidad de evaluar nuevos grupos de insecticidas bajo condiciones adversas, las cuales son muy frecuentes en campo, con el fin de determinar si la toxicidad de estos insecticidas se ve afectada de manera significativa, de modo que se puedan aportar conocimientos que permitan su integración y uso correcto en programas de control de vectores, particularmente mosquitos. En la siguiente sección se abordan los resultados obtenidos a partir de bioensayos con spinosad y spinetoram sobre *Aedes aegypti*, bajo diferentes condiciones de temperatura, contenido de materia orgánica y pH.

LITERATURA CITADA

Alkenani N. A. 2018. Semi-field assessment of Spinosad in combination with Altosid briquetand Dudim DT tablets against *Aedes aegypti* mosquito larvae reared in pond water. Saudi Journal of Biological Sciences. Doi: https://doi.org/10.1016/j.sjbs.2018.06.006

Aponte, H. A., R. P. Penilla, F. Dzul-Manzanilla, A. Che-Mendoza, A. D. López, F. Solis, P. Manrique-Saide, H. Ranson, A. Lenhart, P. J. McCall, and A. D. Rodríguez. 2013. The

pyrethroid resistance status and mechanisms in *Aedes aegypti* from the Guerrero state, Mexico. Pesticide Biochemistry and Physiology 107:226–234.

Arivoli, S., R. Raveen, S. Tennyson, M. Sakthivadivel. 2015. Adult emergence inhibition activity of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaf extracts against *Aedes aegypti* (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). Int. J. Mosq. Res. 2: 24–28.

Benelli, G. 2015. Research in mosquito control: current challenges for a brighter future. Parasitology Research, 114: 2801–2805.

Benelli, G. 2016. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance. Parasitology Research, 115: 23-34.

Bhatt, S, P. W. Gething, O. J. Brady, J. P. Messina1, A.W. Farlow, C. L. Moyes, J. M. Drake, J. S. Brownstein, A. G. Hoen, O. Sankoh, M. F. Myers, D. B. George, T. Jaenisch, G.R. W. Wint, C. P. Simmons, T. W. Scott, J. J. Farrar, and S. I. Hay. 2013. The global distribution and burden of dengue. Nature, 496: 504-507.

Bourtzis, K., R.S. Lees, J. Hendrichs, M.J.B. Vreysen. 2016. More than one rabbit out of the hat: radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. Acta Trop. 157: 115–130.

Brady, O. J, P. W. Gething, S. Bhatt, J. P. Messina, J. S. Brownstein, A. G. Hoen, C. L. Moyes, A. W. Farlow, T. W. Scott, S. I. Hay. 2012. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Neglected Tropical Diseases, 6: 1-15.

Carvalho, D.O., A.R. McKemey, L. Garziera, R. Lacroix, C.A. Donnelly, L. Alphey, A. Malavasi, M.L. Capurro. 2015. Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. PLoS Neglected Tropical Diseases. DOI: 10.1371/journal.pntd.0003864.

Chino-Cantor, A., H. Sanchez-Arroyo, L. D. Ortega-Arenas and E. Castro-Hernández. 2014. Insecticide Susceptibility of *Aedes aegypti* L. (Diptera: Culicidae) in Guerrero, Mexico. Southwestern Entomologist, 39(3): 601-612

Darriet, F., S. Marcombe, M. Etienne, A. Yébakima, P. Agnew, Marie-Michelle Yp-Tcha, V. Corbel. 2010. Field evaluation of pyriproxyfen and spinosad mixture for the control of insecticide resistant *Aedes aegypti* in Martinique (French West Indies). Parasites & Vectors 3: 88.

Dourado, C. W. M., J. de C. Apolinário C., K. D. Saraiva B., W. A. Starke B. 2017. Biological control of *Anopheles darlingi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae using shrimps. Parasite Epidemiology and Control. 2: 91-96.

Flores A, W. Albeldaño-Vásquez, I. Fernández-Salas, M. Badii, H. Loaiza-Becerra, G. Ponce-García, S. Lozano-Fuentes, W. Brogdon, W. Black and B. Beaty. 2005. Elevated α-esterase levels associated with permethrin tolerance in *Aedes aegypti* (L.) from Baja California, México. Pesticide Biochemistry and Physiology, 82: 66-78.

Flores A. E., J. S. Grajales, I. F. Salas, G. P. Garcia, M. H. Becerra, S. Lozano, W. G. Brogdon, W. C. Black WC and B. Beaty. 2006. Mechanism of insecticide resistance in field populations of *Aedes aegypti* (L.) from Quintana Roo, Southern Mexico. Journal of the American Mosquito Control Association, 22(4):672-677.

Flores A. E., G. Ponce, B. G. Silva, S. M. Gutierrez, C. Bobadilla, B. López, R. Mercado and W. C. Black. 2013. Wide spread cross resistance to pyrethoids in *Aedes Aegypti* (Diptera: Culicidae) from Veracruz state Mexico. Journal of Economic Entomology, 106(2): 959-569.

Flores, A. E. 2016. Detección de resistencia a insecticidas en mosquitos con énfasis en *Aedes aegypti*. Artrópodos y Salud 1:21–36.

Griffin, L. 2014. Laboratory evaluation of predation on mosquito larvae by Australian mangrove fish. Journal of Vector Ecology 39: 197–203.

Huang, K., L. Xia, Y. Zhang, X. Ding, J. A. Zahn. 2009. Recent advances in the biochemistry of spinosyns. Applied Microbiology and Biotechnology, 82: 13-23.

Jayapriya, G., S. F. Gricilda. 2015. Bioefficacy of leaf extracts of *Rhinacanthus Nasutus* Linn (Acanthaceae) against *Aedes Aegypti* Linn and *Culex Quinquefasciatus* Say. International Journal of Pharmaceutical Research and Bio-Science 4, 219–233.

Lacey, L.A. 2007. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. Journal of the American Mosquito Control Association. 23: 133–163.

Mertz F.P, Yao R.C.1990 *Saccharopolyspora spinosa* sp. Nov. isolated from soil collected in sugar mill rum still. International Journal of Systematic Bacteriology37:19–22

Naqqash, M.N., A. Gökçe, A. Bakhsh, M. Salim. 2016. Insecticide resistance and its molecular basis in urban insect pests. Parasitology Research. 115: 1363–1373.

Owusu, H. F., N. Chitnis & P. Müller. 2017. Insecticide susceptibility of *Anopheles* mosquitoes changes in response to variations in the larval environment. Scientific Reports DOI:10.1038/s41598-017-03918-z.

Ringu-Perez, J.G., G.G Clark, D. J. Gubler. 1997. Dengue and Dengue hemorrhagic fever. Lancet, 7: 352–971

Sistema Nacional de Vigilancia Epidemiológica (SNVE). 2018. Panorama Epidemiológico de Dengue 2018 - Semana Epidemiológica 52. Disponible en: https://www.gob.mx/salud/documentos/panorama-epidemiologico-de-dengue-2018-semana-epidemiologica-52. (Ultimo acceso 06 de febrero de 2019)

Thomas, D.D., C. A. Donnelly, R. J. Wood, L.S Alphey. 2000. Insect population control using a dominant, repressible, lethal genetic system. Science 287: 2474–2476.

World Health Organization (WHO). 2009. Dengue: Guidelines for diagnosis, treatment, prevention and control. DOI: https://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf. (Ultimo acceso 06 de febrero de 2019).

World Health Organization (WHO). 2011. Operational guide for assessing the productivity of *Aedes aegypti* breeding sites DOI: https://www.who.int/tdr/publications/documents/sop-pupal-surveys.pdf. (Ultimo acceso 06 de febrero de 2019).

World Health Organization (WHO). 2012. Global strategy for dengue prevention and control 2012-2020. DOI: http://apps.who.int/iris/bitstream/10665/75303/1/9789241504034_eng.pdf. (Ultimo acceso 06 de febrero de 2019).

World Health Organization (WHO). 2015. Biosafety for human health and the environment in the context of the potential use of genetically modified mosquitoes (GMMs) 2008-20011. DOI: https://apps.who.int/iris/bitstream/handle/10665/180388/9789241549271_eng.pdf?sequence=1. (Ultimo acceso 06 de febrero de 2019).

World Health Organization (WHO). 2017. Vector borne-diseases. Disponible en: https://www.who.int/en/news-room/fact-sheets/detail/vector-borne-diseases. (Ultimo acceso 06 de febrero de 2019).

CHAPTER I. EFFECT OF TEMPERATURE, ORGANIC MATTER AND pH IN THE TOXICITY OF SPINOSAD AND SPINETORAM AGAINST LARVAE OF *Aedes aegypti* (DIPTERA: CULICIDAE)

RESUMEN

El mosquito, *Aedes aegypti* L. (Diptera: Culicidae), por su capacidad para transmitir una amplia variedad de arbovirus, es la especie más peligrosa para el ser humano. El objetivo de este estudio fue evaluar el efecto de la temperatura, contenido de materia orgánica y pH en la toxicidad de spinosad y spinetoram en larvas de la población de referencia New Orleans de *Ae. aegypti*. El método de bioensayo utilizado fue el propuesto por la Organización Mundial de la Salud. Inicialmente se determinaron las tasas de supervivencia de esta especie a un rango de valores de estas variables. Posteriormente, se realizaron dentro de estos rangos, bioensayos para estimar la toxicidad de ambos insecticidas. Temperatura: la CL_{50} varió de 0.041 (1.0×; 25 °C) a 0.053 mg L^{-1} (1.29×; 30 °C) y de 0.10 (0.83×; 30 °C) a 0.12 mg L^{-1} (1.0×25 °C) para spinosad y spinetoram, respectivamente. Materia orgánica (MO): la CL_{50} de spinosad fluctuó entre 0.064 (1.0×; 0% MO) y 0.22 mg L^{-1} (3.43×; 13% MO); en spinetoram, la menor toxicidad se observó con 0% de materia orgánica (CL_{50} =0.095 mg L^{-1}; CL_{95} =0.36 mg L^{-1}); los límites fiduciales al 95% se traslaparon para el resto de concentraciones tanto a nivel de CL_{50} como de CL_{95}. pH: la mayor toxicidad del spinosad se observó a valores de 7, 8, 9 y 10; mientras que para spinetoram, la mayor toxicidad se observó a pH = 12 (CL_{50} = 0.040 mg L^{-1}; CL_{95} = 0.13 mg L^{-1}). Se estima que, a pesar de que en algunos casos se observaron diferencias estadísticas, es poco probable que se reflejen faltas de control en campo.

Palabras clave: Bioensayo, mosquitos, larvicida, control químico, spinosinas.

ABSTRACT

The mosquito, *Aedes aegypti* L. (Diptera: Culicidae), for its ability to transmit a wide variety of arboviruses, is the most dangerous species to humans. This study aimed to evaluate the effect of temperature, organic matter content, and pH on spinosad and spinetoram toxicity on early fourth instar larvae of the New Orleans strain of *Ae. aegypti*. The bioassay method used was the one proposed by the World Health Organization. Initially, the survival rate of this species to a range of values of these variables were determined. Subsequently, between these rates, bioassays were carried out to estimate the toxicity of both insecticides. Temperature: the LC_{50} ranged from 0.041 (1.0×; 25 °C) to 0.053 mg L^{-1} (1.29×; 30 °C) and from 0.10 (0.83×; 30 °C) to 0.12 mg L^{-1} (1.0×; 25 °C) for spinosad and spinetoram, respectively. Organic matter (OM): the spinosad LC_{50} fluctuated between 0.064 (1.0×; 0% OM) and 0.22 mg L^{-1} (3.43×; 13% OM); in spinetoram, the lowest toxicity was observed at 0% organic (LC_{50} = 0.095 mg L^{-1}; LC_{95} =0.36 mg L^{-1}), the fiducial limits at 95% in the rest of concentrations overlapped at both LC_{50} and LC_{95}. pH: the highest toxicity of spinosad was observed at pH of 7, 8, 9 and 10, as the pH was lower than 7 or higher than 10, the LC_{50} values increased.; while for spinetoram, the highest toxicity was observed at pH =12 (LC_{50} = 0.040 mg L^{-1}; LC_{95} = 0.13 mg L^{-1}). It is estimated that even though, in some cases, statistical differences were observed, it is unlikely to reflect field control failures.

Keywords: Bioassay, mosquitoes, larvicide, chemical control, spinosyns.

1.1 INTRODUCTION

The mosquito, *Aedes aegypti* L., (Diptera: Culicidae), has become the most dangerous animal species for humans due to the frequency and severity of the viral diseases it transmits (Darriet et al. 2010). This species is widely distributed in tropical and subtropical areas of Mexico that are located at altitudes below 1700 AMSL (Ibáñez-Bernal 1987). However, in September 2015, the presence of larvae was detected in two locations in Mexico City, at 2,250 meters above the average sea level (Kuri-Morales et al. 2017). Therefore, it is expected that in the short term this species may represent a new threat to nearly 20 million people living in this city.

Despite control actions, the management of this vector poses important challenges such as the outbreaks of diseases to the human being, development of resistance to insecticides, and the expansion of the geographical zones that it infests (Arivoli et al. 2015; Benelli et al. 2017). In Mexico, combating this species is mainly based on the destruction of the larval habitat and the use of insecticides. Currently, society demands the use of effective insecticides with less adverse effects on health and the environment (Benelli et al. 2017). An alternative to these demands is the use of bioinsecticides such as spinosad and spinetoram. Spinosad is an insecticide composed of a mixture of two metabolites (Spinosyn A and D) that are derived from the fermentation of the bacteria (Actinomycete), *Saccharopolyspora spinosa* Mertz & Yao, 1977 (Salgado 1998). Spinetoram is a molecule of the Spinosyn family that is effective against different pest species (Sparks et al. 2008; Vassilakos et al. 2012; Vassilakos & Athanassiou 2013) and consists of a mixture of two synthetically modified spinosyns (Spinosyn J and L). Both insecticides share the same mode of action and act on the nicotinic acetylcholine receptor (nACh), which is different from that of neonicotinoids (Watson et al. 2010, Dripps et al. 2011). However, to optimize the performance in the field of these insecticides, it is important to know the impact that

environmental variables have on their toxic potency. Therefore, this study aimed to evaluate the toxicity of spinosad and spinetoram on early fourth instar larvae of *A. aegypti* in different conditions of temperature, organic matter content, and pH.

1.2 MATERIALS AND METHODS

1.2.1 Experimental locations

The study was conducted, under laboratory conditions, from Mar 2018 to Oct 2018, at the facilities of the Toxicology Area of the College of Postgraduate, Campus Montecillo, State of Mexico.

1.2.2 *Aedes aegypti*.

We used the New Orleans strain of *Ae. aegypti*, which is characterized by being susceptible to insecticides. The Faculty of Biological Sciences of the Autonomous University of Nuevo Leon, Mexico, provided this population.

1.2.3 Insecticides

Two commercial formulations of insecticides were evaluated: Natular® (Spinosad, emulsifiable concentrate, 20.6%, 239 g of a.i. L^{-1}, Clarke Mosquito Control Products, Inc., Mexico) and Exalt® (Spinetoram, concentrated suspension, 5.87%, 60 g of a.i. L^{-1}, Dow AgroSciences, Mexico). Serial dilutions were prepared using distilled water.

1.2.4 Study variables.

Before the bioassays with insecticides, the capacity range of the larvae to support, without visible adverse effects, different temperature degrees, organic matter content, and pH levels, was

evaluated. In containers of 127 mL capacity, with 100 mL of drinking water (Bonafont®), 20 early fourth instar larvae were added. After 24 h of exposure to the respective treatment, the mortality percentage was evaluated. In total, five replications of each treatment were carried out, and the experimental units were maintained in controlled environmental conditions: photoperiod 12:12 h L: D and 25 ± 2 °C. In the evaluation of the effect of temperature, the indicated photoperiod was maintained at different temperature values.

1.2.4.1 Temperature.

Bioclimatic chambers (Thermo Scientific®) were individually adjusted to the desired temperature. Plastic disposable transparent cups with 100 mL of drinking water, were set up inside the bioclimatic chambers, for six hours. To verify the temperature values, a thermometer (Hanna instruments®, HI98128) was placed inside these cups. Initially, the survival of larvae was evaluated for a period of 24 h, at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C of temperature.

1.2.4.2 The Content of Organic Matter.

A commercial vermicomposting was used, which is sold in bulk without a trademark. The compost was homogenized with a No. 25 mesh of 0.707 mm.). The following concentrations (weight / volume) of organic matter were prepared, using drinking water as diluent: 0 (control), 1.0, 4.7, 9.0, 13.0, 16.6 and 23%.

1.2.4.3 pH.

The pH was measured with a potentiometer (Hanna instruments®, HI98128). To adjust the pH, phosphoric acid (H_2PO_4, 85%, Mexichem S.A.B. de C.V. Mexico) or sodium hydroxide (NaOH, 98%, J. T. Baker, Sweden) was used. The survival of larvae of *Ae. aegypti* at pH values of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 was recorded.

1.2.5 Bioassays.

Bioassays were carried out with the indicated insecticides, at different values of temperature, organic matter content, and pH, within the survival ranges *Ae. aegypti* larvae, using the methodology of the World Health Organization (WHO 2005). Initially, the range of concentrations in which there was zero and 100% mortality was determined (biological response window). Subsequently, seven intermediate equidistant concentrations covering that range were included.

In containers with a capacity of 127 mL, with 100 mL of drinking water and 20 larvae of the fourth early instar larvae, 1 mL of the required concentration of insecticide was added. After 24 h of exposure, the mortality percentage was evaluated. A larva was considered dead if it lacked mobility or it exhibited abnormal movements (WHO 2005; Chino-Cantor et al. 2014). In total, five replications were performed on a different day, and each replication included an untreated control. The maximum accepted mortality for the control was 10%, and this was adjusted with Abbott´s formula (1925). The experimental units were kept under controlled environmental conditions: 25 ± 2 °C and a photoperiod of 12:12 h L:D. For the case of evaluations at different temperatures, the experimental units were kept at the required temperature (± 2 °C) and a photoperiod of 12:12 h L:D.

1.2.6 Statistical Analysis.

To estimate the lethal concentration at 50 (LC_{50}) and 95% mortality (LC_{95}), and their respective fiducial limits at 95% reliability, a Probit analysis was performed using the PROC PROBIT procedure of the statistical software SAS 9.4 (SAS Institute 2016). The larval response was considered different when the fiducial limits did not overlap (Robertson & Preisler 1992). The susceptibility index (SI), was obtained by dividing the $LC_{50\ (95)}$ of the respective variable value, between the $LC_{50\ (95)}$ of the value that was considered as the basis of comparison. For temperature, organic matter content and pH, the reference values were 25 °C, 0%, and 7, respectively. The criterion of Mazzarri and Georghiou (1995) was used to estimate if the differences observed in the IS_{50} and IS_{95}, may reflect significant deviation in the response that could impair field efficacy. This criterion considers that a variation <5× would not affect the effectiveness of the insecticide in the field; between 5 and 10× there would be a moderate resistance and >10× there would be no control in the field due to the expression of high resistance.

1.3 RESULTS

1.3.1 Temperature.

At temperatures of 5 °C or above 40 °C, 100% mortality of larvae was obtained (Table 1). At 10 and 15 °C, there was no mortality, but the larvae showed a significantly reduced mobility, and this condition could interact with the toxic potency of spinosad and spinoteram; therefore, they were not included in the evaluation of insecticides. Therefore, Spinosad and Spinetoram were evaluated at 20, 25, 30 and 35 °C (Table 1).

1.3.2 The Organic Matter Content.

No adverse effects of the organic matter were observed on all the evaluated concentrations: 0 (control), 1.0, 4.7, 9.0, 13.0, 16.6 and 23% (weight / volume). It was not possible to evaluate higher concentrations of organic matter because there was not enough free water left for larval development.

1.3.3 pH.

Mortality of larvae at the pH of 2, 3, 13 and 14 was observed (Table 2). There was no evidence of visible adverse effects in the pH range of 4 to 12. Therefore, the toxicity of spinosad and spinetoram in the following pH values was evaluated: 4, 5, 6, 7, 8, 9, 10, 11 and 12.

1.3.4 Bioassay with insecticides

1.3.4.1 Temperature.

Spinosad. The LC_{50} value for spinosad ranged from 0.042 to 0.053 mg L^{-1}, and overlap of fiducial limits was observed at 20 and 25 °C (Table 3). The values of the LC_{50} at 30 and 35 °C overlapped each other and were statistically different from those observed at 20 and 25 °C. A variation in IS_{50} values from 1.0 to 1.29× was observed (Table 3). At the LC_{95} level, the variation in response was between 0.17 and 0.19 mg L^{-1}, the fiducial limits, at all the temperature levels evaluated, overlapped and the variation of IS_{95} values ranged from 1.0 to 1.05× (Table 3).

Spinetoram. The LC_{50} for spinetoram varied from 0.10 to 0.12 mg L^{-1} and overlap of fiducial limits was observed in the four temperature levels evaluated, with values of IS_{50} between 0.83 and 1.0× (Table 4). A Similar situation was observed at the level of the LC_{95}, which ranged from 0.35 to 0.50 mg L^{-1}, and the IS_{95} values were between 0.7 and 1.0×.

1.3.4.2 The Organic Matter Content.

Spinosad. The LC_{50} of spinosad fluctuated between 0.064 and 0.22 mg L^{-1}, which corresponds to ranges of IS_{50} from 1.0 to 3.43× (Table 5). The lowest toxic potency was observed at 13% of organic matter (LC_{50} = 0.22 mg L^{-1}, IS_{50} = 3.43×). At the LC_{95} level, values were between 0.23 and 0.92 mg L^{-1} (Table 5) and the lowest toxicity was also observed at 13% organic matter (LC_{95} = 0.92 mg L^{-1}; IS_{95} = 4.0×), with IS_{95} values that ranged from 1 (0% organic matter) to 4.0× (13% organic matter) (Table 5).

Spinetoram. The lowest toxicity values were observed at 0% organic matter, both at the LC_{50} (0.095 mgL^{-1}) and the LC_{95} (0.36 mg L^{-1}) levels (Table 6). The 95% fiducial limits for the rest of the evaluated levels of organic matter overlapped, at both LC_{50} and LC_{95}, indicating that there was no significant difference between them (Table 6).

1.3.4.3 pH.

Spinosad. The highest toxicity was observed at pH values of 7 (LC_{50} = 0.032 mg L^{-1}), 8 (LC_{50} = 0.031 mg L^{-1}), 9 (LC_{50} = 0.026 mg L^{-1}) and 10 (LC_{50} = 0.028 mg L^{-1}). The IS_{50} presented values between 0.81 and 1.0× (Table 7). As the pH was lower than 7 or higher than 10, the LC_{50} values increased, indicating a decrease in the toxic potency of spinosad (Table 7). At the LC_{95} level, the highest toxicity was observed at pH 6 (LC_{95} = 0.15 mg L^{-1}), 7 (LC_{95} = 0.11 mg L^{-1}), 8 (LC_{95} =

0.12 mg L^{-1}), 9 (LC_{95} = 0.10 mg L^{-1}) and 10 (LC_{95} = 0.11 mg L^{-1}). With pH values lower or higher than indicated, the spinosad toxicity decreased. The variation of IS_{95} was 1.0 to 2.72× (Table 7).

Spinetoram. The LC_{50} values obtained varied between 0.040 mg L^{-1} (pH = 12) and 0.2 mg L^{-1} (pH = 4). The IS_{50} ranged between 0.33 and 1.66× (Table 8). The LC_{95} values were in the range of 0.13 mg L^{-1} (pH = 12) and 0.71 mg L^{-1} (pH = 4). The IS_{95} ranged from 0.33 to 1.82×. In both mortality levels (50 and 95%), establishing the pH of 7 as the reference, the toxicity showed a tendency to reduce as the pH approached 4 and to increase as the pH approached 12 (Table 8).

Although in some cases, a reduction in the toxic potency of both spinosad and spinetoram at different temperature levels, organic matter content and pH was observed, it is considered that is not high enough to cause an alert of their biological field effectiveness against larvae of *A. aegypti*. In those cases, the reduction in toxicity is not comparable to that experienced by insecticide resistant populations, according to the criteria of Mazzarri and Georghiou (1995).

1.4 DISCUSSION

Su & Cheng (2014a) selected a population of *Culex quinquefasciatus* Say, with spinosad for 45 generations, and a significant increase in the proportion of resistance was observed. The LC_{50} went from 1,415 to 2,229×, while the LC_{90} rose from 9,613 to 17,062×. In this case, the evaluated population was considered resistant to spinosad and these bioassays values correlated with field failure, in agreement with the criteria of Mazzarri & Georghiou (1995).

The environmental variables not only can reduce the toxic potency of insecticides, but also increase the severity of the pest or facilitate extending its geographical range. For example, the

increase in temperature over time tends to be more severe in urban areas due to the "heat island" effect, where building materials, and vehicle use which release pollutants, further raise the temperature in comparison of similar places covered with vegetation (Rosenthal et al. 2007). This scenario increases the risk of *Ae. aegypti* invasion to new geographical areas or increase the frequency of disease transmission where it is already present (Vieira et al. 2015). In Mexico City, Barradas (1991) confirm differences of up to 5.6 °C between urban areas with vegetation and built-up areas, and this may be the factor that recently allowed *Ae. aegypti* to invade this huge urban area (Kuri-Morales et al. 2017).

It has been shown that the soil temperature correlates with better development of larvae of this pest, which translates into more adults and therefore more frequent diseases transmitted (Vieira et al. 2015). The temperature may significantly modify field efficacy of some insecticides (Johnson 1990; Vieira et al., 2015). For example, the toxicity of organophosphorus and pyrethroid type II insecticides increases with increasing temperature, while the opposite occurs with the type I pyrethroids (Johnson 1990). In other cases, temperature does not affect the toxic potency of an insecticide. Athanassiou et al. (2008), as well as Vassilakos & Athanassiou (2013), indicated that in *Rhyzopertha dominica* Fabricius; *Sitophilus oryzae* L. and *Tribolium confusum* Jacquelin du Val, the temperature has little effect on the toxicity of spinosad and spinetoram, in agreement with this study.

The bodies of water, where larvae of *Ae. aegypti* develop, are characterized by being stagnant and having low organic matter contents (Leyva et al., 2016). However, it cannot be ruled out that this species expands its distribution zone, developing under conditions of the higher amount of organic matter as happened with *C. quinquefasciatus* (Salazar & Moncada 2004; Leyva et al. 2016). Studies conducted by Li et al. (2018) on the absorption coefficient of clothianidin and

thiamethoxam (neonicotinoids) indicate that the higher the organic carbon (organic matter) content and soil pH, the higher the adsorption coefficient. Ping et al. (2010) documented that, in soils treated with exogenous humic acids, imidacloprid adsorption was greater at low pH and temperature. In the present study, a slight decrease in spinosad toxicity was observed in water with high contents of organic matter, indicating a certain degree of adsorption; but in the case of spinetoram, no significant effect of organic matter was observed, as was the case with the study conducted by Ping et al. (2010) with imidacloprid.

The pH can have important effects on the toxicity of several insecticides. In studies conducted by Aktar et al. (2010) with methomyl, they had observed slightly higher dissipation rates with at an alkaline pH (9.2); thus, faster degradation and reduction of useful half-life under field conditions. Liu and Li (2004) indicated that the photodegradation of spinosyn A and D was slower in an aqueous acidic solution, compared to an alkaline solution. In this research, the toxicity of spinetoram was not affected by the pH levels evaluated, which agrees with Dripps et al. (2008).

The pH values of the water infested with larvae, of this and other species, may have important variations. For example, preliminary observations indicated that the bodies of water of Lake Texcoco, State of Mexico, have a pH of 8.7 to 9.7. We estimate that this pH value would not be an obstacle for *Ae. aegypti* to invade this area, which is currently at a distance of no more than 20 km from Mexico City. Under this condition, we also expect that the biological performance of both spinosad and spinetoram is not affected. However, spinosad and spinoteram are highly prone to resistance (Su & Cheng 2014b). Therefore, their use must be rational and within an integrated pest management program that considers the appropriate rotation of insecticide groups that are effective and lack of cross-resistance. Su & Cheng (2014b) documented that in *C. quinquefasciatus,* the presence of spinosad resistance does not affect the performance of *Bacillus*

thuringiensis israelensis (Bti), methoprene, pyriproxyfen, diflubenzuron, novaluron, temephos, and imidacloprid.

It is possible that a field application of spinosad or spinoteram at different conditions of temperature, organic matter content, and pH, may affect the length of the protection. However, it is unlikely to affect the intensity of their toxic potency since the range of response in all cases were <5×, which coincides selection studies with spinosad (Su & Cheng, 2014a) and spinetoram (Su & Cheng, 2014b). However, other conditions could also affect the field performance of the biological effectiveness of the insecticides used to combat *Ae. aegypti*. According to Vassilakos & Athanassiou (2013), the protection period is also affected by the quality of the commercial formulation and the resistance level of the target pest. Therefore, the use of chemicals against this pest must be based on the results of samplings, and field evaluations of the insecticides intended to be used. Laboratory bioassays generate early warnings of possible adverse effects of environmental variables on the toxic potency of the insecticides that are integrated into pest control programs.

Spinosad and Spinetoram are promising alternatives for the control of *A. aegypti* larvae, given its efficacy in the field, which can last from six to 13 weeks (Marina et al. 2011) and the fact that it does not affect pollinators or biological control agents (Williams & Valle 2003).

1.5 CONCLUSION

The response to spinosad and spinoteram in the fourth early instar larvae of *Ae. aegypti*, under a wide range of temperature (20-35 °C) organic matter (0-28 % p/v) content and pH (4-12), showed a small variation as reflected on susceptibility index <5×. Thus, the toxic potency of both insecticides is not significantly influenced by the evaluated variables.

1.6 ACKNOWLEDGE

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1.7 TABLES

Table 1. Effect of temperature on the mortality of the fourth early instar larva of the New Orleans strain of *Aedes aegypti* L.

Temperature	Mortality (%)						Average
	I	II	III	IV	V	Total	mortality ± EE[a]
5	100	100	100	100	100	100	100
10	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0
40*	5	10	10	5	5	7	7 ± 1.2
45	100	100	100	100	100	100	100
50	100	100	100	100	100	100	100

n= Number of larvae treated per replication =20; [a] Standard error

* Adverse effect on the larva of the fourth instar in the value of 40 °C was observed

Table 2. Effect of pH on the mortality of the fourth early instar larva of the New Orleans strain of *Aedes aegypti* L.

pH	Mortality (%)						Average mortality ± EE[a]
	I	II	III	IV	V	Total	
2	100	100	100	100	100	100	100
3*	50	50	35	65	60	52	52 ± 5.1
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
13*	100	80	95	95	90	92	92 ± 3.4
14	100	100	100	100	100	100	100

n = Number of larvae treated per replication = 20; [a] Standard error

* Adverse effect on the larva of fourth instar was observed in the values of 3 and 13

Table 3. Toxicity of spinosad on early fourth instar larvae of the New Orleans strain of *Aedes aegypti* L. at different temperature levels.

Temperature (°C)	n^a	gl^b	b±EE[c]	LC_{50}[d] (95% LF[f]) mg L^{-1}	LC_{95}[e] (95% LF[f]) mg L^{-1}	Pr > χ^{2}[g]	[h]IS_{50}	[h]IS_{95}
20	700	5	2.51±0.17	0.042 (0.038-0.046)	0.19 (0.15-0.24)	0.21	1.02	1.11
25	700	5	2.64±0.16	0.041 (0.037-0.045)	0.17 (0.14-0.21)	0.65	1.00	1.00
30	700	5	3.05±0.19	0.053 (0.048-0.058)	0.18 (0.15-0.22)	0.33	1.29	1.05
35	600	4	3.22±0.21	0.053 (0.049-0.058)	0.17 (0.14-0.21)	0.42	1.29	1.00

[a]Total larvae treated; [b]Degrees of freedom; [c]Slope ± standard error; [d]Estimated concentration that causes 50% mortality; [e]Estimated concentration that causes 95% mortality, [f]95% fiducial limits, [g]Probability greater than χ^2= fitting test to a straight line, [h]Susceptibility index = $LC_{50\ (95)}$ test at 20 30 and 35 °C / $LC_{50\ (95)}$ of the assay at 25 °C

Table 4. Toxicity of spinetoram on early fourth instar larvae of the New Orleans strain of *Aedes aegypti* L. at different temperature levels.

Temperature (°C)	n^a	gl^b	b±EE[c]	LC_{50}[d] (95% LF[f]) mg L^{-1}	LC_{95}[e] (95% LF[f]) mg L^{-1}	Pr > χ^{2}[g]	[h]IS_{50}	[h]IS_{95}
20	800	6	3.01±0.17	0.11 (0.10-0.12)	0.40 (0.35-0.49)	0.20	0.92	0.80
25	700	5	2.80±0.19	0.12 (0.11-0.14)	0.50 (0.42-0.62)	0.73	1.00	1.00
30	800	6	3.08±0.18	0.10 (0.095-0.11)	0.35 (0.30-0.41)	0.53	0.83	0.70
35	800	6	2.63±0.16	0.10 (0.094-0.11)	0.43 (0.36-0.54)	0.95	0.83	0.86

[a]Total larvae treated; [b]Degrees of freedom; [c]Slope ± standard error; [d]Estimated concentration that causes 50% mortality; [e]Estimated concentration that causes 95% mortality, [f]95% fiducial limits, [g]Probability greater than χ^2= fitting test to a straight line, [h]Susceptibility index = $LC_{50\,(95)}$ test at 20 30 and 35 °C / $LC_{50\,(95)}$ of the assay at 25 °C

Table 5. Toxicity of spinosad on early fourth instar larvae of the New Orleans strain of *Aedes aegypti* L. at different levels of organic matter content.

Organic matter (%)	n^a	gl^b	b±EE[c]	LC_{50}[d] (95% LF[f]) mg L^{-1}	LC_{95}[e] (95% LF[f]) mg L^{-1}	Pr > χ^{2}[g]	[h]IS_{50}	[h]IS_{95}
0.0	700	5	2.89±0.17	0.064 (0.058-0.070)	0.23 (0.20-0.29)	0.62	1.00	1.00
1.0	600	4	3.12±0.21	0.14 (0.12-0.15)	0.47 (0.39-0.58)	0.97	2.18	2.04
4.7	600	4	3.12±0.21	0.18 (0.17-0.20)	0.62 (0.53-0.77)	0.43	2.81	2.69
9.0	600	4	2.70±0.19	0.17 (0.15-0.19)	0.70 (0.57-0.89)	0.71	2.65	3.04
13.0	700	5	2.65±0.17	0.22 (0.20-0.24)	0.92 (0.77-1.15)	0.37	3.43	4.00
16.6	600	4	2.77±0.20	0.17 (0.16-0.19)	0.69 (0.57-0.88)	0.29	2.65	3.00
23.0	700	5	2.67±0.17	0.17 (0.15-0.19)	0.71 (0.60-0.88)	0.15	2.65	3.08

[a]Total larvae treated; [b]Degrees of freedom; [c]Slope ± standard error; [d]Estimated concentration that causes 50% mortality; [e]Estimated concentration that causes 95% mortality, [f]95% fiducial limits, [g]Probability greater than χ^2= fitting test to a straight line, [h]Susceptibility index = $LC_{50\ (95)}$ of organic matter / $LC_{50(95)}$ test without organic matter.

Table 6. Toxicity of spinetoram on early fourth instar larvae of the New Orleans strain of *Aedes aegypti* L. at different levels of organic matter content.

Organic matter (%)	n^a	gl^b	b±EE[c]	LC_{50}[d] (95% LF[f]) mg L^{-1}	LC_{95}[e] (95% LF[f]) mg L^{-1}	Pr > χ^{2}[g]	[h]IS_{50}	[h]IS_{95}
0.0	800	6	2.80±0.17	0.095 (0.087-0.0104)	0.36 (0.31-0.44)	0.93	1.00	1.00
1.0	800	6	2.71±0.16	0.060 (0.055-0.065)	0.24 (0.20-0.30)	0.99	0.63	0.67
4.7	800	6	2.71±0.16	0.061 (0.055-0.066)	0.24 (0.20-0.30)	0.15	0.64	0.67
9.0	800	6	2.92±0.17	0.054 (0.050-0.059)	0.20 (0.17-0.24)	0.61	0.57	0.56
13.0	700	5	3.85±0.24	0.069 (0.064-0.074)	0.18 (0.16-21)	0.90	0.73	0.50
16.6	700	5	3.93±0.24	0.065 (0.061-0.070)	0.17 (0.15-0.20)	0.90	0.68	0.47
23.0	700	5	4.05±0.25	0.068 (0.063-0.072)	0.17 (0.15-0.20)	0.88	0.72	0.47

[a]Total larvae treated; [b]Degrees of freedom; [c]Slope ± standard error; [d]Estimated concentration that causes 50% mortality; [e]Estimated concentration that causes 95% mortality, [f]95% fiducial limits, [g]Probability greater than χ^2= fitting test to a straight line, [h]Susceptibility index = $LC_{50(95)}$ of organic matter / $LC_{50(95)}$ test without organic matter.

Table 7. Toxicity of spinosad on early fourth instar larvae of the New Orleans strain of *Aedes aegypti* L. at different pH levels.

pH	n^a	gl^b	b±EE[c]	LC_{50}[d] (95% LF[f]) mg L^{-1}	LC_{95}[e] (95% LF[f]) mg L^{-1}	Pr > χ^2[g]	[h]IS_{50}	[h]IS_{95}
4	800	6	2.92±0.17	0.083 (0.076-0.092)	0.30 (0.25-0.37)	0.76	2.59	2.72
5	700	5	2.94±0.19	0.065 (0.059-0.072)	0.23 (0.19-0.30)	0.10	2.03	2.09
6	700	5	3.17±0.19	0.046 (0.042-0.051)	0.15 (0.13-0.18)	0.65	1.43	1.36
7	700	5	3.12±0.19	0.032 (0.030-0.035)	0.11 (0.095-0.13)	0.13	1.00	1.00
8	600	4	2.77±0.21	0.031 (0.028-0.034)	0.12 (0.10-0.15)	0.48	0.96	1.09
9	600	4	2.76±0.20	0.026 (0.023-0.029)	0.10 (0.086-0.13)	0.58	0.81	0.90
10	700	5	2.77±0.17	0.028 (0.025-0.030)	0.11 (0.093-0.13)	0.78	0.87	1.00
11	700	5	2.73±0.17	0.048 (0.043-0.052)	0.19 (0.15-0.24)	0.29	1.50	1.72
12	700	5	2.69±0.17	0.035 (0.032-0.038)	0.14 (0.12-0.17)	0.58	1.09	1.27

[a]Total larvae treated; [b]Degrees of freedom; [c]Slope ± standard error; [d]Estimated concentration that causes 50% mortality; [e]Estimated concentration that causes 95% mortality, [f]95% fiducial limits, [g]Probability greater than χ^2= fitting test to a straight line, [h]Susceptibility index = $LC_{50\ (95)}$ test at different pH levels / $LC_{50\ (95)}$ with pH = 7.

Table 8. Toxicity of spinetoram on early fourth instar larvae of the New Orleans strain of *Aedes aegypti* L. at different pH levels.

pH	n^a	gl^b	b±EE[c]	LC_{50}[d] (95% LF[e]) mg L^{-1}	LC_{95}[d] (95% LF[e]) mg L^{-1}	Pr > χ^{2}[f]	[g]IS_{50}	[g]IS_{95}
4	800	6	3.09±0.18	0.20 (0.19- 0.22)	0.71 (0.61-0.85)	0.14	1.66	1.82
5	800	6	3.19±0.18	0.17 (0.16-0.19)	0.57 (0.49-0.68)	0.45	1.42	1.46
6	700	5	3.23±0.20	0.16 (0.15-0.17)	0.52 (0.44-0.63)	0.76	1.33	1.33
7	700	5	3.22±0.20	0.12 (0.11-0.13)	0.39 (0.33-0.47)	0.96	1.00	1.00
8	700	5	3.43±0.21	0.10 (0.092-0.108)	0.30 (0.26-0.36)	0.62	0.83	0.76
9	700	5	3.52±0.22	0.11 (0.105-0.123)	0.33 (0.28-0.40)	0.21	0.91	0.85
10	700	5	3.38±0.21	0.096 (0.089-0.104)	0.29 (0.25-0.35)	0.86	0.80	0.74
11	700	5	3.08±0.19	0.049 (0.045-0.053)	0.16 (0.14-0.20)	0.25	0.41	0.41
12	800	6	3.16±0.18	0.040 (0.037-0.043)	0.13 (0.11-0.15)	0.76	0.33	0.33

[a]Total larvae treated; [b]Degrees of freedom; [c]Slope ± standard error; [d]Estimated concentration that causes 50% mortality; [e]Estimated concentration that causes 95% mortality, [f]95% fiducial limits, [g]Probability greater than χ^2= fitting test to a straight line, [h]Susceptibility index = $LC_{50\ (95)}$ test at different pH levels / $LC_{50\ (95)}$ with pH = 7.

REFERENCES CITED

Abbott WS. 1925. A Method of Computing the Effectiveness of an Insecticide. Journal of Economic Entomology 18: 265–267.

Aktar W, Alam S, Sengupta D, Chowdhury A. 2010. Degradation dynamics of an oxime carbamate insecticide (methomyl) in aqueous medium of varying pH under laboratory simulated condition. The Electronic Journal of Chemistry 2: 225-234

Arivoli S, Raveen R, Tennyson S, Sakthivadivel M. 2015. Adult emergence inhibition activity of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaf extracts against Aedes aegypti (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). International Journal of Mosquito Research 2: 24-28.

Athanassiou CG, Kavallieratos NG, Yiatilis AE, Vayias BJ, Mavrotas CS, Tomanovic Z. 2008. Influence of temperature and humidity on the efficacy of spinosad against four stored-grain beetle species. Journal of Insect Science 8:1-9.

Barradas VL. 1991. Air temperature and humidity and human comfort index of some city parks of Mexico City. International Journal of Biometeorology 35: 24-28.

Benelli G, Caselli A, Canale A. 2017. Nanoparticles for mosquito control: Challenges and constraints Nanoparticles for mosquito control. Journal of King Saud University-Science 29: 424-435.

Chino-Cantor A, Sánchez-Arroyo H, Ortega-Arenas LD, Castro-Hernández E. 2014. Insecticide Susceptibility of *Aedes aegypti* L. (Díptera: Culicidae) in Guerrero, México. Southwestern Entomologist 39: 601-612.

Darriet F, Marconbe S, Etienne M, Yebakima A, Agnew P, Yp-Tcha M, Corbel V. 2010. Field evaluation of pyriproxyfen and spinosad mixture for the control of insecticide resistant *Aedes aegypti* in Martinique (French West Indies). Parasites & Vectors 3: 88.

Dripps JE, Boucher RE, Chloridis A, Cleveland CB, DeAmicis CV, Gomez LE, Paroonagian DL, Pavan LA, Sparks TC, Watson GB. 2011. The spinosyn insecticides. In: Lopez O, Fernandez-Bolanos JG. (Eds.), Green Trends in Insect Control. Royal Society of Chemistry, Cambridge, UK, pp. 163-212.

Dripps J, Olson B, Sparks T, Crouse G. 2008. Spinetoram: how artificial intelligence combined natural fermentation with synthetic chemistry to produce a new spinosyn insecticide. Plant Health Progress. doi:10.1094/PHP-2008-0822-01-PS. (last accessed 15 Jan 2019).

Ibáñez-Bernal S. 1987. Nuevo registro altitudinal de *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera: Culicidae) en México. Folia Entomológica Mexicana 72: 163-164.

Johnson DL. 1990. Influence of temperature on the toxicity of two pyrethroids to grasshoppers (Orthoptera: Acrididae). Journal of Economic Entomology 83: 366-373.

Kuri-Morales P, Correa-Morales F, González-Acosta C, Sánchez-Tejeda G, Dávalos-Becerril E, Juárez-Franco MF, Díaz-Quiñonez A, Huerta-Jimenéz H, Mejía-Guevara MD, Moreno-García M, González-Roldán JF. 2017. First report of *Stegomyia aegypti* (= *Aedes aegypti*) in Mexico City, Mexico. Medical and Veterinary Entomology 31: 240-242.

Leyva IM, María DC, Montada LD. 2016. Segregation of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) niche under laboratory conditions. Revista Cubana de Medicina Tropical 64: 206-211.

Li Y, Su P, Li Y, Wen K, Bi G, Cox M. 2018. Adsorption-desorption and degradation of insecticides clothianidin and thiamethoxam in agricultural soils. Chemosphere 207: 708-714.

Liu S, Li QX. 2004. Photolysis of spinosyns in seawater, stream water and various aqueous solutions. Chemosphere 56: 1121-1127.

Marina CF, Bond JG, Casas M, Muñoz J, Orozco A, Valle J, Williams T. 2011. Spinosad as an effective larvicide for control of *Aedes albopictus* and *Aedes aegypti*, vectors of dengue in southern Mexico. Pest Management Science 67: 114-121.

Mazzarri MB, Georghiou GP, 1995. Characterization of resistance to organophosphate, carbamate, and pyrethroid insecticides in field populations of *Aedes aegypti* from Venezuela. Journal of the American Mosquito Control Association 11: 315-322.

Ping L, Zhang C, Zhu Y, Wu M, Dai F, Hu X, Zhao H. 2010. Imidacloprid adsorption by soils treated with humic substances under different pH and temperature conditions. African Journal of Biotechnology 9: 1935-1940.

Robertson JL, Preisler HK. 1992. Pesticide Bioassays with Arthropods. CRC, Boca Raton, FL. 127 p.

Rosenthal JK, Sclar ED, Kinney PL, Knowlton K, Crauderueff R, Brandt-Rauf PW. 2007. Links between the built environment, climate and population health: interdisciplinary environmental change research in New York City. Annals Academy of Medicine Singapore 36: 834-46.

Salazar MJ, Moncada LI. 2004. Ciclo de vida de *Culex quinquefasciatus* Say, 1826 (Diptera: Culicidae) bajo condiciones no controladas en Bogotá. Biomedica 24: 385-92

Salgado VL. 1998. Studies on the Mode of Action of Spinosad Insect Symptoms and Physiological Correlates. Pesticide Biochemistry and Physiology 60: 91-102.

SAS Institute. 2016. SAS User's Manual 9.4. SAS Institute, Cary, North Carolina, USA

Sparks TC, Crouse GD, Dripps JE, Anzeveno P, Martynow J, DeAmicis CV, Gifford J. 2008. Neural network-based QSAR and insecticide discovery: Spinetoram. Journal of Computer-Aided Molecular Design 22: 393-401

Su T, Cheng M. 2014a. Laboratory Selection of Resistance to Spinosad in *Culex quinquefasciatus* (Diptera: Culicidae). Journal of Medical Entomology 51: 421-427.

Su T, Cheng M. 2014b. Cross Resistances in Spinosad-Resistant *Culex quinquefasciatus* (Diptera: Culicidae). Journal of Medical Entomology 51: 428-435.

Vassilakos TN, Athanassiou CG, Saglam O, Chloridis AS, Dripps JE. 2012. Insecticidal effect of spinetoram against six major stored grain insect species. Journal of Stored Products Research 51: 69-73.

Vassilakos TN, Athanassiou CG. 2013. Effect of temperature and relative humidity on the efficacy of spinetoram for the control of three stored product beetle species. Journal of Stored Products Research 55: 73-77.

Vieira AR, Roberto AM, Costa-da-Silva AL, Suesdek L, Soares FNC, Marçal BN, Katz G., Cardoso VA, Castro BC, Lara CM, Cardoso AVLA. 2015. São Paulo urban heat islands

have a higher incidence of dengue than other urban areas. The Brazilian Journal of Infectious Diseases 9: 146-155.

Watson GB, Chouinard SW, Cook KR, Geng C, Gifford JM, Gustafson GD, Hasler JM, Larrinua IM, Letherer TJ, Mitchell JC, Pak WL, Salgado VL, Sparks TC, Stilwell GE. 2010. A spinosyn-sensitive *Drosophila melanogaster* nicotinic acetylcholine receptor identified through chemically induced target site resistance, resistance gene identification, and heterologous expression. Insect Biochemistry and Molecular Biology 40: 376-384.

WHO (World Health Organization). 2005. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization 1–41. http://apps.who.int/iris/bitstream/handle/10665/69101/WHO_CDS_WHOPES_GCDPP_2005.13.pdf;jsessionid=4C43ADD825F47999B817E65F466DC3A0?sequence=1. (last accessed 15 Jan 2019).

Williams T, Valle J, Viñuela E. 2003. Is the Naturally Derived Insecticide Spinosad® Compatible with Insect Natural Enemies? Biocontrol Science and Technology 13: 459–475.

CONCLUSIONES GENERALES

A partir de la evaluación de pruebas de sobrevivencia a los factores de estudio se observó que *Ae. aegypti* sobrevive a rangos amplios de temperatura (10-35), materia orgánica (>23 % p/v) y pH (4-12).

Después de la observación de resultados de mortalidad obtenidos a partir de bioensayos y analizados mediante modelo Probit se determinó que en Índice de Susceptibilidad (IS) obtenido en función de la CL_{50} y CL_{95} no es significativo, ya que en todos los casos el IS fue menor a 5, lo cual indica que los factores evaluados a diferentes niveles no ejercen efecto significativo sobre la toxicidad de spinosad y spinetoram, por lo que se espera que en campo no se observe efecto de estos factores, de modo que estas moléculas pueden aplicarse a las dosis recomendadas sin necesidad de la implementación de alguna medida correctiva.

Derivado de la presencia de poblaciones resistentes de mosquitos a carbamatos, organofosforados y piretroides en diferentes regiones de México, el uso de spinosad y spinetoram representa una alternativa eficiente para el control de larvas, las cuales por su habito se encuentran mas localizadas. A pesar de que su uso en programas de Salud Pública es reciente, debe de considerarse un uso adecuado de estas moléculas incorporándolas en programas de rotación de insecticidas ya que existe riesgo de desarrollo de resistencia al tratarse de ingredientes homólogos.

Se recomienda también la aplicación de estas moléculas en combinación con reguladores crecimiento como piriproxifen, metopreno y diflubenzuron así como insecticidas microbiales como *Bacillus thuringiensis* var *israelensis* pues numerosos estudios sugieren que se prolonga el periodo de control de larvas limitándola emergencia de adultos. La implementación de estas estrategias de control debe de realizarse cuando se observe presencia de fuentes de desarrollo larval

lo cual coincide con épocas de lluvia debido a que el costo de implementación es elevado además debe combinarse con estrategias de control para adultos esto con el fin de prolongar la vida útil de estos insecticidas dentro de los programas de control de vectores.