EVALUATION OF THE POTENTIAL ACARICIDAL OF THE HEXANIC EXTRACT OF *Trichilia pallida* SW. (SAPINDALES: MELIACEAE) LEAVES AND ESSENTIAL OIL OF *Sesamum indicum* LINN. (LAMIALES: PEDALIACEAE) SEEDS AGAINST LARVAL INSTAR OF *Rhipicephalus sanguineus* (LAT.) (ACARI: IXODIDAE)

AVALIAÇÃO DO POTENCIAL ACARICIDA DO EXTRATO HEXÂNICO DE FOLHAS DE *Trichilia pallida* SW. (SAPINDALES: MELIACEAE) E DO ÓLEO ESSENCIAL DE SEMENTES DE *Sesamum indicum* LINN. (LAMIALES: PEDALIACEAE) CONTRA LARVAS DE *Rhipicephalus sanguineus* (LAT.) (ACARI: IXODIDAE)

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RESUMO

Este trabalho objetiva verificar os potenciais das plantas *Trichilia pallida* e *Sesamum indicum* na prospecção de acaricidas botânicos, para serem utilizados como nova alternativa para controle do ixodídeo *Rhipicephalus sanguineus*. O extrato hexânico (e.h.) de folhas de *T. pallida* e o óleo essencial de sementes de *S. indicum* foram obtidos em laboratório, a partir de amostras coletadas, respectivamente, em uma área de Mata Atlântica e em fazendas de cultivo no Brasil. Larvas de *R. sanguineus* foram obtidas a partir de fêmeas ingurgitadas, coletadas em ambientes urbanos naturalmente infestados. Os bioensaios foram realizados em quadruplicata, a 27 ± 1^oC, RH ≥ 80% e fotoperíodo de 12 horas. Cerca de 50 larvas, de 14 a 21 dias de idade, foram acondicionadas em envelopes de papel-filtro, impregnados com diferentes concentrações das substâncias vegetais, obtidas a partir da diluição de soluções-padrão. Em cada bioensaio, foram utilizados quatro envelopes impregnados com cada concentração testada. O grupo controle usou a mesma quantidade de larvas, submetidas a: 1. Envelopes sem nenhum tratamento; 2. Envelopes tratados com água destilada e, 3. Envelopes com solventes utilizados na preparação de soluções vegetais. Pela ação do e.h. de folhas de *T. pallida* e do óleo de *S. indicum*, após 24 horas de exposição, obtiveram-se, respectivamente, CL_{50} de 4.660 ppm e 107.729 ppm e CL_{99} de 14.217 ppm e 279.912 ppm. Após 48h, obtiveram-se, respectivamente, CL_{50} de 1.555 ppm e 78.880 ppm e CL_{99} de 3.431 ppm e 221.255 ppm. Mortalidade larval significativa não foi observada no grupo controle (P < 0,05).
Palavras-chave: *Rhipicephalus sanguineus;* carrapato-vermelho-do-cão; acaricidas botânicos; baga-de-morcego, sésamo.

ABSTRACT

This paper aims at verifying the potentials of *Trichilia pallida* and *Sesamum indicum* in the prospection of botanical acaricide, to be used as a new alternative to *R. sanguineus* control. Hexanic extract (h.e.) of *T. pallida* leaves and essential oil from seeds of *S. indicum* were obtained in laboratory, from samples collected, respectively, in an area of Atlantic forest and in agricultural farms, in Brazil. *R. sanguineus* larvae were obtained from engorged females collected in naturally infested urban environs. Bioassays were carried out in quadruplicate, at 27 ± 1^oC, RH ≥ 80% and photoperiod of 12 hours. About 50 larvae, from 14 to 21 days of age, were conditioned in filter paper envelopes, impregnated with different concentrations of vegetable substances, obtained from dilution of stock solutions. In each bioassay, four envelopes impregnated with each tested concentration were used. The control group used the same amount of larvae, submitted to: 1. envelopes with no treatment; 2. envelopes with distilled water and, 3. envelopes having solvents used in the preparation of vegetable solutions. For the action of h.e. leaves of *T. pallida* and *S. indicum* oil, after 24h of exposure, it was obtained, respectively, LC_{50} of 4,660 ppm and 107,729 ppm and LC_{99} of 14,217 ppm and 279,912 ppm. After 48h, it was obtained, respectively, LC_{50} of 1,555 ppm and 78,880 ppm and LC_{99} of 3,431 ppm and 221,255 ppm. There was no significant mortality rate within the control group (P < 0.05).
Keys words: *Rhipicephalus sanguineus;* brown dog tick; botanical acaricides; gaita; sesame.

INTRODUCTION

Rhipicephalus sanguineus (Latreille, 1806) (Acari: Ixodida: Ixodidae) is a tick that firstly sponges mainly the domestic dog in urbanized areas and secondarily other mammals, birds and reptiles. With an epicenter of dispersion in the Afrotropical region, this ixodid was dispersed mainly by domestic dogs that accompanied the people in their migrations and, secondarily, by wild free-living canids (1, 2). The *R. sanguineus* complex is composed of species with diverse geoclimatic distribution, as well as some morphological and genotypic aspects, and possibly presenting variations in the vectorial competence for different species and genotypes of animal and human pathogens. Phylogeographic studies currently divide it into two clades: The lineages Tropical and Temperate. The tropical lineage, present in Brazil, is found in other countries located between latitudes 35°N and 20°S, in areas with an annual mean temperature > 20°C (3).

R. sanguineus is responsible for causing severe anemia (in solid infestations that happen in the domestic dog, that seems not to develop resistance to the tick), discomfort because of the bites, and for transmitting from several pathogens to their hosts, during its habit (4). Among the transmitted pathogenic agents, in several places of the world, by different lineages of *R. sanguineus* to their hosts, including the man, there are mainly *Ehrlichia canis* and *Babesia canis*, but also *B. caballi*, *B. gibsoni*, *Hepatozoon canis*, *Haemobartonella canis*, *Theileria equi*, *Rickettsia conorii*, agent of the "fever exanthematic of Mediterranean", and *R. rickettsii* from the etiological group of the "spotted fever". As a matter of fact, in some areas of Mexico, for instance, it is believed that *R. sanguineus* is a more important vector of this illness, known at that country as "fiebre manchada", than *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), main vector in the Neotropical area (4-6) Besides, recent studies demonstrated that *R. sanguineus* also acts as reservoir of *Leishmania chagasi*, etiological agent of visceral leishmaniasis. (7).

Due to the growth of reports of accidental parasitism in humans, mainly for their immature forms, in the USA, Spain, Uruguay, Mexico, and in Brazil (6, 8), it is believed that *R. sanguineus* is becoming more anthropophilic, thus, it can come to cause the increase in the ehrlichiosis incidence, babesiosis and spotted fever, such as emerging antropozoonose. Reinforcing the fact that due to the absence of pathognomic clinic signs, these illnesses in humans a lot of times are confused with other diseases with similar clinic profile, leading to a late clinical diagnosis of the patient which may result in his death (4-6, 8).

The continuous and a lot of times inadequate use of synthetic chemical insecticides, main products used in the control of this vector, has been favoring the resistance development to these products, inefficiency in the control measures, sanitary and economic damages, besides environmental damages caused by the accumulation of these drugs in the environment (9-10).

For decades in Brazil, the combat to this tick *R. sanguineus* was just made through baths or pulverization of the dogs infested with acaricide and insecticides, in dosages of the most varied, mainly developed for use in cattles or for environmental disinfestations (9-10). This historic of incorrect use has probably led to significant reduction in sensibility of the target site of these acaricides, i.e., sodium channels of the nervous system, producing *Knockdown resistance* (Kdr) (11-12). It is believed that the populations of existent *R. sanguineus* have developed two different mechanisms of resistance, because these manifested loss of the efficiency of cypermethrin, resistance to deltamethrin, but not to permethrin, all pyrethroid (10). A similar situation was observed in Panama for this tick, whose resistance was characterized by an increase in the esterase levels as well as Kdr (11). A possible explanation for this phenomenon would be that the specific tick formulations for use in dogs, using permethrin base and other chemical groups, were introduced at the Brazilian commercial market, and adopted by the dog breeders, only many years after the use of the deltamethrin and cypermethrin in

formulations for domissanitary use or in cattle (10). It is important to observe that the resistance of adults and larvae of *Amblyomma cajennense* to deltamethrin and other synthetic acaricides was also recently diagnosed in Brazil (13). Later, resistance of *R. sanguineus* to synthetic chemical acaricide was also diagnosed at other countries (11, 14, 15).

These factors, combined to the notable biotic potential of the species and the complexity of the environments infested by the same (16), come to propose the accomplishment of studies for development of new control strategies for *R. sanguineus*. The use of vegetable substances with acaricide properties has been proved a promising alternative for control of ticks (17-28). The great success obtained with the pyrethrum, an isolated molecule of *Chrysanthenum* spp, and its derivatives, and the great biological diversity of botanical species in the world, stimulate the accomplishment of future researches to find new insecticidal substances extracted from other plant species. The market for plant-based acaricidal products is very promising when we consider the very large losses caused by ticks and the diseases they transmit, the high levels of consumption of synthetic chemical acaricides, the worldwide desire for prevention food contamination and the environment and the possibility of using organo-natural acaricides in the organic livestock, and the need to establish new acaricidal products on the market for which there are still no resistant strains of ticks (17).

In this sense, the present study aimed to evaluate the potential of two plants found in Brazil, being *Trichilia pallida* Swartz, 1788 (Sapindales: Meliaceae) and *Sesamum indicum* Linnaeus, 1753 (Lamiales: Pedaliaceae) for the search of botanical acaricide, to be used as alternative measure of control for *R. sanguineus*. These plants were chosen for the study mainly with base in the previous verification of their harmful activity on insects of agricultural importance.

Trichilia pallida Swartz, popularly known as "bat berry" and "gaita", in Brazil and the USA respectively, have demonstrated has insecticidal and repellent properties against several insects of agricultural importance, such as "variegated cutworm" *Peridroma saucia* Hübner (Lepidoptera: Noctuidae), the "tobacco cutworm" *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), the "fall armyworm" *S. frugiperda* (Lepidoptera: Noctuidae), and "striped cucumber beetle" *Acalymma vittatum* F. (Coleoptera: Chrysomelidae), the "tomato pinworm" *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), and the "whitefly" *Bemisia tabaci* (Hemiptera: Aleyrodidae) (29-31). It was found that extracts and essential oils from branches and leaves of *T. pallida* are rich in terpenoids, among other bioactive chemicals (30). Simmonds et al. (2001) extracted from the roots of *T. pallida* a molecule of tetranortriterpene with antifeedant activity against larvae of four lepidopteran species: *Helicoverpa armigera*, *Heliothis virescens*, *Spodoptera exigua,* and *S. littoralis* (32). The terpenoids, natural substances of vegetable origin, have been reported for their ecological importance as natural plant defense against insect predation, causing repellency, suppression of appetite, and other toxic effects (33).

Sesamum indicum, a plant of the Pedaliaceae family, is popularly known as "sesame", in reference to the famous phrase literary "Open Sesame", because its fruits if open fully when mature, letting their seed fall to the ground. This plant was chosen for this study by having presented, in previous studies, deleterious action of its leaves on anthills, when used as food, altering the breathing metabolism of *Atta sexdens rubropilosa* (Formicidae) (34) and exercising inhibitory action on its fungi symbiotic *Leucoagaricus gongilophorus* (Singer) Möller (Agaricales: Basidiomycota) (35). It was also observed that lignin isolated from *S. indicum* essential oil are poisonous for labor ants *Atta sextens* L. (36) Other factor that has influenced this choice was the previous verification that composts of this plant, sesamin and sesamolin, when in insecticide formulations, show synergistic activity, increasing the toxicity of pyrethroids used in the control of *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) and others arthropods (37).

METHODS

Obtaining vegetable solutions

The methods used to obtain the tested substances were adapted from traditional methods of extracting substances of vegetable origin (31, 37). Leaves of *T. pallida* were collected in March 2002 and November 2003 at the ESALQ/USP Campus, a small area of Atlantic Forest in the municipality of Piracicaba, São Paulo state, Brazil. The exsicata of the plant is deposited in the herbarium of the Department of Biological Sciences of ESALQ/USP, under the number ESA 81288. The leaves were taken to the laboratory of chemistry for processing of its extract. These leaves were dried in forced ventilation greenhouse to 40°C with reduction of the pressure for the achievement of a fine powder. The extraction was made by maceration in hexane with three repetitions, leaving it in rest for 72 h. Soon afterwards, the filtrate was put in a rotate evaporator for the achievement of the hexanic extract (h.e.). Seeds of *S. indicum* were collected in August 2005 on farms of cultivation belonging to the municipality of Amaralina, located in the northern state of Goiás, Brazil. These were initially cold pressed. After that, the obtained oily pulp was saturated with hexane, which that dissolved the whole present essential oil, staying in rest for 6 hours. The resulting mass was then filtered through vacuous and, the evaporated solvent of the essential oil for heating about reflow for 2.5 hours at a boiling temperature of 68°C. Soon afterwards this was submitted to a distillation process for extraction of the essential oil. After the condensation of the steam, the mixture of water with the essential oil was stored in decantation collectors, coupled to the exit of the coil. The time of decantation was about 12 hours. Later, the oil was filtered to eliminate impurities. The yields of the essential oil extracted from the seeds of *S. indicum* and the hexanic extract from the leaves of *T. pallida* were 52.6% and 4%, respectively.

The preparation of different concentrations of the tested plant substances was carried out by adapting the previously described methodologies (20-24). For the preparation of the stock solutions of the vegetable extracts, these were weighted in analytical scale, accurately 0.0001 g. In each bioassay a stock solution with h.e. of the leaves of *T. pallida* were prepared in the concentration of 5,000 ppm, diluting 0.75 g of this extract in distilled water, 19,5 ml of the solvent alcohol methanolic P.A. and 4,5 ml of the tenso-active Tween[®] 80. This period in rest for about 24 h to facilitate the dissolution, was homogenized in magnetic agitator for approximately 15 minutes and it had its adjusted volume with water distilled for 150 ml. Stock solutions of the essential oil of the seeds of *S. indicum* were prepared to 250,000 ppm, diluting 37,5 g of the essential oil in a similar way, however, using as solvent the alcohol ethanol P.A. (30 ml), being added 6,0 ml of Tween[®] 80 and being completed its volume to 150 ml. With the objective of making possible the verification of the biocide activity of different concentrations of the vegetable extracts, it was obtained by dilution of the stock solutions with distilled water other solutions, at concentrations of 4,000, 3,000, 2,500, 2,000 and 1,500 ppm of h.e. of *T. pallida* and 230,000, 200,000, 180,000, 150,000 and 100,000 ppm of the essential oil of *S. indicum*.

Obtaining of ixodidae larvae

Engorged females of *R. sanguineus* were collected in environments frequented by dogs naturally infested, in several neighborhoods of Goiânia, capital of the Brazilian state of Goiás. These were stored in glass flasks with proper cover and then sent to the laboratory, where they were washed in distilled water, dried on paper towels. With aid of a stereoscope it was identified taxonomically and selected engorged female in perfect anatomical conditions (10). For the accomplishment of the oviposition, the engorged females were adhered dorsally with doubled sided tape on slides, inversely disposed on the base of a dish Petri. Later on they were conditioned in an incubator of the type B.O.D., acclimatized to 27±1°C, RH ≥ 80% and photoperiod of 12 hours. The daily

oviposition were collected and gathered in a single tube of polyethylene (3 x 9 cm) with proper cover, constituting a pool of eggs. After the appearance, each tube of polyethylene was sealed then with adhesive ribbon to avoid the escape of larvae.

Methodology for verification of larval sensibility to vegetable extracts

The methodology of Fernandes (2000) (10) was used to evaluate the larval sensitivity. This method was developed from the *larval packet test* (*lpt*) - the FAO method (38), with methodological modifications aimed at reducing costs, increasing its practical use, however, without loss of efficiency. This methodology was previously tested with satisfactory results in the evaluation of the acaricidal activity of other botanical substances (20-24).

The bioassays were accomplished in a biological camera, acclimatized 27±1°C and RH ≥ 80%, photoperiod of 12 h and, especially built for studies with botanical acaricide. For the contention and exhibition of the larvae to the tested solutions in filter paper envelopes (≈ 327 cm^2) containing micropores to provide ventilation in their interior. The internal surfaces of each envelope were impregnated with 2 ml of each tested solution, distributed evenly with aid of a pipette. It was used in the bioassays just larvae with 14 to 21 days of age, aiming at making possible the comparison of the results with other authors' studies. A bracket of these larvae was put in the center of a sheet of white paper, fastened on the bench with doubled sided tape. From these at least 50 larvae with good motility were hoisted with a brush n° 4 of clear hair, previously moistened in the tested solution, and put in each envelope. The remaining larvae were eliminated with ribbon crape. The envelopes were physically sealed, by bending its opening (border with 1 to 2 cm) and tightening the same with aid of metallic fasteners. These were hung in form supports in order to be suspended in the air, without contact with any surface, in order to avoid losses of the solution or contamination. Under the envelopes, in the benches, humidifiers were used to maintain appropriate the acclimatization of the biological camera.

The bioassays were made in quadruplicate, for each bioassay a new solution stock of the vegetable extract was prepared. In each bioassay, for each tested concentration, four envelopes were used. All of the experiments were accompanied by a control group, containing the same number of larvae in each envelope and, prepared in the following way: 1. dry envelopes (without treatment); 2. envelopes with distilled water and, 3. envelopes impregnated with the solvents and used tenso-active, in the same concentrations used for the group test.

The envelopes were open and inspected by the stereoscope after 24 and 48 hours of exhibition, for the registration of alive and dead larvae and possible observed toxicological effects. To make possible the comparison of the results with other authors studies, the percentile ones medium of mortality were calculated considering also killed the larvae without capacity of locomotion. In case of an eventual bioassay with mortality of the group control larger than 5%, it would be discarded and repeated.

Aiming the determination of the Lethal Concentrations (LC), especially LC_{50} and LC_{99}, and their respective Confidence interval (CI) of 95%, it was interpolated the percentile medium mortality rate obtained by the action of the different tested concentrations, through regression analysis *Probit*, using the software System for Statistical Analyses (SAEG)$^{®}$ version 9.0 the qui-square test was Applied (X^2) at the level of 5% of significance to verify the influence of the concentration in the larvicide efficiency.

RESULTS

The h.e. from leaves of *T. pallida* and the essential oil of *S. indicum* presented larvicide activity for *R. sanguineus*. The medium indexes of mortality obtained by the extract of *T. pallida* at concentrations of 1,500, 2,000, 2,500, 3,000, 4,000 and 5,000 ppm, were

respectively of 0, 6.66, 9.7, 17.4, 31.3 and 60.6% of the larvae, after 24 hours of exhibition. At the 48th hour, for the same concentrations, the mortality of 50%, was verified in 0, 72, 89.28, 99.9, 100 and 100% of the larvae. The mortality results observed after 24 and 48 hours of exhibition to the different concentrations of the extract of *T. pallida* were demonstrated in the Figure 1.
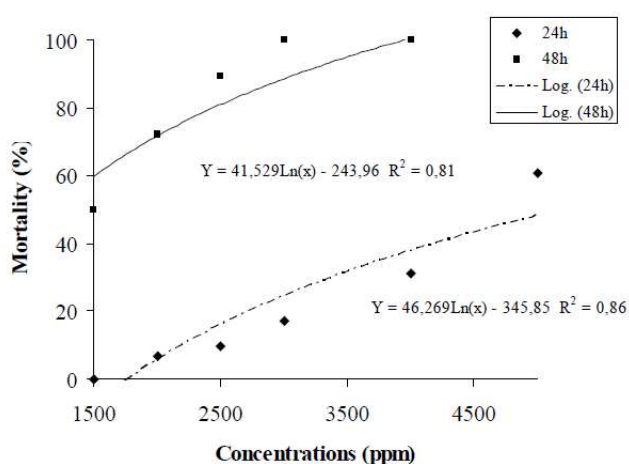

Figure 1. Mortality of larvae of *Rhipicephalus sanguineus* for action of different concentrations of the hexanic extract of *Trichilia pallida* leaves, observed at the 24th and 48th hour of exposure. The trend line is derived from logarithmic regression (Probit analysis). Y = Equation of the straight line.
R^2 = determination Coefficient.

The essential oil of *S. indicum* at concentrations of 100,000, 150,000, 180,000, 200,000, 230,000, 250,000 ppm, caused the mortality of 50%, respectively 68.1, 88, 93, 99.7 and 100% of the larvae, after 24 hours of exhibition. At the 48th hour the mortality of 75, 84, 98, 99.8, 99.9 and 100% of the larvae were observed, respectively, for the same concentrations. The larval susceptibility of *R. sanguineus* to the different concentrations of the essential oil of *S. indicum* is demonstrated in the Figure 2. There was not significant

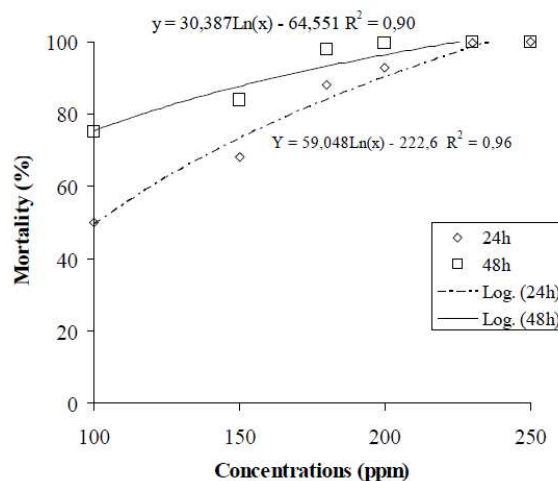mortality (larger than 5%) in the control group, in none of their treatments (P < 0.05).

Figure 2. Susceptibility of larvae of *Rhipicephalus sanguineus* to different concentrations of the essential oil of *Sesamum indicum* seeds, observed after 24 and 48 hours of exposure. Concentrations values (x 1000). The trend line is derived from logarithmic regression (Probit analysis). Y = Equation of the straight line.
R^2 = Determination coefficient.

After 24 hours of exposition to the extract of *T. pallida*, it was obtained the lethal concentrations LC_{50} and LC_{99} of 4,660 ppm and 14,217 ppm, respectively. By the action of the essential oil of *S. indicum* it was obtained on this same schedule of observation LC_{50} of 107,729 ppm and LC_{99} of 279,912 ppm. The lethal concentrations obtained after 48 hours of exhibition to e.h of *T. pallida* (X^2 = 5.92 > X^2.05 (1) = 3.84) and to the essential oil of *S. indicum* (X^2= 14.09 > X^2.05 (1) = 3.84) are in the Table 1. The values of X^2 obtained allow rejection the hypothesis that the mortality of the larvae does not depend on the concentration of the vegetable solutions.

Table 1. Susceptibility of larvae of *Rhipicephalus sanguineus* to the hexanic extract of *Trichilia pallida* leaves and to the essential oil of the *Sesamum indicum* seeds, observed after 48th hour of exposure by the method of *larval packet test* (*lpt*).

Vegetable extracts	Lethal Concentrations – LC (ppm) (95% Confidence Interval)				X^2	R^2
	LC_{25}	LC_{50}	LC_{75}	LC_{99}		
Trichilia pallida	1239 ppm (1087–1357)	1555ppm (1433–1656)	1953 ppm (1851–2059)	3431 ppm (3085–4006)	6.00	0.81
Sesamum indicum	58636 ppm (41381–71124)	78880 ppm (62830–90075)	106113 ppm (93767–116059)	221255 ppm (189837–289657)	15.00	0.90

R^2: Determination coefficient values; LC_{25}: Concentration required to kill 25% of larvae (LC_{50}: 50%; LC_{75}: 75%; LC_{99}: 99%); Confidence Interval (CI) of 95% significance; X^2: Chi-square values; $p < 0.05$.

DISCUSSION

A harmful action on insects was previously also observed in extracted substances of other vegetable species, such as acid crisantemic – extracted from the flowers of the "pyrethrum", *Chrysanthemum cinerariafolium* (Asteraceae) (17) –, that have insecticide action; rotenona – extracted from roots of the "timbó", *Derris* spp. (Fabaceae) –, that have action fagoinibitora and deterrente; nicotine – of the leaves of the "tobacco", *Nicotiana* spp. (Solanaceae), that have insecticide action and growth inhibitor); quassina – of the stem of the "corner", *Quassia amara* (Simaroubaceae) –, with insecticide action; and azadiractina – extracted from the *Azadirachta indica* (Meliaceae), also known as "neem" or "amargosa", that have insecticide action, of inhibition of feeding and growth of the insect. This latter, stands out as one of the best known, because of their insecticide properties, having been registered its action on more than 400 species of insects and acaridae, besides action against mushrooms, bacteria and nematode (30-34, 37-38). The high potential of this plant was one of the reasons that motivated the choice for the present study of another species from Meliaceae family, *Trichilia pallida* Swartz.

In addition to the selection by taxonomic similarity, and for its repellent and insecticidal properties against several insects of agricultural importance, *T. pallida* was chosen for the study also because it possesses bioactive substances with simpler molecular structures and, therefore, with economically viable synthesis (29-37).

Results encouraging the use of substances extracted from plants to control ticks have been obtained by different researchers. Souza Chagas et al. (26, 27) testing the oil essential three species of *Eucalyptus* genus (Myrtaceae) on larva of the bovine tick, obtained 100% of larval mortality by the action of the concentrations of 10% (≈ 100,000 ppm) of the essential oil of *E. staigeriana* and of *E. citriodora*, and of 20% (≈ 200,000 ppm) of the oil of *E. globulus*. Borges et al. observed that hexanic and chloroformic extracts of mature fruits of another species of Meliaceae family, *Melia azedarach* Linn., at concentration after 0.25%, caused 98% and 100% larval mortality, respectively, to the "cattle tick", *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) (Acari: Ixodidae). It was also observed that these extracts produced significant inhibition of the reproductive efficiency of females (100 and 91.5%, respectively) of this tick (18, 19). Fernandes et al. (23, 24) verified the larvicide activity of the crude ethanolic extract (c.e.e.) from the peel of the stem of *Sapindus saponaria* L. (Sapindaceae) against *R. (B.) microplus* (LC_{99}: 6,360 ppm ≈ 0.64%) and *R. sanguineus* (3,922 ppm ≈ 0.39%). As well as of c.e.e. from the peel of the stem of another soapberry, *Magonia pubescens* St. Hil, against these same species of tick (20, 21). These authors observed 99% of mortality (LC_{99}) of larvae of *R. microplus* and of *R. sanguineus* submitted respectively to the concentrations of 4,000 ppm (≈ 0.4%) and of 9,991 ppm (≈ 1,0%) of this e.b.e. Fernandes and Freitas (22) observed larvicide action of the oleoresin of an Amazonian species of *Copaifera*, *Copaifera reticulata* (Leguminosae: Caesalpinioideae), on the "cattle tick", *R. microplus, obtaining* LC_{50} and LC_{99} values were 1579 and 3491 ppm (≈ 1,6% and ≈ 3,5%), respectively. And, more recently, Thanh Hai and Atsushi (28) also observed promising larvicidal activity for *R. sanguineus* by action of *Camellia sasanqua* (Theaceae) thumb seed oil (LC_{50} ≈ 5.43% and LC_{90} ≈ 9.50%). Ghosh et al. (25) verified acaricidal and reproductive

inhibitory effects on engorged females of *R. microplus* by action of" ethanolic extracts of *Datura metel* (Solanaceae) fruits and of the *Argemone mexicana* (Papaveraceae) whole plant ", which determined respectively the LC_{99} values of 7.13 and 11.3%. The lethal concentrations of the vegetable substances observed by the foregoing authors, opposed to the results obtained in the present work, ratify the potentiality of *T. pallida* for investigation of botanical acaricide, once this determined the mortality of 99% of the larvae (LC_{99}) of *R. sanguineus* by the action of a smaller concentration (3,431ppm ≈ 0.34%).

In the present study, it was observed that the extract of *T. pallida* presents a larger toxicity to the larvae than the essential oil of *S. indicum*. However, it is believed that in the analysis of the potentiality for development of organonatural acaricide, it should also be considered the largest viability and smaller cost of the last in relation to the first, given the domain of the techniques of cultivation of *S. indicum* in wide scale, quite spread in the agriculture (37). The results obtained stimulate investments for research development and preservation of the studied plants, especially *T. pallida* in the areas of Atlantic Forest, its natural environment.

On the biocidal potential of *S. indicum*, it is relevant to report that, in addition to the toxic activity of *S. indicum* for the ants (34, 36), researchers have found that sesamin extracted from its seeds has bactericidal activity; and that chlorosamone, extracted from the roots of this plant, presents antifungal action (35, 37).

FAO (37), in studies for verification of the susceptibility and/or resistance of arthropods to the insecticides or acaricide, authorizes the correction of the medium indexes of mortality of the group tests, using the "formula of Abbott", in case there is mortality of the group controls between 5 and 10%. However, in the present study it was opted to use a more rigid criterion of evaluation, discarding and repeating any bioassays, in which was observed mortality above 5% in the control group. This care was taken aiming to eliminate any possibility of interference of the solvents or of any unexpected factors

in the observed larval mortality, propitiating a pure verification of larvicide activity of the vegetable substances tested.

The results of the present study allow the recommendation of the tested solvents and tenso-active, for the preparation of substances acaricide organonatural, especially for control of *R. sanguineus*, because in the tested concentrations they didn't cause larval mortality. Preceding the beginning of the present study, bioassays of larval tolerance with these products were realized. These have demonstrated through the methodology of larval packet test (lpt), that the Tween® 80, in concentrations of until 5%, and the ethanol and methanol in concentrations of up to 25%, can be used with safety in the preparation of the stock solutions, once they have not caused any mortality to the larvae, after 48 hours of exposition. The absence of mortality of larvae contained in the dry envelopes demonstrates the absence of toxicity of the filter paper used. The absence of significant mortality in none of the three treatments of the group controls allow the recommendation of the methodology used in this work for studies of evaluation of the susceptibility of larvae from ticks to acaricide.

Studies accomplished by different authors demonstrated that extracts obtained by different vegetable organs produce toxicological effects of greater or lesser intensity, in the submitted arthropods (30, 31). These data may suggest the accomplishment of new studies, aiming at to investigating the larvicide efficiency on *R. sanguineus*, of extracts produced with other vegetable organs from *T. pallida* and *S. indicum* plants.

CONCLUSION

The larvicidal activities observed in the present study by the tested concentrations of the h.e. extract of leaves of *T. pallida* and the essential oil of seeds of *S. indicum* allows to conclude that these botanical species present potential for the supply of bioactive substances aimed to compose new organonatural acaricides, to be used as alternative of more ecofriendly

control, to be added to the measures of integrated control of the *R. sanguineus* tick.

The results of the present work also allow us to conclude that the continuity of this research is necessary and justified, aiming at the isolation of bioactive fractions and sub-fractions of the extract and essential oil tested, with monitoring of the sensibility of *R. sanguineus* to them, endeavoring the development of botanical acaricide of smaller environmental impact than the Commercial Synthetic Acaricides.

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