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ENVIRONMENTAL PROGRESS & SUSTAINABLE ENERGY

ORIGINAL RESEARCH

Remediation and Treatment

From waste to resource: Garlic peel-derived proteases (*Allium sativum*) for nematode control

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Abstract

This study *aimed* to prepare a protease-rich extract from garlic peel (*Allium sativum*) and evaluate its nematicidal potential. The garlic peel was crushed, suspended in distilled water, and the obtained extract was subjected to filtration, centrifugation, and dialysis to concentrate the proteases. The nematicidal activity was tested in vitro on *Panagrellus* sp. and *Meloidogyne incognita* nematodes using both active and denatured extracts. Additionally, the effectiveness of the extract was evaluated in soil simulations and in a greenhouse trial with beans (*Phaseolus vulgaris*). In the in vitro tests, a 72.38% reduction in *Panagrellus* sp. and complete elimination of *M. incognita* were observed with the active extract. In the soil simulation, the extract reduced nematode populations by up to 100%. In the greenhouse trial, a 93% reduction in *M. incognita* eggs in bean roots was noted, along with improvements in plant growth and an increase in pod number. This is the first study to report the nematicidal effect of garlic peel on these species, suggesting its potential for nematode control.

KEYWORDS
garlic peel, *Allium sativum*; nematicidal activity; proteases

1 | INTRODUCTION

United Nations (UN) estimates that population growth will increase the demand for food by 70% by 2050, and as a consequence, more agro-industrial waste will be produced.[1–3] Brazil stands out for its expressive agricultural production, being a major producer of garlic (*Allium sativum*), producing around 300 000 tons per year.[4] However, the high production is linked to the generation of a residue, garlic peel, which is commonly discarded improperly.

Garlic peels are an abundant and cost-effective source of enzymes, including proteases, which have potential applications in the biochemical control of pests, offering an environmentally friendly alternative to chemical pesticides. Despite this potential, the instability of these enzymes and challenges associated with large-scale extraction limit their practical use. Nonetheless, garlic peels present valuable material for research due to their rich enzymatic content.[5,6]

Enzymes are proteins that have catalytic action and are essential for numerous biochemical reactions. Such molecules can be applied in several areas, with emphasis on the chemical industry. In this context, we highlight protease, one of the most commercialized enzymes worldwide.[7,8]

Proteases are enzymes of the hydrolases class (EC 3.4) whose function is to catalyze the hydrolysis of peptide bonds in proteins.[9]

These enzymes are produced by plants, animals, and microorganisms and are indispensable for any organism. They act in the synthesis of biomolecules that control the size, shape, and composition of fundamental proteins.[10]

The hydrolysis of peptide bonds in the proteins of nematode cuticles, catalyzed by protease, results in cuticle degradation and the death of the organisms.[11,12] Thus, this enzyme plays a crucial role in the process of cuticle destruction in nematodes. It is important to highlight that proteases also play a key role in regulating the life of insects and agricultural pests and have the potential to be used as biopesticides.[13,14]

Nematodes are parasitic organisms that cause significant problems in agriculture, infecting plants and causing reduced productivity.[15,16] The most used control method against nematodes is the application of chemical products to the soil, known as nematicides. However, the use of agrochemicals has been questioned due to their adverse effects on human health and the environment.[17,18] Therefore, there is a need for nematode control to be carried out in alternative and efficient ways that cause less environmental impact, have a low toxicological risk, but, at the same time, are economically viable.[19,20]

In this study, the nematodes *Panagrellus* sp. and *Meloidogyne incognita* were chosen due to the distinct characteristics that both present. The free-living nematodes *Panagrellus* sp. are widely used as an experimental model, making them ideal for evaluating enzymatic action under controlled conditions.[21] On the other hand, *Meloidogyne incognita* is a nematode with a significant economic impact on agriculture, responsible for causing substantial damage to plants[22]

Research in this area is important as it seeks to reduce the environmental impact caused by pesticides, offering a biochemically based option, still little discussed, for pest control and contributing to more sustainable agricultural practices. The objective of this study was, therefore, to investigate the action of proteases present in garlic peel on these two species, using different in vitro and in vivo assays.

2 | MATERIALS AND METHODS

2.1 | Preparation of the enzymatic extract

Garlic peels (*Allium sativum*) were kindly provided by BBC Agrícola, located in São Gotardo, Minas Gerais, Brazil. The extract was prepared according to the methodology of Ferreira et al.[23] Five grams of garlic peels were crushed and suspended in 75 mL of distilled water. The mixture was then taken to a rotary shaker and remained under stirring for 12 h. The extract was then filtered and centrifuged at 10,000 g for 15 min. The supernatant was collected and named crude extract.

2.2 | Protease activity assay

Proteolytic activity was determined according to the methodology proposed by Braga et al.[24] with some modifications. In this experiment, 100 µL of extract were incubated at 50 °C in the presence of 500 µL of 1% gelatin (w/v) and 400 µL of 50 mM Tris–HCl buffer (pH 7.00) for 1 h. The reaction was stopped by adding 1 mL of 10% (w/v) trichloroacetic acid (TCA) solution. A reaction zero was prepared under the same conditions as the enzymatic assay; however, the reaction was stopped by TCA before the extract containing enzymes was inserted. Then, the samples were centrifuged at 10,000 g for 10 min, and the supernatant was used to read the absorbance at 280 nm. One protease unit was defined as the amount of enzyme required to release 1 µg of tyrosine per minute under the reaction conditions described above.

2.3 | Enzyme concentration

Proteases were precipitated by ammonium sulfate ($(NH_4)_2SO_4$). Initially, ammonium sulfate at a concentration of 70% (w/v) was added to the crude enzymatic extract from garlic peels, and after precipitation, the solutions were centrifuged at 10,000 g for 10 min. Sequentially, the precipitated extract was dialyzed against water for 24 h on a dialysis membrane (12 KDa, 1.3 inches wide, MWCO 11,331 Sigma-Aldrich).[23] The entire process of extract preparation and purification can be observed in Figure 1.

2.4 | In vitro nematicidal activity

The nematicidal effect of concentrated enzymes obtained from garlic peel extract was evaluated on juveniles of *Panagrellus* sp. and *Meloidogyne incognita* (J_2). The free-living nematode *Panagrellus* sp. was obtained commercially and cultured according to the methodology described by Sufiate et al.[25] *Meloidogyne incognita* was multiplied in tomato plants (*Solanum lycopersicum*), Santa Clara cultivar, grown in a greenhouse. Eggs were extracted from the tomato roots using the technique described by Hussey and Barker.[26] Second-stage juveniles (J2) were obtained using the Baermann funnel technique.[27] For the in vitro assays, J2 collected 48 h after the onset of incubation in the hatching chamber were used. Assays were carried out in three different groups in microtubes, according to the methodology of Braga et al.[28]

In the first group, control, 20 µL of distilled water and approximately 50 nematodes were used. In the second group, 20 µL of crude extract (precipitated and subsequently dialyzed, which underwent the process of denaturing its enzymes by heating at 100 °C for 2 h) and approximately 50 nematodes were used, and finally, the third group

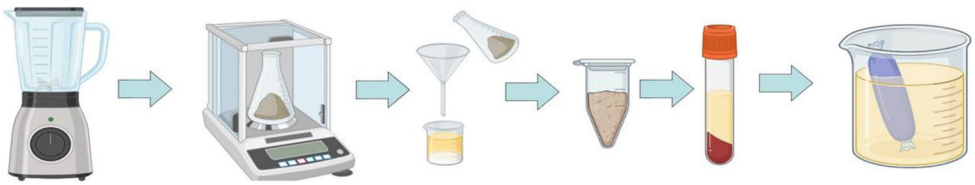

FIGURE 1 Schematic preparation of garlic peel extract rich in proteases.

contained 20 µL of active crude extract (precipitated and subsequently dialyzed) and approximately 50 nematodes. The described procedure was carried out on 50 juveniles (mixed culture) of Panagrellus sp. and 50 s stage juveniles (J_2) of *M. incognita*.

All groups were designed with 6 replicates per treatment, and the microtubes were kept at 25 °C for 24 h. After incubation, the amount of intact nematodes present was counted in each group using optical microscopy according to the methodology of Braga et al.[28] The whole experiment was repeated at least 2 times.

2.5 | Nematicidal activity under simulated soil conditions

The nematicidal effect of concentrated enzymes from garlic peel was studied on two nematode species: *Panagrellus* sp. and *Meloidogyne incognita* (J_2). The assays were conducted in Petri dishes containing 2 g of commercial substrate (Tropstrato HT Hortaliças®) to simulate soil conditions. Approximately 1000 juveniles were used for *Panagrellus* sp., while about 500 juveniles were used for *M. incognita* (J_2).

For both experiments, three groups were established. In the control group, 200 µL of distilled water was added to the juveniles. In the second group, the juveniles were exposed to 200 µL of crude extract, which had been precipitated, dialyzed, and subjected to enzyme denaturation by heating at 100 °C for 2 h. In the third group, the juveniles were treated with 200 µL of active crude extract, which was also precipitated and dialyzed, but without the denaturation process. Each group included 6 replicates per treatment.

The Petri dishes were maintained at 25 °C for 24 h. Following this period, nematode counting was performed after extraction using the Baermann method.[27] The entire experiment was repeated at least twice to ensure the reproducibility of the results.

2.6 | Nematicidal activity under greenhouse conditions

The experiment was conducted in the greenhouse of the Plant Pathology Department at UFLA (21°13'35.4″S and 44°58'31.5″W). The design employed was completely randomized. The treatments utilized were denatured extract and extract. All groups included 6 replications per treatment. Prior to the experiment, a germination and vigor assay of the seeds was performed to ensure the appropriate number of seeds was sown per pot, following the seed analysis rule (RAS).[29]

For the implementation of the experiment, 12 pots of 0.8 dm^3 containing commercial substrate (Tropstrato HT Hortaliças®) were used. The sowing of the experiment was conducted manually, with 3 seeds of bean (*Phaseolus vulgaris*) from the BRS Pérola cultivar per pot. After germination, thinning was performed, leaving only one plant per pot. The sowing fertilization was carried out according to Alvarez et al.[30] to meet all the nutritional requirements of the crop and avoid interference from nutrient deficiency on the product results.

After thinning, a solution containing 1000 eggs of *M. incognita* was added to each pot near the plant's roots, followed by the inoculation of 1 mL of active extract and 1 mL of denatured extract. Crop management was performed as needed.[31] Irrigation was carried out daily to maintain the substrate moisture at its field capacity, avoiding water shortage or excess.

2.6.1 | Plant analysis

All subsequent evaluations were conducted after 60 days of the experiment.

Total pod count

The pods present on each plant were counted individually and manually.

Measurement of bean plant height

Using a measuring tape, the height of each plant was measured from the apical meristem of the aerial part to the apex of the root system (cm).

2.6.2 | Extraction of *M. incognita* eggs

Eggs of *M. incognita* were extracted from the roots of the bean plants. These roots were washed and cut into small pieces. They were then blended for 30 s in a 0.5% (w/v) sodium hypochlorite solution. The resulting mixture of eggs and roots was washed with distilled water through two different mesh sieves. The roots were retained on the 200-mesh sieve, while the eggs were retained on the 500-mesh sieve, following the method described by Hussey and Barker.[32] After extraction, the egg count was performed using a bench-top optical microscope.

2.7 | Statistical analysis

The data were statistically analyzed through analysis of variance (ANOVA), with significance levels of 1% to 5%, followed by Tukey's mean comparison test for all parameters (Length of the Root System to the Apex, Total Pod Count, Juveniles of *Panagrellus* sp., J2 of *M. incognita*, and eggs of *M. incognita*). The mean percentage reduction was calculated for each parameter (juveniles of *Panagrellus* sp., J_2, and eggs of *M. incognita*) using the following formula:

$$\%Reduction = \frac{(Control\ Mean - Treatment\ Mean)}{Control\ Mean} \times 100$$

3 | RESULTS

3.1 | Enzyme concentration

After the concentration of the crude extract showed the highest proteolytic activity (6.43 U/mL), the second concentration step was dialysis, and as can be seen in Table 1, the best specific activity results were obtained after this process.

TABLE 1 Concentration of garlic (*Allium sativum*) peel proteases.

Concentration Steps	Activity (U/mL)	Protein (mg/mL)	Specific activity (U/mg)	Yied (%)	Purification (fold)
Crude Extract	4.39	0.74	5.92	100	1
Precipitation	6.43	0.64	9.98	146.6	1.7
Dialyzed extract	7.32	0.51	14.32	166.8	2.4

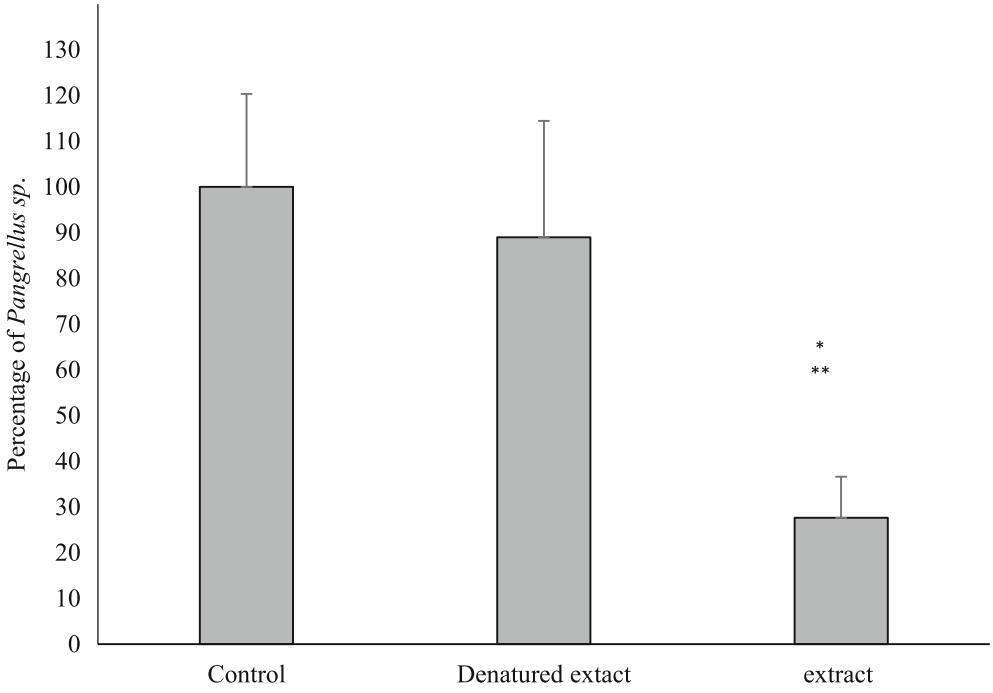

FIGURE 2 Average percentages of juveniles of *Panagrellus* sp. in the 2 groups (denatured extract and active extract) and in the control group (containing only distilled water and *Panagrellus* sp.) after incubation for 24 h at 25 °C. The group represented with an asterisk (*) showed a significant difference ($p < 0.05$) in relation to the control group (Tukey's test), while the representation with two asterisks (**) means that there was a statistically significant difference ($p < 0.05$) between the denatured extract and the active extract.

3.2 | In vitro nematicidal activity on *Panagrellus* sp.

Results show (Figure 2) that there was a significant difference in the number of juveniles of P*anagrellus* sp. between the group with active enzymes, the group with denatured enzymes, and the control ($p < 0.05$). On the other hand, there was no significant difference between the control group and the denatured group. This strongly suggests that the nematicidal action was due to enzyme activity. The average reduction percentages of *Panagrellus* sp. in the experimental groups, in comparison with the control, were 10% for the denatured extract and 72.38% for the extract (Figure 2).

The percentage reduction is associated with the fact that the protease acts on the digestion of the nematode cuticle, which can be seen in Figure 3.

3.3 | In vitro nematicidal activity on *Meloidogyne incognita*

Figure 4 presents the results regarding the in vitro nematicidal activity on *Meloidogyne incognita*. The results show that there was nematicidal activity of the crude extract (precipitated and subsequently dialyzed) in the assay with *M. incognita*, with a significant difference between the group with active enzymes, the group with denatured enzymes, and the control ($p < 0.05$). On the other hand, as in the assay with *Panagrellus* sp., there was no significant difference between the control group and the denatured group. Once again, this result strongly suggests that the nematicidal action was due to enzyme activity. Furthermore, it suggests that proteases affect the cuticles of different nematodes. The average percentages of reduction of *M. incognita* (J_2) in the experimental groups, in comparison with the control, were 56.73% for the denatured extract and 100% for the active extract (Figure 4).

Figure 5 illustrates a second-stage juvenile (J_2) of *M. incognita* degraded during the in vitro experiment by the action of garlic peel proteases, reinforcing the obtained results and highlighting the nematicidal effect of the active proteases in the extract.

3.4 | Nematicidal activity on *Panagrellus* sp. under simulated soil conditions

There was a nematicidal activity of the active crude extract (precipitated and later active dialysate) on *Panagrellus* sp. juveniles, with a significant difference between the group with active enzymes, the group with denatured enzymes, and the control ($p < 0.05$). The average

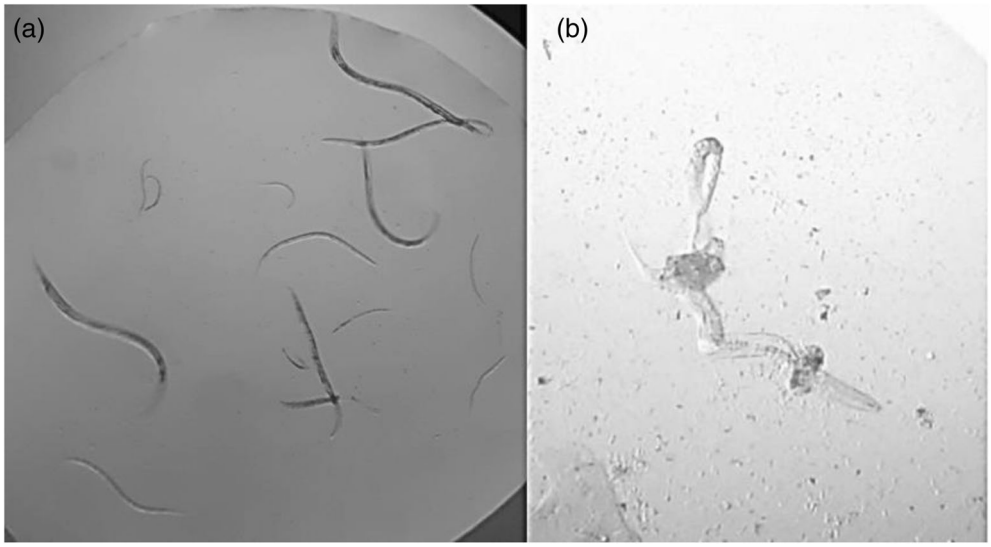

FIGURE 3 (a) *Panagrellus* sp. juveniles in the control group. (b) Garlic peel extract proteases degrading the cuticle of the free-living nematode *Panagrellus* sp.

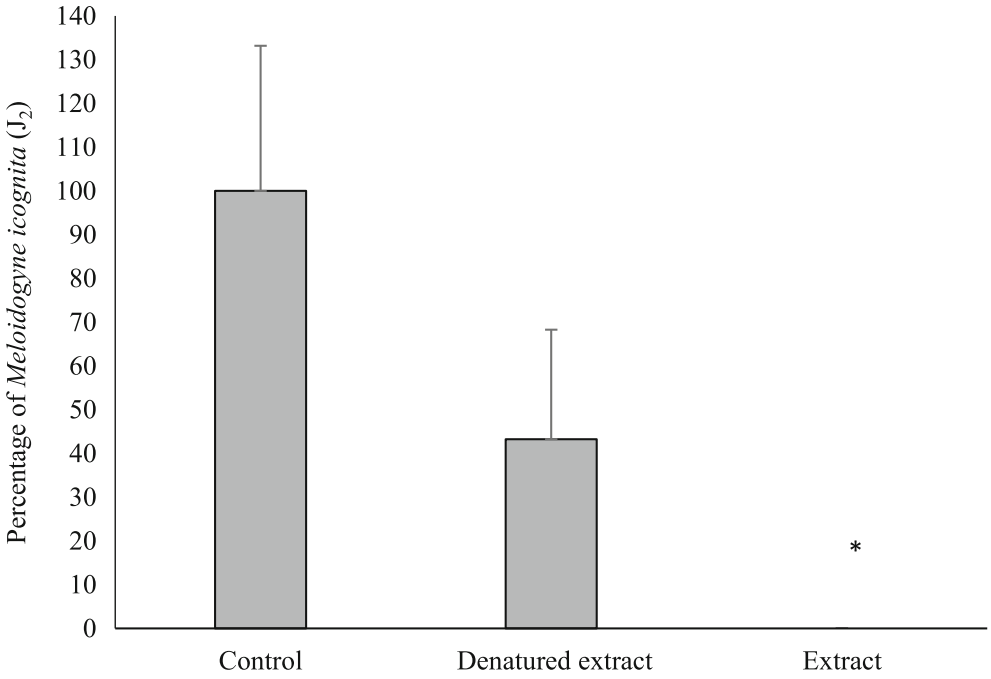

FIGURE 4 Average percentages of juveniles of *Meloidogyne incognita* (J_2) in the 2 groups (denatured extract and active extract) and in the control group (containing only distilled water and J_2) after incubation for 24 h at 25 °C. The group represented with an asterisk (*) showed a significant difference ($p < 0.05$) in relation to the control group according to the Tukey test.

percentages of reduction after recovery of *Panagrellus* sp., in relation to the control, were 0.77% for the denatured extract and 45.45% for the active extract (Figure 6).

3.4.1 | Nematicidal activity on *M. incognita* under simulated soil conditions

There was a significant nematicidal activity of the active crude extract (precipitated and later active dialysate) on *M. incognita* (J_2), with a significant difference between the group with active enzymes, the group with denatured enzymes, and the control ($p < 0.05$). The average percentages of reduction after recovery of *M. incognita*, in relation to the control, were 57.9% for the denatured extract and 100% for the active extract (Figure 7).

3.4.2 | Length of the root system to the apex

A significant difference in plant growth was observed between those treated with denatured extract and those that received garlic peel extract with active enzymes. The plants treated with the denatured extract exhibited an average length that was 10.9 cm shorter compared to those that received the garlic peel extract with active enzymes. Thus, it is evident that the plants treated with the active extract experienced superior growth, as shown in Table 2.

3.4.3 | Total pod count

The plants treated with the denatured extract produced an average of 11.8 pods, while those receiving the garlic peel extract with active

enzymes produced approximately 15.8 pods (Table 2). This demonstrates that treatment with the active extract resulted in 4 additional pods compared to the denatured extract, indicating an improvement in production yield.

3.4.4 | Egg extraction

The in vivo results demonstrated a nematicidal activity of the active crude extract on *M. incognita*, evidenced by a significant reduction in the number of eggs after the application of the active extract compared to the control group of denatured enzymes ($p < 0.05$). The reduction was 93.52%, confirming the efficacy of the active extract.

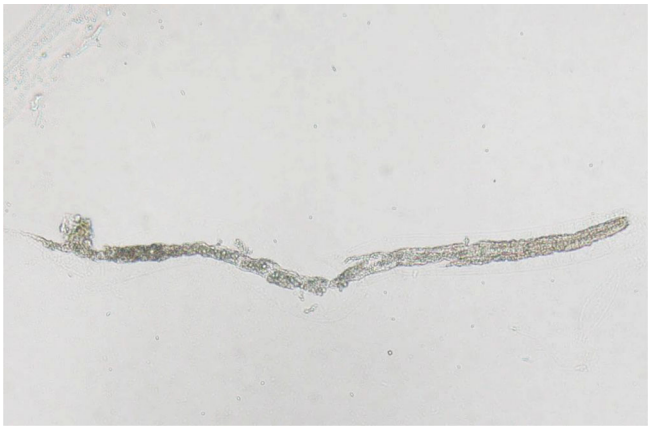

FIGURE 5 Second-stage juvenile of *Meloidogyne incognita* completely degraded by the action of garlic peel proteases.

4 | DISCUSSION

A study conducted by Singiri et al.[5] investigated the biochemical and biological properties of garlic peel, revealing the presence of 67 proteins. Among the identified proteins, proteases, known for their role in protein modification and degradation, proved particularly relevant.

Proteases play a fundamental role in various biological processes, including nematode control. These enzymes catalyze the breakdown of peptide bonds in proteins, resulting in the degradation of the parasites' cuticle. The cuticle, primarily composed of proteins and collagen, is responsible for maintaining structural integrity and protecting the nematode against external threats. When degraded by proteases, this protective layer is weakened, making nematodes significantly more vulnerable to environmental and biological factors.[33] The proteases found in garlic peel have shown significant potential in this context, proving effective in inhibiting and controlling nematodes, thereby offering a promising approach for the biochemical management of pests in crops.[23]

The crude extract of garlic peel showed protease activity of 4.39 U/mL, as shown in Table 1. After precipitation, this value was 6.43 U/mL, and later with dialysis, this result increased to 7.32 U/mL. Thus, there was a concentration of proteases contained in the extract after the processes were carried out. At the same time, the specific activity increased following the same trend, with the dialyzed extract having the highest value and the highest recovery rate.

The analysis of the percentage reduction of nematodes in the in vitro test in microtubes showed high efficiency of the proteases present in the garlic peel, with a 100% reduction of *M. incognita* in relation to the control (Figure 3). These results are in line with the work by Soliman et al.,[34] in which the researchers found a 100%

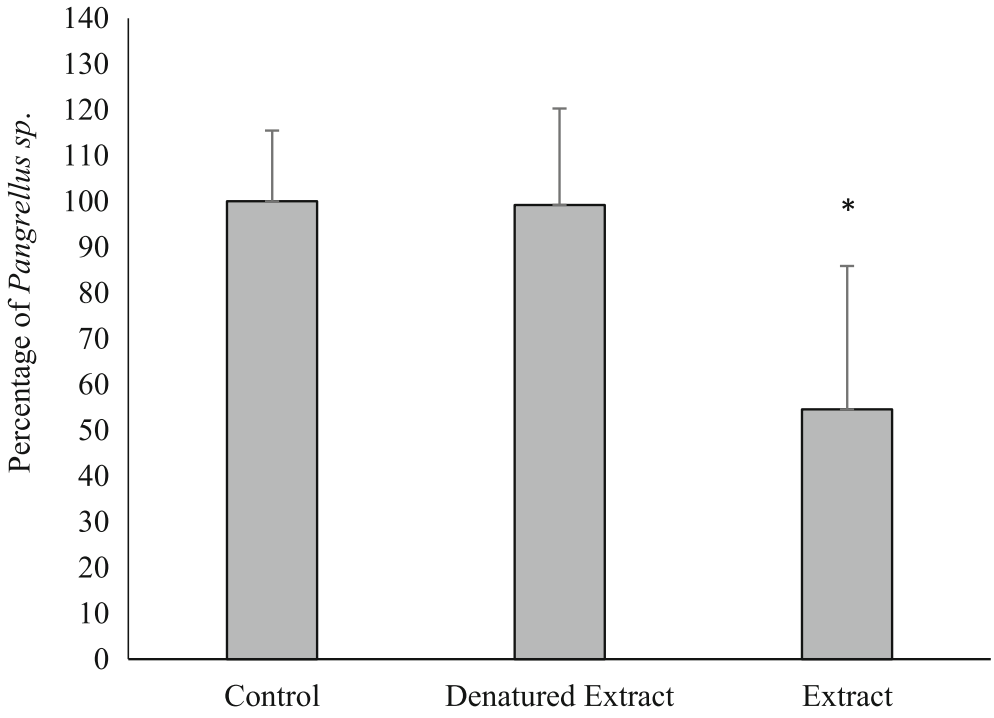

FIGURE 6 Average percentages of recovered juveniles of *Panagrellus* sp. in the 2 groups (denatured extract and active extract) and in the control group (containing only distilled water and *Panagrellus* sp.) after incubation for 24 h at 25 °C. The group represented with an asterisk (*) showed a significant difference ($p < 0.05$) in relation to the control group according to the Tukey test.

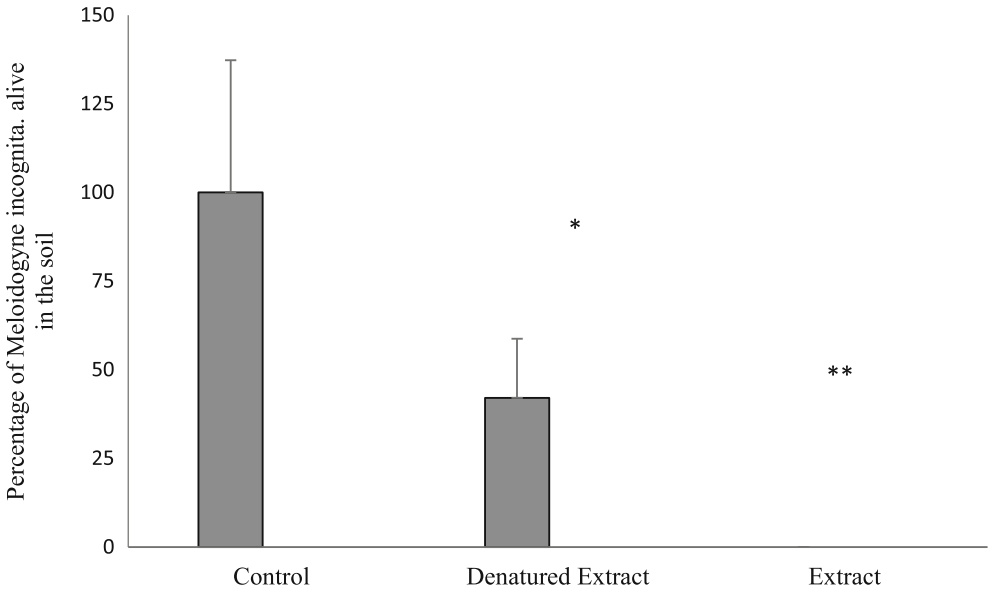

FIGURE 7 Average percentages of *Meloidogyne incognita* (J_2) in the 2 groups (denatured extract and active extract) and in the control group (containing only distilled water and *Meloidogyne incognita*.) after incubation for 24 h at 25 °C. The group represented with an asterisk (*) showed a significant difference ($p < 0.05$) in relation to the control group (Tukey's test), while the representation with two asterisks (**) means that there was a statistically significant difference ($p < 0.05$) between the denatured extract and the active extract.

TABLE 2 Length of the root system to the apex of the aerial part meristem of bean plants in the experiment with the application of garlic peel extract and bean plants with denatured extract.

Treatment	Denatured Extract	Extract	CV (%)
Length of root system to apex	80.9 b	91.8 a	7.89
Total number of pods	11.8 b	15.8 a	19.88

reduction in the same nematodes but using proteases produced by the bacterium *Paenibacillus polymyxa*. In this context, Afzal et al.[35] evaluated aqueous extracts of *Amaranthus viridis*, *Chenopodium album*, *Solanum nigrum*, *Carica papaya*, and *Euphorbia hirta* on *Meloidogyne* spp., and all selected plant species caused mortality, with a maximum reduction of 33% obtained with the extract of leaves of *C. album*.

Using the crude latex extract of *Euphorbia trigona* as research material, Sufiate et al.[36] obtained a 96% reduction in the number of live juveniles of *M. incognita*. This result was due to the presence of three proteases present in this latex, a result close to that found in the present study. Also, in relation to plant extracts rich in protease, Filgueiras et al.[37] evaluated the action of latex from the fruit *Carica papaya* L. on larvae and females of the tick *Rhipicephalus microplus*. The authors observed 92.36% (100 mg/mL) of tick control, being the first study that associated the acaricidal effect with the proteolytic activity of vegetable latex on the tick cuticle.

Still, according to Figure 1, the reduction obtained for Panagrellus sp. was 72% compared to the control, which is higher than the result found by Lubian et al.[34] Lubian et al.[38] observed a 66% reduction in the number of *P. redivivus* in vitro using *C. papaya* extract, as well as observed that plant extracts of *E. milii*, *Psychotria carthagenensis*, *Clusia variegate*, and *Zamioculcas zamiifolia* reduced by 37.0%, 25.0%, 22.0%, and 21.5%, respectively, the number of *P. redivivus* compared to the control group. In another interesting research, Gomes et al.[39] obtained similar results to the present study, in which there was a 72% reduction in the number of *P. redivivus*. However, in that study, latex from the *Synadenium grantii* plant was used. This result was due to the proteolytic action presented by the latex.

In the present study, the authors sought to mimic the conditions closest to those found in the original soil. Thus, the nematicidal assay, whose results are shown in Figure 4, was carried out in Petri dishes containing an environment similar to the soil, and the results showed a 45% reduction in *Panagrellus* sp. However, it is worth noting that the conditions were close to natural, requiring further studies to be carried out in the future.

A study conducted by Krif et al.[40] analyzed four bionematicides for efficacy against *M. javanica* in tomato plants in a greenhouse. One of the evaluated bionematicides was the aqueous extract of nettle and horsetail. This aqueous extract of nettle and horsetail reduced the population of second-stage juveniles (J_2) by 11.84%, a value lower than that obtained in this article from the extract produced from garlic peel, demonstrating that the extract from garlic peel is considerably promising.

We observed after the completion of the greenhouse experiments that there was overall greater growth in the plants, both in the root system and in the aerial part of the plants treated with the protease-rich extract obtained from garlic peels. This vigorous development allows the bean plants to better withstand stress conditions such as diseases, pests, water deficits, and high temperatures, among other environmental challenges.[41]

Increased growth may be directly related to the effective control of nematode infestation and multiplication in the soil, enabling plants to absorb better water, nutrients, and oxygen.[42] This beneficial effect is reflected in the increased number of pods, a crucial component for high bean productivity, as there is a strong correlation between the number of pods and final yield.[43]

The main components influencing bean production include the number of plants per meter, the number of pods per plant, the number of seeds per pod, and the weight of the seeds, which determine the yield per hectare.[44] Among these components, the total number of

pods is significant for producers seeking high productivity, as it directly affects the weight of the seeds that will develop in the subsequent stages of the bean cycle.

The results obtained in this study, which demonstrate a reduction in the number of nematode eggs due to the enzymes, are consistent with the work conducted by Asaturova et al.[45] with *Bacillus velezensis* strains BZR 86 and BZR 277 for the control of *M. incognita* in greenhouses. The evaluated bacteria demonstrated significant nematicidal activity, resulting in up to a 76% reduction in nematode infestation compared to the control group. Furthermore, the treatments led to increases in plant height, leaf area, and root mass, along with improvements in yield. Those results suggest that *B. velezensis* may be a promising basis for the development of bionematicides. The authors also highlight that the nematicidal mechanism of action of these bacteria is often associated with the synthesis of extracellular enzymes such as proteases, lipases, and chitinases. These enzymes can compromise the physical and physiological integrity of the eggs and cuticle of nematodes, demonstrating the ability to damage the egg membrane. On the other hand, this study shows that garlic peel-derived proteases have a nematicidal action, even without the presence of cells.

Similarly, endophytic bacteria have also the potential to control *Meloidogyne* spp., with pivotal enzymatic action.[46] For example, the study conducted by Tran et al.[47] investigated the effectiveness of endophytic bacteria in controlling *Meloidogyne* spp. in black pepper, highlighting that five *Bacillus* strains demonstrated 100% mortality of J2 nematodes. The strain *Bacillus megaterium* DS9 stood out, reducing infestation by 81.86% in the soil and 73.11% in the roots, in addition to promoting plant growth. The study also detected enzymatic activities such as chitinase and protease, which are associated with the biological control of *Meloidogyne* sp. The results indicate that these strains of endophytic bacteria have the potential as promising candidates for biological control of *Meloidogyne* sp.

In this sense, the study by Youssef et al.[48] evaluated the nematicidal potential of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces lilacinus* against *M. incognita* using cowpea (insert species) in a greenhouse. The crude extract of *P. lilacinus* exhibited the highest efficacy, with an 84.5% reduction in the nematode population, followed by *B. bassiana* with 81.1% and *M. anisopliae* with 78.5%. When applied as spores, *P. lilacinus* achieved a reduction of 85.3%, while *M. anisopliae* and *B. bassiana* reduced by 83.6% and 77.1%, respectively. In addition to controlling nematodes, all treatments promoted plant growth, indicating that these antagonistic fungi are promising alternatives for nematode management in sustainable agricultural systems. Also, de Paula et al.[49] demonstrated that papaya latex and pure papain lead to *Meloidogyne javanica* J2 mortality. Latex reached 100% mortality at the highest concentration (1%). Similarly, in this study, we obtained a 93% reduction in *M. incognita* eggs.

5 | CONCLUSION

This is the first study to report the nematicidal effect of enzymes extracted from garlic peels on *Panagrellus* sp. and *M. incognita*. Nematicidal action was mainly observed in the in vitro assays, where a significant reduction in nematodes was found. Additionally, a beneficial effect on plant growth was noted, with a 93% reduction in *M. incognita* eggs in plant roots in the greenhouse environment, indicating that these enzymes not only contribute to nematode control but also promote healthier plant development. The extract demonstrated greater efficacy in reducing *M. incognita* in vitro and decreasing its eggs, making it a promising alternative for nematode management. Therefore, further studies are recommended to refine the application of these enzymes, with the goal of developing innovative products that can contribute to sustainable nematode control.

AUTHOR CONTRIBUTIONS

Conceptualization, Cecilia Baldoino Ferreira and Filippe Elias de Freitas Soares; Methodology, Cecilia Baldoino Ferreira; Micaela Rodrigues de Sousa and Filippe Elias de Freitas Soares; Investigation, Cecilia Baldoino Ferreira; Paulo Henrique Frois Correa Barros; Ruth Celestina Condori Mamani; Data Curation, Carlos Henrique Milagres Ribeiro and Ana Carolina Silva and Julia Carvalho Araújo; Writing – Original Preparation of Drafts, Cecilia Baldoino Ferreira; Writing – Proofreading and Editing, Filippe Elias de Freitas Soares and Cecilia Baldoino Ferreira; All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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