Quasi-Field and Laboratory Evaluations of Biorational Agricultural Insecticides against Eggs, Larvae, and Pupae of *Aedes aegypti* (Diptera: Culicidae)[1]

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Abstract The aim of this study was to evaluate the ovicidal, larvicidal, and pupicidal activities of various commercial insecticides against *Aedes aegypti* (L.) (Diptera: Culicidae), a primary vector of several human diseases. Laboratory bioassays were used to evaluate fenpyroximate, etoxazole, spinetoram, pyriproxyfen, flufenoxuron, spinosad, neem oil, soybean oil, and spiromesifen to identify promising treatments. Quasi-field tests followed to assess the practical applicability of these insecticides. None of the tested compounds exhibited significant ovicidal activity. The most effective larvicidal treatments were spinosad, spinetoram, pyriproxyfen, and neem oil, with 95% lethal concentration (LC_{95}) values of 0.029, 24.3, 95.3, and 103.8 mg/L, respectively. Neem oil exhibited the lowest LC_{95} value against pupae (28.3 mg/L) and was considered the most promising treatment of the nine compounds tested to manage larvae and pupa of *Ae. aegypti*. In stagnant water, neem oil at 264 mg/L provided 96.9–100% larval mortality over 28 d, with and without 10% water exchange (i.e., simulated rain-induced dilution). At this concentration, pupal mortality exceeded 99% at 1 and 7 d after treatment (72 h of exposure). Neem oil, a natural and cost-effective insecticide, has significant potential for controlling the larval and pupal stages of *Ae. aegypti*.

Key Words yellow fever mosquito, neem oil, dengue, chemical control

Human interaction with mosquitoes dates back to our African origins (Powell 2018). The yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae), feeds mainly on human blood (Halstead 2008) and has developed the capacity to transmit disease pathogens (Powell 2018), such as dengue and its hemorrhagic variants Zika, chikungunya, yellow fever, and Mayaro (WHO 2009). Because of the occurrence of this mosquito in urban environments, the risk of disease transmission to humans has increased (Lindsay et al. 2017). There is no vaccine or specific

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medical treatment for most of these diseases other than supportive care (Lubinda et al. 2019).

The management of this vector focuses on eliminating larval developmental sites and applying insecticides (WHO 2005). Current biological larvicides, such as insect growth regulators and bacteria aimed at the larval stage, have little or no effect on pupae (Prabhu et al. 2011). Consequently, pupae are highly likely to reach adulthood and severely threaten human health.

The incidence of dengue, a human disease transmitted by this vector, is increasing. Since the beginning of 2024, over 13 million dengue cases have been documented worldwide, and approximately 8,500 deaths have occurred (ECDPC 2024). During 2023, in Mexico there were 56,333 confirmed cases of dengue; by October 2024, this number had reached 80,866 cases, and deaths increased from 88 to 200 (week 41) (CENAPRECE 2024a). Most countries affected by *Ae. aegypti* do not have the economic resources to use friendly, low-risk, but expensive insecticides. Moreover, these chemicals are ineffective against eggs or pupae. Thus, the search for low-cost alternatives against larvae and pupae of this vector is urgent. Therefore, this research was conducted to evaluate commercial insecticides under laboratory and quasi-field conditions to identify treatments that can be used against the immature stages of *Ae. aegypti*.

Materials and Methods

Susceptible strain. In these studies we used the insecticide-susceptible New Orleans strain of *Ae. aegypti* provided by the Universidad Autónoma de Nuevo León, Mexico. The rearing method used was that suggested by the World Health Organization (WHO; 2005).

Insecticides. Nine commercial insecticides with previous evidence of toxicity against immature stages of the yellow fever mosquito or other agricultural pest species (Table 1) were assessed against *Ae. aegypti* eggs, larvae, and pupae. The insecticides were diluted in tap water to prepare the required concentrations.

Bioassays with eggs. The ovicidal activity was assessed in groups of 24–164 eggs (experimental unit) that were 7–15 d old. We used two types of insecticide exposure: (a) eggs laid on Kraft papers (KRAFT ROLL®, APSA S.A. de C.V., Ciudad de México, Mexico) were dipped for 24 h in 100 ml of the required concentration of insecticide, and (b) eggs laid on Kraft paper were directly sprayed by using a Potter tower (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, U.K.) with 2 ml of the appropriate concentration for 5 s at a pressure of 1.39 kg/cm^2. The Potter tower was used to simulate field spraying for immature stages of this vector.

After treatment, the egg papers were dried at room temperature for 24 h and then dipped in a cup containing 100 ml of previously boiled (10 min) commercial bottled water (Epura®, Mexico) and cooled to room temperature (25°C) according to the methodology of Nelson (1986). Each set of insecticidal concentrations had an untreated control, consisting of tap water and the tested eggs. All eggs were placed in bioclimatic chambers (TFFU2065FWA, Thermo Fisher Scientific, Waltham, MA) at 27 $\pm$ 2°C and a 12:12 h (light:dark) photoperiod. The percentage reduction in egg hatch in the experimental units treated with insecticide compared

Table 1. Insecticides used to evaluate ovicidal, larvicidal, and pupicidal activity against the New Orleans strain of *Aedes aegypti* L.

Commercial Name	Active Ingredient	Active Ingredient Formulation (g/L or kg)	Biological activity	Reference(s)	Company
Tetrasan	Etoxazole	CS* (110)	Ovicide in mites	Minakuchi et al. 2006	Valent de México
Avolant	Fenpyroximate	CS* (50.9)	Ovicide in mites	Minsik et al. 2005	Arysta Lifescience México
Knack	Pyriproxyfen	EC** (103)	Ovicide in Diptera	Suman et al. 2013	Valent de México
Cascade	Flufenoxuron	F† (100)	Ovicide in mites, Coleoptera	Suman et al. 2013, Salokhe et al. 2003	Arysta Lifescience México
Exalt	Spinoteram	CS† (60)	Larvicide in Lepidoptera, Diptera	Nedal and Hassan 2009, Su et al. 2019	Corteva Agriscience México
Plasma Power	Neem oil	PNE‡ (1,000)	Ovicide and larvicide in Diptera	Suman et al. 2013	Hortitec Internacional México
Tracer Edge	Spinosad	G‡‡ (360)	Larvicide in Lepidoptera, Diptera	Pérez et al. 2007, Díaz-Martínez et al. 2016	Corteva Agriscience México
Golden Pest	Soybean oil	EC** (924)	Larvicide in Diptera, Hemiptera	Amer and Mehlhorn 2006, Fenigstein et al. 2001	Stoller México S.A. de C.V.
Oberon	Spiromesifen	CS* (240)	Ovicide and adulticide in mites	Saryazdi et al. 2013	Bayer de México SA de CV

* Concentrate solution.
** Emulsifiable concentrate.
† Flowable.
‡ 100% pure neem extract.
‡‡ Granules.

with the untreated control was evaluated 24 h after treatment with the following formula:

$$\%\,\text{hatching change} = [(\%\,\text{hatch insecticide treatment} * 100/\%\,\text{hatch untreated control}) - 100]$$

Bioassays with larvae and pupae. Larval and pupal bioassays were conducted using the WHO (2005) method. Larval bioassays consisted of 20 third instars in 127-ml plastic cups (Plásticos Adheribles del Bajío S.A. de C.V., León, Guanajuato, Mexico) containing 99 ml of tap water. For pupal bioassays, 0–24-h-old individuals (20 per cup) were used. Subsequently, 1 ml of the treatment mixture was added to each experimental unit. Mortality was evaluated 24 h after treatment. Larvae without the typical diving reaction when the water was shaken were considered dead (Flores 2014). Pupal mortality was determined by the absence of movement when submerged with the aid of a brush.

Quasi-field evaluation. Quasi-field evaluations were conducted via the methodology proposed in the Official Mexican Norm 032 (NOM-032-SSA2-2014 2015) and the WHO (2005). Neem oil was assessed under quasi-field conditions because of its lower concentration need to kill 99% of the treated individuals (LC_{99}). When this treatment did not reach the field efficacy established by NOM-032-SSA2-2014 (>98% acute mortality with residual effects for >3 wk), it was re-evaluated at twice the respective LC_{99} value. Neem oil was assessed with three treatments: (a) without water exchange (WEW), (b) with 10% water exchange (10% WE), and (c) with 30% water exchange (30% WE). Water exchanges were conducted at 7, 14, and 21 d after setting up the experiment (DAS) to simulate a reduction in insecticide concentration due to rainwater dilution, as suggested by Mexican regulations (NOM-032-SSA2-2014 2015).

Containers with a capacity of 200 L (Soluplastic Empaques Retornables S.A. de C.V., Monterrey, Nuevo León, México) containing 100 L of tap water were treated with the desired concentration of neem oil. Larvae and pupae were exposed to treatments in separate 127-ml plastic cups. The bottom of each cup was removed, and the top part was covered with organza cloth (Capital Textil S.A. de C.V., Centro, Mexico) secured with a rubber band. The plastic cups (two per container) were positioned inside the float holes and arranged within the containers to permit approximately 100 ml of the treated water to enter. Mortality was evaluated at 24, 48, and 72 h postexposure.

Statistical analysis. For the laboratory bioassays, probit analysis (Finney 1971) was used to estimate the LC_{50} (concentration lethal to 50% of treated individuals), LC_{95} (concentration lethal to 90% of treated individuals), and LC_{99} values, 95% confidence intervals (CIs), slope (± standard error), and χ^2 value ($Pr > \chi^2$) (SAS Institute 2013). The maximum mortality accepted in untreated controls was 10%, and treatment mortality was corrected with Abbott's formula (Abbott 1925).

A completely randomized experimental design with four treatments and four replications was used for the quasi-field experiments. Before the statistical analysis, the percentage data were transformed with the arcsine function of the square root of the response divided by 100 to achieve normality. The transformed data were subsequently subjected to an analysis of variance and a Tukey comparison of means ($\alpha = 0.05$; SAS Institute 2013).

Results

Bioassays with eggs. When eggs were exposed to treated water, hatching occurred with only four of the insecticides tested: fenpyroximate (−25.3% compared with control; 1.0 mg/L), etoxazole (−15.2%; 1.0 mg/L), spinosad (−10.3%; 10 mg/L), and spiromesifen (−45.5%; 10,000 mg/L) (Table 2). It was impossible to evaluate higher concentrations of fenpyroximate, etoxazole, and spinetoram because of the formation of a precipitate. When eggs were treated with the Potter tower, a decrease in hatch rates was observed with only spiromesifen (−19.2%; 10,000 mg/L) (Table 3).

Bioassays with larvae. Spiromesifen was nontoxic to larvae, and larval toxicity depended on the concentrations for the other treatments (Table 4). The lowest LC_{99} values were observed for spinosad (0.043 mg/L), followed by spinetoram (37.6 mg/L) and neem oil (132 mg/L). Spinosyns have been used as larvicides against *Ae. aegypti* for more than 6 yr (CENAPRECE 2018, 2024b), so they were not further considered for quasi-field evaluations.

Bioassays with pupae. Of the insecticides tested, neem oil had the lowest LC_{50} (12.1 mg/L), LC_{95} (22.0 mg/L), and LC_{99} (28.3 mg/L) values against pupae (Table 5). Therefore, neem oil was evaluated via quasi-field trials.

Quasi-field experiments. A 132 mg/L (LC_{99}) neem oil WWE concentration resulted in high larval mortality (99.7%) 7 DAS. Similar mortality occurred with 10% WE. However, with 30% WE in all the evaluations, the biological efficacy was unsatisfactory (54.2% mortality) against this developmental stage. Pupal mortality was acceptable only at 7 DAS (>95%) in the treatments with 10% WE or WWE.

Considering that 132 mg/L of neem oil was unsatisfactory in all treatments, we evaluated it at 264 mg/L (twice the LC_{99}) (Table 6). At this concentration (28 DAS), larval mortality was >99% (WWE) and >96% (10% WE; 24, 48, and 72 h of exposure). With 30% WE, the biological effectiveness of neem oil was >99% at 7 DAS.

At 264 mg/L neem oil, pupal mortality (72-h of exposure) was 100% for WWE at 1 and 7 DAS (Table 7). At 10% WE, the pupal mortality was >99% (72 h of exposure). In the treatment with 30% WE (72 h of exposure), pupal mortality was elevated (>96%) at 1 and 7 DAS.

Discussion

Our results agree with those of Argueta et al. (2011), who found a slight reduction in the mean hatching percentage of *Ae. aegypti* eggs (6.6–8.2% inhibition) at 10 ppm spinosad and concluded that this insecticide had no significant ovicidal effects. Fenigstein et al. (2001) evaluated soybean oil (Sigma, Jerusalem, Israel) emulsified with Tween 80® (Sigma) and reported a similar effect (1% hatching inhibition) at 27,300 mg/L. Suman et al. (2013) evaluated pyriproxyfen at 1.0 mg/L on *Aedes albopictus* (Skuse) and *Ae. aegypti* and found hatching inhibition of 80.6% and 47.3%, respectively. Díaz-Martínez et al. (2016) estimated an ovicidal LC_{50} of 28.6 mg/L for spinosad.

Suman et al. (2013) studied the potential of azadirachtin (in neem oil) as an ovicide at an application rate of 1.0 ppm to freshly laid eggs of *Ae. aegypti*

Table 2. Eggs hatching for New Orleans strain of *Aedes aegypti* L. exposed for 24 h to water containing insecticides at various concentrations.

		1.0 (mg/L)		10 (mg/L)		100 (mg/L)		1,000 (mg/L)		10,000 (mg/L)	
Insecticide	Control (0 (mg/L) Hatching % (M ± SE)*	Hatching % (M ± SE)	% Change**	Hatching % (M ± SE)	% Change	Hatching % (M ± SE)	% Change	Hatching % (M ± SE)	% Change	Hatching % (M ± SE)	% 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

* Mean ± standard error.

** The symbols + and − indicate an increase or decrease, respectively, in the hatching percentage compared with the untreated control.

† Concentration was not evaluated.

Table 3. Eggs hatching for New Orleans strain of *Aedes aegypti* L. treated with various insecticides via Potter's tower.*

Insecticide	Hatching %(M ± SE)**	% Change†
Fenpyroximate	38.3 ± 5.50	+69.9
Etoxazole	33.2 ± 13.29	+46.8
Spinetoram	49.2 ± 5.10	+117.9
Pyriproxyfen	49.1 ± 5.61	+117.3
Flufenoxuron	23.8 ± 4.38	+5.6
Spinosad	34.3 ± 7.19	+51.7
Neem oil	51.8 ± 11.63	+129.2
Soybean oil	57.8 ± 9.53	+156.0
Spiromesifen	18.2 ± 5.36	−19.2
Untreated control	22.6 ± 6.02	

* Potter's tower application was 2 ml at 10,000 mg/L for 5 s at a pressure of 7.5 lb/in (134 kg/m).
** Mean ± standard error.
† The symbols + and − indicate an increase or decrease, respectively, in the hatching percentage compared with the untreated control.

and *Ae. albopictus.* They reported 15.7% and 42.9% hatching inhibition, respectively. This ovicide level differs from our data, possibly due to differences in neem oil extraction methodology, as Fernandes et al. (2019) suggested.

Demba et al. (2007) estimated an LC_{50} for neem oil that was lower than the 2 mg/L we observed against *Ae. aegypti* larvae. Shanmugasundaram et al. (2006), using neem oil against larvae of the same species, estimated LC_{50} and LC_{95} values of 2,900 and 16,000 ppm, respectively. After treatment of *Ae. aegypti* larvae and pupae with neem oil (Brahmastra Ayurvedic Products, Lucknow, Uttar Pradesh, India), Kaura et al. (2019) reported LC_{50} values of 7,852 and 19,059 ppm and LC_{90} values of 10,092 and 19,952 ppm, respectively.

The methods used to combat larvae of *Ae. aegypti* include many low-risk insecticides, such as *Bacillus thuringiensis* Beliner var. *israelensis,* insect growth regulators, and juvenile hormone mimics (CENAPRECE 2018), but these chemicals are expensive. Of these, only pyriproxyfen had slight adverse effects on pupae (Hustedt et al. 2020). The desirable features of insecticides for combating these mosquito larvae and pupae include acceptable biological efficacy, low cost, environmental compatibility, and human health and safety. Neem oil has all these characteristics, and there is no documented case of resistance in *Ae. aegypti* or any other insect pest species (Mota-Sanchez and Wise 2020). Thus, countries affected by *Ae. aegypti* should consider the use of neem oil against the larval and pupal stages of this vector.

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

Insecticide	*N**	df**	*b* ± SE†	LC_{50}†† mg/L (95% FL)‡‡	LC_{95}†† mg/L (95% FL)	LC_{99}†† mg/L (95% FL)	Pr > χ^2
Fenpyroximate	900	7	3.51 ± 0.19	91.8 (85.5–98.4)	270.0 (239.5–311.8)	422.2 (360.6–511.8)	0.80
Etoxazole	800	6	3.71 ± 0.22	531.7 (498.5–567.4)	1475.0 (1301.0–1721.0)	2250.0 (1907.0–2768.0)	0.58
Spinetoram	900	7	3.59 ± 0.19	8.5 (7.9–9.0)	24.3 (21.6–27.9)	37.6 (32.2–45.3)	0.49
Pyriproxyfen	700	5	2.77 ± 0.23	24.3 (20.2–29.6)	95.3 (69.7–150.9)	167.7 (112.3–207.6)	0.15
Flufenoxuron	900	7	3.50 ± 0.19	302.3 (282.9–323.0)	890.8 (787.9–103)	1394.0 (1184.0–1700.0)	0.33
Spinosad	800	6	3.87 ± 0.23	0.0107 (0.0100–0.0115)	0.029 (0.0254–0.0329)	0.043 (0.0367–0.0519)	0.028
Neem oil	600	4	6.35 ± 0.41	57.2 (54.6–59.9)	103.8 (95.9–114.5)	132.0 (119.9–151.6)	0.51
Soybean oil	900	7	2.41 ± 0.15	838.9 (768.0–915.1)	4016.0 (3315.0–5119.0)	7683.0 (5922.0–10719.0)	0.29

* Total treated larvae or pupae.
** Degrees of freedom.
† Slope ± standard error.
†† Concentration that kills 50, 95, and 99% of the treated individuals.
‡‡ Fiducial limits (95%).

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

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

* Total treated larvae or pupae.
** Degrees of freedom.
† Slope ± standard error.
†† Concentration that kills 50, 95, and 99% of the treated individuals.
‡‡ Fiducial limits (95%).

Table 6. Mortality of third instar larvae of the New Orleans strain of *Aedes aegypti* L. treated with twice the LC_{99} of neem oil (264 mg/L).

Treatment	Exposure (h)	% Mortality (Mean ± SE)*				
		1 DAS	7 DAS	14 DAS	21 DAS	28 DAS
Without water exchange	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
10% water exchange	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
30% water exchange	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 ± 0.63b	69.4 ± 8.19b	6.9 ± 2.58b	0.0 ± 0.00b
	72	100.0 ± 0.00a	99.4 ± 0.63b	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

* Data are from a field experiment with a complete randomized block design and four replicates. Within a column, mean values with the same letter are not significantly different ($P > 0.05$). DAS = days after the experiment was set up.

Table 7. Mortality of pupae (0–24 h old) of the New Orleans strain of *Aedes aegypti* L. treated with twice the LC_{99} of neem oil (264 mg/L).

Treatment	Exposure (h)	% Mortality (Mean ± SE)*				
		1 DAS	7 DAS	14 DAS	21 DAS	28 DAS
No water exchange	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 ± 5.4 a	99.4 ± 0.63a	69.4 ± 18.30a
10% water exchange	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
30% water exchange	24	99.2 ± 0.83a	50.0 ± 11.86b	30.3 ± 2.17c	1.9 ± 1.20b	0.6 ± 0.63b
	48	99.1 ± 0.83a	93.7 ± 1.61b	41.4 ± 3.95c	2.5 ± 2.39b	0b
	72	99.1 ± 0.83a	96.9 ± 1.57b	43.9 ± 3.54c	1.8 ± 2.39b	0b
Untreated control	24	0.8 ± 0.83b	0c	1.2 ± 1.25c	0b	0b
	48	0.8 ± 0.83b	0c	1.9 ± 1.20c	1.2 ± 0.72b	0b
	72	0.8 ± 0.83b	1.9 ± 1.20c	1.9 ± 1.20c	1.9 ± 1.20b	0b

* Data are from a field experiment with a complete randomized block design and four replicates. Within a column, mean values with the same letter are not significantly different ($P > 0.05$). DAS = days after the experiment was set up.

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