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Extracts of plant residues from soursop peels in the management of palm red mite

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Abstract
Raoiella indica Hirst, 1924 (Prostigmata: Tenuipalpidae), is one of the main pest mites of palm and banana trees, causing significant damage in producing regions. Thus, the objective of this work was to evaluate the acaricidal effect of soursop peel extract (*Annona muricata* L.) on *R. indica* adults. The experiment was conducted in a completely randomized design using soursop peel extract, with 7 replications and 12 individuals of *R. indica* per replication. The experimental units consisted of discs of coconut palm leaves (4 cm in diameter), with cotton moistened at the bottom of the Petri dish (10.0 x 1.2 cm) and around the disc to maintain turgor and prevent mites from escaping. The application was carried out using an airbrush, connected to a calibrated compressor with a constant pressure of 1.3 psi and 1 mL of solution per plate. The acaricidal effect was evaluated 24, 48, and 72 hours after spraying. Mortality data were corrected and subsequently submitted to probit analysis ($p \leq 0.05$) using the statistical program R, with the LC_{50} and LC_{90} of the extract being calculated. The soursop peel extract at the maximum concentration used (15%) showed mortality of 80% of individuals of *R. indica*. The LC_{50} was 1.934%. It was concluded that extracts from soursop peel have acaricidal potential on *R. indica* in the laboratory.

Keywords: *Annona muricata, Raoiella indica,* alternative control, agro-industrial residues

Introduction

Initially described in India and then in other countries in Northeast Africa and the Middle East, the red palm mite *Raoiella indica* Hirst, 1924 (Prostigmata: Tenuipalpidae), is a species that lives mainly in plants of the Arecaceae and Musaceae family, mainly in palm trees and banana trees [1]. The pest species arrived in Brazil in 2009 through the state of Roraima, quickly dispersing throughout the other Brazilian states [2, 3]. In 2018, it was reported in Espírito Santo in coconut and pygmy date palm (*Phoenix roebelenii* O'Brien) in the municipalities of Guarapari, Vila Velha and Vitória, becoming a problem for the producing regions of the state [4].

R. indica is considered an extremely severe pest species. The habit of feeding on the abaxial surface of the leaf, through the stomata of the host, interferes with the photosynthetic and respiratory processes of the plant and consequently causes initial tanning of the leaf, which can later lead to necrosis of plant tissues and, in extreme cases, to the death of young plants, in addition to significantly reducing the productivity of adult plants [3].

Despite its importance, there are few official control methods registered for this pest species. In Brazil, the predatory mite *Neoseiulus barkeri* (Acari: Phytoseiidae) is registered as a biological control and has few synthetic chemicals [5]. This leads many producers to misuse unregistered synthetic chemicals, without guaranteeing their efficiency, contributing to increased environmental contamination, and causing negative interference in the population of natural enemies, which directly hinders pest management, in addition to promoting the increase of populations resistant to numerous active principles of pesticides.

In this context, the use of plants with acaricidal and/or insecticidal principles and, mainly, plant by-products from residues of agro-industrial processes, such as fruit peel, emerge as a possible alternative for controlling individual pests through secondary metabolites of plants [6].

In addition, this alternative fits with the principles of the Sustainable Development Goals (SDGs) that are part of the UN Agenda 2030 and aim to guarantee a more prosperous, equitable, and healthy planet by the year 2030.
Among many examples of species of fruit plants that are used in the agro-industrial sector and that generate residues with insecticidal/acaricidal characteristics, there are the plants of the Annonaceae family. Soursop, *Annona muricata* L., for example, is commonly used by industries in the production of ice cream and its derivatives, however, other fields also use its properties, such as the pharmaceutical industries, which use it for natural medicine. In the management of agricultural pests, soursop is used because it has among its main active components annonaceous acetogenins, in addition to being a varied source of phenolic compounds, essential oils, alkaloids, flavonol triglycosides, megastigmanes, and several minerals, including Mg, Fe, Cu, K and Ca [7].
Thus, this study aimed to evaluate the acaricidal effect of extracts obtained from the peel of *A. muricata* on adults of *R. indica*.

Materials and Methods

The experiment was carried out at the Agricultural Entomology and Acarology Laboratory of the Federal Institute of Education, Science, and Technology of Espírito Santo - Campus Itapina (IFES-Campus Itapina), located in the municipality of Colatina, with the geographic coordinates of 19°29'52.7" S 40°45'38.5"W (Fig 1).

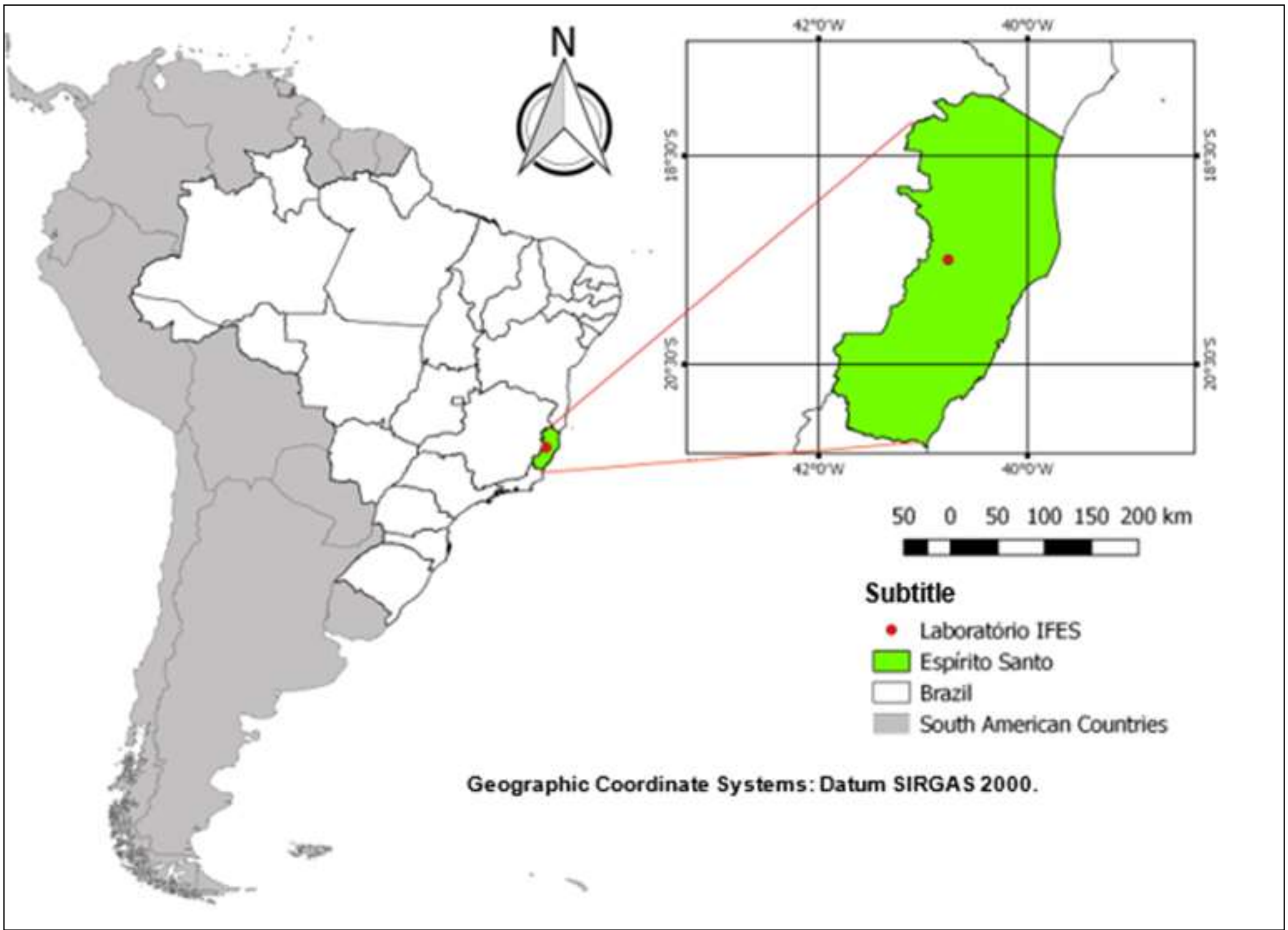

Fig 1: Geographical Map of Agricultural Entomology and Acarology Laboratory of the Federal Institute of Education, Science, and Technology of Espírito Santo - Campus Itapina (IFES-Campus Itapina).

Breeding and maintenance of the palm red mite *R. indica*

Palm leaflets infested with the pest species were collected in the experimental area of the IFES-Campus Itapina, identified through a stereoscopic microscope, and placed on dwarf coconut seedlings (*Cocos nucifera* L. Var. Nana), planted in 26-liter pots, so that multiplication of *R. indica* occurred, according to the methodology of Pinheiros & Vasconcelos [8].
Maintaining the rearing of the red mite on palm trees consisted of caring for the coconut seedlings, paying attention to regular irrigation, and exposure to the sun. When the seedlings reached a high degree of infestation, seedlings without *R. Indica* infestation were placed in contact with the infested seedlings so that the migration of *R. indica* individuals to these new seedlings could occur, thus completing the cycle and continuing the creation performed in the laboratory.

Obtaining the solutions

Soursop peel was obtained from the fruit pulp processing sector of the IFES-Campus Itapina. These residual peels were cleaned in the Agricultural Entomology and Acarology laboratory at IFES-Campus Itapina with a 5% sodium hypochlorite solution. After cleaning, the soursop peels underwent a drying process in an oven at a temperature of 50 ºC for 7 days, following the methodology by Paz, *et al*. [9]. Then, this material was ground in a Willey knife mill, obtaining a powder. The crushed material was stored in Becker-type glassware sealed with plastic film and aluminum foil to avoid contact with the external area and with the intensity of light photons.

Bioassays

To carry out the bioassays, 12 adult females of *R. indica* were removed from mass rearing developed in dwarf coconut seedlings, as described above, and transferred to Petri dishes (14.0 x 1.5 cm) with palm tree leaves (4 cm in diameter) placed under moistened cotton to maintain leaf turgor, as well as around these discs to prevent the mites from escaping. The Petri dishes were kept in B.O.D type climatized chambers (Bio-Oxygen Demand) at a temperature of 25±1 ºC, Relative Humidity of 70±10%, and Photophase of 12 h.

Direct Application Test

Initially, a 15% soursop peel extract solution was applied to observe the mortality of *R. indica*. Having observed mortality of 95% of the mite individuals, a logarithmic scale interval was schematized, obtaining concentrations of 1.0 1.72, 2.95, 5.07, 8.73, and 15.0% by arithmetic progression, following the model suggested by Carvalho, *et al*. [10].

For the dilution of each concentration of the soursop peels extracts, the amount corresponding to the percentage found on the logarithmic scale in grams of the fine powder of the aforementioned extract was transferred to an Erlenmeyer flask (100 mL) containing distilled water, 0.5 mL of alcohol as a solubilizer and 1 mL of Tween® 80 adhesive spreader (0.05% v/v) in quantities previously calculated to correspond to the final concentration of the extract solution. Subsequently, the concentrations were maintained under homogenization in a transverse shaker (240 rpm) for a period of 24 hours. Each concentration was applied to adult females of *R. indica*, obtained from mass rearing, as previously described. Each experimental unit consisted of a Petri dish, with palm leaf discs, as described above, and each treatment consisted of 7 Petri dishes (10.0 x 1.2 cm) as repetition and with 12 adult mite individuals.

Spraying was performed using an Alfa 2 airbrush, connected to a calibrated compressor with a constant pressure of 1.3 psi and 1 mL of a solution of each concentration for each plate. Distilled water, alcohol, and the adhesive spreader Tween® 80 (0.05% v/v) were used as a control treatment. The experimental units were kept in acclimatized chambers at a temperature of 25 ± 1 ºC, Relative Humidity of 70 ± 10%, and Photophase of 12 h. The acaricidal effect was evaluated 24, 48, and 72 hours after spraying, and at the end of this period, the cumulative total number of dead *R. indica* individuals was counted.

Statistical analysis and obtaining the LC_{50} and LC_{90}

The design used was completely randomized. *R. indica* mortality data were corrected using the Abbott formula [11], and subsequently submitted to Probit analysis ($p \leq 0.05$), using the R statistical program. From the equations obtained, the LC_{50} and LC_{90} were calculated for the extract applied to the red spider mite of palm trees.

Physical-chemical characterization of the compounds present in the extracts

Content of total phenolic compounds

The quantification of total phenolic compounds followed the methodology described by Swain and Hills [12], with adaptations. The crude extract was diluted in distilled water (1 mg/mL). In test tubes, 0.5 mL of diluted extract, 8 mL of distilled water, and 0.5 mL of Folin-Ciocalteau reagent were added. The solution was vortexed, and after 3 min 1 mL of 7.5% sodium carbonate solution was added, and the mixture was vortexed again. After 1 hour of rest in the dark, absorbance readings were taken in triplicate in a spectrophotometer at 750 nm. Gallic acid was used as a standard to construct a calibration curve. From the equation of the straight line obtained, the calculation of the content of total phenolic compounds was performed, expressed in mg EAG/g extract (milligrams of gallic acid equivalent per gram of extract).

Total tannin content

Total tannins were quantified according to Pansera [13]. The crude extract was diluted in distilled water (1 mg/mL). In a test tube, 1 mL of the diluted extract and 1 mL of the Folin-Denis reagent were added. The solution was homogenized and, after 3 minutes, 1 mL of 7.5% sodium carbonate solution was added and the mixture was vortexed. After 1 hour of rest in the dark, the reaction tubes were centrifuged at 2000 RPM for 5 min. The supernatant was subjected to absorbance reading in a spectrophotometer at 750 nm. Gallic acid was used as a standard to construct a calibration curve. From the equation of the straight line obtained, the calculation of the total tannin content expressed in mg EAG/g extract (milligrams of gallic acid equivalent per gram of extract) was carried out.

Total flavonoid content

The total flavonoid content was determined by the colorimetric method with aluminum chloride (AlCl3), performed according to Perdigão [14] with modifications. A 2 mL aliquot of the extract was diluted in distilled water (1 mg/mL) and transferred to a 25 mL volumetric flask. Then, 0.6 mL of glacial acetic acid, 10 mL of pyridine and water solution (1:4, v/v), and 2.5 mL of aluminum chloride solution 7.5% (w/v) were added, completing the volume to 25 mL with distilled water. After 30 min, the samples were read at 420 nm in the spectrophotometer. A blank was also prepared using all of the above reagents except the aluminum chloride sample. The quercetin concentrations used to establish the standard curve were 5, 10, 20, 30, and 40 mg/mL. All readings were performed in triplicate. The result was expressed in mg EQ/g extract (milligrams of quercetin equivalent per gram of extract).

Results

The toxicity test indicated that as there was an increase in the concentration of the soursop peel extract, there was an increase in the mortality of adult individuals of *R. indica* (Figure 2). It was observed that the maximum concentration used (15%) resulted in 80% mortality of *R. indica* individuals (Figure 1).

The data fit the Probit model $P = \phi\ (4.701+1.044.\ Log(x))$ (X^2=16.03; R^2=0.9970, p>0.05). The slope of the concentration-mortality curve was 1.0446% for soursop peel extract, the slope of the concentration-mortality curve measures the variability of the mite population in response to the application of the extracts. Thus, high values for the slope of concentration-mortality curves indicate greater efficiency of the extract effect with increasing doses. The extracts provided an LC50 of 1.934% ranging from 1.062% to 2.801% (g/mL) (confidence interval) for the soursop peel extract, (Table 1).

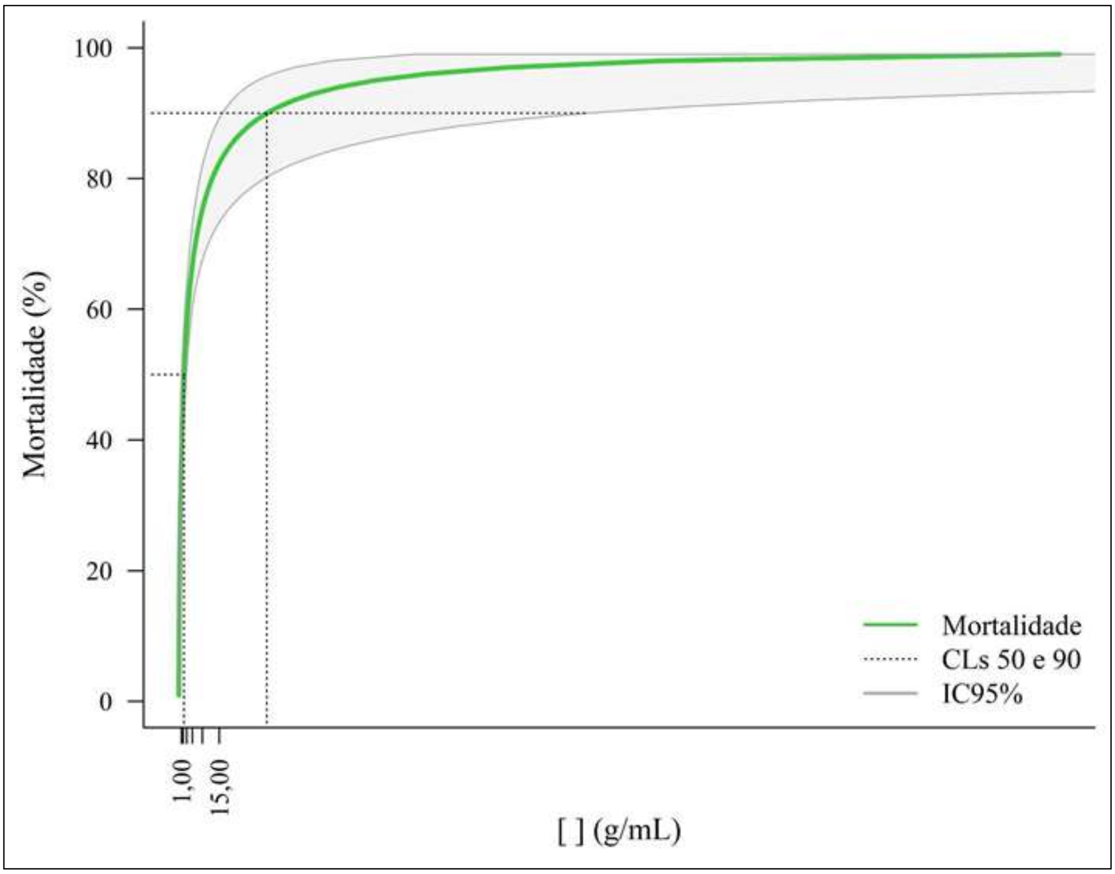

Fig 2: *Raoiella indica* mortality percentage in different concentrations of *Annona muricata* peel extract at 25±1 ºC, 70±10% RH, and 12 h photophase.

Table 1: Mortality curve and respective LC_{50} and LC_{90} of the aqueous extract of *Annona muricata* peel on *Raoiella indica* (Temp.: 25 ± 1° C, RH 70 ±10% and 12 h of phosphatase).

Extract	N	Slope (±EP)	LC_{50} (IC_{95}) mg. L^{-1}	LC_{90} (IC_{95}) mg. L^{-1}	X^2	GL	p-Value
Soursop Peel	504	1,0446 (0,299)	1,934 (1,062; 2,801)	32,60 (16,17; 151,40)	16,03	4	0,9970

N: Number of insects used; CI: Confidence interval; X^2: Chi-square; GL: Degrees of freedom

The toxicity presented by the soursop peel extract on adult females of the red palm mite *R. indica* may be associated with the secondary chemical components present in the solution, which were identified from the chemical characterization (Table 02).

Table 2: Average concentration of secondary compounds present in the aqueous extract of *Annona muricata* peel.

Extract	Total phenolics (mg/g) (±SD)	Total tannins (mg/g) (± SD)	Total flavonoids (mg/g) (± SD)
Soursop Peel	58,408 (0,996)	18,715 (3,935)	0,00141 (0,00006)

SD: Standard deviation

Discussion

Secondary plant compounds, which are concentrated in different plant structures, have phytochemical importance, producing plant compounds rich in metabolites from these plant structures, which act on the biology and behavior of mites and insects [15].

According to the results obtained through chemical analyses, a significant presence of phenolic compounds was observed in the soursop peel extract (58.4 mg/g of extract), in addition to tannins at a concentration of 18.715 mg/g of extract. While for total flavonoids, the values were found in low concentrations of 0.00805 mg/g of the extract (Table 2). The greater or lesser concentration of these compounds in plants is generally determined by exogenous factors such as light, precipitation, place of cultivation, spacing, and sun, as well as by endogenous factors such as plant age and genetic variability in populations [8].

Phenolic compounds cause physiological disturbances in the growth of insects and mites and their development processes [16]. Carvalho *et al.*, [17] evaluating the larvicidal and pupicid effect of substances extracted from *Anacardium occidentale* against the mosquito *Aedes aegypti* (Diptera: Culicidae), observed that the compounds extracted and tested, mainly the phenolic compounds, contributed to higher mortality of individuals compared to the witnesses.

Tannins, for example, a secondary compound obtained in this work, have anti-food and food-dissuading properties. This compound can reduce the efficiency of digestion in phytophagous arthropods through its chemical bonds [18]. Djilali *et al.*, [19] evaluated the insecticidal effect of tannins extracted from *Cydonia oblonga* against *Tribuliumm confusum* (Coleoptera: Tenebrionidae) and observed dose-dependence of the concentrations studied, where with the increase in the concentration of tannins, there was an increase in insect mortality.

Flavonoid compounds act by inhibiting the metabolism of insects and mites by altering enzymatic and hormonal activity, blocking biochemical pathways, and consequently reducing the assimilation of essential substances and nutrient storage [20]. Studies carried out by Kariyat *et al*., [21] found significant mortality and deleterious effects on corn and sorghum aphids treated with an artificial diet supplemented with the flavonoid 3-Deoxyanthocyanidin obtained from sorghum plants, confirming the hypothesis that these compounds may be acting in the mortality rate of individuals of *R. indica*.

In addition to the aforementioned compounds, according to Ezemuoka *et al*. [22] leaves and stem peel of *A. muricata*, have the presence of substances from groups, such as saponins, alkaloids, and triterpenes, which would be causing mortality of larval individuals of *Aedes aegypti* (Diptera: Culicidae). Likewise, Maciel *et al*. [23], when using *A. muricata* extracts to assess the mortality of *Tetranychus urticae* (Acari: Tetranychidae), found the presence of acetogenins, these substances act as mitochondrial electron transport inhibitors and thus affect the survival of the mites [24]. Amakiri *et al*. [25], using leaf and stem peel extracts of *A. muricata*, found insecticidal activity in *Anopheles gambiae* larvae (Diptera: Culicidae) due to the presence of phenolic compounds, tannins, saponins, and alkaloids. These compounds mentioned above by several authors could, therefore, together with the other metabolites found in the chemical analyses of the present research, be acting, in a toxic way, on adults of *R. indica*.

Furthermore, the form of application of the soursop peel extract on *R. indica* may be favoring the intoxication of the mites. By spraying the solution on the mites, as well as on the arena, which is their food, it may be having action by contact and by ingestion simultaneously acting on such individuals. Alimohamadian *et al*., [26] tested the insecticidal potential of silica nanoparticles against *Spodoptera exigua* Hubner (Lep: Noctuidae) on 3 application forms. The authors observed that the direct spray method on the individuals and their food caused higher mortality rates than the immersion of the leaves with the solution and spraying only the insects, which were later transferred to the test plate.

It was observed that over time after spraying, a thick film formed on the organisms. This film composed of pectin is crucial for the texture of fruits and vegetables in general, during their growth, ripening, storage, and processing [27] and, according to the observed results, they help in the stoppage and mortality of the mite species in the study.

Even the individuals of *R. indica* that, theoretically, continued to feed and move, did not lay postures during the evaluation period, indicating that the secondary compounds, even though they did not cause the individual's mortality, negatively interfered with reproduction, as well as in posture.

Conclusion

Extracts from the peel of *A. Muricata* were efficient, at the laboratory level, in the mortality of the red palm mite *R. indica*.

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