



ARE PAPAYA SEEDS AT DIFFERENT STAGES OF DEVELOPMENT EFFECTIVE IN CONTROLLING THE RED PALM MITE?

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ABSTRACT

Objective: To evaluate the acaricidal potential of papaya fruit seed (*C papaya L.*) extracts at different phenological stages of development on the red palm mite.

Methods: Extracts from green and ripe seeds, diluted at different concentrations, were sprayed on adult mites in a completely randomized design using Petri dishes with discs of dwarf coconut leaf. The acaricidal effect was assessed at intervals of 12 hours up to 72 hours after spraying.

Results and Conclusion: The mortality of *Roiella indica* was proportional to the increase in extract concentrations for both green and ripe papaya fruit seeds. It was concluded that aqueous extracts from green and ripe papaya fruit seeds exhibit acaricidal activity against adult red palm mites under laboratory conditions.

Research Implications: Papaya seeds at different stages effectively controlled red palm mites under laboratory conditions.

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Originality/Value: This research contributes by exploring the potential of an agricultural residue as a pest control agent in agriculture and demonstrating its potential for more sustainable agricultural practices.

Keywords: *Carica Papaya*, *Raoiella Indica*, Alternative Management, Botanical Extracts.

AS SEMENTES DE MAMÃO EM DIFERENTES ESTÁGIOS DE DESENVOLVIMENTO SÃO EFICAZES NO CONTROLE DO ÁCARO DE PALMA VERMELHO?

RESUMO

Objetivo: Avaliar o potencial acaricida de sementes de frutos de mamão (*Carica papaya* L.) em diferentes estádios fenológicos de desenvolvimento sobre o ácaro vermelho das palmeiras.

Métodos: Os extratos de sementes verde e maduro, diluído em diferentes concentrações, foram pulverizados sobre fêmeas adultas de ácaros em placa de Petri com discos de folha de coco anão em delineamento inteiramente casualizado. O efeito acaricida foi avaliado em intervalos de 12 horas até completar 72 horas após a pulverização.

Resultado e conclusão: A mortalidade de *Roiella indica* foi proporcional ao aumento da concentração dos extratos para sementes de frutos de mamão verdes e maduro. Concluiu-se que os extratos aquosos de sementes de frutos verdes e maduros de mamão (*C. papaya* L.) apresentam atividade acaricida sobre adultos do ácaro vermelho das palmeiras, em condições de laboratório.

Implicações da pesquisa: As sementes do mamão, em diferentes estádios, controlaram o ácaro vermelho das palmeiras a condições laboratoriais.

Originalidade/valor: Esta pesquisa contribui ao explorar o potencial de um resíduo agrícola como controle de pragas na agricultura e demonstrando seu potencial para as práticas agrícolas mais sustentáveis.

Palavras-chaves: *Carica Papaya*, *Raoiella Indica*, Manejo Alternativo, Extratos Botânicos.

¿SON EFECTIVAS LAS SEMILLAS DE PAPAYA EN DIFERENTES ETAPAS DE DESARROLLO PARA CONTROLAR EL ÁCARO ROJO DE LA PALMA?

RESUMEN

Objetivo: Evaluar el potencial acaricida de semillas de papaya (*Carica papaya* L.) en diferentes estadios fenológicos de desarrollo sobre el ácaro rojo de las palmeras.

Métodos: Extractos de semillas verdes y maduras, diluidas en diferentes concentraciones, fueron rociadas sobre ácaros hembras adultas en placa de Petri con discos de hoja de coco enano en diseño completamente casualizado. El efecto acaricida se evaluó a intervalos de 12 horas hasta completar 72 horas después de la pulverización.

Resultado y conclusión: La mortalidad de *Roiella indica* fue proporcional al incremento en la concentración de extractos para semillas de papaya verde y madura. Se concluyó que los extractos acuosos de semillas de frutos verdes y maduros de papaya (*C. papaya* L.) muestran actividad acaricida en adultos de palma roja en condiciones de laboratorio.

Implicaciones de la investigación: Las semillas de la papaya, en diferentes etapas, controlaron el ácaro rojo de las palmeras bajo condiciones de laboratorio.

Originalidad/valor: Esta investigación contribuye a explotar el potencial de un residuo agrícola como control de plagas en la agricultura y demostrar su potencial para prácticas agrícolas más sostenibles.

Palabras clave: *Carica Papaya*, *Raoiella Indica*, Manejo Alternativo, Extractos Botánicos.

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1 INTRODUCTION



The red palm mite, *Raoiella indica* Hirst, 1924 (Prostigmata: Tenuipalpidae), is a phytophagous species found on coconut and banana trees (Barroso et al., 2019; Cuzzuol et al., 2023). This mite stands out for having high reproductive capacity and rapid dissemination and adaptation to new hosts (Navia et al., 2016). This species has the habit of feeding on the abaxial surface of the leaves, through the introduction of stylets into the stomata through the ostiole, causing yellowing, dark spots, and drying of the leaves. In high populations, this species can cause severe attacks and significant damage (Navia et al., 2016; Piffer et al., 2023).

Synthetic chemicals have been the usual form of control against the red palm mite. In Brazil, two synthetic chemical products are officially registered for this pest species (AGROFIT, 2024). However, the damage caused by synthetic chemicals is a topic of great relevance, especially concerning the action of these products on non-target organisms, thus leading to a search for alternative methods for controlling pest organisms. (Ruiz-Jimenez et al., 2023). The predatory mite *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) has been a biological product that has shown significant performance in controlling this pest organism (AGROFIT, 2023). However, other alternative and sustainable forms of control emerge as possibilities to be applied to pest species.

The use of plants with insecticidal/acaricidal properties, such as seeds and plant barks, appears as a possible and promising alternative for controlling pest individuals (Carriço et al., 2024). In this sense, papaya *Carica papaya* L. (Caricaceae) is well known for its nutritional and pharmaceutical properties throughout the world. Parts such as roots, leaves, bark, fruits, and seeds have nutritional and therapeutic significance and several studies have been developed in this field (Dotto & Abihudib, 2021; Ugboogu et al., 2023). Furthermore, plants produce secondary defense metabolites that during the development stages can be more or less concentrated and directly influence the toxicity of plant extracts on arthropods (LI et al., 2020; Holtz et al., 2020). Papaya seed extract was studied by Ogbonna et al. (2021) on *Plutella xylostella* L. (Lepidoptera: Plutellidae) and *Brevicoryne brassicae* L. (Hemiptera: Aphididae), with mortality rates of up to 100% and a repellency effect on individuals being observed.

In this context, given the economic importance of *R. indica* and the growing search for more sustainable management methods in agriculture, this work aims to test the efficiency of aqueous extracts obtained from seeds of papaya fruits in stages of green and mature development on the red spider mite of palm trees.

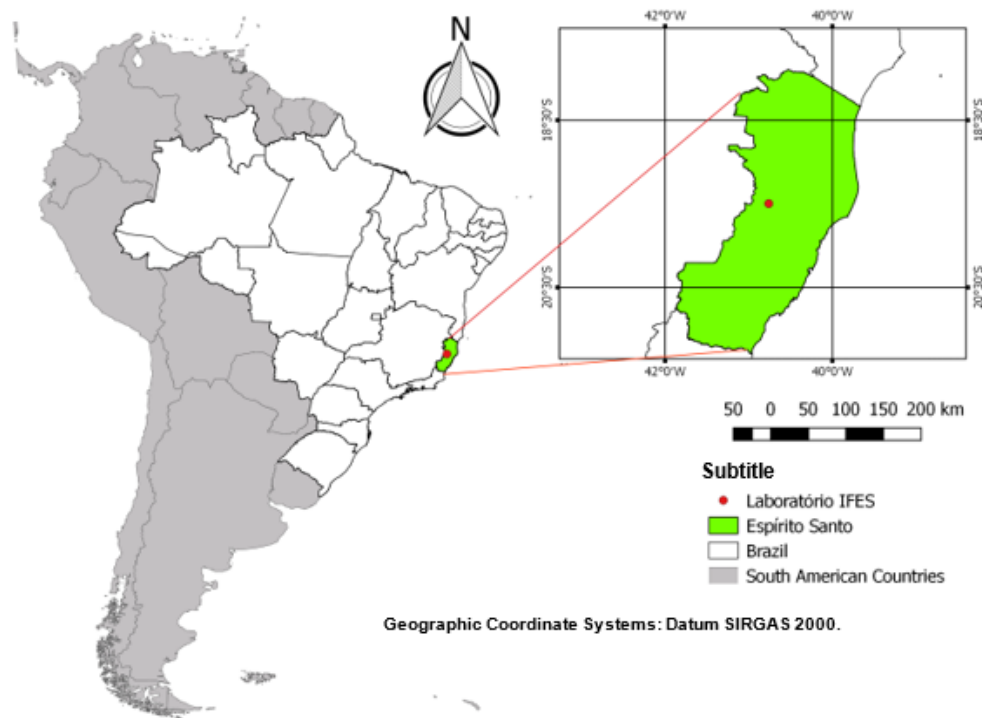
2 MATERIAL AND METHODS



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

Figure 1

Map of the place where the experiments were carried out.



Source: adaptad from Piffer et al., (2023).

The mite was reared on dwarf coconut (*Cocos nucifera* L.) seedlings, following the methodology of Piffer et al. (2023). For initial infestation, leaflets infested with *R. indica* were placed in contact with dwarf coconut seedlings. When plants showed symptoms of high mite infestation, they were placed in contact with healthy plants for new colonization of the other plants. No chemicals were used to control pests and diseases.

To prepare the extracts, papaya fruits in the green and mature phenological stages were collected in a planting area free from agrochemical contamination at IFES – Campus Itapina. Subsequently, the fruits, in the two different phenological stages, were taken to the laboratory, cleaned and the seeds of the green fruits and the ripe fruits were separated. These materials were placed, separately by phenological stage, in a forced air circulation oven at a temperature



of 50° for 72 hours to dry. After the material had completely dried, it was subjected to grinding using a knife mill, obtaining a fine powder that was used to prepare the solutions.

The direct application test of the extracts on the mites was carried out according to the methodology suggested by Carvalho et al. (2017). Initially, a solution of each of the above-mentioned extracts was applied at 10% to observe the mortality rate of *R. indica* at 95%. After this procedure, a logarithmic scale interval was drawn up, and concentrations were obtained: 1.58, 2.51, 3.98, 6.31, and 10.0% for the green papaya seed extract and 0.5, 0.75, 1.14, 1.73, 2.62 and 3.98% for the hand seed pod. Extract dilutions were carried out corresponding to the percentage found on the logarithmic scale in grams for green and mature phenological stage fruits. Distilled water was used as a solvent and Tween® 80 adhesive spreader (0.05% v/v) in amounts previously calculated to correspond to the final concentration of the extract solution. Subsequently, the material was maintained under homogenization on a transversal shaker (240 rpm) for 30 minutes.

Each experimental unit was composed of a Petri dish (10.0 x 1.2 cm), with palm leaf discs measuring approximately 4 cm in diameter, with moistened cotton around it to maintain the turgidity of the leaf and avoid mite escape. Each solution was applied to *R. indica* adults aged 25 days, obtained from breeding, with each treatment consisting of 7 replications, with 12 individuals. Spraying was carried out using an Alfa 2 model airbrush, connected to a calibrated compressor with constant pressure of 1.3 psi and 1 mL of solution of each formulated for each repetition of both the mature seed extract and the green seed extract. Only distilled water and the adhesive spreader Tween® 80 (0.05% v/v) were used as control treatments. The experimental units were kept in air-conditioned chambers at a temperature of $25 \pm 1^\circ\text{C}$, relative humidity of $70 \pm 10\%$, and a 12-hour photophase. The acaricidal effect was evaluated 24, 48, and 72 hours after spraying.

For the chemical characterization of the extracts, the quantification of total phenolic compounds followed the methodology described by Swain & Hills (1959), with adaptations. The crude extracts were diluted in distilled water (1 mg/mL). 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, after 3 min, 1 mL of 7.5% sodium carbonate solution was added and the mixture was vortexed again. After 1 h of rest away from light, triplicate absorbance readings were taken on a spectrophotometer at 750 nm. Gallic acid was used as a standard to construct a calibration curve. From the straight-line equation obtained, the content of total phenolic compounds was calculated, expressed in ug EAG/mg extract (micrograms of gallic acid equivalent per milligram of extract).



Total tannins were quantified according to Pansera (2003). The crude extracts were diluted in distilled water (1mg/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 min, 1 mL of 7.5% sodium carbonate solution was added and the mixture was vortexed. After resting for 1 h away from light, the reaction tubes were centrifuged at 2000 RPM for 5 min. The supernatant was subjected to absorbance reading on a spectrophotometer at 750 nm. Gallic acid was used as a standard to construct a calibration curve. From the straight-line equation obtained, the total tannin content expressed in ug EAG/mg extract (micrograms of gallic acid equivalent per milligram of extract) was calculated.

The total flavonoid content was determined by the colorimetric method with aluminum chloride (AlCl₃), carried out according to Perdigão (2012) 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 7.5% aluminum chloride solution (w/v) were added completing the volume to 25 mL with distilled water. After 30 min, the samples were read at 420 nm using a 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 µg/mL. All readings were performed in triplicate. The result was expressed as ug EQ/mg extract (micrograms of quercetin equivalent per milligram of extract).

For each aqueous extract, the design used was randomized. The mortality data were corrected using the Abbott formula (1925) and subsequently subjected to Probit analysis ($p \leq 0.05$), using the statistical program R Core Team (2024). From the equations obtained, the LC50 and LC90 were calculated for each extract applied to *R. indica*. For chemical characterization data, the Tukey test was performed at 5% probability using the statistical program R Core Team (2024).

3 RESULTS AND DISCUSSION

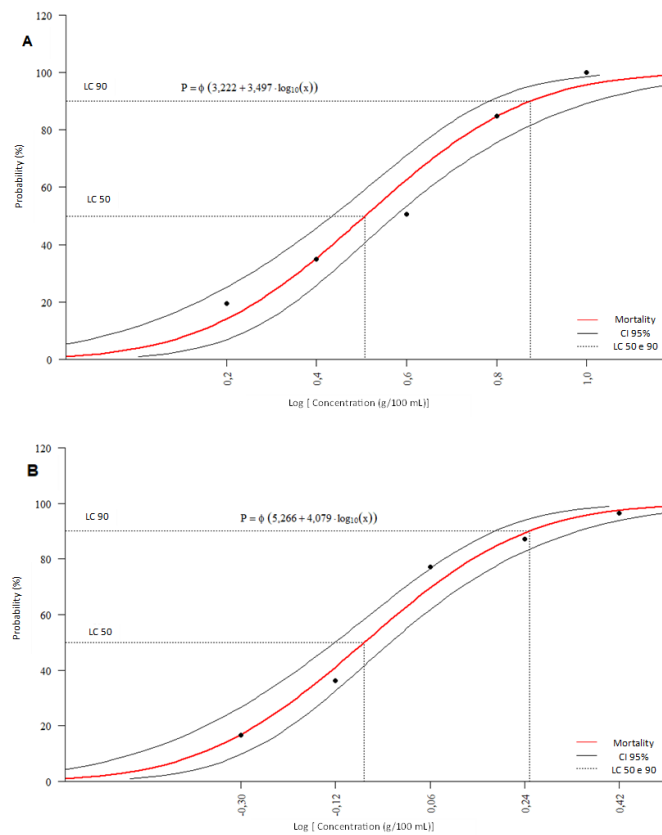
In this study, a mortality rate with an increasing trend was obtained for both the green papaya seed extract and the ripe papaya extract, indicating toxicity for the target pest organism (Figure 2). The slope coefficient of the straight line of the mortality curve presented a value of



3.49% and 4.07% for the green and mature seed extract respectively. These values obtained suggest that the smallest increase in the concentration of extracts allows for an increase in *R. indica* mortality. The 50% lethal concentrations obtained in bioassays were estimated between 3.22 and 1.77%, and the 90% lethal concentrations were between 7.49 and 3.22% for green and ripe papaya seed extracts respectively in adults of *R. indica* (Table 1).

Figure 2

Mortality curve of Raiiella indica in different concentrations of extract from green papaya (A) and ripe papaya seeds (B). Temp.: 25 ± 1°C, RH 70 ± 10% and 12 hours of photophase.



Source: author (2024).



Table 01

Parameters of the mortality curve of R. indica sprayed with green and ripe papaya seed extract.

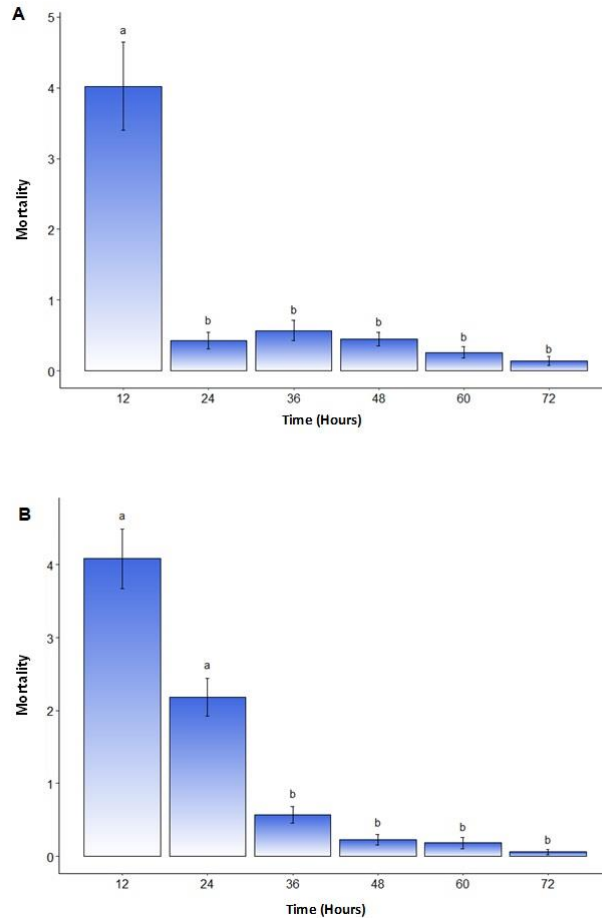
Extract	N	Slope(\pm EP)	LC ₅₀ g/100 mL (%)	(CI95)	LC ₉₀ g/100 mL (%)	(CI95)	X ²	Df	p-value
Green Verde	504	3.497 \pm 0.575	3.22	(2.72; 3.75)	7.49	(6.07;10.40)	11.13	3	0.988
Ripe Seed	504	4.079 \pm 0.347	1.77	(1.52; 2.19)	3.22	(2.72; 3.75)	4.41	4	0.647

N: number of individuals, CI: Confidence Interval, X²: Chi-square, GL: Degrees of freedom.
Source: author (2024).

The highest mortality rates occurred within the first 12 hours of evaluation for the green papaya seed extract (Figure 3A) and between the first 12 and 24 hours of evaluation for the ripe papaya seed extract (Figure 3B).

Figure 3

Mortality rate of Raoiella indica in different concentrations of green papaya (A) and ripe papaya (B) seed extract. Temp.: 25 \pm 1°C, RH 70 \pm 10% and 12h photophase



Source: author (2024).



The quantification of secondary compounds present in extracts from green and ripe papaya seeds is presented in Table 02. The extracts from green papaya seeds showed a statistical difference in the concentration of phenolic compounds and tannins concerning the green and ripe seed extract. However, the flavonoids present did not differ statistically (Table 02).

Table 02

Chemical analysis of green and ripe papaya seed extract.

Extract	Phenolics ($\mu\text{g EAG/g}$)	Tannins ($\mu\text{g EAG/g}$)	Flavonoids ($\mu\text{g EQ/g}$)
Green Seed	109.5667 a	9.8257 a	0.2194 a
Ripe Seed	10.05603 b	8.3151 b	0.2161 a

Lowercase letters in the columns do not differ statistically from each other using the Tukey test at 5% probability. Source: author.

The findings in this study report the efficiency of the aqueous extract of papaya seeds at the green and mature phenological stage in the management of the red mite of palm trees. The mortality of *R. indica* is attributed to secondary compounds present in green and ripe papaya seeds. Secondary metabolites are related to plant defense compounds and may be present in different concentrations depending on the structure of the plant used or its degree of maturation, the form of cultivation, and climatic conditions (Ukoroije & Otoyoy, 2020; Piffer et al., 2023; Carriço et al., 2024).

Seeds as a propagation vehicle have a varied composition of secondary metabolites (Daoudi et al., 2022), the main compounds associated with individual mortality were phenolic compounds and tannins. These compounds showed greater quantification in the seeds of papaya fruits at the green stage of development than in mature papayas, however, although in both extracts these compounds were contributing significantly to the greater mortality of individuals, lower concentrations of the papaya seed extract were required. ripe papaya (0.5, 0.75, 1.14, 1.73, 2.62, and 3.98%) to cause mortality of individuals than the green papaya seed extract (1.58, 2.51, 3.98, 6.31, and 10.0%). This may be related to the synergism of compounds present in ripe fruits that developed throughout the maturation process (Menezes Filho, et al., 2020). Papaya as a climacteric fruit, in its green stage of development, does not have all the final compounds present, while ripe fruits have already developed all the compounds during the ripening process. therefore, the synergism of the components may be greater and cause mortality at lower concentrations (Fenn & Giovannoni, 2021).

Among the compounds observed, phenolics are compounds that cause acute toxicity in insects, with antifeedant properties, growth inhibitors, and anti-ovipositional activity. Multivariate analyses prove that high concentrations of these compounds can inhibit the



enzymes acetylcholinesterase and butyrylcholinesterase, causing lethal oxidative damage to the cells of organisms and therefore interfering with the growth and development processes of herbivores (Devrnja et al., 2022; Bini et al., 2023). During this experiment, significant mortality was observed in a short period (Figures 3A and 3B) and no oviposition was observed in the arenas with green and ripe papaya seed treatments. This observation is attributed to the high concentration and presence of phenolic compounds, similar to what was found by Rodríguez et al. (2022) for corn weevil treated with ten different types of tested compounds. Tannins, being a group extracted from phenolic compounds, also act as digestion inhibitors with an anti-feeding function and also interfere with the synthesis of integumentary proteins and cause deregulation of the growth and development of herbivores (Ukoroije & Otoyayor, 2020). From the reported acaricidal properties of tannins in mites treated with green and ripe papaya seeds, it was observed that *R. indica* lost their characteristic reddish glow, the mites had contracted palps and legs and some were stuck to the leaves, having their capacity reduced movement and feeding and as a result they died within a few hours of assessment.

Flavonoid compounds, although in minimal quantity, may also have contributed to mite mortality, as it has a feeding deterrent function (Ukoroije & Otoyayor, 2020). In addition to the compounds quantified in this study, papaya contains papain and chymopapain, enzymes that increase intestinal transit time and have antiviral, antifungal, and antibacterial properties (Ugbogu et al., 2023).

Another hypothesis related to the acaricidal activity of the tested extracts is also associated with the method of direct exposure of the extracts to the mite and its food. The mites are likely becoming intoxicated both through direct contact with the extracts through the spiracles, as well as through the digestive route (HOLTZ et al. 2023). Abouelatta et al. (2022) found mortality rates of up to 100% when carrying out contact toxicity testing of commercial vegetable oils on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (F.) (Bostrychidae: Coleoptera), confirming that the method of direct exposure may be influencing the mortality of individuals.

4 CONCLUSION

Aqueous extracts of green and ripe papaya seeds showed an acaricidal effect on the red palm mite. The highest mortality rates were found between 12 and 24 hours for both extracts. Furthermore, extracts from green and ripe papaya seeds contained secondary compounds in



their composition, such as phenolics, tannins, and flavonoids, which have proven acaricidal activity.

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