

Larvicidal activity of aqueous extracts of *Ilex paraguariensis* and *Ilex theezans* on *Aedes aegypti* (L.)

Atividade larvicida de extratos aquosos de *Ilex paraguariensis* e *Ilex theezans* sobre *Aedes aegypti* (L.)

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ABSTRACT

Aedes aegypti is the main vector of Dengue, Yellow Urban Fever, Chikungunya fever and Zika virus fever. The strategies for its control include synthetic products that cause damage to the environment and other organisms. This study evaluated the larvicidal activity of aqueous extracts of leaves and fruits of *Ilex paraguariensis* and *Ilex theezans* on *Aedes aegypti* mosquito larvae. The bioassays were conducted at the Ecological Entomology Laboratory, Unochapecó, under controlled conditions of temperature and photoperiod. The extracts of fresh and dried leaves of *I. theezans* showed greater larvicidal activity when compared to extracts of fruits of the same plant. Variation in larvicidal activity was also observed during exposure periods. The results suggest the use of extracts of these plants in the control of *A. aegypti* and the prospection of substances that can be used as an alternative to synthetic products. They point to the possibility of using yerba mate tissues that are not used commercially.

Keywords: Yerba mate; Yerba caúna; Natural products; Larval susceptibility

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RESUMO

O mosquito *Aedes aegypti* é o principal vetor da dengue, febre amarela urbana, febre Chikungunya e febre do Zika vírus. Dentre as estratégias para o controle deste vetor são frequentemente utilizados produtos sintéticos que podem causar danos ao ambiente e outros organismos. Este estudo avaliou a atividade larvicida de extratos aquosos de folhas e frutos de *Ilex paraguariensis* e de *Ilex theezans* sobre as larvas do mosquito *A. aegypti*. Os bioensaios foram realizados no Laboratório de Entomologia Ecológica da Unochapecó sob condições controladas de temperatura e fotoperíodo. Os extratos de folhas in natura e secas de *I. theezans* demonstraram maior atividade larvicida quando comparados aos extratos de frutos da mesma planta. Os resultados sugerem o uso de extratos destas plantas no controle de *A. aegypti* e a prospecção de substâncias que possam ser utilizados como alternativa frente aos produtos sintéticos. Apontam para a possibilidade de utilização de tecidos vegetais da erva mate que não são utilizados comercialmente.

Palavras-chave: Erva-mate; Erva-caúna; Produtos naturais; Suceptibilidade larval.

1 INTRODUCTION

Population control of vector insects has become a growing challenge considering the impact of control actions on human health, non-target species and the selection of resistant populations (FEITOSA; SOBRAL; DE JESUS, 2015). Urban environments, where are conducted the control activities of the *Aedes aegypti* mosquito (Linnaeus, 1762), are complex as regards the supply of resources for the proliferation of this insect, however, social, economic and environmental factors determine the effectiveness of control mechanisms. With respect to the use of insecticides, both larval and adult control, synthetic products such as pyrethroids and carbamates are used, which are little selective, toxic to humans and the environment, can select resistant populations and remain in the environment for a long period (GUARDA et al., 2016).

Aedes aegypti (Linnaeus, 1762) is one of more than 500 species of the genus *Aedes* (Diptera: Culicidae), the main vector of dengue, urban yellow fever, chikungunya fever and Zika virus (ZHOULI et al., 2016). *A. aegypti* infestation has increased significantly due to disordered demographic expansion and city planning failures (FEITOSA; SOBRAL; DE JESUS, 2015). The poor basic sanitation conditions create favorable environments for mosquito breeding sites and its dissemination throughout the country (FLAUZINO, SOUZA-SANTOS; DE OLIVEIRA, 2011). For population control of this insect, the World Health Organization (WHO) established strategies, including the use of synthetic products integrated with environmental management programs aimed at eliminating larval stages and adult insects (MATIAS, 2017). Among the threats to the environmental balance, the continuous use of synthetic insecticides may present undesirable effects, such as contamination of water, food (JAYARAJ; MEGHA; SREEDEV, 2016), as well as the mortality of non-target species.

The management of vectors with the use of natural products is less impacting to natural systems than conventional insecticides (synthetic), since they present a faster degradation, resulting in less occupational exposure and reduced contamination of the environment (FOSTER, 2016). The botanical diversity existing in Brazil provides the possibility of studies from plant extracts. In this sense, the expectation is to find substances with insecticidal properties and simultaneously selective to be used in new formulations of products for the control of vectors (MATIAS, 2017).

Ilex paraguariensis A. St. Hill (yerba mate) is an arboreal plant belonging to the family Aquifoliaceae and the genus *Ilex*, native to South America and native to temperate regions. It is commonly consumed in Argentina, Brazil, Paraguay and Uruguay. From the dried leaves of this plant, the *tererê* and the *chimarrão* are produced (drinks based on dry and ground leaves elaborated with cold or hot water, respectively) and with the toasted leaves, the mate tea is prepared (MACHADO et al., 2007). In countries where the species is cultivated, it has economic and social importance due to the habit related to the culture of these regions. Although the chemical properties of the present compounds are already well known, there is little

literature about the fruits of *I. paraguariensis*. The leaves are used commercially, fruit have not aroused as much interest of the researchers as for their chemical components.

Ilex theezans (Mart. Ex Reissek), Aquifoliaceae, is an abundant perennial tree in the southern region of Brazil. This plant is popularly known as yerba caúna, and is little used commercially, but often described as an adulterant of *I. paraguariensis*. The leaves of this plant have as chemical constituents, triterpene saponins, flavonoids, phenolic acids and xanthines, however, there are few studies on the pharmacological activities of the plant.

Recently, ethanolic leaf and fruit extracts were tested on *A. aegypti* larvae, where 90.6% efficiency was observed for leaf extract of *I. theezans* (KNAKIEWICZ et al., 2016). However, the larvicidal evaluations of the aqueous extracts mimicking the popular use of these species are scarce. Given the prospectivity of products from commercially unused plant tissues of *I. paraguariensis*, the ecological and economical importance of this species in the southern region of Brazil and the constant demand for more selective household cleaning products, less toxic to the environment and human health, it is justified to carry out studies to increase the knowledge about the properties of this plant. The lack of studies involving another species of the genus, *I. theezans*, also justifies the study. In this context, we evaluated the larvicidal activity of aqueous extracts of *I. paraguariensis* and *I. theezans* against larvae of *A. aegypti*.

2 MATERIAL AND METHODS

2.1 Plant material

The leaves and fruits of *I. paraguariensis* and *I. theezans* (native and cultivated) were collected in the district of Marechal Bormann (27°19'05"S, 52°65'11"W), Chapecó, State of Santa Catarina, in December 2015. The plant material was identified by the curator of the Herbarium of the Community University of the Chapecó Region (Unochapecó) where samples were registered.

Samples of plant material (leaves and fruits) of both species were transferred to the Pharmacognosy Laboratory of Unochapecó, reduced to small fragments and part of the material was dried at room temperature, milled with a knife mill (Ciemlab[®], CE430), selected in 425 µm sieves (35 Tyler/Mesh) and stored protected from direct light and humidity for subsequent extract production. Samples were also used in the preparation of fresh extracts.

2.2 Extract production

For the preparation of the extracts, it was used plant material (leaves and fruits), fresh and dry (20 g) and distilled water (200 mL), yielding 20 aqueous extracts (Table 1), identified as fresh and dried leaves and fruits of *I. paraguariensis* (native and cultivated) and fresh and dried leaves and fruits of *I. theezans*. After filtration by Büchner funnel, the extracts were rotary evaporated under reduced pressure, lyophilized, weighed and stored in a freezer at -20°C for further testing (KNAKIEWICZ et al., 2016).

2.3 Experimental procedure

The collection of *A. aegypti* eggs was carried out in the city of Chapecó, State of Santa Catarina, from November 2015 to April 2016. Fifteen egg traps were installed and for egg laying, a seed germination paper piece was used, with the white color cut into 29.7 x 10 cm strips (KNAKIEWICZ et al., 2016).

To obtain the larvae, the papers with eggs were placed in 20 x 30 cm white plastic trays containing 2 liters of untreated water. Larvae were fed with fish meal (Holiday[®]) and remained in the trays until reaching the L2 instar (second stage larvae), whose period was three days. Bioassays were carried out under laboratory conditions (Laboratory of Ecological Entomology - LABENT-Eco of Unochapecó), under a temperature of 28±3°C and a 12-hour photoperiod.

2.4 Experimental design

Experimental microcosms consisted of transparent plastic cups with a capacity of 300 mL. Each microcosm was added with a volume of 80 mL of one of the different concentrations of plant extracts ranging from 50 to 2000 µg/mL and 20 active second instar larvae of *A. aegypti*. Control microcosms received only 80 mL untreated water, food and 20 mosquito larvae. Each treatment was replicated three times and all the larvae used in the experiment were given fish feed only at the beginning of the trial (GUARDA et al., 2016). The larval susceptibility was evaluated at intervals of 24, 48 and 72 hours after exposure to solutions. Larvae were counted in each period, thus obtaining cumulative mortality. After the end of the experiment, the remaining larvae were sacrificed in boiling water and discarded.

2.5 Data analysis

Data were evaluated using analysis of variance (one-way ANOVA) on the number of live larvae, and the means were grouped by the Duncan's test at a level of 5% of error probability. For the analysis, the software SANEST (ZONTA; MACHADO, 1987) was used. The efficiency of the treatments tested was calculated by the ABBOTT (1925) equation.

3 RESULTS

The survival of *A. aegypti* larvae was significantly affected by the treatment ($F = 82.9$, $p = 0.00001$), by exposure time ($F = 869.3$, $p = 0.00001$) and also by interaction between treatments and time ($F = 14.9$, $p = 0.00001$).

The results showed that the aqueous extracts of fresh and dried leaves and fruits of *I. paraguariensis* presented greater larvicidal activity. The extract of native fresh fruit of *I. paraguariensis* (1000 µg/mL) presented the highest efficiency (100%), followed by dried fruit extracts (750 µg/mL) of *I. paraguariensis* (90%). The extracts of fresh and dried leaves of *I. theezans* also had larvicidal activity (> 65%) from the

concentration of 750 µg/mL to 2000 µg/mL. All other extracts presented efficiency less than or equal to 35% (Table 1).

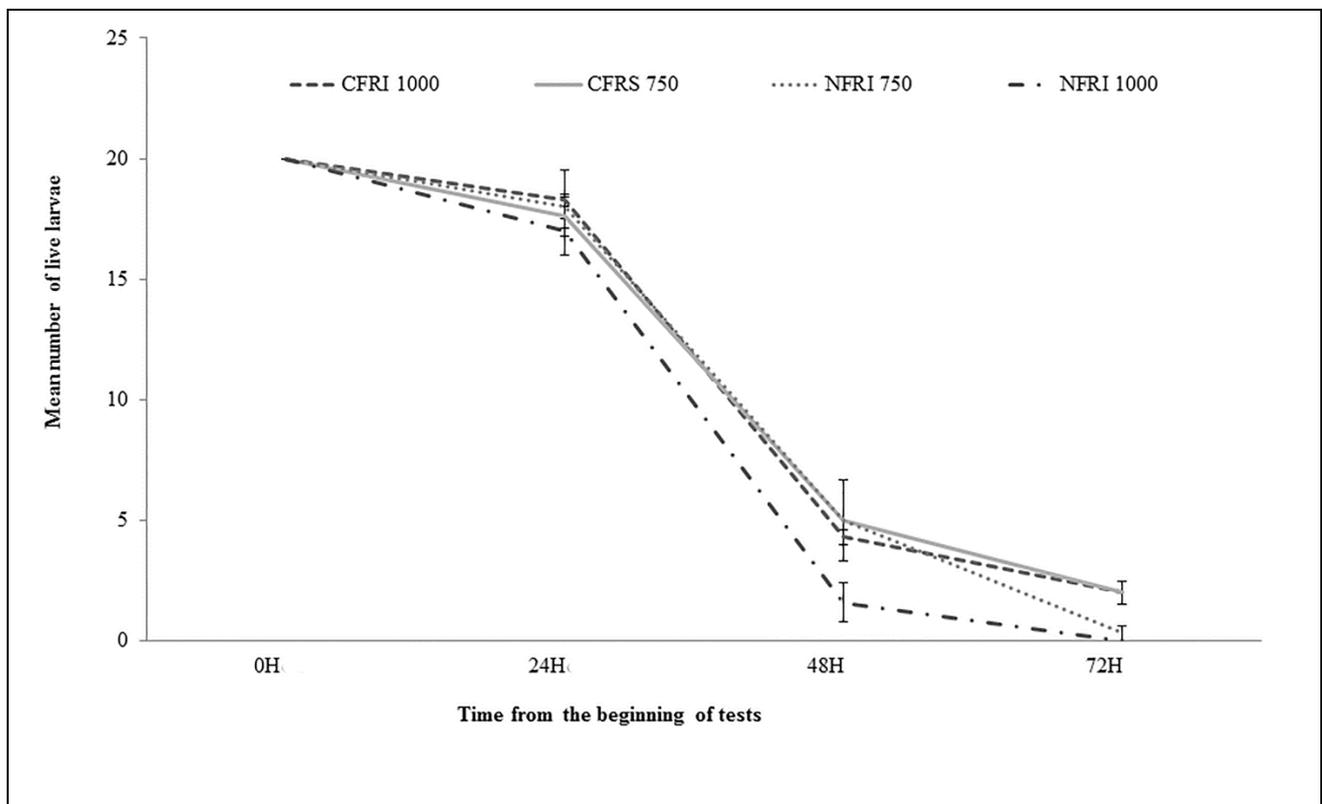
Table 1 – Number of live larvae of *Aedes aegypti* after 72 hours of exposure to aqueous extracts of *Ilex paraguariensis* and *Ilex theezans*. Note: - no activity. Different letters in the number of live larvae differ statistically ($p < 0.05$; one-way ANOVA and Duncan's test)

Treatments (µg/ml)	Live larvae of <i>A. aegypti</i> (mean ± error)	% Efficiency (72h)
<i>I. paraguariensis</i> Native Fresh Fruit 1000	0±2.73 HI	100
<i>I. paraguariensis</i> Native Fresh Fruit 750	0.33±2.69 GHI	98.4
<i>I. paraguariensis</i> Cultivated Dried Fruit 750	2±2.47 G	90
<i>I. paraguariensis</i> Cultivated Fresh Fruit 1000	2±2.58 G	90
<i>I. paraguariensis</i> Cultivated Fresh Fruit 750	3±2.24 GH	85
<i>I. paraguariensis</i> Native Dried Fruit 250	3.66±2.12 FG	81.7
<i>I. paraguariensis</i> Native Dried Fruit 100	4.66±2.32 F	76.7
<i>I. theezans</i> Fresh leaves 750	5±2.54 F	75
<i>I. theezans</i> Fresh leaves 2000	6.66±1.37 F	66.7
<i>I. paraguariensis</i> Cultivated Dried Fruit 1000	13±1.08 E	35
<i>I. theezans</i> Fresh leaves 1000	13.66±1.82 DE	31.7
<i>I. theezans</i> Fresh Fruit 2000	17.33±0.52 CDE	13.4
<i>I. theezans</i> Dried Fruit 1000	18.33±0.44 BC	8.4
<i>I. paraguariensis</i> Native Fresh leaves 2000	18.33±0.30 BC	8.4
<i>I. theezans</i> Dried leaves 750	18.33±0.30 ABC	8.4
<i>I. theezans</i> Dried Fruit 2000	19±0.24 AB	5
<i>I. theezans</i> Fresh leaves 2000	19.33±0.29 AB	3.4
<i>I. paraguariensis</i> Cultivated Fresh leaves 2000	19.33±0.17 AB	3.4
<i>I. paraguariensis</i> Cultivated Dried leaves 2000	19.66±0.16 AB	1.7
Control	20 ± 0 A	-

The larvicidal effect of the four most efficient extracts of *I. paraguariensis* was not accentuated in the period between 0 and 24 hours of exposure. Although activity was observed in this period, the most intense activity was found in the period between 24 and 48 hours, reducing again between 48 and 72 hours. The extract of fresh cultivated fruit of *I. paraguariensis* (1000 µg/mL) (CFRI 1000) was the most efficient within 48 hours of exposure and similar to the extract of native fresh fruit *I. paraguariensis* (750 µg/mL) (NFRI 750) at 72 hours (Figure 1).

The most efficient aqueous extracts of *I. theezans* also showed activity after 24 hours of exposure. The extracts that had the most intense larvicidal activity were those from dry leaves of *I. theezans* (2000 µg/mL) from the first 24 hours and from fresh leaves of *I. theezans* (750 µg/mL), between 24 and 48 hours

Figure 1 – Larvicidal activity of *I. paraguariensis* extracts over 72 hours of exposure. CFRI 1000: *I. paraguariensis* Cultivated fresh fruit (1000 µg/mL); NFRI 750: *I. paraguariensis* Native fresh fruit (750 µg/mL); CFRS 750: *I. paraguariensis* Cultivated dried fruit (750 µg/mL); NFRI 1000: *I. paraguariensis* Native fresh fruit (1000 µg/mL). Vertical bars indicate the Standard error of the mean



4 DISCUSSION

The results showed that the aqueous extracts of both native and dried fruits of native and cultivated *I. paraguariensis* presented larvicidal activity at the concentrations evaluated, except for the concentration of 50 µg/mL, those of 750 µg/mL and 1000 µg/mL were the most efficient. The native fresh fruit extract of *I. paraguariensis* at 1000 µg/mL was the most efficient (100%) in 72h (Table 1). The dried fruit extract of this species, obtained from cultivated plants, presented 90% efficiency, in which the highest activity was observed in the period between 24 and 48 hours of exposure (Figure 1).

Fresh and dried fruit of *I. paraguariensis* showed greater activity in relation to the leaves of the same species, which can be attributed to the higher concentrations of saponins present in fruits of yerba mate, compared to the leaves, which are a defense against herbivory and other forms of consumption and predation. After ripening, there is a predominance of substances that facilitate the consumption and dispersion of seeds, such as sugars, proteins, fats and carbohydrates (COGOI et al., 2013). Saponins present in fresh fruit of *I. paraguariensis* may have been responsible for the larvicidal activity on *A. aegypti*. The effect of saponin present on fruit of several plant families is known to be deleterious to the development of pests on maize monocrops, such as *Spodoptera frugiperda* (Smith, 1797) and *Helicoverpa zea* (Boddie, 1850) (DOWD et al., 2011).

In the study by BUSATO et al. (2015) on leaf extracts of *I. paraguariensis*, there was variation in larval mortality in periods of exposure between 24 and 48 hours. At 48 hours exposure, aqueous extracts of fresh leaves showed 100% efficiency at the concentration of 2000 µg/mL. This result corroborates the hypothesis that the concentration of extracts may interfere with larvicidal activity over the exposure time. Despite the low larvicidal efficiency (<10%) of the leaf extracts of *I. paraguariensis*, different from the results obtained by BUSATO et al. (2015), it is suggested to perform additional research, encompassing different methods of extraction, isolating compounds and testing the residual effect.

The commercial interest for *I. paraguariensis*, besides its cultural and gastronomic aspects, is due to the presence of xanthic bases. According to BORGES et al. (2013), the main constituents of the yerba mate leaves used for teas include the methylxanthines, caffeine, theobromine and traces of theophylline. Theophylline, caffeine and theobromine are methylated xanthines, or methylxanthines. Xanthines are nitrogenous bases of the same class (alkaloids), which include atropine, cocaine, ephedrine, morphine, quinine, nicotine and several others, all related to the great variety of physiological actions. Studies have demonstrated the different properties presented by yerba mate like antioxidant activity, protective effect against DNA-induced damage, vasodilation activity, thermogenic effects, effect on the improvement of glucose tolerance and anti-inflammatory effects (PEREIRA et al., 2012; PIMENTEL et al., 2013).

Considering shaded environments with specific microclimatic conditions, such as intensity and duration of solar radiation, and preestablished genetic variations and even by the presence or absence of endophytic microorganisms, differences are expected as to the chemical composition of native and cultivated plant extracts. Due to the structure of the plant, yerba mate responds directly to environmental conditions, such as light intensity and nutrient contents, and may alter its morphology and physiological processes (MELO; AZEVEDO, 1998). Studies on the phytochemical composition of yerba mate show that its compounds can vary in qualitative and quantitative terms, according to the type of cultivation, climate, agronomic conditions, plant age, methodology of analysis and industrial processing (KNAKIEWICZ et al. 2016). These variations may explain the efficiency differences of extracts obtained from plant tissues of native and cultivated plants. Fruit extracts from native plants presented higher efficiencies. In this context, the results corroborate the need for a broader phytochemical characterization of native species that are also commercially grown.

Leaves of *I. theezans* are used mixed in beverages to increase bitterness for containing a higher concentration of saponins. The main biological activities of saponins are related to their complexing, hemolytic and toxic properties (MAHATO;

SARKAR; PODDAR, 1988). In this study, the aqueous extracts of *I. theezans* also presented larvicidal efficiency, but smaller compared to *I. paraguariensis*. The fresh leaf extract of *I. theezans* at the concentration of 750 µg/mL was the most efficient (75%). Fresh and dried leaves of *I. theezans* showed greater activity in relation to fruits of the same species. These results differ from those reported by KNAKIEWICZ et al. (2016) with hydroalcoholic extracts, in which this plant showed larvicidal activity in all concentrations of extracts evaluated and in a higher percentage in relation to extracts of *I. paraguariensis*. This result indicates that the method of extraction of the compounds results in differences in larvicidal activity.

5 CONCLUSION

Aqueous extracts of fresh and dried fruit of *I. paraguariensis* present high larvicidal potential on *A. aegypti*. Subsequent studies focused on the evaluation of residual effects in laboratory conditions and in the field, using extracts of these two plants will confirm the possibility of using residues from these plants (fruit) or leaves (after use) as methods of population control of *A. aegypti* mosquito. The results contribute to the knowledge about the properties of *I. paraguariensis* and *I. theezans*. They point to the possibility of prospecting products with larvicidal properties from fruit of *I. paraguariensis*, not commercially used.

REFERENCES

- ABBOTT WS. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*. 1925; 18(2): 265-267.
- BORGES ACP, DARTORA N, RIL FT, GOLÇALVES TL, VALDUGA AT. Avaliação do conteúdo de cafeína na oxidação de folhas de *Ilex paraguariensis* st. Hil. *Revista de Ciência e Tecnologia*. 2013; 15 (20): 38-43.
- BUSATO MA, VITORELLO J, LUTINSKI JA, DAL MAGRO J, SCAPINELLO J. Potencial larvicida de *Melia azedarach* L. e *Ilex paraguariensis* A. St.-Hil. no controle de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Ciência e Natura*. 2015; 37(2): 277 – 282.

- COGOI LM, GIACOMINO MS, PELLEGRINO N, ANESINI C, FILIP R. Nutritional and Phytochemical Study of *Ilex paraguariensis* Fruits. *Journal of Chemistry*. 2013; 2013(750623): 1-6.
- DOWD PF, JOHNSON E, VERMILLION KE, BERHOW MA, PALMQUIST DE. Coconut leaf bioactivity toward generalist maize insect pests. *Entomologia experimentalis et applicata*. 2011; 141(3): 208-215.
- FEITOSA FRS, SOBRAL IS, DE JESUS EN. Indicadores socioambientais como subsídio à prevenção e controle da dengue. *Electronic Journal of Management, Education and Environmental Technology (REGET)*. 2015; 19(3): 351-368.
- FLAUZINO RF, SOUZA-SANTOS R, DE OLIVEIRA RM. Indicadores socioambientais para vigilância da dengue em nível local. *Saúde e Sociedade*. 2011; 20(1): 225-240.
- FOSTER K. Vector Control Tools and Resources for Environmental Health Professionals. *Journal of Environmental Health*. 2016; 78(10): 44-46.
- GUARDA C, LUTINSKI JA, ROMAN-JUNIOR WA, BUSATO MA. Atividade larvicida de produtos naturais e avaliação da susceptibilidade ao inseticida temefós no controle do *Aedes aegypti* (Diptera: Culicidae). *Interciencia*. 2016; 41(4): 243-247.
- JAYARAJ R, MEGHA P, SREEDEV P. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinar Toxicology*. 2016; 9(3-4): 90-100.
- KNAKIEWICZ AC, LUTINSKI JA, GUARDA C, PARIS A, BELOTTI A, BUSATO MA, ROMAN JUNIOR, WA, SIMÕES DA. Larval susceptibility of *Aedes aegypti* (L.) (Diptera: Culicidae) to extracts of *Ilex paraguariensis* and *Ilex theezans*. *Revista Brasileira de Ciências Ambientais*. 2016; 42: 113-120.
- MACHADO CCB, BASTOS, DHM, JANZANTTI NS, FACANALI R, MARQUES MOM, FRANCO MR B. Determinação do perfil de compostos voláteis e avaliação do sabor e aroma de bebidas produzidas a partir da erva-mate (*Ilex paraguariensis*). *Química Nova*. 2007; 30(3): 513-518.
- MAHATO SB, SARKAR SK, PODDAR G. Triterpenoid saponins. *Phytochemistry*. 1988; 27(10): 1185-1236.
- MELO IS, AZEVEDO JL. Controle Biológico. Jaguariúna: Embrapa Meio Ambiente. 1998.
- MATIAS NG. Avaliação da atividade larvicida de extratos vegetais frente ao *Aedes aegypti*. Pindamonhangaba-SP: FAPI Faculdade de Pindamonhangaba; 2017. 47p.
- PEREIRA DF, KAPPEL VD, CAZAROLLI LH, BOLIGON AA, ATHAYDE ML, GUESSER SM, DA SILVA EL, SILVA FR. Influence of the traditional Brazilian drink *Ilex paraguariensis* tea on glucose homeostasis. *Phytomedicine*. 2012; 19(10): 868-877.

PIMENTEL GD, LIRA FS, ROSA JC, CARIS AV, PINHEIRO F, RIBEIRO EB, OLLER DO NASCIMENTO CM, OYAMA LM. Yerba mate extract (*Ilex paraguariensis*) attenuates both central and peripheral inflammatory effects of diet-induced obesity in rats. *The Journal of nutritional biochemistry*. 2013; 24(5): 809-818.

ZAHOULI JBZ, UTZINGER J, ADJA MA, MÜLLER P, MALONE D, TANO Y, KOUDOU BG. Oviposition ecology and species composition of *Aedes* spp. and *Aedes aegypti* dynamics in variously urbanized settings in arbovirus foci in southeastern Côte d'Ivoire. *Parasit Vectors*. 2016; 9(523): 1-14.

ZARA ALSA, DOS SANTOS SM, FERNANDES-OLIVEIRA ES, CARVALHO RG, COELHO GE. Estratégias de controle do *Aedes aegypti*: uma revisão. *Epidemiologia e Serviços de Saúde*. 2016; 25(2): 391-404.

ZONTA EP, MACHADO AA. SANEST - Sistema de análise estatística para microcomputadores. Pelotas: DMEC/IFM/UFPel, 1987. 138p.