Scientific Research Publishing

Agricultural Sciences, 2023, 14, 541-552
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

# Palm Red Mite Management with Soursop Seed Plant Residue Extracts

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**How to cite this paper:** Piffer, A.B.M., Bolsoni, E.Z., Fornaciari, I.M., Holtz, A.M., Marchiori, J.J.P., De Carvalho, J.R., Aguiar, R.L., Fontes, P.S.F. and Magnani, B.O. (2023) Palm Red Mite Management with Soursop Seed Plant Residue Extracts. *Agricultural Sciences*, **14**, 541-552.
https://doi.org/10.4236/as.2023.144036

**Received:** March 15, 2023
**Accepted:** April 22, 2023
**Published:** April 25, 2023



## Abstract

*Raoiella indica* Hirst, 1924 (Prostigmata: Tenuipalpidae), is one of the leading pest mites in palm and banana trees, however, there are few control methods available for this pest species. Therefore, this work aimed to evaluate the acaricidal effect of soursop seed extract (*Annona muricata* L.) on *R. indica* adults. The experiment was conducted in a completely randomized design using soursop seed extract with 7 replicates and 12 individuals of *R. indica* per replicate. 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 × 1.2 cm) and around the disc to maintain turgor and prevent mites from escaping. The application was performed using an airbrush, connected to a calibrated compressor with a constant pressure of 1.3 psi and 1 mL of solution per repeat plate. The acaricidal effect was evaluated 24, 48, and 72 hours after spraying. Mortality data were corrected and submitted to Probit analysis ($p \leq 0.05$) using the statistical program R, with the $LC_{50}$ and $LC_{90}$ calculated for the extract. At the maximum concentration (15%), the soursop seed extract showed mortality of 70% of individuals of *R. indica*, and the $LC_{50}$ was 6.58%. It was concluded that the soursop seed extract showed acaricidal potential on *R. indica* in the laboratory.

## Keywords

*Annona muricata*, *Raoiella indica*, Alternative Control, Agro-Industrial Residue

## 1. Introduction

The species *Raoiella indica* Hirst, 1924 (Prostigmata: Tenuipalpidae), known as the red palm mite, is a pest mite that lives in plants of the Arecaceae and Musaceae family, especially in palm trees and banana trees [1]. The first record of this species was in India, in coconut trees (*Cocos nucifera* L.) and later it was found in the northeast and south of Africa and the Middle East. In these cited regions, the main host plants were coconut and date palm. Although quarantine measures were taken, these organisms quickly dispersed in the Central American regions, arriving in Brazil in 2009 through the state of Roraima, and dispersing through the other Brazilian states [2]. In 2018, the pest was reported in the coastal region of Espírito Santo on pygmy coconut and date palms (*Phoenix roebelenii* O'Brien) in the municipalities of Guarapari, Vila Velha, and Vitória [3].

*R. indica* is considered extremely invasive and of a severe attack. This species of mite has the habit of feeding on the abaxial face of the leaf, through the host's stomata, and may therefore interfere with the photosynthetic and respiratory processes of the plant, by leaving the leaf, at first with a tanning appearance, and subsequently leading to plant tissue necrosis and, in extreme cases, to the death of young plants, in addition to significantly reducing the productivity of adult plants [2].

The treatment strategy for the red mite of palm trees in other countries is largely represented by the use of synthetic agrochemicals and a few biological products. Despite being widely spread around the world, this mite is still considered new in several Brazilian regions. The official control methods registered in Brazil for this mite are the use of biological control with the predatory mite *Neoseiulus barkeri* (Acari: Phytoseiidae), and synthetic chemical control using the products Vertimec 18 EC and Abamectin 72 EC Nortox, both products registered only for coconut cultivation [4]. Despite this context, many producers make improper use of 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 in population resistant to numerous active ingredients in pesticides.

However, other alternatives arise to enable the control of this pest species, so that they do not cause damage to the spheres of the environment and health. 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 seeds, emerge as a possible alternative for controlling pest individuals through secondary metabolites of plants.

This is because the residual plant parts discarded in industrial processes may still contain secondary metabolites whose main function is to protect the plant against herbivores [5]. Despite the name, substances derived from "secondary" pathways are vital for plants, acting as attractants or repellents of pollinators, deterrents to herbivory, protection against UV radiation and pollution, water stress, intraspecific signaling, and allelopathy, among others. functions [6].

Among many examples of species of fruit plants that are used in the agro-industrial sector and that generate residues with insecticidal/acaricidal characteristics [7] [8], there are the plants of the Annonaceae family [9]. 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 annonaceous acetogenins among its main active components. In addition to containing a varied source of phenolic compounds, essential oils, alkaloids, flavonol triglycosides, megastigmans, and various minerals, including Mg, Fe, Cu, K, and Ca [10].

Thus, this study aimed to evaluate the acaricidal effect of the extract obtained from the seeds of *A. muricata* on adults of *R. indica*.

## 2. Methodology

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 (**Figure 1**).

### 2.1. 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,

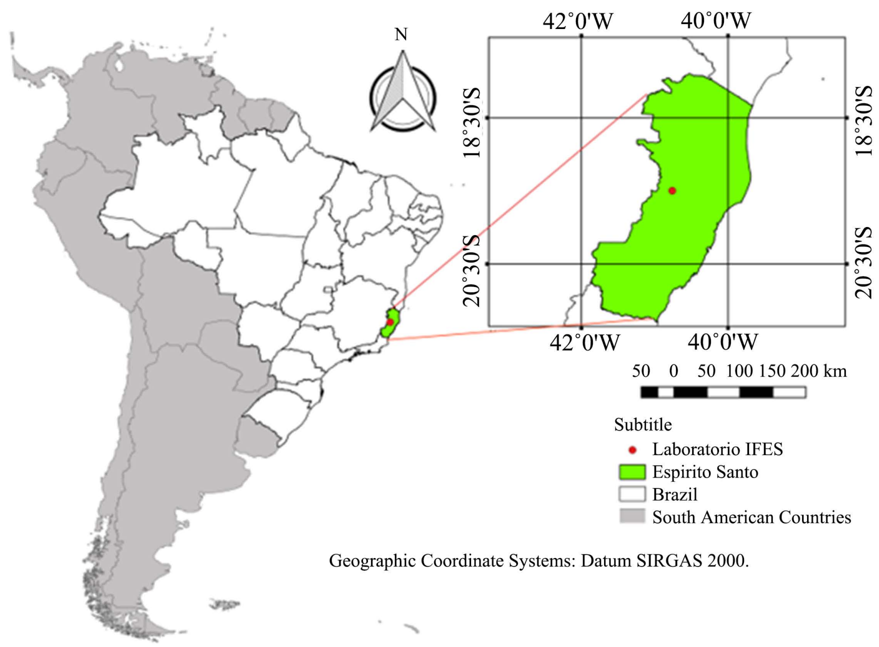


**Figure 1.** Map of the geographical position of agricultural entomology and acarology laboratory of the federal institute of education, science, and technology of Espírito Santo-Campus Itapina (IFES-Campus Itapina).

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 [11].

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.

### 2.2. Obtaining the Solutions

Soursop seeds were obtained from the fruit pulp processing sector of the IFES-Campus Itapina. These residual seeds were cleaned in the Agricultural Entomology and Acarology laboratory at IFES-Campus Itapina with a 5% sodium hypochlorite solution. After cleaning, the soursop seeds underwent a drying process in an oven at a temperature of 50˚C for 7 days, following the methodology by Paz *et al.* [12]. 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.

### 2.3. Bioassays

First, confirm that you have the correct template for your paper size. 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 × 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˚C ± 1˚C, Relative Humidity of 70% ± 10%, and Photophase of 12 h.

### 2.4. Direct Application Test

Initially, a 15% soursop seed 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%, 1.72%, 2.95%, 5.07%, 8.73%, and 15.00% by arithmetic progression, following the model suggested by Carvalho *et al.* [13].

For the dilution of each concentration of the soursop seed extract, 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 × 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˚C ± 1˚C, 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.

## 2.5. 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 [14], 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.

## 2.6. Physical-Chemical Characterization of the Compounds Present in the Extracts

### 2.6.1. Content of Total Phenolic Compounds

The quantification of total phenolic compounds followed the methodology described by Swain and Hills [15], 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).

### 2.6.2. Total Tannin Content

Total tannins were quantified according to Pansera [16]. 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.

#### 2.6.3. Total Flavonoid Content

The total flavonoid content was determined by the colorimetric method with aluminum chloride ($AlCl_3$), performed according to Perdigão [17] 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).

## 3. Results and Discussion

The toxicity test indicated that as there was an increase in the concentration of soursop seed extract, there was an increase in the mortality of adult individuals of *R. indica*. It was observed that at the maximum concentration used for the soursop seed extract (15%), a mortality rate of 70% was obtained for the individuals of the red mite of palm trees (**Figure 2**). The data fit the Probit model P = (3.828 + 1.431 × log10($x$)) ($\chi^2$ = 10.85; $R^2$ = 0.9718, p > 0.05) and the slope of the concentration-mortality curve was 1.431% (**Table 1**). The slope of the concentration-mortality curve measures the variability of the mite population in response to the application of the extract. Thus, high values for the slope of the concentration-mortality curve indicate greater efficiency of the extract effect with increasing doses. The extract provided an estimated $LC_{50}$ of 6.58%, ranging from 5.01% to 9.41% (g/mL) (Confidence Interval) (**Table 1**).

**Table 1.** Concentration-mortality curve and respective $LC_{50}$ and $LC_{90}$ of the aqueous extract of *Annona muricata* seeds on *Raoiella indica* (Temp.: 25˚C ± 1˚C, RH 70% ± 10%, and 12 h of Photophase).

| Extract | N | Slope (±EP) | $LC_{50}$ ($IC_{95}$) mg·L$^{-1}$ | $LC_{90}$ ($IC_{95}$) mg·L$^{-1}$ | $\chi^2$ | GL | p-Value |
|---|---|---|---|---|---|---|---|
| Soursop Seed | 504 | 1.431 (0.259) | 6.58 (5.01; 9.41) | 51.78 (27.70; 161.25) | 10.85 | 4 | 0.9718 |

N: Number of insects used; IC: Confidence interval; $\chi^2$: Chi-square; GL: Degree of freedom.

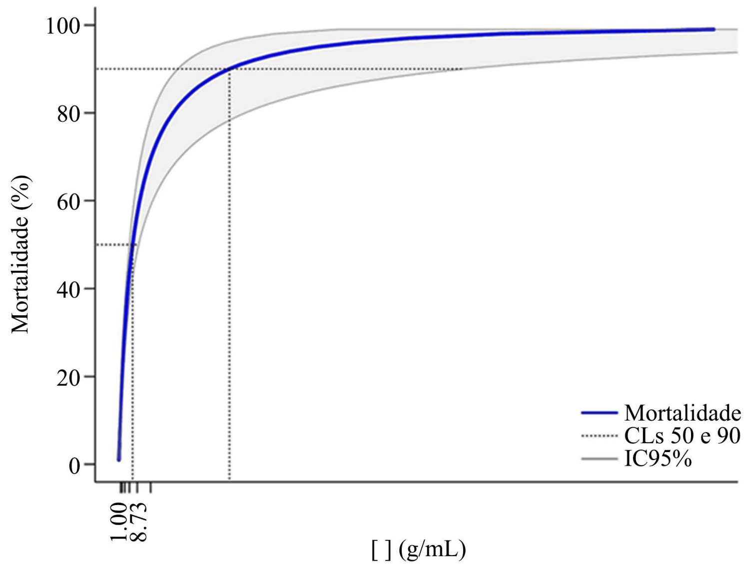


**Figure 2.** Percentage of *Raoiella indica* mortality in different concentrations of *Annona muricata* seed extract. Temp.: 25˚C ± 1˚C, RH 70% ± 10%, and 12 h of photophase.

The toxicity presented by the soursop seed extract in adult females of the red mite *R. indica* may be associated with the secondary chemical components present in the solution, which were identified from the chemical characterization (**Table 2**).

The secondary compounds of plants, which are concentrated in different plant structures, such as leaves, bark, stems, roots, flowers, and seeds [18], have phytochemical importance, producing essential oils, crude oils, etc. resins or plant compounds that act by altering the biology and physiological behavior of mites and insects [19].

According to the results obtained in the chemical analyses, the most significant presence of phenolic compounds was observed in the seed extract at a concentration of 7.90 mg/g of the extract, followed by the presence of tannins at a concentration of 18.078 mg/g. While for total flavonoids, the values were in insignificant amounts (**Table 2**). Most of the plant's defense mechanisms are concentrated in the seeds, given their great importance as a vehicle for the propagation and survival of the species, which is why there is probably the highest concentration of total phenolics in the soursop seed extract [20]. The higher or lower concentration of these compounds 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 [11].

Of the mentioned compounds, phenolic compounds can act as a toxin for herbivores, after being oxidized; they become toxic peroxidases or polyphenolic metabolites that cause physiological disturbances in the growth of insects and mites and their development processes [21]. As an example of phenolic compounds, tannins play important roles in pest organisms, such as anti-food and

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

| Extract | Total phenolics (mg/g) (±SD) | Total tannins (mg/g) (±SD) | Total flavonoids (mg/g) (±SD) |
|---|---|---|---|
| Soursop Seed | 7.907 (3.485) | 18.078 (0.362) | 0.00805 (0.00022) |

SD: Standard Deviation.

food deterrent properties, as they reduce the efficiency of digestion in phytophagous arthropods through their chemical bonds [22]. Mostafa *et al.* [23] studying the allelopathic effect of *Alhagi maurorum* extract as a phytocaricide against the mite *Panonychus citri* (Acari: Tetranychidae), found an increase in the mortality rate of individuals due to the presence of tannin compounds.

Flavonoid compounds, although observed in smaller amounts in this study, act on 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 Stec *et al.* [24], using ethanolic solutions of the flavonoids apigenin, daidzein, genistein, and kaempferol against the soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae), found changes in the behavior of the studied aphids. An increase in the period of duration of derailment of stylet mechanics and in the ingestion of xylem sap and limitation in the ingestion of phloem sap of plants by aphids was observed.

Thus, the biological mechanisms of the red palm mite will be suppressed by the secondary compounds present in the seeds of *A. muricata*, acting from the interruption of the assimilation of nutrients, affecting the digestive system, and blocking important biochemical pathways for the survival of the mite.

Another factor that may be related to the mortality of individuals of *R. indica* was the form of application of the extracts because when spraying the solution on the adult individuals of the mite, as well as on the arena, which is its food, the action by contact and by ingestion acted simultaneously on such individuals, which may have favored the mortality of *R. indica*. In this sense, Neto *et al.* [25] studied the efficiency of different methods of application of insecticides on aphids and found significant efficiency of the direct spray form on the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). The authors observed that the form of direct spraying on the organisms exceeded the estimated lethal concentration of 90% considered for the study, attributing that this method resulted in additional intoxication in aphids due to both ingestion and contact with the insecticide.

In addition, it was observed over time after spraying, the formation of a thick film characteristic of pectin, a substance that makes up soursop seeds [26], on the mites, which prevented their movement along the leaf blade. This layer of pectin also prevented the mites from feeding and could be affecting their detoxifying capacity. Detoxification can be overcome when certain genes of their physiological system act producing specific coding enzymes that promote the ability to overcome and acquire resistance to toxic substances present in plants [27] [28]. Some of these affected enzymes are acetylcholinesterase, *α*- and *β*-carboxylesterase, and glutathione S-transferase. Parthiban, *et al.* [29] analyzing the alterations caused by the soursop seed extract in the biochemical components of proteins and two groups of biomarker enzymes (esterase and phosphatase) of the mosquito *Aedes aegypti* (Diptera: Culicidae), found a significant reduction in the action of the acetylcholinesterase enzyme and high susceptibility of the *α*- and *β*-carboxylesterase enzymes to total inhibition of their functionality. With the inactivation of these enzymes, the tested organisms had recorded mortality of up to 100% of their population. Thus, the xenobiotic response of soursop with the pellicle formed by pectin probably promotes the stoppage and mortality of the mite species under 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 in the biological cycle of the mite, such as laying eggs.

## 4. Conclusion

*A. muricata* seed extracts showed significant mortality rates on *R. indica* the mediate that increased the extract concentration. The $LC_{50}$ was established at 6.58%, thus it was concluded that *A. muricata* seed extracts demonstrate acaricidal potential, at the laboratory level, in the mortality of the red mite *R. indica*. Significant amounts of phenolic compounds and tannins were found in the *A. muricata* extract. Semi-field and field tests must be carried out to prove the acaricidal effectiveness/efficiency of soursop seed extracts on *R. indica*.

## Acknowledgements

This project was made possible by the Federal Institute of Espírito Santo, the Research Support Foundation of the State of Espírito Santo, and the National Council for Scientific and Technological Development.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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