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LARVAL SUSCEPTIBILITY OF *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE) TO EXTRACTS OF *ILEX PARAGUARIENSIS* AND *ILEX THEEZANS*

SUSCETIBILIDADE LARVAL DE *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE) AOS EXTRATOS DE *ILEX PARAGUARIENSIS* E *ILEX THEEZANS*

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ABSTRACT

Aedes aegypti is the main vector of dengue, Chikungunya and Zika virus worldwide. The strategies for the population control involve the use of synthetic insecticides, which may cause toxicity problems and environmental contamination. This study evaluated the larvicide effect of hydroalcoholic extracts of leaves and fruits *Ilex paraguariensis* and *Ilex theezans* on *A. aegypti*. The bioassays were conducted under controlled conditions. Fresh and dry fruits of *I. paraguariensis* showed the higher larvicidal activity on *A. aegypti* than the leaves of the same species. Mosquito larvae showed increased susceptibility from dried leaf extracts, fresh fruit and leaf of *I. theezans*. It was also observed a variation in larval mortality during periods of exposure. The larvicide efficiency of *I. theezans* extracts on *A. aegypti* are promising results and widen the possibility for further studies of the use of extracts of this plant.

Keywords: vector control; dengue; yerba mate; larvicide; natural products.

RESUMO

Aedes aegypti é o principal vetor da dengue, febre Chikungunya e Zika vírus em escala global. As estratégias para controle populacional deste vetor recorrem à utilização de inseticidas sintéticos, que podem acarretar problemas de intoxicação e contaminação ambiental. Este estudo avaliou o efeito larvicida de extratos hidroalcoólicos de folhas e frutos de *Ilex paraguariensis* e de *Ilex theezans* sobre *A. aegypti*. Os bioensaios foram conduzidos sob condições controladas. Os frutos *in natura* e secos de *I. paraguariensis* demonstraram maior atividade larvicida sobre *A. aegypti* em relação às folhas da mesma espécie. As larvas do mosquito demonstraram maior susceptibilidade frente aos extratos de folhas secas, *in natura* e frutos verdes de *I. theezans.* Também foi observada variação na mortalidade larval nos períodos de exposição. A eficiência larvicida dos extratos de *I. theezans* são resultados promissores e abrem a possibilidade para estudos subsequentes do uso de extratos desta planta.

Palavras-chave: controle vetorial; dengue; erva-mate; larvicida; produtos naturais.

INTRODUCTION

The epidemiological situation of dengue, Chikungunya fever and Zika virus in Brazil has been increasingly complex as its main vector. The mosquito *Aedes aegypti* (Linnaeus 1762) is spread by almost all the national territory, where epidemics have been recurrent (BARRETO & TEIXEIRA, 2008). The *A. aegypti* is one of more than 500 species of the *Aedes* genus (Diptera: Culicidae), considered as the main vector of these viruses and also of urban Yellow Fever. It uses water tanks to lay its eggs and consequently develop their larvae. An important evolutionary feature that allowed the conquest of a large proportion of the globe is that its eggs are resistant to desiccation, remaining viable in the absence of water for more than one year (TAUIL, 2002; POWELL & TABACHNICK, 2013). When it is in contact with water, the larvae emerges, going through four stages (L1, L2, L3 and L4) and subsequently originating the pupae and, soon after, adult mosquitoes (NEVES, 2011).

Among the strategies for population control of this vector, there is also the use of synthetic insecticides, such as Pyrethroids Deltamethrin and the Carbamate Bendiocarb (GUARDA *et al*., 2016). The use of these insecticides may cause toxicity problems and environmental contamination, leading to risks to local biodiversity and human health. Synthetic insecticides have low selectivity and can select genes that cause resistance in populations of *Aedes* (CALDAS, 2000; SANTIAGO *et al*., 2005). The vector management with the use of natural products is less intrusive than using the conventional insecticides (synthetic), having a more rapid degradation, resulting in lower occupational exposure and less environmental pollution (VALLADARES; DEFAGO; PALACIOS, 1997; BARRETO, 2005).

Studies have shown the effectiveness of natural products for the control of embryonic development, larval, pupa and adult emergence of *A. aegypti* (BRUNHEROTTO & VENDRAMIM, 2001; ROSSI *et al*., 2007; BUSATO *et al*., 2015). A perspective arises from the study of Busato *et al*. (2015) successfully testing the larvicide efficiency to the ethanol extracts of leaves of *Ilex paraguariensis* A. St.-Hill (Aquifoliaceae) on *A. aegypti*.

The species *I. paraguariensis* is from South America, popularly known as yerba mate or congonha, being grown and consumed as tea or mate in Argentina, Brazil, Paraguay, and Uruguay. After processing, its leaves and branches make the drinks appreciated by the population of these countries (SOUZA, 2009; LORENZI & MATOS, 2002). In the botanical description of Kricun (1983) and Giberti (1995), it is an evergreen tree, with height about 3 to 5 m (in growing forests) and up to 12 m high and 70 cm in diameter, in a native forest environment. Besides its cultural and gastronomic aspects, the great commercial interest in *I. paraguariensis* is due to the presence of caffeine and theobromine, recognized for exercising stimulating effects on cardio-circulatory systems as coffee, cocoa and guarana (CASTALDELLI *et al*., 2011). The plant also has vitamins, amino acids and triterpene saponins of nutritional and medicinal interest in its chemical constitution (RATES, 2004; PIOVEZAN-BORGES *et al*., 2016).

The Ilex *theezans* Mart species Ex Reissek (Aquifoliaceae) is popularly known as Cauna and little used commercially, but common in vegetation types in the South of Brazil. It is an evergreen tree, early secondary or late secondary species. Its height reaches up to 20 m, has a diameter of 70 cm and the chemical properties of the extracts of this plant are still poorly known.

In this context, this study aimed to evaluate the larvicidal effect of hydroalcoholic extracts of leaves and greens and dried fruit of *I. paraguariensis*, native and cultivated and *I. theezans* on *A. aegypti*.

MATERIAL AND METHODS

Plant material

The leaves and fruits collection of *I. paraguariensis*, native and cultivated and *I. theezans* were held in Marechal Bormann district (27°, 19’05”S,52°,65’11”W), Chapecó (SC), on December 2015. The collected plant material was identified by the curator of the Herbarium of the Universidade Comunitária da Região de Chapecó.

These materials were transferred to the Pharmacognosy Laboratory, reduced to small fragments, and submitted to drying at room temperature, protected from direct light and moisture. Subsequently, the dried plants were ground in a knife mill (Ciemlab®, CE430) selected in a filter of 425 µm (35 Tyler/Mesh), identified and stored away from the light.

Extracts production

For the preparation of extracts by turbolisis (5 days), fresh and dried leaves and fruits, were follow protocol used as recommended by the Brazilian Pharmacopoeia (FB 5, 2010). There were 12 extracts prepared: fresh and dry leaves, as well as the fruits of *I. paraguariensis* (native and cultivated); leaves (fresh and dried) and fruit of *I. theezans*. After their filtration through Büchner funnel, the extracts were concentrated by rotary evaporation under reduced pressure, lyophilized, weighed and stored in a freezer at -20°C for further assays.

Experimental procedure

The collection of eggs of *A. aegypti* was conducted from November 2015 to April 2016. There were 15 egg-traps characterized as plastic containers of black color, with a capacity of 500 mL, with 200 mL of water into each trap and monitored every 7 days. For laying the eggs and for the collection of the field, a white crop seed germination paper cut into strips of 29.7 x 10 cm was used (GOMES; SCIARICO; EIRAS, 2006). The cut was placed around the inside of the trap so that approximately four centimeters were submerged in the solution. The egg traps were distributed at different points of the city of Chapecó, in establishments previously known by the mosquito infestation, according to data from the Programa Municipal de Controle da Dengue (city program for dengue control).

The papers with the eggs were placed in white plastic trays 20 x 30 cm containing 2 L of water without treatment to obtain the larvae. The larvae were fed with fish feed (Holiday®) and remained in the trays until the L2 stage with a period of three days. The bioassays were carried out in laboratory conditions (Ecological Entomology Laboratory - LABENT-Eco of the Community University of Chapecó Region), with a temperature of 28 ± 3°C and a photoperiod of 12 hours.

Experimental design

The experimental microcosms were in transparent plastic cups with 300 mL capacity. Each microcosm had a volume of 80 mL of vegetable extracts concentrations ranging from 500 to 2,000 Experimental g/mL and 20 active *A. aegypti* larvae of the second stage. The control microcosms received only 80 mL of untreated water and 20 mosquito larvae. Each treatment was repeated three times and all larvae used in the experiment were fed with fish feed – only at the beginning of the test. Larval susceptibility was evaluated at intervals of 24, 48 and 72 h after exposure to the solutions. The living larvae were counted in each period. After the experiment ends, the remaining larvae were sacrificed in boiling water and discarded.

Statistical analysis

The data evaluation was performed by the analysis of variance (ANOVA, one-way) on the number of living larvae, where the means were grouped by the Duncan test at 5% probability of error. The efficiency of the treatments tested was calculated by Abbott equation (1925), used to compare the treatments with respect to control and defined by the formula %Efficiency = (T-t)*100/T, where "T" represents the average number of living larvae in Control, and "t" is the average of surviving larvae in each treatment.

RESULTS

Larval susceptibility to *A. aegypti* was significantly affected by the treatment variable, by the time variable and also by the interaction between treatment and time (Table 1).

The extracts of dried leaves of *I. theezans* at a concentration of 750 µg/mL showed the highest larvicide efficiency from the first 24 hours. There was an efficient success in extracts of fresh leaves and fruits (1,000 µg/mL) and dried fruits (2,000 µg/mL) of *I. theezans*. The native dried fruit extract of *I. paraguariensis* also showed greater than 50% activity in concentration of 1,000 µg/mL. All extracts of *I. paraguariensis* matched to the control ($p < 0.01$) (Table 2).

Table 1 – Variance analysis of the number of *Aedes aegypti* living larvae exposed to treatments with hydroalcoholic extracts of *I. paraguariensise* and of *I. theezans* under laboratory conditions (temperature 28°C and 12 hours photoperiod).

Variation cause	Degrees of freedom	Average square	Probability
Treatment	28	0.82	p<0.01
Hours	3	3.93	p<0.01
Treatments x hours	84	0.26	p<0.01
Residue	232	0.04	
Total	347		

Coefficient of variation = 6.8%

Table 2 – Treatments, average of *A. aegypti* alive after 72 hours of exposure to treatment with hydroalcoholic extracts of *I. paraguariensise* of *I. theezans* followed by standard error and efficiency percentage in laboratory conditions (temperature 28°C and 12 hours' photoperiod). Means followed by capital letters differ by Duncan test (p < 0.01).

Treatments	Alive larvae	% Efficiency (72 h)
Control	20 ± 0 A	0%
I. paraguariensis fresh native fruits 750 µg/mL	20 ± 0 A	0%
I. paraguariensis fresh native leaves 1,000 µg/mL	20 ± 0 A	0%
I. paraguariensis fresh cultivated leaves 1,000 µg/mL	20 ± 0 A	0%
I. paraguariensis fresh cultivated fruits 1,000 µg/mL	20 ± 0 A	0%
I. paraguariensis fresh native leaves 2,000 µg/mL	20 ± 0 A	0%
I. paraguariensis dry native leaves 1,000 µg/mL	20 ± 0 A	0%
I. paraguariensis dry native leaves 2,000 µg/mL	20 ± 0 A	0%
I. paraguariensis dry cultivated leaves 2,000 µg/mL	20 ± 0 A	0%
I. paraguariensis dry cultivated leaves 1,000 µg/mL	19.66 ± 0.08 A	1.7%
I. paraguariensis dry native fruits 1,000 µg/mL	19.33 ± 0.77 A	3.35%
I. paraguariensis fresh cultivated fruits 2,000 µg/mL	18.66 ± 0.11 A	6.7%
I. paraguariensis fresh cultivated leaves 2,000 µg/mL	18.66 ± 0.23 A	6.7%
I. paraguariensis dry cultivated fruits 750 µg/mL	16.03 ± 0.65 AB	19.85%
I. paraguariensis fresh native fruits 1,000 µg/mL	14.93 ± 0.57 AB	25.35%
I. paraguariensis dry native fruits 2,000 µg/mL	13.93 ± 0.98 AB	30.35%
I. theezans freen leaves 750 µg/mL	13.85 ± 0.69 AB	30.75%
I. paraguariensis fesh native fruits 2,000 µg/mL	12.82 ± 0.84 AB	35.9%
I. paraguariensis dry cultivated fruits 1,000 µg/mL	9.76 ± 1.37 AB	51.2%
I. theezans dry fruits 1,000 µg/mL	8.78 ± 1.41 B	56.1%
I. theezans fresh fruits 2,000 µg/mL	8.58 ± 1.43 BC	57.1%
I. theezans fresh leaves 1,000 µg/mL	6.23 ± 2.03 CD	68.85%
I. theezans fresh fruits 1,000 µg/mL	5.64 ± 1.74 CD	71.8%
I. theezans dry leaves 750 µg/mL	1.88 ± 2.36 E	90.6%

The most effective larvicidal effect of the extracts was observed after 24 hours of exposure. Although it was observed activity in the period between 0 and 24 hours for the fresh leaf extract of *I. theezans* (1,000 µg/mL), the stronger activity of the other was observed in the period between 24 and 48 hours and less intense between 48 and 72 hours (Figure 1).

DISCUSSION

The results showed that the hydroalcoholic extracts of leaves and fruits, both fresh or dry of *I. theezans* showed larvicidal activity at all concentrations tested, and concentrations above 750 µg/mL were the most efficient. The extract of dried leaves of *I. theezans* at a concentration of 750 µg/mL was the most efficient (Table 2). In 72 hours, it showed the efficiency of 90.6%. The fresh fruits extract of this species presented 71.8% efficiency. The highest activity was observed in the period between 24 and 48 hours of exposure (Figure 1).

Ilex theezans is an early successional species, considered one of the most frequent in Mixed Rain Forest and according to Viani and Vieira (2007) it usually coexists with *I. paraguariensis*. The *I. theezans* leaves are mixed in the mate to increase the bitterness, having a higher concentration of saponins. The main biological activities of saponins are related to their active tense, complexing, haemolytic and toxic properties (MAHATO; SARKAR; PODDAR, 1988). The fact that *I. theezans* had larvicide effectiveness against *A. aegypti* may be related to the presence of a higher concentration of saponins usually used to differentiate species of *Ilex*. However, they would require additional studies on their composition.

It was noticed that the extracts of *I. theezans* showed the highest efficiency between 24 and 48 hours. After this period, the activity was still observed, although with a reduction in the effect of the extracts on *A. aegypti* larvae. For Garcia (2014), a product is commercially efficient and recommended if it shows lethality inferior to 80%, so that there is a selection of genes causing resistance. One of the treatments reached the parameter of the extract of dried leaves of *I. theezans* (750 µg/mL).

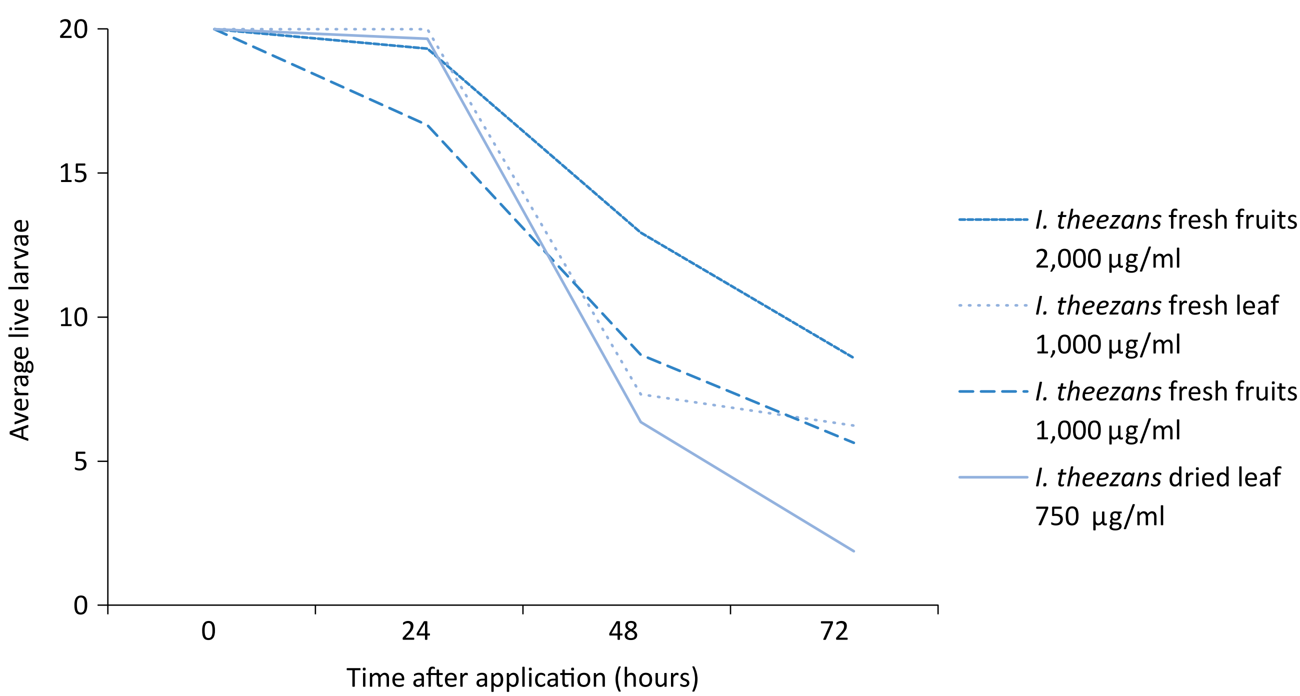

Figure 1 – Survival of *A. aegypti* larvae exposed to different concentrations of *I. theezans* extracts, evaluated every 24 hours under laboratory conditions (temperature 28°C and 12 hour photoperiod).

Fruit and fresh and dry leaves extracts of *I. paraguariensis*, native and planted, from the concentration of 1,000 μg/mL showed a relatively low larvicide efficiency when compared to extracts of *I. theezans* (Table 2). The fact of being native or planted did not result in differences in this study. For more favorable, shaded and with specific microclimatic condition environments, such as intensity and duration of sun radiation, and the predetermined genetic variations (COELHO; MARIATH; SCHENKEL, 2002) and even the presence or absence of endophytic microorganisms (MELO & AZEVEDO, 1998), differences in the activity of the extracts were expected. Studies regarding the phytochemical composition of yerba mate demonstrated that their compounds may vary in quantitative and qualitative terms, such as the type of crop, climate, agronomic conditions, plant age, methodology analysis and industrial processing (DUTRA, 2009). In the study of Busato *et al*. (2015), in a bioassay using a hydroalcoholic extract of *I. paraguariensis* leaves, using the drying method in an oven for 48 hours, larvicide efficiency was checked on *A. aegypti*, with 100% mortality at a concentration of 2,000 μg/mL. Comparing the results of Busato *et al*. (2015), it is possible to infer that the extracts of the preparation method may have influenced the results for *I. paraguariensis*.

The fresh and dry fruits of *I. paraguariensis* showed greater activity than the leaves of the same species. The fresh fruits of yerba mate, compared to the leaves, showed higher concentrations of saponins, which are present as a defense against herbivores and other forms of consumption and predation (PAVEI *et al*., 2007). After ripening, there is a predominance of other nutrients, which facilitate the use and dispersal of seeds, such as sugar, proteins, fats and carbohydrates (RAVEN; EVERT; EICHHORN *et al*., 1996). Probably these saponins in fresh fruits of *I. paraguariensis* were lethal to *A. aegypti* larvae. The effect of this saponin in fruits of various families of plants is known to be deleterious to the development of pests of maize monocultures such as *Spodoptera frugiperda* (Smith, 1797) and *Helicoverpa zea* caterpillars (Boddie 1850) (DOWD *et al*., 2011).

The great commercial interest in *I. paraguariensis* as well as its cultural and gastronomic aspects is due to the presence of xanthine bases. According to Borges *et al*. (2013), one of the main constituents of yerba mate leaves used for teas are methylxanthines, caffeine, theobromine and theophylline traits.

These results open perspectives in replacement of synthetic insecticides by natural products in the control of *A. aegypti*, by their abundant presence in southern Brazil. These results are promising, creating the possibility for further studies regarding the use of extracts of these plants as larvicides of *A. aegypti*, as an alternative to the synthetic products. For the treatments carried out, the method of extraction and preparation of I. paraguariensis extracts proved to be little efficient. One possibility is that not all compounds were released during the process of preparation. For the species *I. theezans,* the method was efficient, widening the perspective for further studies of this yet little used species.

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