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Original Article

Phytochemical prospection, hemagglutinating and insecticidal activity of saline extracts from the seeds of Tamboril (*Enterolobium contortisiliquum*) Vell. Morong (Fabaceae) on *Aedes aegypti* (Diptera: Culicidae)

Prospecção fitoquímica, atividade hemaglutinante e inseticida dos extratos salinos das sementes de Tamboril (*Enterolobium contortisiliquum*) Vell. Morong (Fabaceae) sobre o *Aedes aegypti* (Diptera: Culicidae)

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Abstract

This study evaluated the insecticidal activity of crude extracts from *Enterolobium contortisiliquum* (Vell.) seeds on eggs and larvae of *A. aegypti*, and also verified the phytochemical profile and the presence of lectins in the extract. The 0.15 M NaCl saline solution was used as the extracting substance. For tests with eggs and larvae, the crude extract was used in its raw form (RCE) and boiled at 100º C for 5 min (BCE). Concentrations of 4.68; 9.37; 18.75; 28.13; 37.13 and 46.89 mg/mL, with distilled water as a negative control. Assays were performed in triplicate. The results were subjected to analysis of variance, Tukey's test and Log-Probit analysis to determine LC_{50} and LC_{90}. BCE showed better results on eggs than RCE, managing to prevent the hatching of larvae in 81.66% ± 10.40 of treated eggs, at a concentration of 46.89 mg/mL. The LC_{50} and LC_{90} were set at 35.95 and 52.67 mg/mL, respectively. In tests with larvae, concentrations of 46.89 and 37.13 mg/mL, for RCE and BCE, caused 100% mortality in 24 hours of exposure. Larval mortality at the other concentrations increased with exposure time extending to 48 h. RCE, at 48 h exposure is the most promising extract on larvae (E = 72.77%, LC_{90} = 10.86 mg/mL). In RCE, the presence of lectins and secondary metabolites: flavonoids, xanthones and phenols, were detected. The results demonstrate the potential of *E. contortisiliquum* seed extracts with ovicidal and larvicidal action on *A. aegypti.*

Keywords: mosquito vector, plant extracts, phytochemistry, secondary metabolites.

Resumo

Esse estudo avaliou a atividade inseticida dos extratos brutos das sementes de *Enterolobium contortisiliquum* (Vell.) sobre ovos e larvas do *A. aegypti*, verificou também o perfil fitoquímico e a presença de lectinas no extrato. A solução salina de NaCl 0,15 M foi utilizada como substância extratora. Para os ensaios com ovos e larvas, o extrato bruto foi utilizado na forma crua (RCE) e fervida a 100º C por 5 min (BCE). Foram testadas as concentrações de 4,68; 9,37; 18,75; 28,13; 37,13 e 46,89 mg/mL, tendo a água destilada como controle negativo. Os ensaios foram realizados em triplicada. Os resultados foram submetidos à análise de variância, Teste de Tukey e análise Log-Probit para determinar CL_{50} e $_{90}$. O BCE apresentou melhores resultados sobre os ovos do que o RCE, conseguindo impedir a eclosão das larvas de 81,66% ± 10,40 dos ovos tratados, na concentração de 46,89 mg/mL. As respectivas CL_{50} e $_{90}$, foram definidas em 35,95 e 52,67 mg/mL, respectivamente. Nos testes com larvas, as concentrações de 46,89 e 37,13 mg/mL, para RCE e BCE, causaram 100% de mortalidade em 24 horas de exposição. A mortalidade larval nas demais concentrações aumentou com o tempo de exposição estendendo-se para 48 h. RCE, com 48 h de exposição é o extrato mais promissor sobre as larvas (E = 72,77%, CL_{90} = 10,86 mg/mL). Em RCE, a presença de lectinas e os metabólitos secundários: flavonoides, xantonas e fenóis, foram detectadas. Os resultados demonstram o potencial dos extratos das sementes de *E. contortisiliquum* com ação ovicida e larvicida sobre o *A. aegypti.*

Palavras-chave: mosquito vetor, extratos vegetais, fitoquímica, metabólitos secundários.

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1. Introduction

The *Aedes aegypti* mosquito (Linnaeus, 1762) is the main vector of dengue (DENV), yellow fever (YFV), zika (ZIKV) and chikungunya (CHIKV) viruses (Scalvenzi et al., 2019; Silva et al., 2019). These viruses cause the main arboviruses that plague tropical countries (Hameed et al., 2015; Näslund et al., 2021). These are diseases that have high rates of morbidity and mortality and can cause severe clinical manifestations (Luz et al., 2020), such as dengue Hemorrhagic Fever (DHF) (Näslund et al., 2021), Guillain-Barré Syndrome and microcephaly (Scalvenzi et al., 2019; Näslund et al., 2021; Mukhtar and Ibrahim, 2022).

The combination of factors such as lack of urban planning, deficient environmental sanitation measures and the climatic conditions of tropical countries are considered responsible for promoting the ideal environmental conditions that favored the development of *A. aegypti* in the Americas (Gregianini et al., 2017).

The elimination of *A. aegypti* breeding sites to avoid the development of larvae, associated with the use of synthetic chemical insecticides in populations of larvae and adults, is a fundamental measure for the control of arboviruses (Pineda-Cortel et al., 2019; Sombié et al., 2019). However, synthetic insecticides have limitations in use because they affect non-target species (Pineda-Cortel et al., 2019), tend to accumulate in the environment (Pineda-Cortel et al., 2019) and are increasingly likely to selection of mosquitoes resistant to current formulations (Moyes et al., 2017; Alkenani et al., 2022), which limits their use (Luz et al., 2020).

Other ways to combat mosquitoes include the use of microbial insecticides containing the bacteria *Bacillus thuringiensis israelensis* (Bti), educational campaigns aimed at eliminating breeding sites, implementation of traps to capture eggs and adults, in addition to biological control using the bacteria *Wolbachia* spp., in addition to the use of *A. aegypti* predators (Baldacchino et al., 2015; Weeratunga et al., 2017; Ogunlade et al., 2021).

With the limitations in the use of inorganic insecticides and the reduction of formulations available for the control of *A. aegypti* (WHO, 2006), the search for alternatives that enable its control ecologically and sustainably has increased. In this sense, plants have proven to be excellent sources of substances and molecules with insecticidal potential (Barbosa et al., 2014; Pavela, 2015; Silva et al., 2018; Pineda-Cortel et al., 2019; Scalvenzi et al., 2019; Luz et al., 2020; Alkenani et al., 2022). They have secondary metabolites that can act in defense against insects (Silva et al., 2019). Lectins are a class of proteins present in plants that also can act on insects (Agra-Neto et al., 2014; Coelho et al., 2017; Silva et al., 2019; Alves et al., 2020; Cavalcanti et al., 2021).

Enterolobium contortisiliquum (Vell.) Morong (Fabaceae) known, among other names, as "tamboril", is a plant that has a wide geographic distribution in Brazil, being easily found in the Atlantic Forest, Caatinga, Cerrado (Bezerra et al., 2021) and in other Latin American countries (Lorenzi, 2020). The seeds of this species are remarkably toxic when ingested and can cause severe poisoning in animals (Bonel-Raposo et al., 2008). This toxicity is associated with the presence of saponins, lectins and enterolobin, a 52.9 kDa protein with cytotoxic, cytolytic, inflammatory and insecticide activities (Lima et al., 2007). In addition, EcTI, a powerful protease inhibitor that retards larval development, is present in the seeds of this species (Tabosa et al., 2020).

The toxicological potential on insects of the constituents of the seeds of this species, as well as the scarcity of studies that verified this potential on *A. aegypti*, motivated this research.

This study aimed to evaluate the ovicidal and larvicidal activities of crude and boiled extracts of *E. contortisiliquum* seeds and to verify the presence of secondary metabolites and lectins.

2. Materials and Methods

2.1. Obtaining Aedes aegypti eggs

The eggs were obtained using ovitrap-type traps, consisting of polypropylene vases with a capacity of 400 mL in black color, containing water and two Eucatex straws, with a porous surface, measuring 3x12 cm, and fixation on the vessel wall using staples. Some straws were lined with filter paper to capture the eggs used in the ovicide tests. (Su and Mulla, 1998). Traps were installed in the city of Juazeiro do Norte, Ceará, Brazil. The collected eggs were counted with the aid of a stereoscopic magnifying glass and stored in a dry place at a temperature of 25 °C.

Part of the straws containing *A. aegypti* eggs were placed in white plastic trays with a capacity of 5 liters, containing three liters of clean water. These were kept in a B.O.D. (Biochemical Oxygen Demand) with a temperature of 25 ± 1 °C, relative humidity of 70 ± 10% and a photoperiod of 12 hours. After hatching, the straws were removed and the larvae were fed with Tropical® fish food until they reached the third instar.

2.2. Obtaining the seeds

E. contortisiliquum seeds were obtained from a specimen located in the urban area of the city of Crato, Ceará, Brazil (Coordinates: 7º 13' 41" S and 39º 23' 07" W). After being removed from the fruits, the seeds were stored in plastic containers at room temperature and protected from heat and humidity until the extracts were prepared.

2.3. Preparation of crude extracts

With the aim of promoting the maximum solubilization of secondary metabolites and proteins in the seed extract, it was decided to use saline solution (0.15 M NaCl) as solvent. Two versions of the extract were prepared, one with the Raw Crude Extract (RCE) and another with Boiled Crude Extract (BCE), to verify the participation of bioactive proteins in the insecticide action. To prepare the RCE, a fine powder was obtained by grinding and sieving the seeds without seed coat. 20 g of this flour was added in 200 mL of 0.15 M NaCl solution (1:10 w/v). The solution was homogenized for 4 h at 25 °C in a magnetic stirrer and centrifuged at 10,000 RPM for 30 min at 25 °C to form a precipitate.

This precipitate was discarded and the supernatant was filtered on filter paper for use in the assays. ECB was prepared from 100 mL of RCE submitted to a water bath at 100°C for 5 minutes with subsequent centrifugation at 10,000 RPM for 30 minutes. The precipitate was discarded and the supernatant was filtered and used in the assays.

2.4. Partial chemical composition

A qualitative phytochemical prospection was carried out to verify the participation of secondary metabolites in *E. contortisiliquum* extracts using the method described by Matos (2009). For this, the extract of *E. contortisiliquum* was initially lyophilized (lyophilizer model K105 - Liotop) and a solution (standard solution) was prepared by dissolving 0.3g of this material in 100 mL of 70% ethanol.

2.4.1. Phenol determination

To determine the presence of phenols, in a container, 3 mL of iron chloride ($FeCl_3$) was added to 30 mL of the *E. contortisiliquum* standard solution. After shaking, the variable color change between blue and red indicated the presence of phenols.

2.4.2. Determination of flavones, flavonols, xanthones and flavanones

To detect the presence of flavones, flavonols and xanthones, 30 mL of standard solution of *E. contortisiliquum* was alkalinized to pH 11 by adding sodium hydroxide (NaOH) to a container. The yellow coloration indicated the presence of flavones, flavonols, xanthones.

To determine the presence of flavanones in the extract, the solution was heated for 3 minutes using an alcohol lamp. The presence of flavanones was detected by changing the color of the solution to red-orange.

2.5. Hemagglutination test

A 4% suspension of rabbit erythrocytes was obtained by washing rabbit blood in 15 M saline solution. The animals had free access to food and water and were maintained on a 12/12 h light/dark cycle with temperature controlled at 25°C. The experiments were carried out following the guidelines of the National Institute of Health, for the care and use of research animals, being approved by the Ethics Committee in the use of Animals - CEUA of the Federal University of Cariri, with the following protocol number: 0014/ 2022.

To detect the presence of lectins in *E. contortisiliquum* RCE, the hemagglutination test was performed, based on the method described by Moreira and Perrone (1977). For this, serial dilutions (1/2, 1/4, 1/8, 1/16 and 1/32) of 100 µL of the sample in 100 µL of saline solution with 0.15 mol/L NaCl in 96-well ELISA-type microdilution plates. Then, 100 µL of an erythrocyte suspension was added to each well, with the first column acting as a negative control, filled only with rabbit erythrocytes. The reaction was incubated for 1 h and after this period, direct visualization of the control and non-control wells was performed in the search for agglutination.

2.6. Tests with eggs and larvae of Aedes aegypti

For the ovicidal assay, pieces of filter paper were carefully cut to obtain 21 lots containing 20 *A. aegypti* eggs each. Batches were individually transferred to 21 disposable cups of 50 mL capacity. In each cup, 1 mL of RCE was added, in concentrations that varied from 4.68; 9.37; 18.75; 28.13; 37.13 and 46.89 mg/mL. The eggs remained in contact with the extract for 30 minutes. Then, they were removed and stored for 24 hours under filter paper to absorb excess extract. After this period, each batch was immersed in distilled water, where they remained for seven days, to verify the existence or not of hatching larvae. The same procedure was performed for the BCE. Seven treatments were performed, all in triplicate. 0.15 mol/L NaCl saline was used as a negative control. The hatching rate (HR) was calculated by dividing the number of hatched larvae by the total number of eggs treated and the result multiplied by 100 (Su and Mulla, 1998).

Assays with *A. aegypti* larvae were carried out according to the method recommended by the World Health Organization (WHO, 2006) with some adaptations. 21 polyethylene cups with 50 mL volume each received 10 third instar larvae - L3. Then, each cup received 10 mL of RCE in concentrations ranging from 4.68; 9.37; 18.75; 28.13; 37.13 and 46.89 mg/ml. The larvae were kept in a B.O.D type climatized chamber at a temperature of 25 ± 1 °C, relative air humidity of 70 ± 10% and photoperiod of 12 hours. Dilutions were prepared using the RCE and distilled water. Seven treatments were performed, all in triplicates. The negative control was made using 10 mL of 0.15 mol/L NaCl saline solution. The experiment was repeated using the BCE.

Larvae mortality was verified after 24 and 48 hours of exposure to the extract, by touching the larvae with tweezers, those that did not respond to the mechanical stimulus being considered dead. The percentage efficiency of the extract (E) was calculated by dividing the number of dead larvae by the number of treated larvae and the result multiplied by 100, according to Abbott's formula (Abbott, 1925).

2.7. Statistical analysis

The values of the lethal concentrations of the extracts capable of killing 50% (LC_{50}) and 90% (LC_{90}) of the treated eggs and larvae were obtained through log-probit analysis, using the Graphpad Prism® software, version 9.0, with a confidence interval of 95% (CI 95%). Values were expressed in milligrams per milliliter of dry weight (mg/mL). The PAST software, version 4.03, was used to perform the Shapiro-Wilk test and demonstrate the normality of the data. Significant differences between means were identified using analysis of variance - two-way ANOVA and Tukey's test, with an error of 0.05.

3. Results

The qualitative phytochemical analysis revealed the presence of some secondary metabolism compounds in the *E. contortisiliquum* extract, such as phenols, flavones, flavonols, flavanones and xanthones.

The hemagglutinating activity test was positive for the presence of lectins, since the coagulation of erythrocytes exposed to RCE was verified.

Both extracts showed ovicidal activity. However, the RCE presented very low toxicity for *A. aegypti* eggs, while the BCE obtained a better performance of the ovicidal activity. For the RCE, the concentrations of 46.89 and 37.13 mg/mL were the only ones that showed results that prevented the hatching of 13.33% and 1.66% of the larvae of the treated eggs, respectively. In turn, the process of boiling the crude extract improved the ovicidal activity, since the action was extended up to a concentration of 18.75 mg/mL.

For the BCE, the averages of the ovicidal activity of concentrations 18.75; 9.37 and 4.68, did not differ significantly from the negative control (Figure 1).

Extract concentrations did not statistically significantly influence ovicidal activity ($p = 0.396$).

The results of the sum of the HR of the BCE were 73.3% and the HR of the RCE, 97.5%, which points to the best performance of the BCE on eggs. The hatching rates of eggs treated with different concentrations of BCE ranged from 95 ± 5 to 18.33% ± 10.40%. The respective LC_{50} and LC_{90} were set at 35.95 mg/mL (34.74 - 37.17) and 52.67 mg/mL (50.03 - 55.30). RCE LC_{50} and LC_{90} were not calculated due to its low larvicidal activity. All larvae from eggs treated only with 0.15 M NaCl saline (Negative control) hatched (Table 1).

Both RCE and BCE caused mortality in 100% of the larvae at concentrations of 46.89 and 37.13 mg/mL, respectively, in the first 24 hours. For the other concentrations, the two extracts increased the mortality rate with the extension of the exposure period of 48 hours, when 100% of larval mortality was reached at concentrations of 28.13 and 18.75 mg/mL.

Means of RCE and BCE mortality at concentrations of 9.37 and 4.68 mg/mL do not differ significantly from the control treatment. At the concentration of 18.75 mg/mL, the mean mortality in tests with RCE and BCE for 48 h of exposure are statistically similar to higher concentrations (28.13; 37.13 and 46.89 mg/mL) in maximum mortality was reached (Figure 2).

Concentration ($p < 0.001$) and time ($p < 0.001$) are significant factors for larvicidal activity, as well as the interaction between time and concentration, which proved to be a significant factor for the larvicidal action of the extracts ($p < 0.001$).

The RCE with action for 48h, presented the best total efficiency among the extracts and exposure periods evaluated (E = 72.77%). This extract also showed the lowest LC_{50} and LC_{90}, defined at 10.86 mg/mL (11.44 - 12.61) and 12.11 mg/mL (9.48 - 19.13) respectively (Table 2).

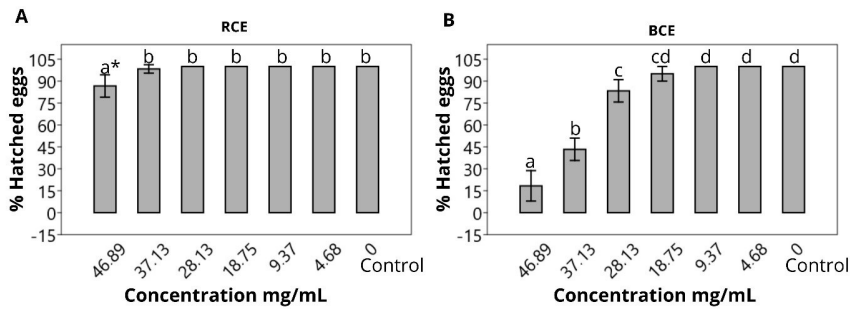

Figure 1. Ovicidal activity (%) of *E. contortisiliquum* extracts. Raw Crude Extract – RCE (A) and Boiled Crude Extract – BCE (B). Results expressed as mean ± standard deviation. *Averages followed by the same letters, do not differ significantly from each other by Tukey's Test at the 5% probability level.

Table 1. Means (M) and standard deviations (SD) of RCE and BCE ovicidal activity.

Extract	Concentration (mg/mL) / M ± SD (%)							HR (%)	LC_{50} mg/mL (CI95%)	LC_{90} mg/mL (CI95%)
	46.89	37.13	28.13	18.75	9.37	4.68	Control			
RCE	86.6 ± 7.63	98.3 ± 2.88	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	97.5	--	--
BCE	18.3 ± 10.4	43.3 ± 7.63	83.3 ± 7.63	95 ± 5	100 ± 0	100 ± 0	100 ± 0	73.3	35.9 (34.74-37.17)	52.67 (50.03-55.30)

Legend: BCE = Boiled crude extract; RCE = Raw crude extract; HR = hatching rate.

Table 2. Means (M) and standard deviations (SD) of larvicidal activity, total efficiency (E) % and minimum inhibitory concentrations (LC_{50} and $_{90}$) of *E. contortisiliquum* extracts (RCE and BCE) for 24 and 48 h on *A. aegypti*.

	Concentration (mg/mL) / Mortality (% M ± SD)									
Extract / Time	46.89	37.13	28.13	18.75	9.37	4.68	Control	E (%)	LC_{50} mg/mL (CI95%)	LC_{90} mg/mL (CI95%)
RCE **24 h**	100 ± 0	100 ± 0	63.3 ± 5.7	20 ± 10	6.6 ± 5.7	6.6 ± 11.5	0 ± 0	49	24.61 (23.03-26.26)	36.17 (32.13-40.68)
RCE **48 h**	100 ± 0	100 ± 0	100 ± 0	100 ± 0	23.3 ± 23	13.3 ± 1.5	0 ± 0	72.7	10.86 (11.44-12.61)	12.11 (9.48-19.13)
BCE **24 h**	100 ± 0	100 ± 0	80 ± 10	26.6 ± 5.7	3.33 ± 5.7	0 ± 0	0 ± 0	51.6	22.13 (21.34-22.97)	31.58 (29.20-34.06)
BCE **48 h**	100 ± 0	100 ± 0	100 ± 0	90 ± 10	26.6 ± 25	0 ± 0	0 ± 0	69.4	11.62 (10.05-13.04)	18.3 (14.30-23.17)

Legend: BCE = Boiled crude extract; RCE = Raw crude extract.

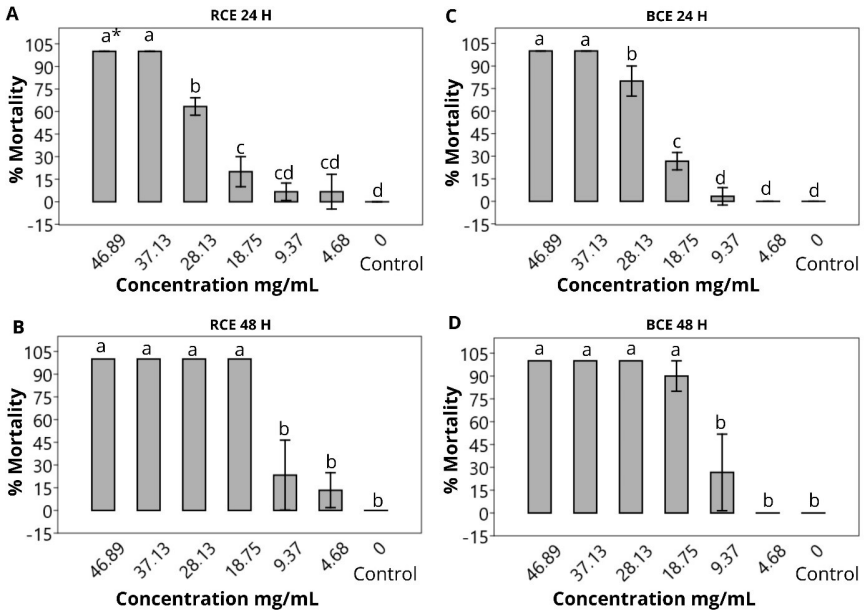

Figure 2. Larvicidal activity (%) of saline extracts of *E. contortisiliquum*. (A) Larvicidal activity of RCE during 24h; (B) Larvicidal activity of RCE during 48h; (C) Larvicidal activity of BCE during 24h; (D) Larvicidal activity of BCE during 48h. *Means followed by the same letters do not differ significantly from each other by Tukey's Test at the 5% probability level.

4. Discussion

Among the secondary metabolites, the class of flavonoids stands out, such as flavones, flavonols and flavanones. Among several biological activities, these metabolites stand out for their insecticidal action. (Simões et al., 2017; Burger et al., 2021).

Flavonoids are important inhibitors of the enzyme acetylcholinesterase (AChE) (Imran et al., 2020). The inhibition of AChE activity is one of the most effective mechanisms of action in insecticidal activity, as it leads to insect death by blocking the transduction of neural signals (Casida and Durkin, 2013). Flavonoids are also capable of reducing the oviposition of A. aegypti females, as well as making their eggs unfeasible (Rajkumar and Jebanesan, 2008).

Although not detected in this research, saponins are secondary metabolites that have already been detected in *E. contortisiliquum* seed extracts (Farias et al., 2010). In addition to flavonoids and saponins, amides, quinones, rotenoids, prenylated, coumarins, alkylphenols, lignans, lactones, monoterpenes, diterpenes, triterpenes, limonins and alkaloids, are examples of secondary metabolites that have proven insecticidal action (Garcez et al., 2013).

In this study, the studied extracts showed ovicidal and larvicidal activity, therefore, it was possible to associate the presence of flavonoids as a determining factor for these findings. This is because this class of secondary metabolites was also detected in other studies on insecticidal activity in *A. aegypti* (Ferreira et al., 2019; Yusuf et al., 2020; Oliveira et al., 2021).

On the other hand, the presence of lectins in the extract reveals that saline extraction was effective in protein solubilization. It is also an indication that other proteins with proven action on insects, such as enterolobin (Lima et al., 2007) and protease inhibitors, such as EcTI (Sasaki et al., 2015; Tabosa et al., 2020) may also have been solubilized. In the research carried out by Farias et al. (2010), lectins and trypsin inhibitors were detected in the aqueous extract of *E. contortisiliquum* seeds. Similar results were obtained in the work by Marques et al. (2017), in which the tested saline extract was also positive for the presence of lectins in its composition. The confirmation of these proteins in an extract makes future investigations feasible with the aim of isolating and testing them on *A. aegypti*.

The increase in larval mortality promoted by plant extracts, by increasing the concentration and extending the exposure time, was also observed in other works on toxicity (Barbosa et al., 2014; Andrade et al., 2020; Yusuf et al., 2020; Oliveira et al., 2021). As extracts enter the organism of *A. aegypti* larvae by ingestion through feeding, a lower concentration of extract requires more time for accumulation to occur in the organism of the mosquito larvae. Extending exposure to 48 h allowed this accumulation and made even the lowest doses effective in killing *A. aegypti* larvae. This characteristic was reflected when the values of LC_{50} and LC_{90} for the larvicidal activity of RCE and BCE were significantly lower for 48 h of exposure. This fact shows the role of the interaction between exposure time and concentration in the effectiveness of the extracts in the mortality of *A. aegypti* larvae.

In turn, *A. aegypti* eggs have a rigid protective shell called the chorion. This barrier hinders the penetration of active molecules and contacts with the embryo (Forattini, 2002). To be effective, a compound considered ovicidal needs to overcome this barrier and the concentration of molecules in an extract is a relevant factor for its ovicidal potential. Given this, it was observed that the ovicidal activity of the BCE was more effective in the highest concentrations.

Heating did not show a significant loss in the performance of BCE on larvae and eggs, this fact is an indication that the toxicity of the extract can be maintained even through mechanisms independent of the action of proteins and enzymes. It is possible that the secondary metabolites present in the BCE maintained the action of the extract, even in the absence of substances that were affected by heat. This action of the bioactivity of the extract may be the result of a synergistic action, or not, of the many molecules present in a vegetable (Chen et al., 1995) and, in the case of secondary metabolites, the temperature is one of the factors that alter the profile of these metabolites (Gobbo-Neto and Lopes, 2007).

In conventional processes for extracting secondary metabolites, high temperatures provide a better yield (Seidel, 2012). Studying the effects of temperature on the yield of secondary metabolites extracted from poplar wood, Todaro et al. (2017) obtained higher yields of flavonoids and polyphenols from wood heated at temperatures between 200 and 220 ºC. In this sense, the increase in temperature may have improved the concentration of secondary metabolites present in the BCE, or even activated mechanisms that facilitated the penetration of metabolites into the chorion of *A. aegypti* eggs. This situation could even explain the improvement in ovicidal action. This hypothesis is also valid to justify the maintenance of larvicidal activity even with the extract proteins being denatured due to heat. A possible increase in the concentration of flavonoids in the BCE would compensate for the absence of these molecules.

The absence of tests using RCE and BCE on pupae and adults of *A. aegypti* may be a limitation of this research, as it is not possible to determine the scope of use of extracts over the entire life cycle of the mosquito. Another possible limitation is the lack of data regarding a positive control in trials using a synthetic insecticide to which *A. aegypti* has not acquired resistance. Thus, it is not possible to compare the efficiency of the plant extract with a synthetic formulation. Finally, the absence of acute toxicity tests can be seen as a limitation of the research, as it does not allow for identifying levels of safety in the use of extracts as a commercial product.

5. Conclusions

Both crude and boiled extracts of *E. contortisiliquum* cause mortality of *A. aegypti* eggs and larvae. However, the boiled crude extract performs better on mosquito eggs. The extracts caused the mortality of the larvae both within 24 and 48h of exposure and the heating did not cause a loss of performance on the morality of the larvae.

The presence of flavonoids and lectins in the crude extract is probably responsible for the ovicidal and larvicidal action of *A. aegypti*. These results demonstrate the potential of the extract of the seeds studied in the control of this vector.

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References

ABBOTT, W.S., 1925. A method for computing the effectiveness of insecticides. *Journal of Economic Entomology*, vol. 18, no. 2, pp. 265-267. http://dx.doi.org/10.1093/jee/18.2.265a.

AGRA-NETO, A.C., NAPOLEÃO, T.H., PONTUAL, E.V., SANTOS, N.D.L., LUZ, L.A., OLIVEIRA, C.M.F., MELO-SANTOS, M.A.V., COELHO, L.C.B.B., NAVARRO, D.M.A.F. and PAIVA, P.M.G., 2014. Effect of *Moringa oleifera* lectins on survival and enzyme activities of *Aedes aegypti* larvae susceptible and resistant to organophosphate. *Parasitology Research*, vol. 113, no. 1, pp. 175-184. http://dx.doi.org/10.1007/s00436-013-3640-8. PMid:24142287.

ANDRADE, T.A.S., SANTANA, I.M., JIMENEZ, G.C., FARIAS, E.T.N., MACEDO, L.O., ALVES, L.C., RAMOS, R.A.N., FAUSTINO, M.A.G. and CARVALHO, G.A., 2020. Larvicidal activity of *Caesalpinia ferrea* Mart. and *Lippia origanoides* Cham. Against *Aedes aegypti* (Linnaeus, 1762) (Diptera: culicidae). *Revista de Patologia Tropical*, vol. 49, no. 1, pp. 145-154. http://dx.doi.org/10.5216/rpt.v49i1.61826.

ALKENANI, N.A., AL-SOLAMI, H.M., ALGHAMDI, A.G., ULLAH, I., JAVEED, K., DAR, S.A. and AHMED, M.M.M., 2022 [viewed 8 March 2023]. Effect of botanicals on Yellow Fever Mosquito *Aedes aegypti* (Diptera: culicidae). *Specialusis Ugdymas* [online], vol. 2, no. 43, pp. 1503-1514. Available from: https://www.sumc.lt/index.php/se/article/view/1459/1192

ALVES, R.R., SOARES, T., BENTO, E.F., ROLDAN-FILHO, R.S., SOUZA, B.S., LIMA, M.K., GONÇALVES, G.G., BRAYNER, F.A., ALVES, L.C., NAVARRO, M.D., NAPOLEÃO, T.H. and PAIVA, P.M., 2020. Ovicidal lectins from *Moringa oleifera* and *Myracrodruon urundeuva* cause alterations in chorionic surface and penetrate the embryos of *Aedes aegypti* eggs. *Pest Management Science*, vol. 76, no. 2, pp. 730-736. http://dx.doi.org/10.1002/ps.5572. PMid:31386279.

BALDACCHINO, F., CAPUTO, B., CHANDRE, F., DRAGO, A., DELLA TORRE, A., MONTARSI, F. and RIZZOLI, A., 2015. Control methods against invasive *Aedes* mosquitoes in Europe: a review. *Pest Management Science*, vol. 71, no. 11, pp. 1471-1485. http://dx.doi.org/10.1002/ps.4044. PMid:26037532.

BARBOSA, P.B.B.M., OLIVEIRA, J.M., CHAGAS, J.M., RABELO, L.M.A., MEDEIROS, G.F., GIORDANI, R.B., SILVA, E.A., UCHÔA, A.F. and FÁTIMA, M.F.M.X., 2014. Evaluation of seed extracts from plants found in the Caatinga biome for the control of *Aedes aegypti*. *Parasitology Research*, vol. 113, no. 10, pp. 3565-3580. http://dx.doi.org/10.1007/s00436-014-4022-6. PMid:25056942.

BEZERRA, J.J.L., PINHEIRO, A.A.V. and LUCENA, R.B., 2021. Phytochemistry and poisoning in ruminants by *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae): a systematic review. *Toxicon*, vol. 201, no. 1, pp. 46-53. http://dx.doi.org/10.1016/j.toxicon.2021.08.008. PMid:34411592.

BURGER, M.C., MATOS, A.P., CUNHA, G.O.S., BERNARDO, A.R., MENEZES, A.C.S., VIEIRA, P.C. and FERNANDES, J.B., 2021. Flavonoides e atividade inseticida sobre *Spodoptera frugiperda* e *Myrsine coriacea* (Primulaceae). *Revista Virtual de Química*, vol. 13, no. 4, pp. 953-958. http://dx.doi.org/10.21577/1984-6835.20210022.

CASIDA, J.E. and DURKIN, K.A., 2013. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annual Review of Entomology*, vol. 58, no. 1, pp. 99-117. http://dx.doi.org/10.1146/annurev-ento-120811-153645. PMid:23317040.

CAVALCANTI, V.L.R., COSTA, R.M.P.B., PONTUAL, E.V., ANDRADE, A.F., ALVES, L.C., PORTO, A.L.F. and BEZERRA, R.P., 2021. *Chlorella vulgaris* lectin kills *Aedes aegypti* larvae. *Algal Research*, vol. 56, no. 1, pp. 102290. http://dx.doi.org/10.1016/j.algal.2021.102290.

CHEN, W., ISMAN, M.B. and CHIU, S.-F., 1995. Antifeedant and growth inhibitory effects of the limonoid toosendanin and *Melia toosendan* extracts on the variegated cutworm, *Peridromasaucia* (Lep., Noctuidae). *Journal of Applied Entomology*, vol. 119, no. 5, pp. 367-370. http://dx.doi.org/10.1111/j.1439-0418.1995.tb01302.x.

COELHO, L.C.B.B., SILVA, P.M.D.S., LIMA, V.L.D.M., PONTUAL, E.V., PAIVA, P.M.G., NAPOLEÃO, T.H. and CORREIA, M.T.D.S., 2017. Lectins, interconnecting proteins with biotechnological/pharmacological and therapeutic applications. *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, no. 1, pp. 1594074. http://dx.doi.org/10.1155/2017/1594074. PMid:28367220.

FARIAS, D.F., CAVALHEIRO, M.G., VIANA, M.P., QUEIROZ, V.A., ROCHA-BEZERRA, L.C., VASCONCELOS, I.M. and CARVALHO, A.F., 2010. Water extracts of Brazilian leguminous seeds as rich sources of larvicidal compounds against *Aedes aegypti* L. *Anais da Academia Brasileira de Ciências*, vol. 82, no. 3, pp. 585-594. http://dx.doi.org/10.1590/S0001-37652010000300006. PMid:21562687.

FERREIRA, M.D.L., FERNANDES, D.A., NUNES, F.C., TELES, Y.C., ROLIM, Y.M., SILVA, C.M.D., ALBUQUERQUE, J.B.L., AGRA, M.F. and SOUZA, M.D.F., 2019. Phytochemical study of *Waltheria viscosissima* and evaluation of its larvicidal activity against *Aedes aegypti*. *Revista Brasileira de Farmacognosia*, vol. 29, no. 5, pp. 582-590. http://dx.doi.org/10.1016/j.bjp.2019.05.008.

FORATTINI, O.P., 2002. *Culicidologia médica: identificação, biologia, epidemiologia*. 2ª ed. São Paulo: Edusp. 860 p.

GARCEZ, W.S., GARCEZ, F.R., SILVA, L.M.G.E. and SARMENTO, U.C., 2013. Naturally occurring plant compounds with larvicidal activity against *Aedes aegypti*. *Revista Virtual de Química*, vol. 5, no. 3, pp. 363-393. http://dx.doi.org/10.5935/1984-6835.20130034.

GOBBO-NETO, L. and LOPES, P.N., 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova*, vol. 30, no. 2, pp. 374-381. http://dx.doi.org/10.1590/S0100-40422007000200026.

GREGIANINI, T.S., RANIERI, T., FAVRETO, C., NUNES, Z.M.A., TUMIOTO GIANNINI, G.L., SANBERG, N.D., DA ROSA, M.T.M. and DA VEIGA, A.B.G., 2017. Emerging arboviruses in Rio Grande do Sul, Brazil: Chikungunya and Zika outbreaks, 2014-2016. *Reviews in Medical Virology*, vol. 27, no. 6, pp. e1943. http://dx.doi.org/10.1002/rmv.1943. PMid:28929534.

HAMEED, M., AHMED, N., KHAN, I.A., SHAH, B., KHAN, A., ALI, M., RASHEED, M.T., JUNAID, K., ADNAN, M., ZAKI, A.B. and SAED, A., 2015. Studies on the efficacy of selected insecticides against *Anopheles* mosquitoes of village Goth Bhoorji (Sindh) Pakistan. *Journal of Entomology and Zoology Studies*, vol. 3, no. 6, pp. 169-173. http://dx.doi.org/10.3390/insects14010025.

IMRAN, M., IRFAN, A., IBRAHIM, M., ASSIRI, M.A., KHALID, N., ULLAH, S. and AL-SEHEMI, A.G., 2020. Carbonic anhydrase and cholinesterase inhibitory activities of isolated flavonoids from *Oxalis corniculata* L. and their first-principles investigations. *Industrial Crops and Products*, vol. 148, no. 1, pp. 112285. http://dx.doi.org/10.1016/j.indcrop.2020.112285.

LIMA, C.M.R., ZANOTTA, P.J., RICART, C.A. and SOUSA, M.V.D., 2007. Presence of the cytolytic protein enterolobin in different developmental stages of *Enterolobium contortisiliquum* seeds. *Brazilian Journal of Plant Physiology*, vol. 19, no. 2, pp. 163-170. http://dx.doi.org/10.1590/S1677-04202007000200008.

LORENZI, H. 2020. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. 8ª ed. Nova Odessa: Plantarum. 385 p.

LUZ, T.R.S.A., MESQUITA, L.S.S., AMARAL, F.M.M.D. and COUTINHO, D.F., 2020. Essential oils and their chemical constituents against *Aedes aegypti* L. (Diptera: Culicidae) larvae. *Acta Tropica*, vol. 212, no. 1, pp. 105705. http://dx.doi.org/10.1016/j.actatropica.2020.105705. PMid:32956639.

MARQUES, F.M.S., MARTINS, M.G.Q., PEREIRA, A.M.G., OLIVEIRA, M.V. and CAJAZEIRAS, J.B., 2017. Determinação de alcaloides, lectina e toxicidade sob náuplios de *Artemia sp*. do extrato aquoso de sementes de *Crotalaria ochroleuca*. *Revista da Universidade Vale do Rio Verde*, vol. 19, no. 1, pp. 206-218. http://dx.doi.org/10.5892/cavalruvrd.v19i1.6381.g10952069.

MATOS, F.J.A., 2009. *Introdução à fitoquímica experimental*. 3ª ed. Fortaleza: UFC. 150 p.

MOREIRA, R.A. and PERRONE, J.C., 1977. Purification and partial characterization of a lectin from *Phaseolus vulgaris*. *Plant Physiology*, vol. 59, no. 5, pp. 783-787. http://dx.doi.org/10.1104/pp.59.5.783. PMid:16659942.

MOYES, C.L., VONTAS, J., MARTINS, A.J., NG, L.C., KOOU, S.Y., DUSFOUR, I., RAGHAVENDRA, K., JOÃO PINTO, J., CORBEL, V., DAVID, J.P. and WEETMAN, D., 2017. Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Neglected Tropical Diseases*, vol. 11, no. 7, pp. e0005625. http://dx.doi.org/10.1371/journal.pntd.0005625. PMid:28727779.

MUKHTAR, M.M. and IBRAHIM, S.S., 2022. Temporal evaluation of insecticide resistance in populations of the major arboviral vector *Aedes Aegypti* from northern Nigeria. *Insects*, vol. 13, no. 2, pp. 187. http://dx.doi.org/10.3390/insects13020187. PMid:35206760.

NÄSLUND, J., AHLM, C., ISLAM, K., EVANDER, M., BUCHT, G. and LWANDE, O.W., 2021. Emerging Mosquito-Borne viruses linked to *Aedes aegypti* and *Aedes albopictus*: global status and preventive strategies. *Vector Borne and Zoonotic Diseases*, vol. 21, no. 10, pp. 731-746. http://dx.doi.org/10.1089/vbz.2020.2762. PMid:34424778.

OGUNLADE, S.T., MEEHAN, M.T., ADEKUNLE, A.I., ROJAS, D.P., ADEGBOYE, O.A. and MCBRYDE, E.S., 2021. A review: *Aedes*-borne arboviral infections, controls and Wolbachia-based strategies. *Vaccines*, vol. 9, no. 1, pp. 1-23. http://dx.doi.org/10.3390/vaccines9010032. PMid:33435566.

OLIVEIRA, A.K.M., PAULIQUEVIS, C.F., MATIAS, R., SILVA, P.G., ZANELLA, D.F.P., ROEL, A.R. and PORTO, K.R., 2021 [viewed 8 March 2023]. Efeito larvicida do extrato etanólico de *Piper umbellatum* sobre o mosquito *Aedes aegypti*. *South American Journal of Basic Education, Technical and Technological* [online], vol. 8, no. 1, pp. 84-101. Available from: https://periodicos.ufac.br/index.php/SAJEBTT/article/view/4524

PAVELA, R., 2015. Essential oils for the development of eco-friendly mosquito larvicides: a review. *Industrial Crops and Products*, vol. 76, no. 1, pp. 174-187. https://doi.org/10.1016/j.indcrop.2015.06.050.

PINEDA-CORTEL, M.R.B., CABANTOG, R.J.R., CAASI, P.M., CHING, C.A.D., PEREZ, J.B.S., GODISAN, P.G.M., LATORRE, C.M.G., LUCERO, D.R. and SALONGA, R.B., 2019. Larvicidal and ovicidal activities of *Artocarpus blancoi* extracts against *Aedes aegypti*. *Pharmaceutical Biology*, vol. 57, no. 1, pp. 120-124. http://dx.doi.org/10.1080/13880209.2018.1561727. PMid:30776315.

RAJKUMAR, S. and JEBANESAN, A., 2008. Bioactivity of flavonoid compounds from *Poncirus trifoliata* L. (Family: Rutaceae) against the dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). *Parasitology Research*, vol. 104, no. 1, pp. 19-25. http://dx.doi.org/10.1007/s00436-008-1145-7. PMid:18810494.

BONEL-RAPOSO, J., RIET-CORREA, F., GUIM, T.N., SCHUCH, I.D., GRECCO, F.B. and FERNANDES, C.G., 2008. Intoxicação aguda e abortos em cobaias pelas favas de *Enterolobium contortisiliquum* (Leg. Mimosoideae). *Pesquisa Veterinária Brasileira*, vol. 28, no. 12, pp. 593-596. http://dx.doi.org/10.1590/S0100-736X2008001200005.

SASAKI, D.Y., JACOBOWSKI, A.C., SOUZA, A.P., CARDOSO, M.H., FRANCO, O.L. and MACEDO, M.L.R., 2015. Effects of proteinase inhibitor from *Adenanthera pavonina* seeds on short-and long term larval development of *Aedes aegypti*. *Biochimie*, vol. 112, pp. 172-186. http://dx.doi.org/10.1016/j.biochi.2015.03.011. PMid:25796215.

SCALVENZI, L., RADICE, M., TOMA, L., SEVERINI, F., BOCCOLINI, D., BELLA, A., GUERRINI, A., TACCHINI, M., SACCHETTI, G., CHIURATO, M., ROMI, R. and DI LUCA, M., 2019. Larvicidal activity of *Ocimum campechianum, Ocotea quixos* and *Piper aduncum* essential oils against *Aedes aegypti. Parasite*, vol. 26, pp. 23. http://dx.doi.org/10.1051/parasite/2019024. PMid:30994444.

SEIDEL, V., 2012. Initial and bulk extraction of natural products isolation. *Methods in Molecular Biology*, vol. 864, no. 1, pp. 27-41. http://dx.doi.org/10.1007/978-1-61779-624-1_2. PMid:22367892.

SILVA, I.M.A., MARTINS, G.F., MELO, C.R., SANTANA, A.S., FARO, R.R.N., BLANK, A.F., ALVES, P.B., PICANÇO, M.C., CRISTALDO, P.F., ARAÚJO, A.P.A. and BACCI, L., 2018. Alternative control of *Aedes aegypti* resistant to pyrethroids: lethal and sublethal effects of monoterpene bioinsecticides. *Pest Management Science*, vol. 74, no. 4, pp. 1001-1012. http://dx.doi.org/10.1002/ps.4801. PMid:29160036.

SILVA, L.L.S., FERNANDES, K.M., MIRANDA, F.R., SILVA, S.C.C., COELHO, L.C.B.B., NAVARRO, D.M.D.A.F., NAPOLEÃO, T.H., MARTINS, G.F. and PAIVA, P.M.G., 2019. Exposure of mosquito (*Aedes aegypti*) larvae to the water extract and lectin-rich fraction of *Moringa oleifera* seeds impairs their development and future fecundity. *Ecotoxicology and Environmental Safety*, vol. 183, pp. 109583. http://dx.doi.org/10.1016/j.ecoenv.2019.109583. PMid:31446169.

SIMÕES, C.M.O., SCHENKEL, E.P., PALAZZO DE MELLO, J.C., MENTZ, L.A. and ROSPETROVICK, P., 2017. *Farmacognosia: do produto natural ao medicamento*. 7ª ed. Porto Alegre: Artmed. 483 p.

SU, T. and MULLA, M.S., 1998. Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: culicidae). *Journal of the American Mosquito Control Association*, vol. 14, no. 2, pp. 204-209. PMid:9673924.

SOMBIÉ, A., SAIKI, E., YAMÉOGO, F., SAKURAI, T., SHIROZU, T., FUKUMOTO, S., SANON, A., WEETMAN, D., MCCALL, P.J., KANUKA, H. and BADOLO, A., 2019. High frequencies of F1534C and V1016I kdr mutations and association with pyrethroid resistance in *Aedes aegypti* from Somgandé (Ouagadougou), Burkina Faso. *Tropical Medicine and Health*, vol. 47, no. 2, pp. 2-8. http://dx.doi.org/10.1186/s41182-018-0134-5. PMid:30787670.

TABOSA, P.M., ALMEIDA FILHO, L.C., FRANCA, R.X., ROCHA-BEZERRA, L.C.B., VASCONCELOS, I.M. and CARVALHO, A.F., 2020. Trypsin inhibitor from *Enterolobium contortisiliquum* seeds impairs *Aedes aegypti* development and enhances the activity of *Bacillus thuringiensis* toxins. *Pest Management Science*, vol. 76, no. 11, pp. 3693-3701. http://dx.doi.org/10.1002/ps.5918. PMid:32453460.

TODARO, L., RUSSO, D., CETERA, P. and MILELLA, L., 2017. Effects of thermo-vacuum treatment on secondary metabolite content and antioxidant activity of poplar (*Populus nigra* L.) wood extracts. *Industrial Crops and Products*, vol. 109, no. 1, pp. 384-390. http://dx.doi.org/10.1016/j.indcrop.2017.08.052.

WEERATUNGA, P., RODRIGO, C., FERNANDO, S.D. and RAJAPAKSE, S., 2017. Control methods for *Aedes albopictus* and *Aedes aegypti. Cochrane Database of Systematic Reviews*, vol. 2017, no. 8, pp. 3895-3904. http://dx.doi.org/10.1002/14651858.CD012759.

WORLD HEALTH ORGANIZATION – WHO, 2006 [viewed 8 March 2023]. *Pesticides and their application for the control of vectors and pests of public health importance* [online]. Available from: https://apps.who.int/iris/handle/10665/69223

YUSUF, Y., EFENDI, K. and DIANTASARI, S., 2020. Larvicidal activity test of ethanolic extract of (*Euphorbia tirucalli* Linn) stem on *Aedes aegypti* Larvae. *Systematic Reviews in Pharmacy*, vol. 11, no. 3, pp. 388-392. http://dx.doi.org/10.5530/srp.2020.3.48.