Larvicidal effect of *Prosopis juliflora* on *Aedes aegypti* (Diptera: Culicidae)

The aim of this work was to evaluate the larvicidal effect of aqueous extracts of *Prosopis juliflora* on *A. aegypti*. Oviposition traps (ovitrap) were used to obtain the insect's eggs. The test was carried out at the Agricultural Entomology Laboratory of the Federal University of Cariri, Crato, Ceará, Brazil, with controlled temperature, humidity and photophase, using larvae in the 3rd instar. The design used was completely randomized with five doses (10, 20, 30, 40 and 50 g L⁻¹) of the aqueous extract plus two control treatments (pyriproxifene at 0.01g L⁻¹ and distilled water). The treatments were evaluated after 24, 48, 72, 96, 120, 144 and 168 h of exposure of the larvae, being considered dead those that did not react to the mechanical stimulus of a clamp. The doses of 40 and 50 g L⁻¹ of *P. juliflora* are effective in the mortality of *A. aegypti* larvae after 48 h of exposure, with greater efficiency (100% mortality) after 120 h. The lowest doses (10 and 20 g L⁻¹) have low mortality efficiency, while the dose of 30 g L⁻¹ causes satisfactory mortality after 148 h of exposure. The aqueous extract of the leaves of *P. juliflora* (in doses of 40 and 50 g L⁻¹) is effective in controlling *A. aegypti*.

Keywords: Algaroba; Botanical Insecticide; Dengue; Secondary Metabolites.

Efeito larvívica de *Prosopis juliflora* sobre *Aedes aegypti* (Diptera: Culicidae)

O objetivo deste trabalho foi avaliar o efeito larvívica de extratos aquosos de *Prosopis juliflora* sobre *A. aegypti*. Armadilhas de oviposição (ovitrap) foram utilizadas para a obtenção dos ovos do inseto. O teste foi realizado no Laboratório de Entomologia Agrícola da Universidade Federal do Cariri, Crato, Ceará, Brasil, com temperatura, umidade e fotofase controladas, utilizando larvas de 3^o ínstar. O delineamento utilizado foi inteiramente casualizado com cinco doses (10, 20, 30, 40 e 50 g L⁻¹) do extrato aquoso mais dois tratamentos controle (piriproxi-feno 0,01g L⁻¹ e água destilada). Os tratamentos foram avaliados após 24, 48, 72, 96, 120, 144 e 168 h de exposição das larvas, sendo considerados mortos aqueles que não reagiram ao estímulo mecânico de uma pinça. As doses de 40 e 50 g L⁻¹ de *P. juliflora* são eficazes na mortalidade de larvas de *A. aegypti* após 48 h de exposição, com maior eficiência (100% mortalidade) após 120 h. As menores doses (10 e 20 g L⁻¹) apresentam baixa eficiência de mortalidade, enquanto a dose de 30 g L⁻¹ causa mortalidade satisfatória após 148 h de exposição. O extrato aquoso das folhas de *P. juliflora* (nas doses de 40 e 50 g L⁻¹) é eficaz no controle de *A. aegypti*.

Palavras-chave: Algaroba; Inseticida botânico; Dengue; Metabólitos secundários.

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INTRODUCTION

Aedes aegypti L., is a mosquito that causes serious problems to human health, transmitting the virus from four important diseases: yellow fever, dengue, chikungunya and Zika (SCOLARI et al., 2019). This vector is widely distributed, carrying out its activities at the same times and environments as the human population (BENNETT et al., 2019). Transmission occurs due to the bite of the female mosquito infected with the DEN virus during blood meal (SCOLARI et al., 2019). Annually, about 50-100 million people worldwide are infected with these four types of viruses and about three billion people are at risk of becoming infected, particularly in tropical countries (WHO, 2011).

The spread of these diseases is considered to be one of the most serious public health problems in the world and can be explained in part by an intensification of conditions favorable to the dispersion and proliferation of *A. aegypti* as a result of global trade and unplanned urbanization; inefficient implementation of vector control programs due to inadequate human, financial and infrastructure capabilities; irregular supply and irregular water storage practices and ineffective waste disposal (LINDSAY et al., 2017).

The main corn producing states are: Mato Grosso, with an index of 20 million tons in the 2015/2016 harvest, followed by Paraná, with 16.2 million tons, Mato Grosso do Sul, with 8.3 million tons, Goiás, with 7.7 million tons, Minas Gerais, with 7.0 million tons and Rio Grande do Sul, with 6.0 million tons (IBGE, 2017).

The prevention or reduction of diseases caused by viruses transmitted by *A. aegypti* continues to depend largely on the control of mosquito populations or the interruption of human-vector contact (ROIZ et al., 2018). The use of chemical insecticides is still the main measure to control vector mosquitoes (ESTRADA et al., 2019), but their use in high doses and number of applications, in addition to causing problems to human and environmental health (POLANCZYK et al., 2013), causes an increase in the resistance of vectors in relation to the insecticides used (ESTRADA et al., 2019).

Vegetable insecticides are promising alternatives for vector control, as they are biodegradable and rich in compounds with diverse biological activities, in addition to offering less risk to human health (VARUN et al., 2013). The mesquite tree [*Prosopis juliflora* (Sw.) DC.] is a tree in the family Leguminosae and subfamily Mimosoideae. This species is cultivated in Brazil mainly in the Northeast region, due to its adaptation to the climatic conditions of the northeastern semiarid, resisting long droughts (RIBASKI et al., 2009). Considered a multi-purpose tree, it has proven importance for reforestation due to its capacity to resist drought, in addition to producing good quality wood for various purposes (PASIECZNIK et al., 2001), providing soil protection against erosion, shading, conservation and improvement of pastures and support for beekeeping (CHAPMAN et al., 2019).

A. aegypti is the cause of several diseases, becoming a social and economic problem, requiring alternatives for its control. Thus, the objective of this work was to evaluate the larvicidal effect of aqueous extracts of *Prosopis juliflora* on *A. aegypti*.

MATERIALS AND METHODS

Installation of traps

Oviposition traps (ovitrams), with water and 10% fermented hay, were installed in ten districts of the city of Crato, Ceara, to obtain the eggs of *A. aegypti*. The traps were removed after five days of installation and the straws with the eggs were immersed in water (from the municipal supply system) for hatching of the larvae. The hatched larvae remained in the trays with water and were fed with fish food (Alcon Pet, Santa Catarina, Brazil) until the third instar.

Obtaining plant material

The aerial parts of *P. Juliflora* were collected at the Xavier site, a rural area in the interior of Paraiba, located in the city of Juru - Paraíba, coordinates Lat. -7.567537/Log. -37.812334, in September 2018 in the morning. The collected material was stored in paper bags and sent to the Chemistry Laboratory (LAQUIM) of the Serra Talhada Academic Unit (UAST), Federal Rural University of Pernambuco (UFRPE). These aerial parts were treated in the New Lab drying oven at a temperature of 45°C for 48h. The dried leaves of *P. Juliflora* were separated from the petiole and then crushed in a domestic blender. The powder was finally obtained after treatment with an ASTM 18 TYLER/MESH 16 granulometric sieve and, soon after, it was stored in a properly closed plastic container.

Preparation of aqueous extract

The larvicidal assay was carried out at the Agricultural Entomology Laboratory of the Federal University of Cariri, in 2018, at the Center for Agricultural and Biodiversity Sciences, in county of Crato, Ceara, Brazil. The experiment was conducted in a B.O.D. (Eletrolab, EL202, São Paulo, Brazil) with controlled temperature (25 ± 1 °C), relative humidity ($70 \pm 10\%$) and photophase (12 h). The species was identified by professor PhD Andre Laurênio de Melo (UFRPE), from the botany department, belonging to herbarium of the Semi-Arid Region of Brazil (HESBRA) at UFRPE/UAST.

The design used was completely randomized with five doses (10, 20, 30, 40 and 50 g L⁻¹) of the aqueous extract of *P. juliflora* plus two control treatments (pyriproxifene of 0.01g L⁻¹, dose recommended by the manufacturer and distilled water), with five repetitions of 10 larvae in each repetition.

The dry powder of the plant and the chemical insecticide were suspended with water from the municipal supply network in the doses already specified and were subjected to mechanical agitation for 1 h. Then, the solutions were transferred to polyethylene cups with a capacity of 50 mL containing 10 larvae in the third instar (L3) for each treatment.

Mortality efficiency

The treatments were evaluated after 24, 48, 72, 96, 120, 144 and 168 hours of exposure of the larvae, being considered dead those that did not react to the mechanical stimulus of a clamp.

The larval mortality efficiency was determined in percentage using the Abbott (1925) formula: $E (\%) = (N_c - N_t) / N_c \times 100$, where: E = mortality efficiency, N_c = number of individuals living in the control treatment and N_t = number of live individuals treated.

Phytochemical analysis of aqueous extract

The phytochemical analysis of the aqueous extract of *P. juliflora* was performed at the Chemistry Laboratory of the Federal Rural University of Pernambuco, Serra Talhada Academic Unit, using 10 g of the dry and crushed extract. The dry extract was placed in an Erlenmeyer and suspended with distilled water to 100 mL of the container. The mixture was stirred for 30 min and then the liquid was collected by means of simple filtration with cotton. Phytochemical tests were performed using 3 mL of the solution, as shown below:

Tannins - 3 drops of 2% gelatin solution saturated with NaCl were added and there was no precipitate.

Saponins - the tube was shaken vertically for 30 min and after 5 min the foam persisted.

Triterpenes - an extraction was performed with CHCl_3 . The organic phase separated from the aqueous phase was added slowly three drops of sulfuric acid. There was no color change.

Steroids - an extraction was performed with CHCl_3 . The organic phase separated from the aqueous phase was added 1 mL of acetic anhydride and, slowly, three drops of sulfuric acid. There was no color change.

Phenolic compounds - three drops of 3% FeCl_3 solution were added. An upper ring with a dark bond was formed.

Flavonoids - Mg shavings were added and then, slowly, five drops of HCl. Dark solution formation was observed.

Alkaloids - three drops of the Dragendorff reagent were added. An orange precipitate was formed.

White (reference).

Statistical analysis

The toxicity data were submitted to probit analysis using the SAS software (SAS Institute, Cary, NC, USA), generating concentration-mortality curves. Mortality data were submitted to ANOVA and Tukey's test using the Statistica 8 software (StatSoft Inc, Tulsa, OK, USA).

RESULTS

The highest mortality of *A. aegypti* larvae was observed in the doses of 40 and 50 g L^{-1} of *P. juliflora* extract after 24 and 48 hours of exposure of the larvae, with 62 and 92% and 92 and 98% mortality, respectively. The dose of 30 g L^{-1} caused 52 and 60% mortality, respectively, after 24 and 48 hours of exposure. The doses of 10 and 20 g L^{-1} did not cause 10% mortality (Table 1), having low efficiency during these periods of exposure.

Table 1: Mortality efficiency (%) of *Prosopis juliflora* extract on *Aedes aegypti* larvae, Crato-CE, 2018.

		Exposure periods (h)						
Treatments	Dosages (g L ⁻¹)	24	48	72	96	120	144	168
		Mortality Efficiency (%)						
Distilled water	0	0	0	0	0	0	0	0
Pyriproxifene	0.01	58	70	75	77	76.5	88.2	95.3
10 mg L ⁻¹ PJE	10	2	4	8.3	16	17	17	29
20 mg L ⁻¹ PJE	20	4	6	16	24	41,2	43,5	50,6
30 mg L ⁻¹ PJE	30	52	60	60.4	63.2	67.1	74.1	85.9
40 mg L ⁻¹ PJE	40	62	92	95.8	97.7	97.6	95.3	96.5
50 mg L ⁻¹ PJE	50	92	98	91.7	97.7	90.6	97.6	97.6

PJE = *Prosopis juliflora* extract.

When analyzing the efficiency of mesquite leaf post-dry, it was noticed a greater efficiency of larval mortality from 40g dosage when compared to pyriproxifene, obtaining a result similar to this chemical from 30g L⁻¹ dosage. It was found that the dosages of 40 and 50g L⁻¹ at the end of the evaluation period of 168 h (seven days), caused an efficiency of larval mortality of 96.3% and 100%, respectively, while pyriproxifene caused 62.9%. There is also a low efficiency of the dried leaves of the mesquite leaf in the dosages of 10 and 20g L⁻¹ (Table 1).

The Probit model was adequate for the treatment dose-mortality data after 96 h of exposure, based on the low value of χ^2 and the high value of P obtained in the post-dry curve of *P. juliflora* leaves on 3rd larvae instar of *A. aegypti*. The value of $\chi^2 = 0.12$ and P = 0.96 was obtained. The lethal doses to cause 50 and 95% mortality of the larvae (LD₅₀ and LD₉₅) were 29.8 and 49.9 g L⁻¹ of water respectively (Table 2). The slope of the curve was (1.92 ± 0.35).

Table 2: Lethal doses of dry powder of *Prosopis juliflora* for *Aedes aegypti* larvae after 96h of exposure, Crato - CE, 2018.

Treatment	LD ₅₀	LD ₉₅	Inclination (±EQM ¹)	X ² (df)	P
Algaroba Extract	29,8	49,9	1.92 ± 0.35	0.12 (5)	0.96

LD= Lethal Dose (g L⁻¹); EQM¹= Mean square error; X² = Chi square; P= Probability; df= degrees of freedom.

Considering 168 h (seven days) of exposure of the larvae submitted to different dosages, it is statistically observed that there was no difference between the dosages of 30, 40 and 50g L⁻¹ which caused an average mortality of 8.8; 9.8 and 10 larvae, respectively. The results of the phytochemical prospecting of the aqueous extract of the dry powder of the mesquite leaf indicate the presence of the metabolites saponin, phenolic, flavonoids and alkaloids (Table 3).

Table 3: Preliminary phytochemical prospecting of the aqueous extract of *Prosopis juliflora*, Serra Talhada-PE, 2018.

Metabolite class	Test	Result
Tannin	Gelatine test	Negative
Saponin	Foam test	Positive
Triterpenes	Salkowski reaction	Negative
Steroids	Liebermann-Burchard reaction	Negative
Phenolics	FeCl ₃ 3%	Positive
Flavonoids	Shinoda (Mg + HCl)	Positive
Alkaloids	Dragendorff Reactive	Positive

Researcher data.

When representing the mortality of *A. aegypti* larvae as a function of the different dosages of the dried leaves of the mesquite leaf in g L⁻¹, it is observed that the increase in the dosage is proportional to the increase in the average mortality. It can also be seen that the peak of mortality starts to verticalize in the

graph from the dosage of 30g L⁻¹, where the mortality efficiency is 62.7% (Figure 1).

Figure 1: Relationship between larvae mortality of *Aedes aegypti* under different dosages of the dry powder of the *Prosopis juliflora* em g/L. Crato-CE, 2018.

DISCUSSION

Secondary metabolites produced by plants for their defense are an alternative for the control of *A. aegypti*. Several studies with plant extracts have been reported with potential ovicides, larvicides and adulticides on *Aedes* (GOVINDARAJAN et al., 2011; RAJAN et al., 2012).

The dose of 30 g L⁻¹ caused 52 and 60% mortality, respectively, after 24 and 48 hours of exposure. The doses of 10 and 20 g L⁻¹ did not cause 10% mortality (Table 1), having low efficiency during these periods of exposure. This can be explained because the increase in the insect mortality rate is proportional to the increase in the concentration of plant extracts and the exposure period (KEHAIL et al., 2017; DANTAS et al., 2019).

In order to cause 90% mortality of *A. aegypti* larvae for the exposure periods of 24 and 48 h, it was necessary to apply 63.2 and 128.1 mg L⁻¹ of fresh leaf extract from the mesquite tree, respectively (BANSAL et al., 2012). In order to cause 90% mortality of *A. aegypti* larvae for the exposure periods of 24 and 48 h, it was necessary to apply 63.2 and 128.1 mg L⁻¹ of fresh leaf extract from the mesquite tree, respectively (BANSAL et al., 2012).

The ether extract of *Lantana Câmara* and *Tridax procumbens* promoted 100% mortality after 48 h. While after 24 h of exposure the mortality rate was 80.60%, respectively. Analyzing the larvicidal effect of methanolic extracts obtained from *Persea americana* seed on *A. aegypti*, Agrela et al. (2014), after 24 hours of exposure of the larvae to the extract in the dosages of 25 and 50 mg L⁻¹, obtained a mortality of 100% of the larvae.

The extract of the leaves and stem of *P. juliflora* showed significant larvicidal action on third stage larvae of *A. aegypti* (BESERRA et al., 2014), which corroborates the results of this research. These larvicidal effects can be explained by the presence of neurotoxins in plant extracts, since phytochemicals derived from plant sources can act as a larvicide, insect growth regulator and repellent (EICH, 2008).

After 72 h of exposure, dry powders at 40 and 50g L⁻¹ dosages caused mortality above 90%, even

eliminating the entire population after 120 h of exposure at 50g L⁻¹ dosage (Table 1). This shows that the dosage of 40g L⁻¹ of the dry powder of the mesquite leaf promotes mortality efficiency similar to that of 50g L⁻¹ and can be used in ecological control programs of *A. aegypti*. Plants can be an alternative source like mosquito larvicides because they are a potential source of bioactive chemicals and are generally free of harmful effects to man. The use of these botanical derivatives in mosquito control instead of synthetic insecticides can reduce the cost and environmental pollution.

The growth regulating insecticide pyriproxifene caused mortality after 72 h of exposure, the morphogenic effects of this insecticide occur mainly in the transition from the larva to pupa phase, where the mechanism is regulated by the increased segregation of the ecdysonium hormone and the absence of the juvenile hormone (INVEST et al., 2008).

Pyriproxifene is a larvicide that has low toxicity to mammals, reduced environmental impact and a long effect of action, to reduce the number of applications and has the particularity of not only interfering in development but also in reproduction (OHBA et al., 2013). The use of pyriproxifene is particularly suitable for mosquito control, and can be applied in artificial breeding sites or even in water containers intended for human consumption (WHO, 2011).

The lethal doses to cause 50 and 95% mortality of the larvae (LD₅₀ and LD₉₅) were 29.8 and 49.9 g L⁻¹ of water respectively. For aqueous extracts of *P. juliflora* leaves at dosages of 10, 20, 50, 100 and 200 ppm, an LC₅₀ of 126.79 and LC₉₀ of 457.32 ppm were found for this vector (TYAGI et al., 2015). For the plant extracts of *Artemisia herba alba* on larvae of *A. aegypti*, LC₅₀ of 542.5 and LC₉₀ of 1,214.0 ppm were found respectively and for *Matricharia chamomilla* extract of 142.1 and 325.8 ppm (MAHYOUB et al., 2014).

The vegetable extract from *Lantana Câmara* showed an LC₅₀ of less than 220 mg mL⁻¹. The LC₅₀ of *Ageratina adenophora* in *A. aegypti* was found in 50 µg mL⁻¹ (RAJMOHAN et al., 2007). *Rubia cordifolia* root extract registered larvicidal activity with LC₅₀ and LC₉₀ values of 102.2 and 350.2 mg L⁻¹ against *A. aegypti* larvae, respectively (MUNUSAMY et al., 2016).

Larvicidal activity of 10% mesquite leaf extracts was effective with an LC₅₀ of 9.3 mg L⁻¹ against *A. stephensi* larvae (SENTHILKUMAR et al., 2009). It's notable that the larvicidal activity of the extract of this plant is variable in different studies. The reason for this variation may be due to the part of the plant used, the geographical location of the plant, photosensitivity of some metabolites, the extraction solvent and the extraction process and, finally, the species responses to the specified extracts (SENTHILKUMAR et al., 2009).

Research has been reported regarding the susceptibility of *A. aegypti* larvae to a wide variety of plant extracts under laboratory conditions, however this susceptibility is dependent on the applied extract dose and the species of plant used (KAUSHIK et al., 2009). The biological properties of these plant extracts may be due to numerous chemical compositions, including phenolics, terpenoids, flavonoids and alkaloids. These constituents can contribute jointly or independently to produce toxic activity against mosquito species (KAMARAJ et al., 2011; MOHANKUMAR et al., 2016).

The dosages of 10 and 20g were less efficient and did not differ statistically from each other, with pyriproxifene being more efficient than the same ones, killing an average of 8 larvae/pupae (Table 4).

Pyriproxyphene does not kill mosquito larvae directly, but it interrupts the normal process of insect development in the fourth larval instar for the beginning of the pupa phase, which leads to pupal mortality and prevention of the emergence of adult mosquitoes (RESENDE et al., 2006).

Table 4: Average number of dead *Aedes aegypti* larvae submitted to different dosages of the post-dry leaf of *Prosopis juliflora*, compared to the chemical insecticide pyriproxifene, after seven days of exposure, Crato-CE, 2018.

Treatments	Dosages (g)	Average dead larvae ¹
Distilled water	0	0
Pyriproxifene	0.01	8.0a
Dry leaf powder	10	4.6b
Dry leaf powder	20	6.2b
Dry leaf powder	30	8.8a
Dry leaf powder	40	9.8a
Dry leaf powder	50	10a
C.V. (%)		14.12

¹Means followed by the same letter in the columns, do not differ significantly by the tukey test at the 5% probability level. C.V.=Coefficient of variation.

The results of the phytochemical prospecting of the aqueous extract of the dry powder of the mesquite leaf indicate the presence of the metabolites saponin, phenolic, flavonoids and alkaloids. The piperidine alkaloids, isolated from the leaves and fruits of this plant are referred to as the most important pharmacologically active compounds, having been tested in different biological systems, being attributed to the therapeutic activities conferred to the plant (SINGH et al., 2012). In addition, it also has anthelmintic activity (BATATINHA et al., 2011) and is an inhibitor of acetylcholinesterase and butyrylcholinesterase, with Ca²⁺ channel blocking activity (CHOUDHARY et al., 2005) which can cause mortality to *A. aegypti*.

The increase in mortality of *A. aegypti* larvae is proportional to the increase in the dose of *P. juliflora* extract. The crude extract obtained from different parts of the *Ipomoea cairica* plant demonstrated late larvicidal activity in the third stage of *A. albopictus* and *A. aegypti* under laboratory conditions. The increase in the dosages of crude extracts caused a proportional increase in the average mortality of the larvae (AHBIRAMI et al., 2014). No mortality was recorded at a concentration of 10 ppm. At the highest concentration (450 ppm) that induced 100% mortality, the larvae reacted with behavioral changes, such as agitated movements for some time, followed by slowness, convulsions and subsequent paralysis at the bottom of the beakers and died slowly (AHBIRAMI et al., 2014).

CONCLUSIONS

The dosages of 40 and 50g L⁻¹ of the dry powder of *P. juliflora* leaves are efficient in the mortality of *A. aegypti* larvae after 48 h of exposure, with greater efficiency after 120 h, causing 100% mortality.

The lower dosages (10 and 20g L⁻¹) have low efficiency in mortality and the dosage of 30g L⁻¹ causes satisfactory mortality after 120h of exposure. *P. juliflora* dry powder has the potential to be used in the ecological control of *A. Aegypti*, however it is necessary to characterize and quantify the constituents of the plant extract followed by bioassays under field conditions, since it was carried out in the laboratory.

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