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Nematicidal and Insecticidal Activity of Proteases from *Carica papaya* and *Ananas comosus*

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Abstract: Plant proteases are well known for their various industrial applications. Papain, present in papaya latex (*Carica papaya*) and pineapple bromelain (*Ananas comosus*), is undoubtedly the most studied and widely used vegetable protease in the food and pharmaceutical industry worldwide. However, its potential as a biopesticide has been little explored. The objective of this study was to evaluate the activity of proteases from *Carica papaya* latex and peel and crown of *Ananas comosus* fruits on agricultural pests. To evaluate proteolytic activity on nematodes, extracts, and approximately 50 juveniles of *Panagrellus* sp. were placed in microtubes. To evaluate the insecticidal effect, larvae and pupae of *Tenebrio molitor* L. were submerged in active and denatured extracts. Additionally, larvae of *T. molitor* were fed an artificial diet at doses of 0, 100, 250, and 500 mg/g of wheat bran. The weight and number of dead larvae were recorded, and feeding behavior was evaluated. The proteases of papaya latex and papain caused reduction ($p < 0.05$) on *Panagrellus* sp. The extracts showed a toxic effect ($p < 0.05$) against the larvae of *T. molitor*. Active papain resulted in the absence of wings in 53.3% of adults from the pupae, and no malformation caused by denatured papain was observed. No mortality was observed in larvae fed an artificial diet. However, there was a strong feed reduction, reduction in the relative rate of consumption, reduction in growth and feed conversion efficiency caused by papaya latex. The results of this study show that plant proteases have the potential for the development of sustainable alternatives for the control of arthropod pests and parasitic nematodes.

Keywords: latex; papain; *Carica papaya*; *Ananas comosus*; *Panagrellus* sp.; *Tenebrio molitor*

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Citation: Castro, H.L.B.; Alves, J.C.d.S.; Gladenucci, J.; Marucci, R.C.; Soares, F.E.d.F. Nematicidal and Insecticidal Activity of Proteases from *Carica papaya* and *Ananas comosus*. *Agriculture* **2023**, *13*, 1119. https://doi.org/10.3390/agriculture13061119

Academic Editors: Yan Wang and Azucena González Coloma

Received: 15 March 2023
Revised: 13 May 2023
Accepted: 23 May 2023
Published: 25 May 2023

1. Introduction

Reducing pest losses is an effective way to increase agricultural production. It is estimated that between 20% and 40% of all crop productivity losses are caused by pests and phytopathogens, respectively [1,2]. On an average, 12.6% worldwide crop losses, which in monetary terms equals to $215.77 billion, has been reported for only the top 20 life-sustaining crops based on the 2010–2013 production figures and prices [3]. In Brazil, the agricultural economy loses about $17.7 billion annually from outbreaks of insects and other arthropod pests in crops [4,5]. There are several pest control strategies. Chemical control is one of the most used, and although it is effective for the control of various types of pests, its indiscriminate use causes negative impacts on the environment [6,7], beneficial organisms such as bees [8] and earthworms [9], and human health [10].

Hence, it is necessary to reduce the use of synthetic agrochemicals and adopt alternative strategies that present a lower risk to the environment and human health [11]. Biopesticides offer a viable substitute to synthetic pesticides, as they possess a lower degree of toxicity towards humans and do not result in the release of harmful residues into the environment or impact beneficial species [7,12–14]. In recent years, proteases have emerged as standard biocatalysts in many industrial processes in different fields, such as pharmaceuticals, medicine, detergent manufacturing, and food science [15]. Among them, papain, present in papaya latex (*Carica papaya*) and pineapple bromelain (*Ananas comosus*) is undoubtedly the most studied and widely used vegetable protease in the food and pharmaceutical industry worldwide [16,17].

Chemical insect control, in addition to harming the environment, has proven to be ineffective against insects that acquire resistance. In this study, *Tenebrio molitor,* a beetle known as the flour grub, was used as a model insect. *T. molitor* is a cosmopolitan pest commonly found infesting stored agricultural produce and products, mainly grains and related products such as bran and pasta, which are found in a variety of facilities [18]. Despite being considered a pest, it has been used as a model insect due to its size and ease of reproduction [19,20].

Although plant proteases are better known in the industry, some authors have studied the efficacy of pineapple bromelain (*Ananas comosus*) in the control of ticks [21] and nematodes [22]. Similarly, papain has been studied in pest control [23,24]. In this context, the objective of this study was to evaluate the in vitro activity of extracts of *C. papaya* and *A. comosus* rich in proteases to control *Panagrellus* sp. and *Tenebrio molitor*.

2. Material and Methods

2.1. Obtaining Pineapple Aqueous Extracts

Ripe pineapple fruits were purchased at the local market in the municipality of Lavras, Minas Gerais, Brazil. The peel and crown of the fruits were removed and then crushed in a blender using distilled water in the ratio of 1:1 (w/v) for the peel and 1:2 (w/v) for the fruit crown. Subsequently, the samples were manually filtered with a sieve and centrifuged at 10,000× g at 4 °C for 10 min, and the supernatant was lyophilized for 48 h. After lyophilization, the resulting powder was stored at −20 °C until use. The powder obtained from the peel and crown of the pineapples was reconstituted with distilled water to concentrations specified in each experiment.

2.2. Collecting and Processing of Papaya Latex

Latex was collected directly from green papaya fruits, according to the methodology of Chandrasekaran et al. [25], with alterations. With the aid of a stainless steel knife, longitudinal incisions were made with a depth of 3 mm on the surface of the fruits. Subsequently, latex was frozen, lyophilized, and stored at −20 °C until further use. The powder obtained was reconstituted with distilled water to concentrations described in each experiment. Papain, one of the enzymes present in papaya latex, was purchased commercially (Contemporary Chemical Dynamics LTDA, Indaiatuba, Brazil) and reconstituted with distilled water to concentrations described in each experiment.

2.3. Enzymatic Activity

The protease activity of pineapple extracts and papaya latex was measured according to the caseinolytic procedure adapted by Gomes et al. [26]. A tyrosine standard curve was used. The tests were carried out in tubes using 450 µL of Tris-HCl buffer 100 mM pH 8.0, 500 µL of casein at 1% (w/v), and 50 µL of sample reconstituted with distilled water to 10% (w/v). The reagents were incubated at 30, 40, 50, 60, and 70 °C for 30 min. After incubation, the reaction was interrupted by the addition of 1 mL of 10% trichloroacetic acid (w/v), the microtubes were centrifuged at 10,000× g to 4 °C for 10 min, the supernatant was collected, and absorbance readings were made in triplicate at 280 nm with the aid of a spectrophotometer. One unit of protease was defined as the amount of enzyme required to release 1.0 µg of tyrosine per minute under the assay conditions.

To determine the specific activity (U/mg), the soluble protein content was measured using the Bradford method [27], using bovine serum albumin (BSA) as the standard.

2.4. Nematicidal Activity

The free-living nematodes *Panagrellus* sp. were acquired commercially and later cultivated following the methodology described by Sufiate et al. [28]. The nematodes were kept in the dark at room temperature (25 °C) for 7 days in jars containing oats and distilled water. To evaluate proteolytic activity on *Panagrellus* sp., a total of 8 groups were formed with 4 groups containing active extract and 4 groups with denatured extract (boiled). Each group consisted of 30 microtubes (6 tubes per treatment) containing the treatments (pineapple peel, pineapple crown, papaya latex, and papain) plus control (distilled water). In order to evaluate the effect of proteases present in the extracts, a group of nematodes was incubated under the same conditions, but with boiled extracts. In each tube were added: 20 µL of extract (active or boiled) 5 mg/mL and 15 mg/mL, plus 10 µL of containing approximately 50 juveniles of *Panagrellus* sp. The tubes containing the extracts and nematodes were incubated at 28 °C in the dark for 24 and 48 h. After these periods, the total number of live juveniles present in each tube was counted by optical microscopy (4×), according to the methodology described by Soares et al. [29]. Immobile larvae with non-intact cuticle were considered dead. The percentage of nematode reduction was calculated according to the following formula:

$$Reduction\ (\%) = \frac{(\overline{x}\ Live\ control\ larvae - \overline{x}\ Live\ treatment\ larvae)}{\overline{x}\ Live\ control\ larvae} \times 100$$

2.5. Insecticidal Activity

2.5.1. Immersion Test with Larvae and Pupae

T. molitor larvae and pupae with homogeneous weights and sizes were acquired from the Entomology Laboratory of the Federal University of Lavras. Larvae with an average weight of 60 mg and an average length of 2 cm were divided into five groups (representing each treatment), according to Rankic et al. [30]. Each group consisted of 90 larvae divided into three vials with 30 larvae each. A 15% solution of each extract reconstituted with distilled water (*w*/*v*) was prepared. The larvae were submerged for 5 s in 30 mL of each extract (150 mg/mL) every 24 h for 3 days. The larvae of the control group were immersed only in distilled water. After immersion, excess moisture from the larvae was removed in paper towels, and the larvae were kept in a room at 25 °C and 65% RH.

To determine the percentage of mortality, the number of dead larvae was recorded every 24 h for 10 days. Larvae that did not respond to tactile stimuli were considered dead. Using the same procedure, an additional experiment was carried out with boiled extracts. To denature the enzymes, the extracts were boiled for 2 h, cooled to room temperature, and stored under the same conditions as the active extracts, until use.

Twenty-four-hour-old pupae with average weights of 90 mg were immersed in the treatments as described in the larval immersion test, but in this experiment, a total of 30 pupae were divided into 3 vials with 10 pupae each. Similarly, an additional experiment was carried out with boiled extracts for two hours as described earlier. Again, the number of pupae killed after immersion in the extracts was recorded. Additionally, the body changes in the metamorphosis of the surviving pupae were recorded, and the percentage of deformation was calculated by recording the absence or malformation of the wings.

2.5.2. Preparation of an Artificial Diet for *Tenebrio molitor* Larvae

The diet was prepared according to an adaptation of the methodology used by Lima et al. [31]. Using wheat bran and distilled water, a solution was prepared at concentrations of 100 mg/g, 250 mg/g, and 500 m/g for each of the extracts, and stirred for 5 min. The control group consisted only of wheat bran and distilled water. Subsequently, 2.5 mL of the solution was pipetted in Petri dishes (with known weight) and allowed to dry overnight at room temperature (18 °C ± 2 °C). The next day, the plates were again weighed, and the weight of the diet contained in each plate was calculated. In triplicate, 15 larvae of *T. molitor* with known weights (60 mg/larva with 2 cm in length) were placed in each Petri dish.

2.5.3. Food Deterrence Index and Nutritional Indices in Larvae

The weights of diet and larvae from day 0 to day 7 were measured [32]. Then, the food deterrence index (ID) was calculated as follows: ID (%) = 100 × (A − B)/(A), where A is the food mass ingested by insects in the experimental control; and B is the food mass ingested by the insects in the test sample. Based on ID, the samples were classified as promoted: without food impediment (ID < 20%), weak food deterrence (20% < ID < 50%), moderate food deterrence (50% < ID < 70%), or strong food deterrence (ID > 70%). The same data were used to calculate the following nutritional indices: (a) relative rate of consumption = C/(D × days), where C is the mass of food ingested in mg; and D corresponds to the initial biomass of insects in mg; (b) relative biomass gain rate = E/(D × days), where E corresponds to biomass gained in mg; and (c) the conversion efficiency of the ingested feed = E/(C × 100).

2.6. Statistical Analysis

The data obtained were analyzed by the statistical program Sisvar and interpreted by analysis of variance at the significance level of 5%. The efficiency of the treatments in reducing the number of nematodes, compared to the control, was performed using Tukey's test at the significance level of 5%.

3. Results and Discussion

3.1. Protease Activity

The results of the proteolytic activity of pineapple peel, pineapple crown, and papaya latex extracts are shown in Figure 1. The pineapple peel extract (Figure 1C) showed maximum activity (0.69 U/mg) at 50 °C. The pineapple crown extract increased its activity with the temperature increase, presenting its maximum activity (1.071 U/mg) at 70 °C, suggesting the presence of other enzymes, compared with the peel extract. It has been reported that pineapple extract (*Ananas comosus*) contains at least eight cysteine proteases, including stem bromelain, fruit bromelain, ananain, and comosain [33]. In addition, the ideal pH and temperature for enzymes present in pineapple may be varied, so for example, the ideal pH and activity temperature for stem bromelain are 6.5–8.0 and 55–60 °C, respectively [34].

The enzymatic activity of papaya latex (Figure 1B) was stable at different temperatures with activity of 8.4 U/mg at 30 °C and 8.095 U/mg at 70 °C. This stability may be related to the presence of other enzymes with different chemical properties [35]. Compared to pineapple extract, papaya latex has higher enzymatic activity, and this is due to plant latex containing, in addition to other substances toxic to pests, several types of enzymes that are produced as a defense mechanism against various types of pests [36]. Specific activity tests were determined according to protein quantification. The higher specific activity of papaya latex is related to the higher concentration of enzymes (102.22 µg/mL), compared with the concentration of enzymes in pineapple peel and crown extracts, 26.18 µg/mL and 34.62 µg/mL, respectively.

3.2. Nematicidal Activity

The extracts (from *C. papaya* latex and peel and crown of *A. comosus* fruits) evaluated in this study caused a reduction ($p < 0.05$) in the number of juveniles of *Panagrellus* sp., compared to the control group, both for the different doses (Figure 2A) and for incubation times (Figure 2B). Similarly, a reduction ($p < 0.05$) in the number of nematodes was observed in the groups treated with active extracts relative to the boiled groups. However, the boiled groups were not different from the control group, suggesting that the boiled groups still contained active proteases or other toxic compounds for *Panagrellus* sp.

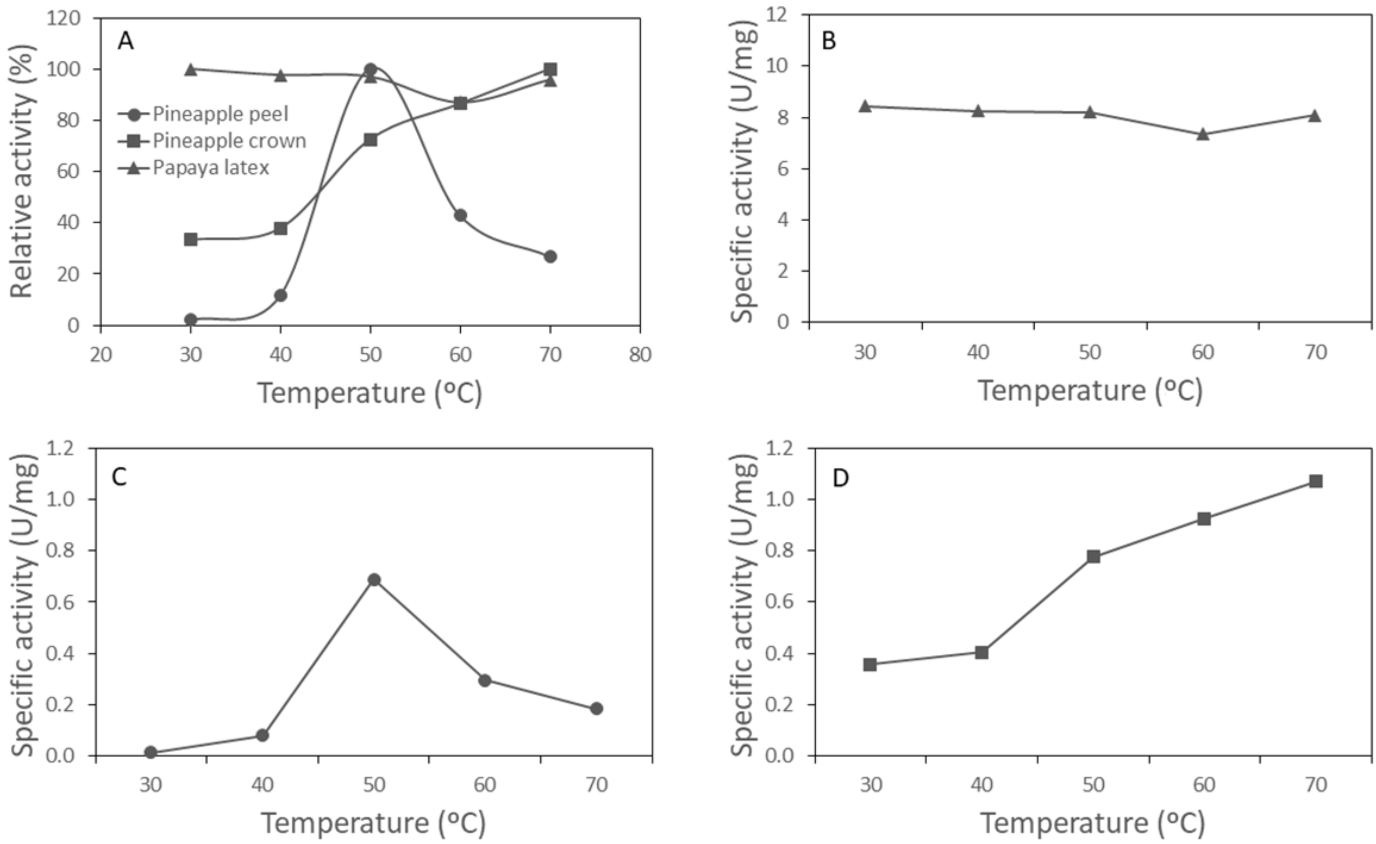

Figure 1. Effect of temperature on the enzymatic activity of three sources of vegetable proteases. (**A**) Comparison of relative activity (%) of different sources of plant proteases. (**B**–**D**) Specific enzymatic activity (U/mg) of papaya latex (**B**), pineapple peel (**C**), and pineapple crown (**D**). The assay was performed with Tris-HCl buffer 100 mM pH 8.0. A standard tyrosine curve was used, and the protein was quantified by the Bradford method to measure specific activity. One unit of protease was defined as the amount of enzyme needed to release 1.0 µg of tyrosine per minute.

In relation to the evaluated doses (Figure 2A), a similar nematicidal effect was observed in pineapple extracts. Pineapple peel extract doses of 5 mg/mL and 15 mg/mL reduced the *Panagrellus* numbers by 40.94% and 57.14%, respectively. Pineapple crown extract doses of 5 mg/mL and 15 mg/mL decreased the *Panagrellus* numbers by 30.02% and 52.78%, respectively. Papaya latex doses of 5 mg/mL and 15 mg/mL reduced the *Panagrellus* numbers by 64.54% and 90.54%, respectively. Regarding papain, although it caused a reduction ($p < 0.05$) in the number of nematodes compared to the control group (72.02% for 5 mg/mL and 70.02% for 15 mg/mL), there was no significant difference between the two concentrations.

The nematicidal effect increased with incubation time, since the period of 24 h after treatment, besides causing reduction ($p < 0.05$) compared with time zero, also caused a reduction ($p < 0.05$) in the number of juveniles of *Panagrellus* sp. compared with 48 h after treatment. This happened with all the extracts evaluated (Figure 2B). After 24 h of incubation, the extracts of pineapple peel, pineapple crown, papaya latex, and papain reduced nematodes by 32.09%, 23.28%, 71.78%, and 60.34%, respectively. Incubation for 48 h, on the other hand, caused reductions of 51.98%, 48.52%, 83.30%, and 81.70%, respectively. Similar behavior was observed by Sufiate et al. [28] in a study of the characterization of proteases present in the latex of *Euphorbia milii*, in which he evaluated the nematicidal potential of these enzymes on *Panagrellus* sp., finding a reduction ($p < 0.01$) in the number of nematodes of 65.59% and 96.46% in 24 h and 48 h, respectively.

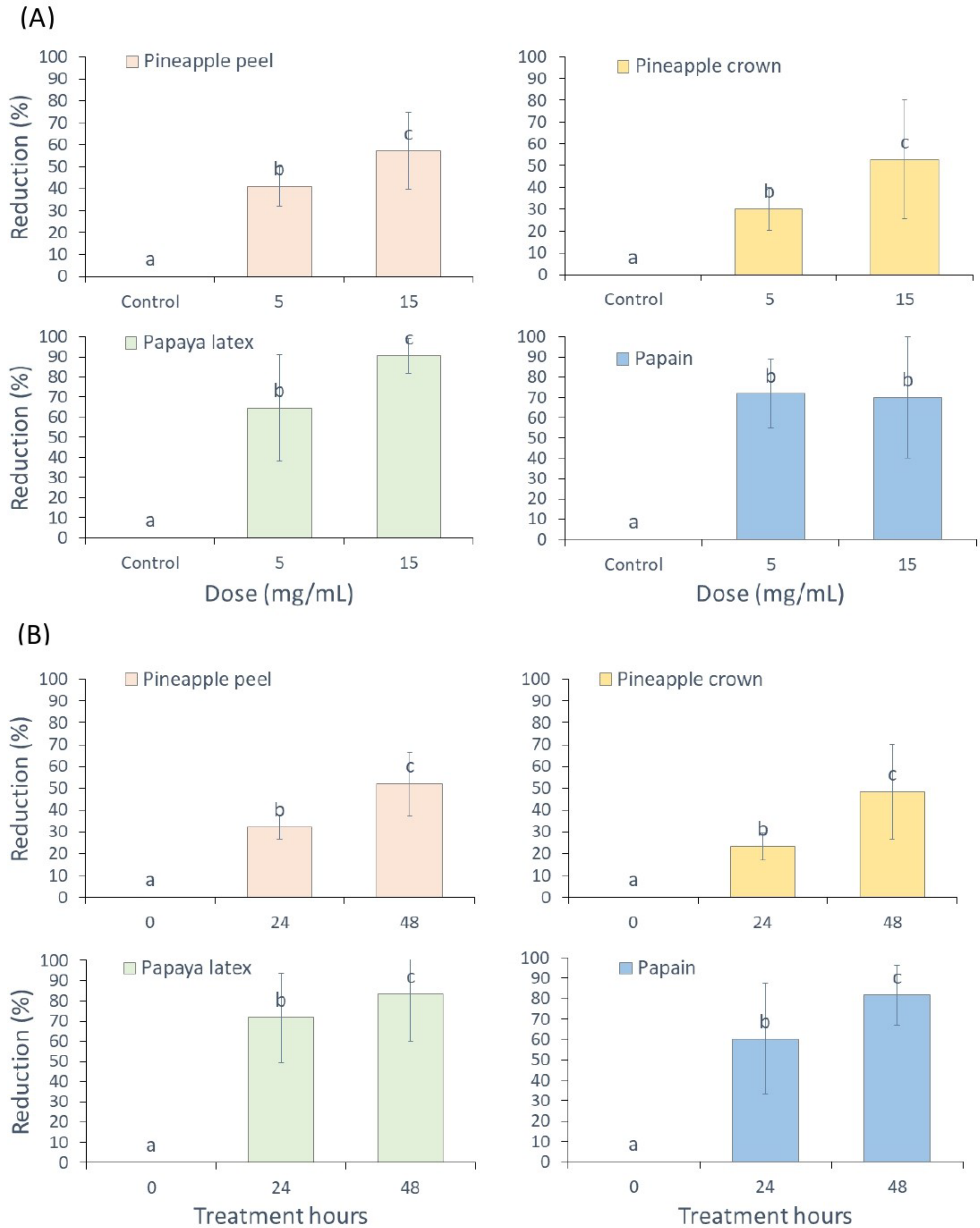

Figure 2. Nematicidal activity caused by different sources of plant proteases on juveniles of *Panagrellus* sp. (**A**) Percentage of reduction in the number of juveniles caused by 5 mg/mL and 15 mg/mL of each source of proteases. (**B**) Percentage of reduction of the number of juveniles after 24 h and 48 h of treatment. The nematodes of the control group were treated only with distilled water. Different letters indicate significant differences ($p < 0.05$) between treatments.

There were no significant differences between the boiled extracts and the control group, but the boiled papaya latex and boiled papain caused a reduction in the number of *Panagrellus* sp. juveniles ($p < 0.05$) different from the reduction caused by active extracts, with reductions caused by boiled and active papaya latex of 62.42% and 92.67%, respectively. Boiled papain caused a reduction of 52.33%, and active papain reduced by 89.71%. Compared to papain, a pure enzyme, papaya latex, in addition to proteases, contains several substances such as alkaloids, terpenoids, tannins, flavonoids, and phenols [25,37] that could have a toxic effect on nematodes, even with denatured enzymes.

Nematodes cuticle is composed of two important types of structural proteins, collagens and cuticulin [38]. As shown in this study (Figure 3), cysteine proteases derived from plants such as papaya, pineapple, fig, and kiwi catalyze the digestion of the nematode cuticle, leading to the death of the worm [39].

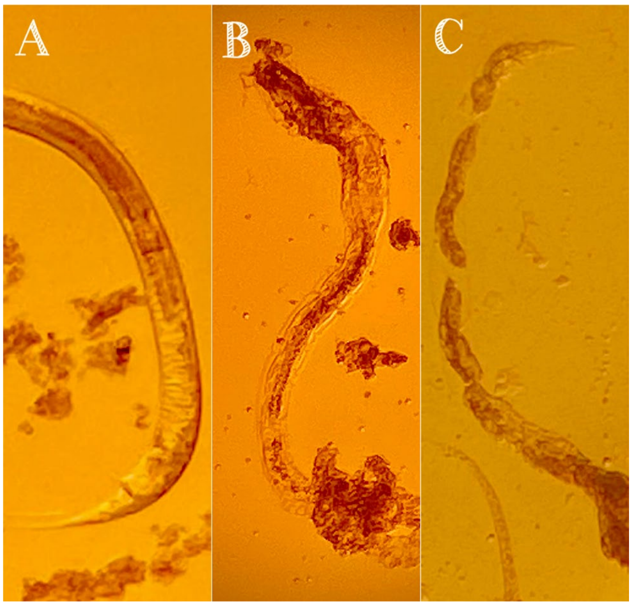

Figure 3. Nematicidal activity caused by different sources of plant proteases on juveniles of *Panagrellus* sp. Degradation of the cuticle and internal content is evident. (**A**)—juvenile without cuticle damage; (**B**)—juvenile after 24 h of incubation with papain; (**C**)—juvenile after 48 h of incubation with papain.

The nematicidal effect caused by the extracts of pineapple peel and crown, papaya latex rich in proteases on free-living nematodes of the genus *Panagrellus* is an indication of action also in other species of nematodes such as phytoparasites or animal parasites as suggested in different studies [28,40,41]. Thus, it is necessary to conduct further research in the field or in vivo level to evaluate the nematicidal effect of the extracts of the peel and crown of pineapple and papaya latex on phytoparasitic nematodes.

3.3. Insecticidal Activity

3.3.1. Mortality of Larvae and Pupae of *Tenebrio molitor*

The effect of proteases present in the extracts of pineapple peel and crown, papaya latex, and papain were evaluated on larvae (Figure 4A) and pupae (Figure 4B–D) of *T. molitor*.

Papaya latex caused higher mortality ($p < 0.05$) compared to other sources of proteases, with an average mortality of 71.11% caused by papaya latex. This can be explained because latex, in addition to proteases, contains other types of enzymes, such as chitinases, and toxic substances against various types of pests [36]. The proteolytic effect of proteases present in pineapple peel and pineapple crown extracts on *T. molitor* larvae was confirmed when compared with boiled extracts, with mortality caused by the active peel extract of 34.45% and active pineapple crown extract of 32.22%, both higher ($p < 0.05$) from the control group and boiled extracts.

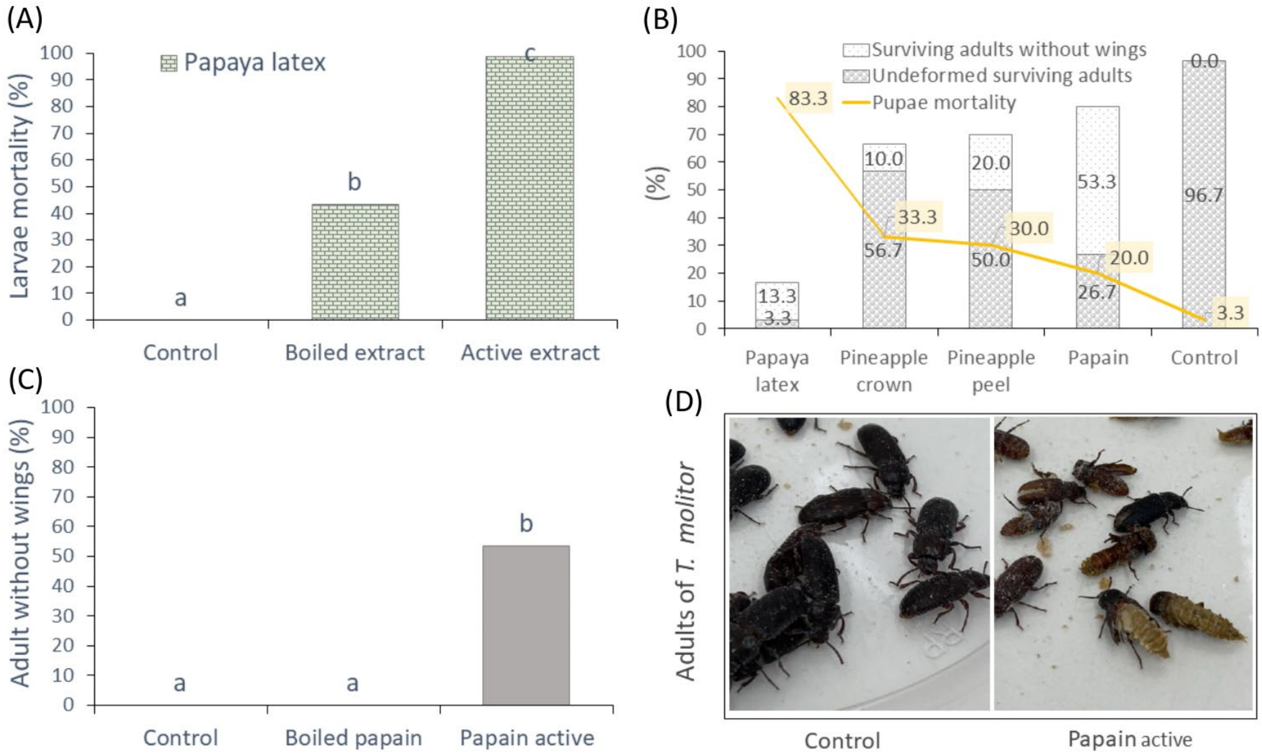

Figure 4. Insecticidal activity caused by 150 mg/mL from different sources of plant proteases in larvae (**A**) and pupae (**B**–**D**) of *Tenebrio molitor*. (**A**) Mortality percentage in larvae of *T. molitor* caused by papaya latex denatured (boiled) and active. (**B**) Percentage of mortality of pupae of *T. molitor* (orange line) and percentage of adults without deformities (gray bars) or with the absence of wings (white bars), from pupae treated from different sources of active proteases. (**C**) Percentage of adults without wings, from the experiment of immersion of pupae treated with papain active. (**D**) Photo of adults from the experiment of immersion of pupae treated with papain active (without wings), compared to adults in the control group (without deformities). The insects of the control group were only immersed in distilled water. The insects were immersed for 5 s in 30 mL of each extract (dose 150 mg/mL) every 24 h for 3 days. Different letters indicate significant differences ($p < 0.05$) between treatments according to the Tukey test at 5% probability.

Pure papain did not cause mortality in *T. molitor* larvae. On the other hand, active latex presented higher ($p < 0.05$) mortality (98.89%), compared to the group with boiled latex, with a mortality of 43.33% (Figure 3A). However, boiled latex still caused higher mortality ($p < 0.05$) than the control group, suggesting that the mortality caused in active latex (55.56%) higher than the mortality caused by boiled extract was due to the action of proteases.

The cuticle of the insect represents part of the external integument of arthropods, formed mainly by linear and relatively rigid polysaccharides, chitin, and structural proteins [42]. It serves both as a skin and as a skeleton (exoskeleton), determines the shape of the animal, and is also the barrier that protects insects from environmental stresses and mechanical damage [43]. The success of entomopathogenic fungi used in the biological control of insects is because they penetrate the cuticle through mechanical pressure and enzymatic degradation of the main components of the cuticle, i.e., proteins, chitin, and lipids [7,44].

Regarding the mean mortality of *T. molitor* pupae, insecticide activity ($p < 0.05$) was observed to be caused by pineapple peel, pineapple crown, and papaya latex, as they were statistically different from the control group, with respective deaths of 21.26%, 31.67%, and 76.67%. However, the mean mortality caused by papain was no different ($p < 0.05$) from the control group. As shown in Figure 3C, active papain affected the development of wings in adults from the pupae in 53.33% of the pupae initially immersed in the solution containing active papain. The absence of wings caused by active papain compared to the normal development of wings in the control group can be observed in Figure 3D, where adults affected by papain had a total or partial absence of wings. A similar effect was observed in adults from pupae treated with active pineapple crown extract, pineapple peel extract, and papaya latex, respectively, at 10%, 13.3%, and 20%. There was no difference ($p < 0.05$) between the mortality caused to the pupae by the active extracts and boiled for none of the extracts evaluated.

Among the cuticular damage in the pupae caused by the evaluated extracts, burns in non-sclerotized tissues, such as intersegmental tissue, stand out. In this regard, there are two main types of cuticles in insects: (1) flexible cuticles, characterized by intersegmental membranes and cuticles of many soft larvae, and (2) solid and sclerotized cuticles that usually cover most of the body surface of insects. Flexible cuticles tend to have relatively high water content and contain almost equal amounts of chitin and protein, and the same or similar proteins are often deposited before and after ecdysis. Sclerotized cuticles contain little water, and the amounts of protein tend to be several times greater than the amount of chitin [45].

3.3.2. Food Deterrence Index (ID) and Nutritional Parameters in Larvae of *T. molitor*

In addition to the immersion experiments, an intake experiment was carried out, in which an artificial diet of wheat bran was prepared for each of the extracts (pineapple peel, papaya latex, and papain), to evaluate larval mortality and feeding behavior through the dietary index deterrence (ID) and nutritional parameters as relative consumption rate, relative weight gain rate, and feed conversion efficiency. The toxicity evaluated with the different extracts present in the wheat bran diet did not cause mortality in *T. molitor* larvae. However, eating behavior was affected.

The average amount of food ingested by *T. molitor* larvae was 48.19 mg/day for the control group (wheat bran only), being equal ($p < 0.05$) to the diet consumption containing pineapple peel extract (60.32 mg/day). The larvae reduced the amount of food ingested when papain was present in the diet, with an average intake of 31.86 mg/day, being different ($p < 0.05$) from the control group. The same behavior was observed in the consumption of foods containing papaya latex, with an average intake of 17.27 mg/day, different ($p < 0.05$) from all groups.

The increase in intake observed in larvae fed the diet containing pineapple peel extract is probably related to high sugar content. However, this increase was not different from the control group ($p < 0.05$), even when the pineapple peel extract dose of 500 mg/g increased to 18.38 mg/day of food consumed by the larvae compared to the control group. Unlike pineapple peel extract, latex and papain caused a reduction in feed intake as the extract dose was increased. The dose of 500 mg/g of latex was reduced to 37.24 mg/day compared to the control. Similarly, except for 100 mg/g of papain, which was equal ($p < 0.05$) to the control, the presence of 500 mg/g of papain in the diet reduced the food intake by the larvae to 25.81 mg/day compared to the control.

As previously reported, plant extracts and an artificial diet with wheat bran present toxicity on stored food pests [31,32,46,47]. However, these studies were carried out in adult insects, and in our experiment, larvae were used, because in preliminary tests cannibalism was observed among adults of *T. molitor*.

The behavior of larvae in terms of food intake is reflected in values of dissuasion index or food repellency (Figure 5A). On average, both papain and papaya latex caused an alimentary deterrence rate of 33.80% and 64.09%, both different ($p < 0.05$) from the control group. The average dietary index deterrence caused by pineapple peel extract (not shown in the figure) was −25.27%, indicating an increase in food consumption by larvae, although it is not different ($p < 0.05$) from the control group. Papaya latex presented an alimentary index deterrence of 46.62%, 68.48%, and 77.16% for doses of 100 mg/g, 250 mg/g, and 500 mg/g, respectively, all statistically ($p < 0.05$) different from the control group (Figure 5A, higher). Papain caused significant dietary deterrence ($p < 0.05$) compared to the control, only at doses of 250 mg/g and 500 mg/g, with rates of 35.71% and 53.62%, respectively (Figure 5A, lower).

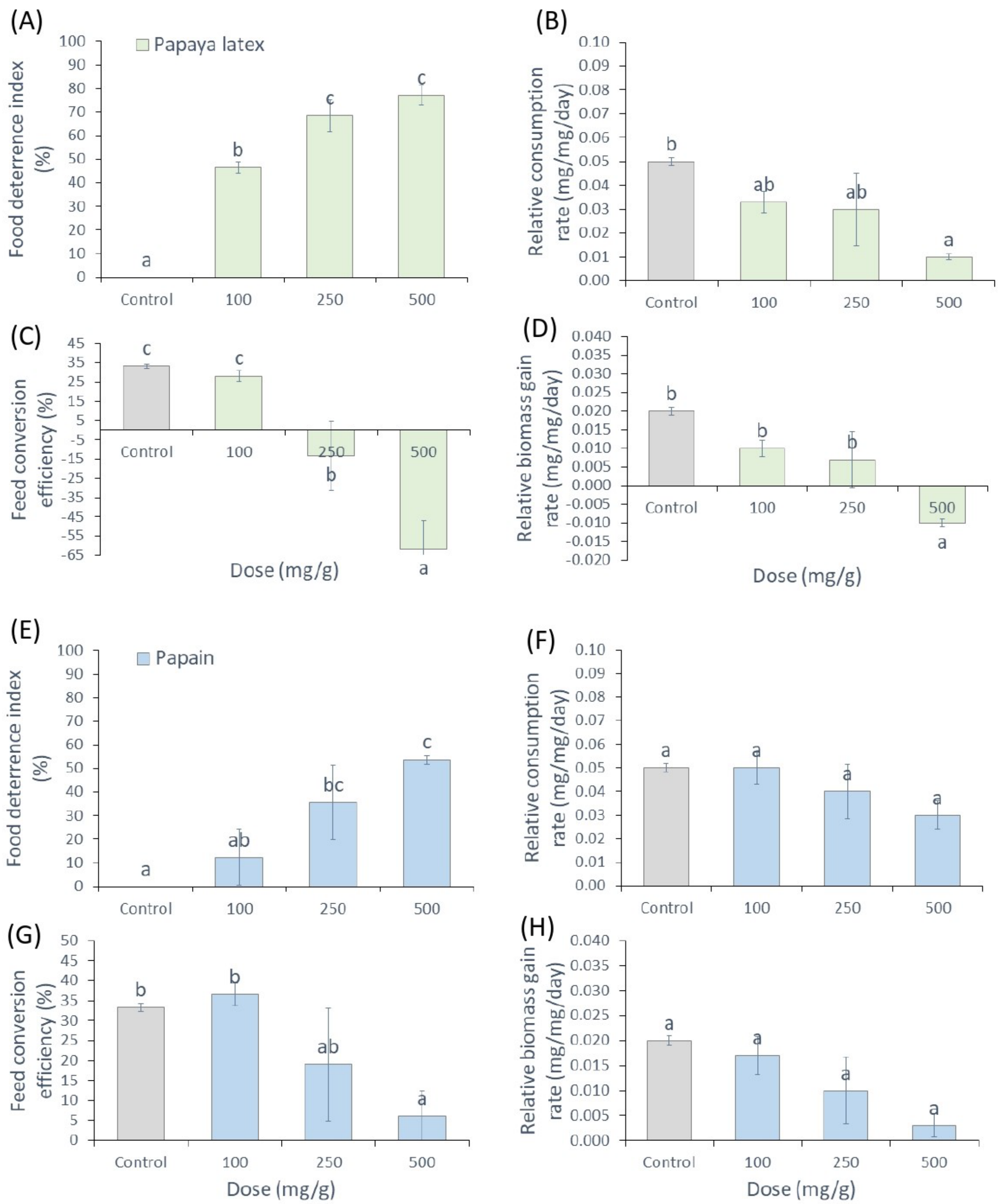

Figure 5. Nutritional indices and feeding behavior measured in larvae of *Tenebrio molitor* seven days after ingestion of an artificial diet of wheat flour (control) mixed with 100 mg/g, 250 mg/g, and 500 mg/g from different sources of vegetable proteases (papaya latex and papain) (**A**,**E**). Food deterrence index (%) (**B**,**F**). Relative consumption rate (mg/mg/day); this parameter indicates the amount of food consumed in mg per mg of insect body weight each day (**C**,**G**). Efficiency of the conversion of ingested food (%); this parameter indicates the amount of food ingested incorporated by insects as biomass (**D**,**H**). Relative biomass gain rate (mg/mg/day); this parameter indicates the amount of biomass gained each day per mg of initial body weight. Different letters indicate significant differences ($p < 0.05$) between treatments according to the Tukey's test at 5% probability.

The relative consumption rate of larvae was reduced only by the presence of papaya latex in the diet (Figure 5B, superior). When 500 mg/g of papaya was present in the diet, the larvae consumed 0.01 mg of food per mg of body weight per day, being statistically different ($p < 0.05$) from the control group. On the other hand, pineapple peel extract caused larvae to consume 0.07 mg of food per mg of body weight per day, being statistically ($p < 0.05$) higher than the control group, with consumption of 0.05 mg/mg/day, and this increase in consumption was stimulated by doses of 500 mg/g (not shown in the figure). Papain did not interfere in the relative consumption rate of larvae (Figure 4A, bottom), suggesting that the weight loss of insects was not significant, in relation to the amount of food ingested, compared to the control group.

The relative biomass gain rate was affected only significantly ($p < 0.05$) by the presence of 500 mg/g of papaya latex in the diet compared to the control group (Figure 4D, higher), indicating significant weight loss probably caused by the strong food dissuasion (77.16%) observed for this same dose of papaya latex. Regarding the different types of extracts, the rate of relative biomass gain caused by pineapple peel was statistically ($p > 0.05$) the same as in the control group, but different ($p < 0.05$) from papain and papaya latex. Indicating that pineapple extract favored larvae growth, probably due to high sugar and carbohydrate content, in comparison to latex and papain.

The amount of food ingested incorporated by insects as biomass (feed conversion), compared to the different types of extracts, was affected only by papaya latex with an average reduction of −15.71%, statistically different ($p < 0.05$) from the control group and the other extracts. In relation to the different doses evaluated, a significant difference was observed only for the dose of 500 mg/g of papaya latex, with a reduction of −61.78% (95.04% lower than the control group, Figure 5C, higher), and 500 mg/g papain, with a reduction in feed conversion of 6.13% (27.14% less than the control group, Figure 5C, bottom).

Pure papain had no effect on larval mortality, probably because the sclerotized cuticle is formed by, in addition to proteins, other compounds such as lipids and chitin, conferring rusticity and protection against adverse factors [42–44]. However, tissue damage related to the development of *T. molitor* wings has been affected by papain, probably because in this growth phase this specific tissue is not yet well developed or the content of substances that confer resistance to adult individuals is still reduced. The pineapple peel present in the diet stimulated the feeding of the larvae, without causing any toxic effect, probably due to the high sugar content and low enzyme content.

4. Conclusions

Both papaya latex and pineapple peel and crown extracts presented nematicidal activity against *Panagrellus* sp. Both papaya latex and papain showed proteolytic activity against *Panagrellus* sp. The evaluated extracts also showed insecticidal activity against *T. molitor*, affecting the feeding behavior of larvae, adult–pupa metamorphoses, and survival of pupae and larvae.

The results of this study show that plant proteases have the potential for the development of sustainable alternatives for the control of arthropod pests and parasitic nematodes. Further studies are needed to identify and characterize the tissue that is affected during the development of the wings in *T. molitor*, caused by papain.

Author Contributions: Conceptualization and visualization, F.E.d.F.S. and H.L.B.C.; material preparation, data collection, and analysis, H.L.B.C., J.C.d.S.A. and J.G.; writing (review and editing), funding acquisition, F.E.d.F.S., R.C.M., J.G. and H.L.B.C.; writing (original draft preparation), H.L.B.C.; supervision, F.E.d.F.S. and R.C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by grants from the Minas Gerais Research Funding Foundation (FAPEMIG) (APQ-02466-21) and National Council for Scientific and Technological Development (CNPq) of Brazil (407265/2021-0).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data will be available upon request.

Acknowledgments: The authors thank the National Council for Scientific and Technological Development (CNPq) and Minas Gerais Research Foundation (FAPEMIG) for financial support.

Conflicts of Interest: The authors declare no conflict of interest.

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