



ALTERNATIVE MANAGEMENT OF *RAOIELLA INDICA* HIRST, 1924 (ACARI: TENUIPALPIDAE) WITH PASSION FRUIT SEED EXTRACT

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ABSTRACT

Objective: The aim of this work was to evaluate the acaricidal potential of the passion fruit seed extract (*Passiflora edulis*) in controlling the red palm mite (*R. indica*).

Theoretical framework: *Raoiella indica* is a pest mite of agricultural interest, considered one of the main pests of banana, coconut, date palm, and ornamental crops.

Method: The experiment was conducted in a completely randomized design using passion fruit seed extract, with 7 repetitions and 12 *R. indica* individuals per repetition. The concentration tested was 1.0; 1.72; 2.95; 5.08; 8.73, and 15.0 %. The experimental units consisted of coconut leaf discs (4 cm in diameter) with moist cotton at the bottom of the Petri dish (10.0 x 1.2 cm) and around the disc to maintain turgidity and prevent the mites from escaping. The application was carried out using an airbrush, connected to a compressor calibrated with a constant pressure of 1.3 psi and 1 mL of solution per plate. The evaluation was conducted at intervals of 24, 48, and 72 hours following the extract's spraying.

Results and discussion: Mortality was observed in the dose-response model, the concentration of 15 % showed mortality rates greater than 98.8 % of individuals. Amounts of phenolics of 10.242, tannins of 14.837, and flavonoids of 9.700 in mg g⁻¹ were found, attributing the mortality of individuals to the presence of these compounds. It was concluded that the passion fruit seed extract has an acaricidal effect on *R. indica* in the laboratory.

Implications of the research: Alternative pest management has grown substantially in recent years, given the demand for more sustainable products in the agricultural field. The use of botanical extracts is beneficial to the

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environment, as it does not promote soil and water contamination and can be an efficient method for controlling agricultural pests.

Originality/value: This article adds value to research areas such as alternative management of agricultural pests, and sustainability and suggests a change in the environmentally conscious behavior of rural producers.

Keywords: Red Palm Mite, Agro-Industrial Residues, Alternative Management, *Passiflora Edulis*.

MANEJO ALTERNATIVO DE RAOIELLA INDICA HIRST, 1924 (ACARI: TENUIPALPIDAE) COM EXTRATO DE SEMENTE DE MARACUJÁ

RESUMO

Objetivo: o objetivo deste trabalho foi avaliar o potencial acaricida do extrato de semente de maracujá (*Passiflora edulis*) no controle do ácaro vermelho da palmeira (*R. indica*).

Referencial teórico: *Raoiella indica* é um ácaro praga de interesse agrícola, considerado uma das principais pragas da bananeira, do coqueiro, da tamareira e de culturas ornamentais.

Método: O experimento foi conduzido em delineamento inteiramente casualizado com extrato de semente de maracujá, com 7 repetições e 12 indivíduos de *R. indica* por repetição. A concentração testada foi 1,0; 1,72; 2,95; 5,08; 8,73 e 15,0%. As unidades experimentais foram constituídas por discos de folhas de coqueiro (4 cm de diâmetro) com algodão umedecido no fundo da placa de Petri (10,0 x 1,2 cm) e ao redor do disco para manter a turgidez e evitar a fuga dos ácaros. A aplicação foi realizada com auxílio de aerógrafo, conectado a um compressor calibrado com pressão constante de 1,3 psi e 1 mL de solução por placa. A avaliação foi realizada em intervalos de 24, 48 e 72 horas após a pulverização do extrato.

Resultados e discussão: A mortalidade foi observada no modelo dose-resposta, a concentração de 15 % apresentou taxas de mortalidade superiores a 98,8 % dos indivíduos. Foram encontradas quantidades de fenólicos de 10.242, taninos de 14.837 e flavonoides de 9.700 em mg g⁻¹, atribuindo a mortalidade dos indivíduos à presença desses compostos. Concluiu-se que o extrato de semente de maracujá apresenta efeito acaricida sobre *R. indica* em laboratório.

Implicações da pesquisa: O manejo alternativo de pragas cresceu substancialmente nos últimos anos dada a demanda por produtos mais sustentáveis na área agrícola. A utilização de extratos botânicos é benéfica ao meio ambiente, pois não promove contaminação do solo e da água e pode ser um método eficiente para controle de pragas agrícolas.

Originalidade/valor: Este artigo agrega valor a áreas de pesquisa como manejo alternativo de pragas agrícolas e sustentabilidade e sugere uma mudança no comportamento ambientalmente consciente dos produtores rurais.

Palavras-chave: Ácaro Vermelho das Palmeiras, Resíduos Agroindustriais, Manejo Alternativo, *Passiflora Edulis*.

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1 INTRODUCTION

The red palm mite, *Raoiella indica* Hirst, 1924 (Prostigmata: Tenuipalpidae) is a pest known to be present in liliopsidas of the Arecaceae family, among other families such as, for example, Musaceae, Heliconiaceae, Strelitziaceae (Pinheiro; Vasconcelos, 2020). This species is reported to attacking mainly coconut plantations, (*Cocos nucifera* L. (Arecaceae)), Data palm (*Phoenix dactylifera* L. (Arecaceae)), and other palm trees in Asia, the Middle East, Africa, and



the Caribbean (Mendonça, Navia, Flechtmann, 2005; Barroso *et al.*, 2019). This pest species was reported in Brazil for the first time in 2009, in the state of Roraima, staying in coconut and banana trees, and in the state of Espírito Santo it was reported in 2018 in the municipalities of Vitória, Vila Velha, and Guarapari in seedlings of ornamental coconut and palm trees (Navia *et al.*, 2011; Marsaro Júnior *et al.*, 2018).

This mite species can promote severe attacks with significant damage at high population levels, mainly due to its polyphagous habit, generally feeding on the abaxial surface of the leaf, through the host's stomata (Ochoa *et al.*, 2011). This specialized feeding habit interferes with the photosynthesis and respiration processes of the host, leaving the leaf, at first, with a tanning appearance, and later, with necrosis of the plant tissues (Kane; Ochoa, 2006; Carrillo *et al.*, 2012; Filgueiras *et al.*, 2020).

The control of the red mite on palm trees in other countries is largely represented by synthetic chemical control of individuals. In Brazil, the synthetic chemical products registered to control this pest are the commercial VERTIMEC® 18 EC and ABAMECTIN 72 EC NORTOX, and the predatory mite *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) is officially registered as biological control (Agrofit, 2023).

The adverse effects caused by the use of conventional synthetic chemical insecticides are cited by Mallmann *et al.* (2021) such as, for example, the development of resistance by pests and the negative environmental impact on fauna and flora, and emphasizes the importance of implementing the use of botanical insecticides as a control alternative, in order to cause less disturbance to agricultural ecosystems (Pereira *et al.*, 2023).

In this context, the use of acaricide plants and, mainly, by-products from agro-industrial residues, such as bark and seeds, emerge as a possible alternative for controlling individual pests. Considering the possibility of extracting oils and seed extracts from these residues, thus contributing to the use of plant residues that would be unusable or discarded, as well as a new pest control methodology (Sathyaseelan *et al.*, 2020). An example of a fruit plant species that is used in the agro-industrial sector, which generates a large amount of waste and which may contain insecticide/acaricide characteristics, is the passion fruit.

The yellow passion fruit (*Passiflora edulis flavicarpa* Degener (Passifloraceae)) is a species native to tropical regions, produced mainly in South America, with the world's largest producers being Brazil, Colombia, and Ecuador (Monteiro *et al.*, 2020). During the processing of passion fruit pulp, more than 60 % of residues from the skin and seeds are discarded (Coelho, 2008). In this way, the passion fruit seed could be used as an alternative control against pest mites, as it contains substances such as linalool, which acts on the sensory activities of these organisms (Aguiar-Menezes, 2005; Oliveira *et al.*, 2012).

Thus, the present work aimed to test the efficiency of the use of passion fruit seed extracts in the control of the red mite of palm trees *R. indica*.

2 LITERATURE REVIEW

The red mite of palm trees (*Raoiella indica*) was described in 1924, and collected in coconut tree leaves in India. Was reported in the Americas for the first time in Martinique in 2004, and despite quarantine measures, spread out fast across the continent (Alcívar, Mesa, Vásquez, 2020; Santos *et al.*, 2020).

In most cases, the pest occurs in coconut and palm tree species (Melo *et al.*, 2018), however, it has been frequently associated with injuries in banana trees and many species from different families of ornamental plants in several countries (Sousa Neto *et al.*, 2021; Cruz *et al.*, 2022). Recently, it has been related to causing damage to açai trees in the Brazilian Northeast (Nuvoloni *et al.*, 2021).



Usually, the control of the mites in other countries is made using synthetic chemical products. However, due to being considered a new pest in Brazil, there are few official control methods, with only two chemical products and one biological, which, however, are registered only for the coconut crop (Agrolink, 2022).

In this context, many rural producers use unregistered products, which may not guarantee control efficiency, in addition to contaminating the environment (Souza *et al.*, 2023), interfering with populations of natural enemies, generating resistant populations, and making management more difficult. Thus, it's necessary to search for new effective methods that don't harm the environment (Holtz *et al.*, 2020).

The yellow passion fruit (*Passiflora edulis*) is a tropical plant widely used in the food industry, to make pulp, juices, candies, jellies, and other products. However, only the pulp is used, while the seeds are discarded, generating waste (Silva, Pessoa, Vega, 2021).

The passion fruit seeds have an oil tenor of approximately 25.7 % (Ramos *et al.*, 2019), therefore it's important to consider using the seed to extract oil, in order to reduce waste generation and find an alternative pest control method.

Seeds from fruits of the *Passiflora* genus, are capable of producing excellent oil quantity when subjected to pressing (24-26 %) (Winitchai, Thanapase, 2007), and are rich in phenolic and nitrogenous compounds, nonpolar compounds, and aromatic hydrocarbons, making the extract a potential bioinsecticide (Schroeder *et al.*, 2022) and antimicrobial (Reis *et al.*, 2023).

3 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 coordinates of 19°29'52.7" S 40°45'38.5" W (Figure 1).

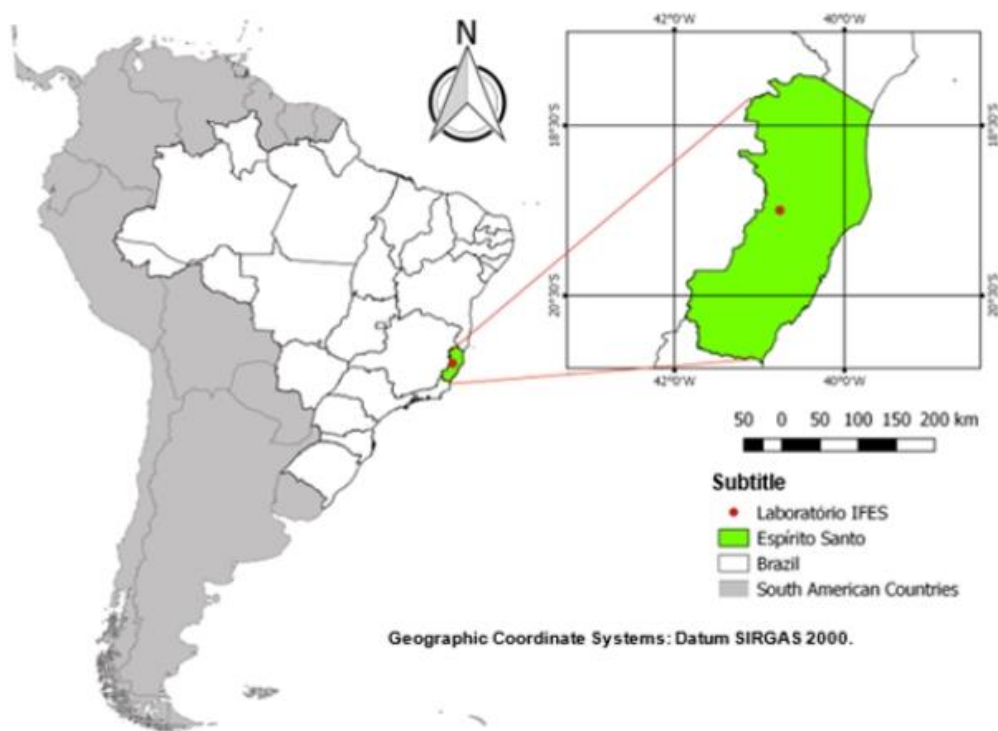


Figure 1 – Site where the experiments were carried out. Adapted from Piffer *et al.*, 2023.

Source: Authors.



3.1 Breeding and Maintenance of the Red Palm Mite *R. Indica*

Mites were maintained on dwarf coconut plants (*Cocos nucifera* L. Var. Nana) grown in 26 L plastic pots. For initial infestation, infested leaflets were placed in contact with coconut seedlings so that *R. indica* individuals migrated for these seedlings, following the methodology of Pinheiro and Vasconcelos (2020).

To carry out the bioassays, 12 adult females of *R. indica* were removed from the palm trees and transferred to Petri dishes (14.0 x 1.5 cm) containing previously sanitized palm leaves (Figure 2). The leaves were arranged under filter paper with cotton moistened around them to maintain leaf turgor and prevent the mites from escaping. The plates were kept in climate-controlled chambers ($25\pm1^\circ\text{C}$, $70\pm10\%$ RH, and 12 h photophase).



Figure 2 – Petri dish with mites collected from breeding for application of the extract.

Source: Authors.

3.2 Obtaining the Extracts

To make the passion fruit seed extracts, agro-industrial residues were collected from the fruit pulp sector of the IFES-Campus Itapina (Figure 1). The residues were sanitized in the laboratory and underwent a drying process in an oven with forced air circulation at 50°C for 7 days. These materials were then ground in a Willey knife mill, obtaining a fine powder. This was stored in Becker-type glassware sealed with plastic film and covered with aluminum foil to avoid contact with the external area and degradation of the material by light.

3.3 Direct Application Test

Initially, a solution of passion fruit seed powder obtained at a concentration of 15% was applied to observe the mortality of *R. indica* at 95 %. Once such individual mortality was



observed, a logarithmic scale interval was outlined, obtaining the concentrations by logarithmic progression, following the model suggested by Carvalho *et al.* (2017). The following concentrations were determined: 1.0, 1.72, 2.95, 5.08, 8.73, and 15.0 %.

For the dilution of each concentration and formulation of the different treatments of the extract, the predetermined amount, in grams, of the fine powder of passion fruit seeds was transferred to an Erlenmeyer flask containing 1 mL of alcohol (solubilizer), 1 mL of adhesive spreader Tween® 80 (0.05% v v⁻¹), completing the volume with distilled water until reaching 100 mL. Subsequently, the extract was kept under homogenization in a magnetic stirrer (240 rpm) for a period of 1 hour, taking care with sealing and sheltering from light.

Each concentration was applied to adult females of *R. indica*, obtained from breeding. Each treatment consisted of 7 petri dishes with 12 individuals in each, each individual being considered a repetition. Each experimental unit consisted of a Petri dish (10.0 x 1.2 cm) with palm leaf discs of about 4 cm in diameter, with moistened cotton around it to maintain turgor and prevent the escape of the mites.

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 solution of each formulation for each plate of each treatment. Distilled water, alcohol, and adhesive spreader Tween® 80 (0.05% v v⁻¹) was used as a control treatment. The experimental units were kept in air-conditioned chambers of the B.O.D. at a temperature of 25 ± 1° C, relative humidity of 70 ± 10%, and a 12 h photophase.

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. The inability of the mites to walk when lightly prodded with a small fine brush was considered a criterion for mortality.

The design used was completely randomized. For each solution, mortality data were corrected using the Abbott formula (1925) and subsequently submitted to Probit analysis (p≤0.05), using the statistical program R Studio. Based on the equations obtained, the LC50 and LC90 were calculated for each solution applied on *R. indica*.

3.4 Physicochemical Characterization of the Compounds in the Extracts

To characterize the phenolic compounds, the methodology described by Swain and Hills (1959) was used, with adaptations. Crude extracts were diluted in distilled water (1 mg mL⁻¹). In a test tube, 0.5 mL of the diluted extract, 8 mL of distilled water, and 0.5 mL of Folin-Ciocalteu 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, triplicate readings of absorbances were performed 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 content of total phenolic compounds was calculated, expressed in mg EAG/g extract (milligrams of gallic acid equivalent per gram of extract).

Total tannins were quantified according to Pansera *et al.* (2003). Crude extracts were diluted in distilled water (1 mg mL⁻¹). 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 min, 1 mL of 7.5% sodium carbonate solution was added and the mixture was vortexed. After 1 h of rest in the dark, the reaction tubes were centrifuged at 2000 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 total tannin content expressed in mg EAG/g extract (milligrams of gallic acid equivalent per gram of extract) was calculated.

The total content of flavonoids was determined by the colorimetric method with aluminum chloride (AlCl₃), performed according to Perdigão (2012), with modifications. A 2



mL aliquot of the extract was diluted in distilled water (1 mg mL^{-1}) 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⁻¹), and 2.5 mL of aluminum chloride solution 7.5% (w v⁻¹) 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 $\mu\text{g mL}^{-1}$. All readings were performed in triplicate. The result was expressed in mg EQ/g extract (milligrams of quercetin equivalent per gram of extract).

4 RESULTS AND DISCUSSION

The slope of the concentration-mortality curve obtained was 2.625 %, estimating an LC90 of 14.5 % (Table 1). The concentration of 15 %, the highest used in the experiment, showed a mortality of 98.8 % of individuals of *R. indica* (Figure 3). The data obtained fit the Probit model ($X^2 = 27.99$; $R^2 = 0.999$, $p > 0.05$). The adjusted model that best explained pest mite mortality as a function of different concentrations of passion fruit seed extract was $P = (3.229 + 2.625 \cdot \log(x))$ (Figure 3).

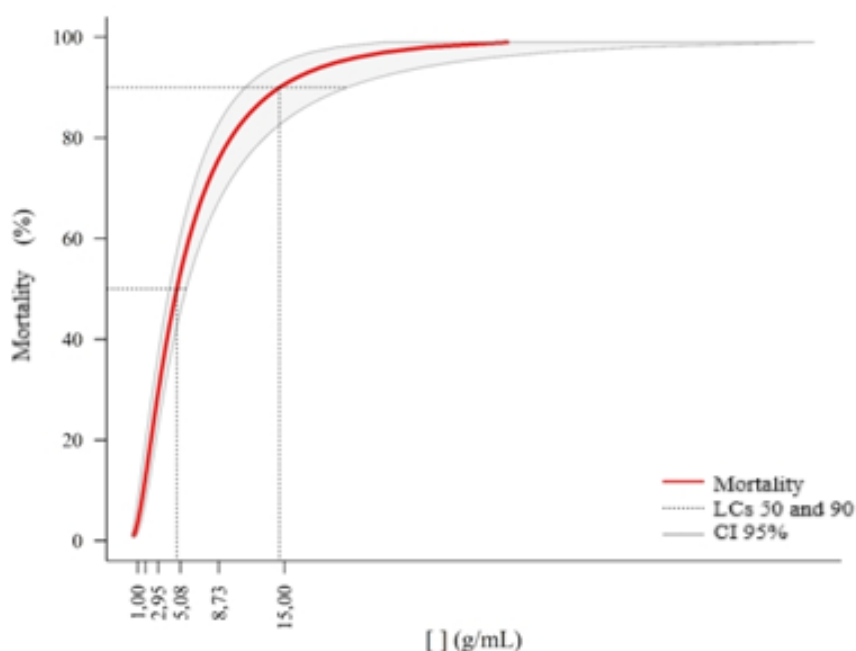


Figure 3 – Mortality of *R. indica* as a function of different concentrations of *Passiflora edulis* seed extract.

Source: Authors.

Table 1 – Concentration-mortality curve, LC50 and LC90 of the aqueous extract of *Passiflora edulis* seeds on *Raoiella indica*.

N	Slope ($\pm$ EP)	LC50 (CI95) g/100 mL (%)	LC90 (CI95) g/100mL (%)	X^2	Df	p-Value
504	2,62 (0,52)	4,72 (4,00; 5,60)	14,53 (11,25; 20,98)	27,99	4	0,99

N = number of individuals; CI = confidence interval; X^2 = chi-square; Df = degree of freedom.

Source: Authors.

The chemical components present in the solution, identified from the chemical characterization, are described in Table 02. Higher concentrations of tannin compounds were quantified, followed by other phenolic compounds and flavonoids.

**Table 2** – Quantitative of compounds present in the aqueous extract of *Passiflora edulis*.

Phenolics	Tannins	Flavonoids
mg/g		
10,242 ± 0,408	14,837 ± 0,212	9,700 ± 0,545

Source: Authors.

From the general observations of the study at the laboratory level, the aqueous extract of yellow passion fruit showed promise in the alternative management of *R. indica*. The high mortality rate obtained in the study may be related to the significant amounts of secondary metabolites present in the botanical extract under study.

Researchers such as Borges and Amorim (2020) claim that, due to their chemical diversity, compounds such as tannins, flavonoids, and some simple phenolic compounds serve as a defense against herbivores and pathogens. Therefore, several authors report the importance of these compounds as plant defenses against attack by phytophagous insects/mites.

According to Silvateam® (2021), tannins, in particular ellagitannins, are endogenous inhibitors of the growth of numerous pest species. They act as an antibiotic substance or as an anti-nutritional deterrent against herbivores. This function is correlated with its ability to complex proteins and decrease food digestibility. This causes negative effects on the endogenous metabolism of organisms.

In view of this, a study on the insecticidal effect of the phytochemical substances of the *Cydonia oblonga* fruit showed significant amounts of tannins and, it was noted that as the concentrations of tannin in the treatments increased, the mortality of the organism *Tribulium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) (Djilali *et al.*, 2021).

Another group characterized in the aqueous extract studied was the group of phenolic compounds. Phenols, which have a simple structure, penetrate microorganisms and cause considerable damage to cellular metabolism. Plant phenolics act as deterrents, repellents, and enzymatic inactivators of phytophages (Kumar *et al.*, 2014). Similarly, Guatam *et al.* (2021), noted that high concentrations of phenolic compounds were detrimental to the development and growth of the second larval instar of the polyphagous pest, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). Thus, it can be concluded that phenolic compounds are effective in controlling pest organisms.

In addition to the compounds characterized in this study, Vilhena, and Faria, through chemical and physical analysis of the extraction of passion fruit seed oil, reported the presence of some fatty acids such as linoleic and palmitic. These were also found in the endosperm of the oil palm *Elaeis guineenses* (Jaquim), and they showed determinant toxicity in the alternative control of the scale cochineal of the cactus pear *Diaspis echinocacti* (Hemiptera: Diaspididae) (Gonçalves *et al.*, 2020). Thus, together with the other compounds, these fatty acids may be contributing to the mortality of individuals.

Besides that, the vegetative stage of seed development may contribute to a smaller or larger amount of these secondary compounds. Holtz *et al.* (2020) observed when analyzing the acaricidal action of the seeds of *Moringa oleifera* Lam., that the different maturation stages influenced the reduction of oviposition and the mortality of the pest mite *Tetranychus urticae* (Acari: Tetranychidae), where the green seeds had greater toxicity acaricidal than mature ones.

Another hypothesis formulated about the mortality of individuals is related to the form of application of the extracts on the mites. The direct application of the extract on the mites and the leaf, which serves as food, may contribute to its mortality. Oliveira *et al.* (2022) studied the modes of action of different insecticides on the whitefly and observed significant mortality rates when the application was performed directly on the studied individuals.

In addition to the expressive mortality, egg laying by the remaining mites was not observed during the evaluation period, which could indicate a means of reducing the population. In studies, Couto *et al.* (2019) observed oviposition inhibition of individuals of *Plutella*



xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae) when using the botanical extract of two species *Annona coriacea* Mart. and *Schinus terebinthifolius*, this inhibition is attributed to the secondary metabolites of the extracts. The same could be happening in the present study, however, further studies should be carried out in order to better understand how the compounds present in the extract and how they act on *R. indica*.

5 CONCLUSION

The aqueous seed extract of *P. edulis* was effective in controlling the red spider mite of *R. indica* palms in laboratory tests. The aqueous extract of passion fruit seeds showed a significant number of tannins and phenolic compounds. Studies must be carried out in order to understand the action of these compounds on *R. indica*.

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