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BÚSQUEDA DE INSECTICIDAS CON PROPIEDADES OVICIDAS, LARVICIDAS Y PUPICIDAS CONTRA *Aedes aegypti* L. (DIPTERA: CULICIDAE)

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BÚSQUEDA DE INSECTICIDAS CON PROPIEDADES OVICIDA, LARVICIDA Y PUPICIDA CONTRA *Aedes aegypti* (DIPTERA: CULICIDAE)

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RESUMEN

Aedes aegypti L. transmite enfermedades a los humanos y no existen tratamientos químicos que se puedan utilizar contra sus huevos o pupas. Se evaluaron insecticidas comerciales con propiedades ovicidas o pupicidas contra este vector u otras plagas: fenpiroximato, etoxazol, espinetoram, piriproxifeno, flufenoxurón, espinosad, aceite de neem, aceite de soja y espiromesifen. Se realizaron bioensayos en huevos (inmersión o aspersión), larvas y pupas. Los tratamientos con CL_{99} relativamente baja se evaluaron en campo, siempre que no se hayan utilizado previamente contra larvas de *Ae. aegypti*. Si el CL_{99} no mostrara efectos deseables, los tratamientos se reevaluarían utilizando el doble del CL_{99} original. Ningún tratamiento tuvo un efecto ovicida significativo. La CL_{99} más baja en las larvas se observó con spinosad (0.043 mg L^{-1}), spinetoram (37.6 mg L^{-1}) y aceite de neem (132.9 mg L^{-1}). Debido a que los dos primeros insecticidas se utilizan contra larvas de *Ae. aegypti*, no se evaluaron en campo. En pupas, la CL_{99} más baja se observó con aceite de neem (28.3 mg L^{-1}), y se evaluó en condiciones de campo a 132.9 mg L^{-1} (CL_{99} en larvas). En contenedores de 200 L, se preparó CL_{99} utilizando agua de la llave. Se utilizó un diseño de bloques completos al azar con tres tratamientos: sin intercambio de agua (LC99-WEW), 10% de intercambio (LC99-10% WE) y 30% de intercambio (LC99-30% WE) y cuatro repeticiones. Las larvas y pupas se expusieron a sus respectivos tratamientos los días 1, 14, 21 y 28. Solo el CL_{99}-WEW fue efectivo a los 28 días, con un 100% de mortalidad de larvas y pupas. En consecuencia, este tratamiento se evaluó a una concentración de 264 mg L^{-1} ($2CL_{99}$ en pupas). A los 28 días, la mortalidad de las larvas y pupas fue la siguiente: 2LC99-WEW, 100 y 69,4%; 2LC99-10% WE, 100 y 78,1%; y 2LC99-30% WE, 0 y 0%. El aceite de neem demostró propiedades larvicidas y pupicidas contra *Ae. aegypti*.

Palabras clave: mosquito de la fiebre amarilla, aceite de neem, dengue, control químico.

RESEARCH OF OVICIDAL, LARVICIDAL AND PUPAECIDAL INSECTICIDES PROPERTIES IN *Aedes aeegypti* L. (DIPTERA: CULICIDAE)

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ABSTRACT

Aedes aegypti L. transmits disease to humans and there are no chemical treatments that can be used against its eggs or pupae. Commercial insecticides with ovicidal or pupicidal properties were evaluated against this vector or other pests: fenpyroximate, ethoxazole, spinetoram, pyriproxyphene, flufenoxuron, spinosad, neem oil, soybean oil and spiromesifen. Bioassays were carried out on eggs (dipping or spraying), larvae and pupae. Treatments with relatively low LC_{99} were evaluated in the field, provided that they had not previously been used against *Ae. aegypti*. If LC_{99} did not show desirable effects, treatments would be re-evaluated using twice the original LC_{99}. No treatment had a significant ovicidal effect. The lowest LC_{99} in the larvae was observed with spinosad (0.043 mg L^{-1}), spinetoram (37.6 mg L^{-1}) and neem oil (132.9 mg L^{-1}). Because the first two insecticides are used against larvae of *Ae. aegypti*, were not evaluated in the field. In pupae, the lowest LC_{99} was observed with neem oil (28.3 mg L^{-1}), and it was evaluated under field conditions at 132.9 mg L^{-1} (LC_{99} in larvae). In 200 L containers, LC_{99} was prepared using tap water. A randomized complete block design was used with three treatments: no water exchange (LC_{99}-WEW), 10% exchange (LC_{99}-10% WE) and 30% exchange (LC_{99}-30% WE) and four repetitions. The larvae and pupae were exposed to their respective treatments on days 1, 14, 21 and 28. Only LC_{99}-WEW was effective at 28 days, with 100% mortality of larvae and pupae. Consequently, this treatment was evaluated at a concentration of 264 mg L^{-1} ($2LC_{99}$ in pupae). At 28 days, the mortality of the larvae and pupae was as follows: $2LC_{99}$-WEW, 100 and 69.4%; $2LC_{99}$-10% WE, 100 and 78.1%; and $2LC_{99}$-30% WE, 0 and 0%. Neem oil demonstrated larvicidal and pupicidal properties against *Ae. aegypti*.

Key words: yellow fever mosquito, neem oil, dengue, chemical control

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CONTENIDO

LISTA DE CUADROS

INTRODUCCIÓN GENERAL

El mosquito *Aedes aegypti* (Diptera: Culicidae) es el animal más dañino para el ser humano, debido a la cantidad de enfermedades que transmite y a la severidad de éstas (WHO, 2009). Para la mayoría de las enfermedades transmitidas por este vector no existe tratamiento médico conocido (Flores-Suarez *et al*., 2016; Lubinda *et al*., 2019). El género *Aedes* es originario de África al igual que los cuatro virus que transmite han causado mayor cantidad de pérdidas humanas a través de los siglos: fiebre amarilla, dengue, chikungunya, virus del Mayaro y fiebre del Zika (Powell, 2018). Los efectos de estas enfermedades son alarmantes, además de ocasionar la muerte, se han llegado a asociar con malformaciones congénitas, como microcefalia tras infecciones por zika (OPS/OMS, 2015).

A. aegypti se introdujo de África al Continente Americano hace 400 - 550 años a través del comercio de esclavos por los europeos (Powell *et al*., 2018). Se ha planteado la hipótesis de que el género *Aedes* ha sido el vector más importante de los virus de la fiebre amarilla, dengue, chikungunya y zika, debido a una historia evolutiva común en África junto con un huésped vertebrado, también africano nativo, que es el *Homo sapiens sapiens* (Powell, 2018).

A. agypti es un insecto antropofílico (Lindsay *et al*., 2017) y se distribuye en todas las zonas urbanas de México (CENAPRESE, 2015), con excepción de los estados de Tlaxcala, Chihuahua y Baja California (NOM-032-SSA2-2014, 2015). Sin embargo, su distribución biogeográfica tiende a la expansión a causa del cambio climático (Ruíz *et al*., 2016). Durante el siglo XX se creía que *Ae. aegypti* se establecía en zonas debajo de los 1700 msnmm, sin embargo, se tiene registro de la especie a 2130 msnmm, en regiones de clima templado (Lozano-Fuentes *et al*., 2014).

Las hembras de *Ae. aegypti* son endofágicas y endofílicas que se alimentan principalmente de sangre humana (Halstead, 2008; Garcia-Rejon *at al*., 2008); necesitan alimentarse varias veces de sangre para poner un lote de huevos, por lo que un solo individuo puede infectar a más de una persona de dengue u otro arbovirus (Scott *et al*., 2000; Benedictis, 2003; Flores-Suares *at al*., 2016). El dengue es la enfermedad arboviral

más importante en el mundo, durante la segunda mitad del siglo XX su incidencia se multiplicó treinta veces con la expansión geográfica de los países (Edelman, 2007). Se estima que hay 390 millones de infecciones por dengue anuales, de los cuales 96 millones (67 a 136 millones) se manifiestan clínicamente (Bhatt *et al*., 2013; WHO 2020), y que existen 3,900 millones de personas, en 128 países, en riesgo de contagio por alguno de los cuatro serotipo de este virus (Brady *et al*., 2012; WHO, 2020). En las últimas décadas ha aumentado la incidencia de dengue en el mundo. La mayoría de los casos son asintomáticos, por lo que el número real es subestimado o mal diagnosticado.

El dengue clásico se presentó por primera vez en el sur de México en 1978, aumentando su presencia en seguimiento de la distribución de *Ae. aegypti* (Icaza, 2003). En México, este vector está presente en 30 estados, exceptuando a Ciudad de México y Tlaxcala (NOM-032-SSA2-2014, 2015; DGE, 2019), dentro de los cuales se reportaron 12,706 casos de dengue durante 2018 (Secretaría de Salud, 2018), 32,690 casos en 2019 (DGE, 2019) y para la semana epidemiológica 38, al 24 de septiembre de 2020, la Secretaría de Salud (2020) reportó 11,228 casos confirmados. Para el control y prevención del dengue, la Organización Mundial de la Salud recomienda, como único método, el combate del vector aplicando estrategias culturales y el uso correcto de insecticidas (WHO, 2020).

El virus del chikungunya es otra enfermedad que transmite *A. aegypti*. Esta enfermedad es endémica de África, donde ha causado grandes epidemias, y se ha expandido rápidamente al continente asiático e islas del Caribe (Montero, 2015). En México, se reportaron 9,000 casos de chikungunya en 2015, con mayor incidencia en los estados de Guerrero, Michoacán, Oaxaca y Veracruz (SS, 2015). La OPS (2017), reportó 123,087 casos confirmados de chikungunya en las Américas. Sumado a lo anterior, desde noviembre de 2015 se han registrado casos positivos de infecciones por el virus zika en México, con un total de 12,892 casos al 4 de noviembre del 2019 y de los cuales 7,129 se han presentado en mujeres embarazadas (DGE, 2019).

En México se ha implementado el programa “Patio limpio” con la finalidad de eliminar el hábitat donde se desarrollan las larvas de *A. aegypti* y disminuir la incidencia del vector (SS, 2001; PAE, 2013; NOM-032-SSA2-2014, 2015). Otros métodos de combate

incluyen la liberación de insectos transgénicos para mitigar la tasa de transmisión de enfermedades (Rall, 2014), así como el uso de entomopatógenos y nematodos parasíticos (Rodríguez *et al*., 2015), no obstante, estas medidas no son suficientes, deben complementarse con el uso de insecticidas (Bisset-Lazcano *et al*., 2009).

En México, el uso de insecticidas es el método primario de combate de *Ae. aegypti*. Las aplicaciones prodcutos como temefos y bifentrina de forma intensiva y por largos periodos de tiempo (más de 30 y 10 años respectivamente) desencadenó en problemas severos de resistencia a insecticidas (Flores, 2014; CENAPRECE, 2014). La Arthropod Pesticide Resistance Database reporta que, en México hasta el 2020, han registrado 53 casos de resistencia a piretroides y organofosforados en *Ae. aegypti*, de un en total 589 casos acumulados mundialmente (Mota-Sanchez *et al*.,, 2020). La resistencia generalizada a piretroides y otros insecticidas (CENAPRECE, 2014) reduce drásticamente las opciones para combate químico, lo que atenta directamente contra la herramienta de combate de mayor peso en México.

El combate químico, así como estudios de susceptibilidad, de *A. aegypti* principalmente se dirige a larvas y adultos, mientras que para los estados de huevo y pupa no existen productos autorizados ni metodología para su combate, además que han sido poco estudiados (Argueta *et al.,* 2011). Por lo anterior, el objetivo del presente trabajo de investigación consistió en la búsqueda de insecticidas con actividad ovicida, larvicida o pupicida para el combate de plagas de artrópodos en *Ae. aegypti*. Dicha información servirá para ampliar el número de productos autorizados para combate de mosquitos vectores, contribuyendo para un mejor manejo de resistencia a insecticidas, para aumentar la vida útil de moléculas y por ende disminuir los contagios por las enfermedades que este vector transmite.

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CHAPTER I. SEARCH FOR INSECTICIDES WITH OVICIDAL, LARVICIDAL AND PUPICIDAL PROPERTIES AGAINST *Aedes aegypti* L. (DIPTERA: CULICIDAE)

1.1. ABSTRACT

Aedes aegypti L. transmits diseases to humans, and there are no chemical treatments that can be used against its eggs or pupae. The following commercial insecticides with ovicidal or pupicidal properties against this vector and other pests were evaluated: fenpyroximate, etoxazole, spinetoram, pyriproxyfen, flufenoxuron, spinosad, neem oil, soybean oil and spiromesifen. Bioassays were carried out on eggs (immersion or spraying), larvae, and pupae. Treatments with a relatively low LC_{99} were evaluated under field conditions, provided that they were not previously used against larvae of *Ae. aegypti*. If the LC_{99} showed no desirable effects, the treatments were re-evaluated using twice the original LC_{99}. No treatment had a significant ovicidal effect. The lowest LC_{99} in the larvae was observed with spinosad (0.043 mg L^{-1}), spinetoram (37.6 mg L^{-1}) and neem oil (132.9 mg L^{-1}). Because the first two insecticides are used against larvae of *Ae. aegypti*, they were not evaluated in the field. In pupae, the lowest LC_{99} was observed with neem oil (28.3 mg L^{-1}), and was evaluated under field conditions at a concentration of 132 mg L^{-1} (LC_{99} on larvae). In 200 L containers, the LC_{99} was prepared using tap water. A randomized complete block design was used with three treatments: without water exchange (LC_{99}-WEW), 10% exchange (LC_{99}-10% WE) and 30% exchange (LC_{99}-30% WE), and four replications. Larvae and pupae were exposed to their respective treatments on day 1, 14, 21 and 28. Only the LC_{99}-WEW was effective 28 days, with 100% mortality of larvae and pupae. Consequently, this

treatment was evaluated at a concentration of 264 mg L^{-1} ($2LC_{99}$ on larvae). At 28 days, the mortality of the larvae and pupae was as follows: $2LC_{99}$-WEW, 100 and 69.4%; $2LC_{99}$-10% WE, 100 and 78.1%; and $2LC_{99}$-30% WE, 0 and 0%. Neem oil demonstrated larvicidal and pupicidal properties against *Ae. aegypti.*
Key Words: yellow fever mosquito, neem oil, dengue, chemical control

1.2. RESUMEN

Aedes aegypti L. transmite enfermedades a los humanos y no existen tratamientos químicos que se puedan utilizar contra sus huevos o pupas. Se evaluaron insecticidas comerciales con propiedades ovicidas o pupicidas contra este vector u otras plagas: fenpiroximato, etoxazol, espinetoram, piriproxifeno, flufenoxurón, espinosad, aceite de neem, aceite de soja y espiromesifen. Se realizaron bioensayos en huevos (inmersión o aspersión), larvas y pupas. Los tratamientos con CL_{99} relativamente baja se evaluaron en campo, siempre que no se hayan utilizado previamente contra larvas de *Ae. aegypti*. Si el CL_{99} no mostrara efectos deseables, los tratamientos se reevaluarían utilizando el doble del CL_{99} original. Ningún tratamiento tuvo un efecto ovicida significativo. La CL_{99} más baja en las larvas se observó con spinosad (0.043 mg L^{-1}), spinetoram (37.6 mg L^{-1}) y aceite de neem (132.9 mg L^{-1}). Debido a que los dos primeros insecticidas se utilizan contra larvas de *Ae. aegypti*, no se evaluaron en campo. En pupas, la CL_{99} más baja se observó con aceite de neem (28.3 mg L^{-1}), y se evaluó en condiciones de campo a 132.9 mg L^{-1} (CL_{99} en larvas). En contenedores de 200 L, se preparó CL_{99} utilizando agua de la llave. Se utilizó un diseño de bloques completos al azar con tres tratamientos: sin intercambio de agua (LC99-WEW), 10% de intercambio (LC99-10% WE) y 30% de intercambio (LC99-30% WE) y cuatro repeticiones. Las larvas y pupas

se expusieron a sus respectivos tratamientos los días 1, 14, 21 y 28. Solo el CL_{99}-WEW fue efectivo a los 28 días, con un 100% de mortalidad de larvas y pupas. En consecuencia, este tratamiento se evaluó a una concentración de 264 mg L^{-1} ($2CL_{99}$ en pupas). A los 28 días, la mortalidad de las larvas y pupas fue la siguiente: 2LC99-WEW, 100 y 69,4%; 2LC99-10% WE, 100 y 78,1%; y 2LC99-30% WE, 0 y 0%. El aceite de neem demostró propiedades larvicidas y pupicidas contra *Ae. aegypti*.
Palabras clave: mosquito de la fiebre amarilla, aceite de neem, dengue, control químico

The interaction between humans and mosquitoes of the genus *Aedes* dates from our origin in Africa (Powell 2016). These arthropods feed mainly on human blood (Halstead 2008; Garcia-Rejon et al. 2008) and, as a result of this coevolution, have developed the capacity to transmit diseases (Powell 2018). The mosquito *Aedes aegypti* (Linnaeus 1762) (Diptera: Culicidae) is the most dangerous animal to humans due to the severity of diseases it transmits (classic dengue, hemorrhagic dengue, Zika, chikungunya, yellow fever, and Mayaro virus) (WHO 2009; Powell 2016). This species of insect establishes its populations in urban and peri-urban areas, which increases the risk of disease transmission (Gloria-Soria et al. 2016; Lindsay et al. 2017), and there is no vaccine or specific medical treatment for these diseases (Flores-Suarez et al. 2016; Lubinda et al. 2019). Worldwide, there are approximately 390 million dengue infections annually (Bhatt et al. 2013; WHO 2020), and 3.9 billion people in 128 countries are at risk of being infected with one of the four dengue serotypes (Brady et al. 2012; WHO 2020).

The fight against this vector focuses on the destruction of breeding sites and the use of insecticides (WHO 2005). Chemical treatment is carried out against larvae and

adults, but there are no options to combat eggs or pupae. The effective destruction of eggs would contribute to reducing the density of larvae. Insecticide applications against larvae have little or no effect on pupae; consequently, the pupae have a high probability of reaching the adult stage and constitute a serious threat to humans. Therefore, the aim of this research was to investigate commercial insecticides to find alternatives that can be used against the eggs, larvae, or pupae of *Ae. aegypti*.

1.3. MATERIALS AND METHODS

LOCATION OF THE EXPERIMENTS

The research was carried out from March to October 2020 at the Colegio de Postgraduados Campus Montecillo facilities in Texcoco, State of Mexico, Mexico.

INSECTS

We used the New Orleans strain of *Ae. aegypti* provided by the Universidad Autónoma de Nuevo León, Mexico. The rearing was carried out following the methodology of the World Health Organization (WHO 2005).

INSECTICIDES

We evaluated nine commercial insecticides that have evidence of ovicidal or pupicidal action against *Ae. aegypti* or other arthropod species (Table 1). Regardless of the criteria used to select a particular insecticide, bioassays were performed on eggs, larvae, and pupae.

BIOASSAYS

For each insecticide, the range of doses at which zero and 100% response (hatching for eggs or mortality for larvae and pupae) occurred, was determined. Subsequently, 6 to 10 concentrations covering this range were introduced. A total of five replications were carried out on consecutive days, and each replication included an untreated control. The untreated control was handled similarly to the rest of the treatments, except for the application of the insecticide.

EGG BIOASSAYS

The evaluation of ovicidal activity was carried out with two types of insecticide exposure: a) ovitraps with 24-164 eggs (7-15 days old) were submerged for 24 h in 100 mL of the required concentration of insecticide, and b) ovitraps with eggs, under the indicated conditions, were sprayed by using a Potter tower (with 2 mL of the appropriate concentration for 5 s at a pressure of 7.5 lb in^{-2}); then, the eggs were dried at room temperature for 24 h and submerged in a receptacle containing 100 mL of drinkable water (Epura, Mexico) at room temperature.

The water was previously boiled for 10 minutes to increase the percentage of egg hatching, as suggested by Nelson (1986). We used tap water at room temperature to prepare the required concentrations of insecticides. All the treatments were placed in bioclimatic chambers (Thermo Scientific Model TFFU2065FWA, Waltham, MA USA) under a 27 ± 2 °C, 12-12 h light-dark photoperiod.

To reduce variation in the response due to the age of the eggs and condition of the females among other factors, the ovitraps were randomly assigned to each concentration used for a given replication. In both types of exposure, the percentage of hatching was evaluated at 24 h, and the rate of change in hatching with respect to the

untreated control was calculated. The percent hatching in the untreated control was considered 100%; based on this value, we estimated the percent change in the eggs treated with insecticides.

BIOASSAYS WITH LARVAE

The larval bioassays were carried out according to the World Health Organization methodology (WHO 2005). The experimental unit consisted of 20 third instar larvae placed in a plastic cup containing 99 mL of tap water. One milliliter of the treatment was added to each experimental unit. Mortality was evaluated 24 h after exposure to the insecticide. Larvae without the typical diving reaction when shaking the water were considered dead (Flores 2014). The maximum mortality accepted in the untreated control was 10% and corrected by Abbott´s formula (Abbott 1925).

PUPAL BIOASSAYS

The pupal bioassays were performed similarly to the larval bioassays, except that we used pupae between 0 and 24 h old. Mortality was evaluated after 24 h of exposure to the insecticide, and pupae without normal mobility when shaking the water were considered dead.

STATISTICAL ANALYSIS OF BIOASSAYS

Probit analysis (Finney 1971) was used to estimate the LC_{50}, LC_{95}, and LC_{99} values, 95% confidence intervals (CI), and slope (± standard error) and to perform a χ^2 adjustment test (Pr> χ^2) using the Proc Probit procedure of the SAS 9.4 statistical program (SAS Institute Inc. 2013). In the untreated control, a maximum of 10% mortality was accepted, and this was corrected by Abbott's formula (Abbott 1925).

FIELD EVALUATION

Field evaluations were carried out with the methodology proposed in the Official Mexican Norm 032 (NOM-032-SSA2-2014 2015) and by the World Health Organization (WHO 2005). The insecticides with the lowest LC_{99} values were evaluated under field conditions, as long as they were not regularly used against larvae of *Ae. aegypti*. If the treatment did not reach the field efficacy established by NOM- 032-SSA2-2014 (2015) (>98% acute mortality or >90% inhibition of emergence, with residual effects for >3 weeks, in which the mortality or inhibition of emergence is >80%), it was re-evaluated under field conditions but using twice the value of the respective LC_{99}. The selected treatment was assessed at three levels: a) without water exchange (LC_{99}-WEW), b) 10% water exchange (LC_{99}-10% WE), and c) 30% water exchange (LC_{99}-30% WE). The water exchanges were carried out at 7, 14, and 21 days after having prepared the respective concentration (DAS) to simulate a reduction in the insecticide concentration due to dilution by rainwater, as suggested by Mexican regulations (NOM-032-SSA2-2014 2015).

In 200 L containers, 100 L of tap water was added and mixed with the respective insecticide to achieve the desired concentration (LC_{99} or twice the LC_{99} observed in the bioassay). The base of 125 mL plastic containers was removed, and covered with organza cloth held with a rubber band. These plastic containers (two per experimental unit) were placed inside float holes in a normal position so that approximately 100 mL of the respective treatment would enter the container. Subsequently, the target biological stage were exposed to the treatments following the methodology described for the respective bioassay. Mortality was evaluated after 24, 48, and 72 h of exposure.

A completely randomized experimental design was used, with four treatments and four replications. Before statistical analysis, to achieve normality, the percentage data were transformed with the arcsine function of the square root of the response/100. Subsequently, the transformed data were subjected to an analysis of variance and comparison of means (Tukey, $\alpha = 0.05$) using the statistical program SAS 9.4 (SAS Institute 2013).

1.4. RESULTS

A reduction in the hatching of eggs treated by immersion occurred in only five insecticides (Table 2): fenpyroximate (-25.3%; 1.0 mg L^{-1}), etoxazole (-15.2%; 1.0 mg L^{-1}), spinetoram (-14.8%; 100 mg L^{-1}), spinosad (-10.3%; 10 mg L^{-1}) and spiromesifen (-45.5%; 10,000 mg L^{-1}). However, as we increased the insecticide concentration, the hatching percentage, with respect to the untreated control, increased. For example, fenpyroximate at a concentration of 1.0 mg L^{-1} reduced hatching by 25.3%, but at 1,000 mg L^{-1}, hatching increased by 343.5% (Table 2). For fenpyroximate, etoxazole, and spinetoram, it was not possible to evaluate higher concentrations due to precipitate formation. When the ovitrap spraying method was applied, a reduction in hatching was observed with spiromesifen (-19.2%) (Table 3).

Spiromesifen was no toxic to larvae of *Ae. aegypti*. There was a larvicidal effect in the rest of the insecticides (Table 4). The lowest LC_{99} values were observed in spinosad (0.043 mg L^{-1}), spinetoram (37.6 mg L^{-1}), and neem oil (132.9 mg L^{-1}). Since spinosyns are used as larvicides against *Ae. aegypti* (CENAPRECE 2018), they were not considered for field evaluations.

The neem oil showed the lowest LC_{50} (12.1 mg L^{-1}), LC_{95} (22.0 mg L^{-1}), and LC_{99} (28.3 mg L^{-1}) values against pupae in all the evaluated insecticides (Table 5). Therefore, neem oil was considered a promising treatment for evaluation under field conditions. However, this product showed a higher LC_{99} value in larvae (132.9 mg L^{-1}) than in pupae (28.3 mg L^{-1}). Thus, we decided to evaluate, under field conditions, neem oil at a concentration of 132.9 mg L^{-1} against larvae and pupae.

In the field trials, the LC_{99} of neem oil (132 mg L^{-1}) without water exchange (LC_{99}-WEW) showed high larval mortality (99.7%) seven days after setting up the experiment (DAS). A similar situation occurred with 10% water exchange (LC_{99}-10% WE), with an average larval mortality of 97.4%. However, with 30% water exchange (LC_{99}-30% WE), the biological efficacy was unsatisfactory (54.2%). From 14 DAS on, the biological effectiveness of all the treatments was low. The effectiveness of LC_{99} (132 mg L^{-1}) against pupae remained >95% for up to 7 days in the treatments without water exchange (LC_{99}-WEW) and with 10% water exchange (LC_{99}-10% WE). At this evaluation date, the treatment with 30% water replacement (LC_{99}-30% WE) did not exceed 68% effectiveness at 72 h of exposure. From 21 DAS, none of the treatments showed acceptable biological effectiveness against the pupae (<30%).

Therefore, the neem oil was again evaluated in the field against larvae and pupae, but at a concentration of twice the LC_{99} observed in the larval bioassays (Tables 6 and 7). In the case of the larvae, twice the LC_{99} (264 ml L^{-1}) maintained >95% mortality for 30 days in the treatments without water exchange ($2LC_{99}$-WEW) and 10% water exchange ($2LC_{99}$-10% WE) (Table 5). However, in the treatment with 30% water

exchange ($2LC_{99}$-30% WE), the biological effectiveness of neem oil was 70% at 14 DAS, and after 28 DAS, there was no mortality (Table 6).

The $2LC_{99}$ (264 mg L^{-1}) against pupae of *Ae. aegypti* (Table 7) exceeded 96% effectiveness at 72 h of exposure in the evaluation at 21 DAS in the $2LC_{99}$-WEW and $2LC_{99}$-10% WE treatments. The $2LC_{99}$-30% WE treatment only maintained acceptable biological effectiveness until 7 DAS (> 96% at 72 h of exposure).

1.5. DISCUSSION

The assessed treatments that demonstrated ovicidal activity against pests of cultivated plants did not show desirable activity against *Ae. aegypti*. In our research, we were unable to reproduce the ovicidal activity documented against this vector in some of the evaluated treatments. For example, Sihuincha et al. (2005) exposed females of *Ae. aegypti* to pyriproxyfen in the residual form (0.003 g a. i. m^{-2}), and they did not observe an effect on the oviposition rate, but egg viability was reduced by 80%. Suman et al. (2013) evaluated pyriproxyfen application at 1.0 mg L^{-1} on *Ae. albopictus* and *Ae. Aegypti* and estimated hatching inhibition of 80.6% and 47.3%, respectively. Díaz-Martínez et al. (2016) assessed the ovicidal effect of spinosad and found an LC_{50} of 28.6 mg L^{-1}.

Our results agreed with those of Argueta et al. (2011), who evaluated the ovicidal potential of spinosad in *Ae. aegypti*, observing a slight reduction in the average hatching rate (6.6-8.2%) at 10 ppm; they concluded that spinosad has no important ovicidal effects. Fenigstein et al. (2001) evaluated soybean oil (Signa Israel, emulsified with Tween 80 from Sigma Israel) in *Ae. aegypti* and found a low ovicidal effect (1% hatching

inhibition) at 27,300 ppm. These authors considered that the variations in response were due to differences in egg morphology and resistance to desiccation.

Suman et al. (2013) studied azadirachtin's potential as an ovicide, finding limited effects on *Ae. aegypti*. When applying 1.0 ppm to freshly laid eggs, the hatching inhibition rates in *Ae. aegypti* and *Ae. albopictus* were 15.7% and 42.9%, respectively. These disagreements with our data may be due to the concentration of azadirachtin in neem oil, which may vary depending on the plant structure and extraction methodology used (Fernandes et al 2019).

The evaluated neem oil showed acceptable larvicidal activity in accordance with the results of other researchers. Demba et al. (2007) evaluated neem oil and neem seed powder and found LC_{50} values lower than those we observed (2 mg L^{-1} for oil and 8 mg L^{-1} for powder). Shanmugasundaram et al. (2006), using neem oil against larvae of this species, documented LC_{50} and $LC_{95\ values}$ of 2,900 and 16,000 ppm, respectively. Kaura et al. (2019) evaluated neem oil (Brahmastra Ayurvedic Products) and documented LC_{50} values of 7,852 and 19,059 ppm in larvae and LC_{90} values of 10,092 and 19,952 ppm in pupae of *Ae. aegypti*. To achieve the same effect, the pupae required higher doses than the larvae. Azadirachtin, the main component of neem oil, is considered a biorational insecticide due to its low environmental impact and lack of adverse effects on human health. For azadirachtin, there is no report of resistance in *Ae. aegypti* or another insect pest species (Mota-Sanchez and Wise 2020). Sihuincha et al. (2005) observed that pyriproxyfen at 0.012 ppm caused 100% mortality in *Ae. aegypti* pupae. In our research, the LC_{50} of pyriproxyfen was 302.3 mg L^{-1}. This difference may be caused by the type and quality of the formulation used.

Field applications of commercial insecticides against the larvae of this vector have little or no effect on its pupae. Thus, pupae have a high probability of reaching the adult stage and dispersing in urban ecosystems, where chemical control is complicated due to the difficulty of efficiently reaching a dispersed population. In addition, there is little time to control pupae. This biological state represents only 5% of the life span of *Ae. aegypti* (Nelson 1986). In contrast, adults occupy 85% (Nelson 1986) of the length of this species' life cycle, and during this time, they represent a severe threat to human health.

Most of the countries that are affected by the diseases transmitted by *Ae. aegypti* have few economic resources needed to use environmentally low risk insecticides that would allow them to reduce the density of this vector. Consequently, they are forced to use inexpensive insecticides with a high propensity for resistance and a high risk to human health. In this research, we found that neem oil can effectively combat larvae and pupae of *Ae. aegypti*. Consequently, the use of this biorational insecticide represents a viable option for countries threatened by yellow fever mosquitoes.

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Table 1. Selected insecticides used to evaluate their ovicide, larvicide, and pupicide activity against the New Orleans strain of *Aedes aegypti* L.

Commercial name	Active ingredient	Formulation	g of a. i. per L o kg	Biological activity	Company
Tetrasan	Etoxazole	CS†	110	Ovicide in mites[1]	Valent de México
Avolant	Fenpyroximate	CS†	50.9	Ovicide in mites[2]	Arysta Lifescience México
Knack	Pyriproxyfen	EC¶	103	Ovicide in Diptera[3]	Valent de México
Cascade	Flufenoxuron	SCθ	100	Ovicide in mites[3], Coleoptera[4]	Arysta Lifescience México
Exalt	Spinetoram	CS†	60	Larvicide in Lepidoptera[5], Diptera[6]	Corteva Agriscience México
Plasma Power	Neem oil	PNE§	1000	Ovicide and larvicide in Diptera[3]	Hortitec Internacional México
Tracer Edge	Spinosad	Ga	360	Larvicide in Lepidoptera[7,8], Diptera[7]	Corteva Agriscience México
Golden Pest	Soybean oil	CE¶	924	Larvicide in Diptera, Hemiptera[9,10]	Stoller México S.A. de C.V.
Oberon	Spiromesifen	CS†	240	Ovicide, adulticide in mites[11]	Bayer de México SA de CV

†Concentrare solution; ¶Emulsifiable concentrate; θFlowable; §100% pure neem extract; aGranules; [1]Minakuchi et al., 2019; [2]Minsik et al., 2005; [3]Suman et al., 2013; [4]Salokhe et al., 2003; [5]Nedal and Hassan, 2009; [6]Su et al., 2019; [7]Pérez et al., 2007; [8]Díaz-Martínez et al., 2016; [9]Amer and Mehlhorn, 2006; [10]Fenigstein et al., 2001; [11]Saryazdi et al., 2013.

Table 2. Eggs hatching in *Ae. aegypti* strain New Orleans exposed 24 h to different concentrations of insecticides.

		Concentration, mg L^{-1}									
		1.0		10		100		1,000		10,000	
Insecticide	Control hatching (%)	Hatching (%)	% change*	Hatching (%)	% change	Hatching (%)	% change	Hatching (%)	% change	Hatching (%)	% change
Fenpyroximate	19.4 ± 0.56	14.5 ±2.31	-25.3	66.9 ± 0.43	+244.7	83.7 ± 0.63	+331.2	86.1 ± 1.23	+343.5		
Etoxazole	20.9 ± 2.28	17.8 ±6.09	-15.2	28.0 ± 6.48	+33.5	51.1 ± 6.27	+143.4	51.6 ± 3.71	+146.1		
Spinetoram	23.1 ± 1.45	24.4 ±0.33	+5.6	23.2 ± 0.9	+0.6	19.66 ± 1.09	-14.8	28.8 ± 2.21	+24.6		
Pyriproxyfen	41.4 ± 7.14			41.5 ± 8.89	+0.1	88.8 ± 7.16	+114.5	90.6 ± 3.6	+118.9	98.7 ± 1.28	+138.4
Flufenoxuron	51.3 ± 1.14			56.2 ± 9.60	+9.4	98.7 ± 0.69	+92.1	97.1 ± 0.6	+89.0	95.7 ± 0.61	+86.3
Spinosad	47.8 ± 5.12			42.9 ± 15.88	-10.3	85.5 ± 1.04	+78.8	96.5 ± 1.12	+101.9	98.7 ± 1.33	+106.4
Neem oil	63.2 ±8.74			88.8 ± 2.36	+40.5	96.5 ± 1.82	+52.7	96.9 ± 2.11	+53.3	98.6 ± 1.39	+56.1
Soybean oil	29.1 ± 4.20			29.6 ± 3.92	+1.8	48.3 ± 2.2	+66.1	55.2 ± 1.81	+90.1	58.2 ± 6.6	+100.3
Spiromesifen	66.8 ± 9.51			87.8 ± 2.71	+31.3	92.4 ± 1.43	+38.2	88.5 ± 3.33	+32.4	36.5 ± 8.25	-45.5

*the symbol + or – indicate increase or decrease in the rate of hatching respect to the untreated control.

Note: blank spaces indicate that the respective concentration was not evaluated.

Table 3. Eggs hatching in *Aedes aegypti* L. (New Orleans strain) treated with different insecticides using the Potter's tower (2 mL at 10,000 mg L^{-1}, 5 s at a pressure of lb in^{-2})

	Hatching (%)			Average hatching	
Insecticide	R1	R2	R3	(%)	% change*
Fenpyroximate	27.7	46.1	41.2	38.3 ± 5.50	+69.9
Etoxazole	22.2	17.6	59.6	33.2 ± 13.29	+46.8
Spinetoram	39.0	54.9	53.7	49.2 ± 5.10	+117.9
Pyriproxyfen	47.2	40.4	59.6	49.1 ± 5.61	+117.3
Flufenoxuron	32.6	20.0	18.9	23.8 ± 4.38	+5.6
Spinosad	22.5	32.9	47.3	34.3 ± 7.19	+51.7
Neem oil	74.7	43.5	37.0	51.8 ± 11.63	+129.2
Soybean oil	45.4	51.4	76.6	57.8 ± 9.53	+156.0
Spiromesifen	9.7	28.1	16.9	18.2 ± 5.36	-19.2
Untreated control	34.5	15.4	17.8	22.6 ± 6.02	

R = replication.

*the symbol + or – indicate increase or decrease in the rate of hatching respect to the untreated control.

Table 4. Probit analysis of the response to insecticides on third instar larvae of the New Orleans strain of *Aedes aegypti* L.

Insecticida	N[†]	*df* [¶]	*b* ± SE[§]	LC_{50}[a] CI 95% [Þ] (mg L^{-1})	LC_{95}[a] CI 95% [Þ] (mg L^{-1})	LC_{99}[a] CI 95% [Þ] (mg L^{-1})	Pr > x^2
Fenpyroximate	900	7	3.51 ± 0.19	91.8	270.0	422.2	0.80
				85.5 – 98.4	239.5 – 311.8	360.6 – 511.8	
Etoxazole	800	6	3.71 ± 0.22	531.7	1475.0	2250.0	0.58
				498.5 – 567.4	1301.0 – 1721.0	1907.0 – 2768.0	
Spinetoram	900	7	3.59 ± 0.19	8.5	24.3	37.6	0.49
				7.9 – 9.0	21.6 – 27.9	32.2 – 45.3	
Pyriproxyfen	700	5	2.77 ± 0.23	24.3	95.3	167.7	0.15
				20.2 – 29.6	69.7 – 150.9	112.3 – 207.6	
Flufenoxuron	900	7	3.50 ± 0.19	302.3	890.8	1394.0	0.33
				282.9 – 323.0	787.9 – 103	1184.0 – 1700.0	
Spinosad	800	6	3.87 ± 0.23	0.0107	0.029	0.043	0.028
				0.0100 – 0.0115	0.0254 – 0.0329	0.0367 – 0.0519	
Neem oil	600	4	6.35 ± 0.41	57.2	103.8	132.9	0.51
				54.6 – 59.9	95.9 – 114.5	119.9 – 151.6	
Soybean oil	900	7	2.41 ± 0.15	838.9	4016.0	7683.0	0.29
				768.0 – 915.1	3315.0 – 5119.0	5922.0 – 10719.0	

[†]*n* = total treated larvae or pupae; [¶]Degrees of freedom; [§]Slope ± standard error; [a]Concentration that kills 50 (95, 99) % of the treated individuals; [Þ]Fiducial limits (95%).

Table 5. Probit analysis of the response to insecticides on pupae (0 to 24 h old) of the New Orleans strain of *Aedes aegypti* L.

Insecticida	N[†]	*df* [¶]	*b* ± SE[§]	LC_{50}[a] CI 95% [Þ] (mg L^{-1})	LC_{95}[a] CI 95% [Þ] (mg L^{-1})	LC_{99}[a] CI 95% [Þ] (mg L^{-1})	Pr > x^2
Fenpyroximate	700	5	3.77 ± 0.24	33.4	91.1	137.9	0.24
				31.0 – 35.8	80.9 – 105.4	117.8 – 168.5	
Etoxazole				>10,000			
Spinetoram	700	5	1.91 ± 0.12	178.4	1289.0	2924.0	0.44
				156.0 – 204.7	992.0 – 1786.0	2074.0 – 4509.0	
Pyriproxyfen	900	7	4.21 ± 0.22	55.9	137.4	199.3	0.58
				52.8 – 59.2	124.4 – 154.8	174.9 – 233.8	
Flufenoxuron				>1000	>1000	>1000	
Spinosad				>1000	>1000	>1000	
Neem oil	1000	8	6.31 ± 0.33	12.1	22.0	28.3	0.48
				11.7 – 12.5	20.6 – 23.9	25.9 – 31.5	
Soybean oil				>1000			

[†]*n* = total treated larvae or pupae; [¶]Degrees of freedom; [§]Slope ± standard error; [a]Concentration that kills 50 (95, 99) % of the treated individuals; [Þ]Fiducial limits (95%).

Table 6. Biological efficacy of 264 mg L^{-1} (twice the LC_{99}) of neem oil on third instar larvae of the New Orleans strain of *Aedes aegypti* L. Data represent mortality (%)[a].

Treatment	Exposure (hours)	1 DAS	7 DAS	14 DAS	21 DAS	28 DAS
$2LC_{99}$-WEW						
	24	100.0 ± 0.00a	100.0 ± 0.00a	98.7 ± 0.72a	99.4 ± 0.63a	99.9 ± 0.63a
	48	100.0 ± 0.00a	100.0 ± 0.00a	99.4 ± 0.63a	100.0 ± 0.00a	100.0 ± 0.00a
	72	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a
$2LC_{99}$-10% WE						
	24	100.0 ± 0.00a	100.0 ± 0.00a	94.4 ± 4.00a	97.5 ± 1.02a	96.9 ± 1.57a
	48	100.0 ± 0.00a	100.0 ± 0.00a	97.5 ± 1.77a	98.7 ± 1.25a	98.7 ± 0.72a
	72	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a	99.7 ± 0.63a	100.0 ± 0.00a
$2LC_{99}$-30% WE						
	24	100.0 ± 0.00a	99.4 ± 0.63a	63.7 ± 7.74b	6.9 ± 3.29b	0.0 ± 0.00b
	48	100.0 ± 0.00a	99.4 ± 0.63a	69.4 8.19b	6.9 ± 2.58b	0.0 ± 0.00b
	72	100.0 ± 0.00a	99.4 ± 0.63a	68.8 ± 8.19b	6.4 ± 2.98b	0.0 ± 0.00b
Untreated control						
	24	0.0 ± 0.00b	0.0 ± 0.00b	0.0 ± 0.00c	0.0 ± 0.00c	0.0 ± 0.00b
	48	0.0 ± 0.00b	0.0 ± 0.00b	0.0 ± 0.00c	1.2 ± 0.72c	0.0 ± 0.00b
	72	1.2 ± 0.72b	1.2 ± .72b	1.9 ± 1.20c	2.5 ± 1.02c	0.0 ± 0.00b

[a]Data are from a field experiment with a complete randomized block design and four replicates.
DAS = Days after setting up the experiment; $2LC_{99}$-WEW = 264 mg L^{-1} without exchange of water; $2LC_{99}$-10% WE = 264 mg L^{-1} with a 10% exchange of water; $2LC_{99}$-30% WE = 264 mg L^{-1} with a 30% exchange of water.

Table 7. Biological efficacy of 264 mg L^{-1} (twice the LC_{99}) of neem oil on pupae (0 to 24 h old) of the New Orleans strain of *Aedes aegypti* L. Data represent mortality (%)[a].

Treatment	Exposure (hours)	1 DAS	7 DAS	14 DAS	21 DAS	28 DAS
$2LC_{99}$-WEW						
	24	98.3 ± 0.96a	85.6 ± 3.44a	84.3 ± 5.04a	64.4 ± 10.33a	33.7 ± 6.25a
	48	100.0 ± 0.00a	99.4 ± 0.63a	89.3 ± 5.04a	93.7 ± 3.31a	55.0 ± 13.58a
	72	100.0 ± 0.00a	100.0 ± 0.00a	91.2 ± 5.45a	99.4 ± 0.63a	69.4 ± 18.30a
$2LC_{99}$-10% WE						
	24	100.0 ± 0.00a	79.4 ± 5.34ab	69.2 ± 9.26a	45.6 ± 6.87a	31.2 ± 8.20a
	48	100.0 ± 0.00a	98.1 ± 1.20ab	78.6 ± 11.84a	79.7 ± 3.68a	58.7 ± 16.50a
	72	100.0 ± 0.00a	99.4 ± 0.63ab	79.9 ± 11.41a	96.8 ± 1.88a	78.1 ± 11.74a
$2LC_{99}$-30% WE						
	24	99.2 ± 0.83a	50.0 ± 11.86b	30.3 ± 2.17b	1.9 ± 1.20b	0.6 ± 0.63b
	48	99.1 ± 0.83a	93.7 ± 1.61b	41.4 ± 3.95b	2.5 ± 2.39b	0.0 ± 0.00b
	72	99.1 ± 0.83a	96.9 ± 1.57b	43.9 ± 3.54b	1.8 ± 2.39b	0.0 ± 0.00b
Untreated control						
	24	0.8 ± 0.83b	0.0 ± 0.00c	1.2 ± 1.25c	0.0 ± 0.00b	0.0 ± 0.00b
	48	0.8 ± .83b	0.0 ± 0.00c	1.9 ± 1.20c	1.2 ± 0.72b	0.0 ± 0.00b
	72	0.8 ± 0.83b	1.9 ± 1.20c	1.9 ± 1.20c	1.9 ± 1.20b	0.0 ± 0.00b

[a]Data are from a field experiment with a complete randomized block design and four replicates.
DAS = Days after setting up the experiment; $2LC_{99}$-WEW = 264 mg L^{-1} without exchange of water; $2LC_{99}$-10% WE = 264 mg L^{-1} with a 10% exchange of water $2LC_{99}$-30% WE = 264 mg L^{-1} with a 30% exchange of water.

CONCLUSIONES GENERALES

No se detectó acción ovicida significativa en los insecticidas evaluados. Se encontraron propiedades larvicidas significativas en todos los productos evaluados. Sin embargo, solo se consideró al aceite de neem como prometedor, ya que con los demás insecticidas, las concentraciones requeridas eran elevadas o ya se utilizan para combate de *Aedes aegypti*. El mejor pupicida fue el aceite de neem con una CL_{50} y CL_{99} de 12.1 y 28.3 mg L^{-1}, respectivamente.

El aceite de neem, en evaluaciones de campo a la dosis de 264 mg L^{-1} ($2CL_{99}$), demostró efecto larvicida y pupicida en *Ae. aegytpi* por el tiempo mínimo necesario que establece la normativa mexicana vigente para insecticidas usados en el combate de vectores: mortalidad aguda >98% y/o inhibición de la emergencia >90%, con efecto residual después de tres semanas. El aceite de neem, es un producto biorracional de origen botánico y bajo impacto ambiental. Además, no tiene efectos adversos a la salud humana y es de baja propensión a resistencia en insectos. En aplicaciones a gran escala, este producto es más barato que los que actualmente se utilizan contra *Ae. aegypti*. En consecuencia es una alternativa barata, de bajo riesgo al ambiente y a la salud humada. Además permite combatir eficazmente larvas de *Ae. aegypti* y por primera vez, mundialmente, se tiene una opción viable contra pupas de este vector de enfermedades al ser humano.