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## Promising nematocidal efficacy of *Verticillium lecanii*, hydrogen peroxide, and melithorin® against root-knot nematode, *Meloidogyne incognita* in the tomato plant

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Plant parasitic nematodes are dangerous pests for many crops including tomatoes. In this work, *in-vitro* and *in-vivo* nematocidal properties of the fungus *Verticillium lecanii* and hydrogen peroxide were compared to the commercial nematicide, melithorin® (90% Fosthiazate) were assessed against root-knot nematode *Meloidogyne incognita*. Results indicated that the application of melithorin® (1ml/L) recorded the highest nematode mortality 89.4% after 96 hours of *in-vitro* application, followed by hydrogen peroxide (150 Mm) and *Verticillium lecanii* (100%) respectively after the same time of application recording 33.7% and 22.1% mortality respectively. In the greenhouse trial, melithorin® recorded the highest reduction in nematode galls, egg masses, females, development stages, and 2<sup>nd</sup> juveniles by (86.15%, 93.89%, 67.81%, 73.22%, and 79.48%) respectively, followed by *Verticillium lecanii* recorded (44.72%, 44.02%, 43.12%, 51.48% and 34.54%) and came next hydrogen peroxide (29.96%, 27.98%, 22.5%, 37.75% and 29.09%) respectively. Infection alleviation was investigated by measuring infected tomato plants' plant growth parameters, photosynthetic pigments, antioxidant enzymes, total proteins, free proline, total phenols, malonaldehyde (MDA), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) contents. Results indicated that the application of melithorin® followed by hydrogen peroxide, then *Verticillium lecanii* showed significant improvements in tomato vegetative growth and biochemical markers. Therefore, it is possible to consider *Verticillium lecanii* and hydrogen peroxide as cheap and efficient nematocidal treatments.

**Keywords:** Nematocidal, *Verticillium lecanii*, mesothelin®, Fosthiazate, H<sub>2</sub>O<sub>2</sub>, and Tomato

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## INTRODUCTION

Plant-parasitic nematodes are considered one of the biggest threats to the production of various crops, including tomatoes. Plant nematodes decrease both yield quality and quantity and can cause up to 60% yield loss (Phani et al., 2021). *Meloidogyne incognita* (MI) is considered one of the most significant species of root-knot nematodes, causing knots in infected roots (Asaturova et al., 2022). Tomato is one of the most consumed fruits, and more than seven billion people all over the world live on it as food. It constitutes about 15% of all vegetables and fruits, with an annual per capita consumption of 20 kilograms (Asaturova et al., 2022). Tomato contains numerous bioactive compounds such as carotenoids, flavonoids, vitamins, and glycoalkaloids (Asaturova et al., 2022). Tomato is susceptible to several harmful nematodes living in different types of soil (Valenzuela et al., 1995). There are multiple methods for controlling plant-parasitic nematodes, including chemical and biological control. Chemical nematicides can significantly reduce nematode numbers in a short period (Barker and Olthof, 1976; Ghorbani et al., 2008). The traditional methods of MI control, which often rely heavily on the use of chemical nematicides, have several disadvantages, including environmental pollution, contamination of soil and water resources, and harm to non-target organisms, including beneficial insects and wildlife (Sikora et al., 2005).

Fosthiazate, the active component of melithorin®, is an organophosphate nematicide used to control plant-parasitic nematodes (Liu et al., 2024). Fosthiazate is known for its effectiveness against a wide range of nematode species, including MI (Saleh, et al., 2022). Exposure to chemical nematocidal residues can pose health risks to farmers, farm workers, and consumers. Repeated use of the same pesticides can lead to the development of resistance in plant pathogens (Hashem et al., 2021; Abdelaziz et al., 2022). On the other hand, the advantages of biological control are its environmental friendliness, targeted pest management, long-term effectiveness, and alignment with sustainable agriculture practices, all while posing lower risks to human health compared to chemical pesticides (Ruberson et al., 1998; Hagler, 2000; Attia et al., 2023). Plant growth-promoting fungi (PGPF) are a diverse group of soil-borne fungi that establish beneficial interactions with plants in the rhizosphere (Yu et al., 2024; Sharma et al., 2024). These fungi can improve crop productivity through various direct and indirect mechanisms. PGPF has been shown to enhance seed germination, root and shoot morphogenesis, flowering, and overall plant growth and yield (Hossain and Sultana, 2020). However, traditional chemical nematicides have many limitations due to their harmful impacts on the environment, humans, and animals, in addition to their high costs (Czaja et al., 2015; Tudi et al., 2021).

Thus, biotic or abiotic alternatives seem appropriate, cheap, and safe for nematode control (Abd-Elgawad, 2020, 2021). Several studies have demonstrated the efficacy of microorganisms as biocontrol agents in reducing the severity of nematode infections and improving plant immunity (Sharaf et al., 2016; Waller, 1999). The fungus, *Verticillium lecanii*, has a wide range of suppressive activities on different types of nematodes (Chen and Dickson, 2004; Sharma et al., 2024). The fungus, *Verticillium lecanii*, showed a significant reduction in root and egg masses infecting tomato plants with MI (Regaieg et al., 2010). Melithorin®, a commercial chemical nematicide with an active compound called fosthiazate, has demonstrated high efficacy against different plant parasitic nematodes (Shalaby et al., 2021). Fosthiazate may be a part of the organophosphate course of pesticides or nematicides and is utilized to control nematode species on vegetables (Li et al., 2020). Hydrogen peroxide has been suggested to have nematocidal properties, potentially suppressing the activity or reproduction of nematodes in the soil (Karajeh, 2008). Hydrogen peroxide is involved in plant defense activation, and it induces the production of reactive oxygen species (ROS), which play a role in signaling pathways that trigger plant defense responses. The present study aims to evaluate the efficacy of *Verticillium lecanii* and hydrogen peroxide compared to the standard chemical nematicide, melithorin®, against MI in tomato plants.

## MATERIALS AND METHODS

### Tomato plant seedlings

Four weeks of tomato seedlings of the 032 variety (*Solanum Lycopersicon L.*) were sourced from the Agricultural Research Center (ARC), the Ministry of Agriculture in Giza, Egypt.

### Root-knot nematode inoculum

A nematode sample *Meloidogyne incognita* (MI) was isolated from infected tomato plants under greenhouse conditions. Eggs of MI were isolated from infected roots using NaOCl 0.5% as defined by Hussey and Barke (Nitao et al., 1999). The isolated eggs were kept in a flask including tap water till hatching. Finally, MI inoculums were fixed at about 20 juveniles/mL for the next experiments.

### Sources of treatments

*Verticillium lecanii* No:3411 was obtained from the Microbial Resource Centre, Cairo mircen. The nematicide melithorin® (90% Fosthiazate),

AGROBEST GROUP- Turkey. was obtained from AL-SALAM International for Development & Agriculture Investment in Egypt. Finally, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was obtained from Sigma Company.

### In vitro study

To determine the impact on the mortality of nematode juveniles, the freshly hatched juvenile suspension (20 juveniles/mL) and three milliliters from each treatment with its concentrations were incubated within sterile Petri dishes at four times (24, 48, 72, and 96 hours). For the control treatment, 3 mL of sterilized water was incubated with 3 mL of a juvenile suspension (20 juveniles/mL). Every treatment was replicated three times. The inactive juveniles were counted using a low-power stereomicroscope, and the mortality percentage was then calculated.

### In vivo study

Fifty tomato seedlings were planted in plastic pots containing autoclaved sandy clay soil by (1 seedling/pot), then divided into five groups in a complete randomized block design as follows: MI + *Verticillium lecanii*, MI + melithorin® (1ml/L), MI + hydrogen peroxide (120 Mm), MI only (positive control), Free of MI (negative control). MI was injected into soil 7 days after planting using 2000 juveniles/pot, while tested treatments were applied 7 days after nematode inoculation. Pots were maintained at 25 ± 5°C, with regular nutrient solutions and water addition as needed. After 60 days of MI injection, tomato plants were uprooted and the nematode galls, egg masses, females, and developmental stages in each 1 g root, as well as the 2<sup>nd</sup> stage juveniles in each 250 g soil, were counted using a light microscope, and the reduction percentages of those MI parameters were calculated in comparison with a positive control.

### Determination of immune responses

The morphological indicators (number of leaves and shoot and root lengths) were assayed. The method outlined by Schwartz and Lorenzo (1990) was used to quantify the amounts of carotenoids, chlorophyll a (Chl a), and chlorophyll b (Chl b) in fresh leaves. To extract photosynthetic pigments from fresh leaves (0.5 g), 50 mL of 80% acetone was used. The extract was filtered, and greenness at 665, 649, and 470 nm was measured. The plant materials used for the estimation of peroxidase (POD) and polyphenol oxidase (PPO) enzymes were the terminal buds in addition to the first and second young leaves. POD

and PPO activities were measured for antioxidant enzyme activities using the Hu and Waller techniques (Matta and Dimond, 1963; Hu, 1974). SOD and CAT activities were measured using a method described by Marklund and Marklund (1974) and Aebi (1984). To ascertain the dried shoot's soluble protein concentration, the method of Lowry et al. (1951) was used. Total dry shoot phenol content was measured using the Dai et al. procedure (1993). Free proline and phenol concentration in plants was altered in response to infection, and thus the content of free proline was assessed by the method of Bates et al. (1973). The method of Hu et al. (2004) was used to determine the amount of MDA in fresh pepper leaves. Fresh pepper leaves were also tested for hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content (Mukherjee and Choudhuri, 1983).

### Statistical analysis

The experimental data were analyzed using one-way analysis of variance (ANOVA) to determine if there were significant differences between the means. The differences between the means were further separated using the least significant difference (LSD) test at a 5% probability level. This analysis was conducted using Co-State software to identify the statistically significant differences between the means.

## RESULTS

### Nematode parameters

**In vitro study:** The data obtained for Table 1 demonstrated that MI mortality increases promptly with increasing therapeutic dosage and application duration, whereas the application of melithorin® (1 mL/L) was the most effective treatment raising MI mortality by 89.4% after 96 hours of application, followed by hydrogen peroxide (150 mM) and then *Verticillium lecanii* after the same time of application recording 33.7% and 22.1% mortality, respectively.

**In vivo study:** In this investigation, the obtained results in Figure (1) showed a significant decline in MI parameters in infected tomato seedlings after application of our tested treatments, especially melithorin® which recorded the highest reduction in MI parameters, including galls, egg masses, females, development stages, and 2<sup>nd</sup> juveniles by 86.15%, 93.89%, 67.81%, 73.22%, and 79.48%, respectively, followed by *Verticillium lecanii* which recorded 44.72%, 44.02%, 43.12%, 51.48%, and 34.54% and hydrogen peroxide recording 29.96%, 27.98%, 22.5%, 37.75%, and 29.09%, respectively.

### Plant growth parameters

Data shown in Figure 2 indicated that all growth parameters as shoot length, root length, and number of leaves of MI infected tomato plants were significantly decreased in comparison with the uninfected healthy control. The application of our elicitors significantly improved the infected tomato growth, especially melithorin® followed by *V. lecanii* and H<sub>2</sub>O<sub>2</sub>, respectively. Melithorin® showed an improvement in shoot lengths, root lengths, and the number of leaves by 96%, 97.3%, and 98.6%, while *V. lecanii* recorded 50%, 74.2%, and 60%, followed by H<sub>2</sub>O<sub>2</sub> which recorded 45%, 69%, and 80%, respectively.

### Photosynthetic pigments

Data in Figure 3 showed that MI infection caused significant decreases in chlorophyll a and chlorophyll b, in addition to significant increases in carotene contents. Regarding the effect of melithorin®, H<sub>2</sub>O<sub>2</sub>, and *Verticillium lecanii* on infected plants, it was found that all treatments alleviated reductions of chlorophyll a and chlorophyll b and enhanced the increases in carotene contents, whereas melithorin® was the highest elicitor alleviating values of chlorophyll a and chlorophyll b by 349% and 307%, followed by H<sub>2</sub>O<sub>2</sub> by 256% and 241% and then *Verticillium lecanii* by 224% and 186%, respectively. On the other hand, it was noticed that application of *Verticillium lecanii* and H<sub>2</sub>O<sub>2</sub> demonstrated the most significant increases in carotenoids by 23.28% and 11.64%, respectively.

### Antioxidant enzymes

The results obtained from Table 2 showed that MI infection triggered significant increases in POD, CAT, PPO, and SOD activities compared to healthy control plants. Moreover, all treatments enhanced these increases in enzymatic activities, whereas melithorin® exhibited the maximum activities of both POD and SOD recording 0.55 and 1.16 U/g F.wt./hr., respectively. Regarding CAT and PPO, the highest activities were achieved using H<sub>2</sub>O<sub>2</sub> which recorded 0.28 and 0.43 U/g F.wt./hr., respectively.

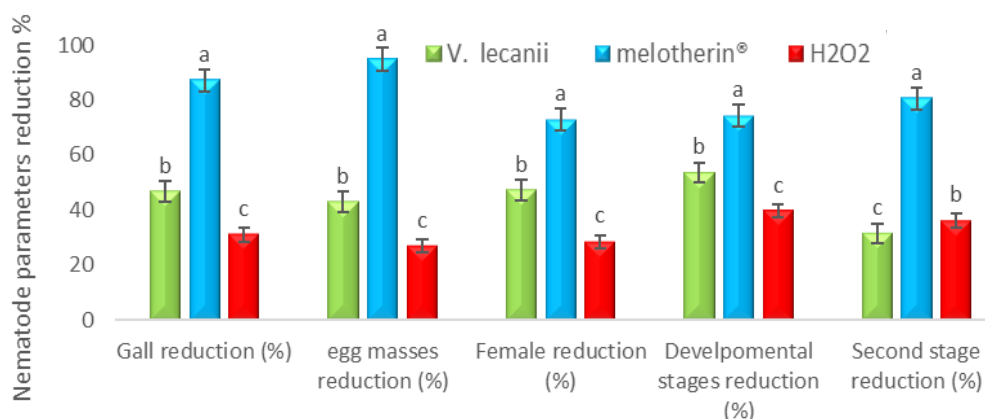
### Biochemical changes

Data generated in Figure 4A showed that protein contents were decreased by 58.98% in MI-infected plants compared to healthy controls and pronounced increases were observed after application of our treatments. Meanwhile, the highest level of proteins

**Table 1.** Effect of *V. lecanii*, melithorin®, and H<sub>2</sub>O<sub>2</sub> on MI juveniles' mortality *in vitro*.

Treatment	Conc.	Incubation period			
		24 hrs.	28 hrs.	72 hrs.	96 hrs.
		Mortality %	Mortality %	Mortality %	Mortality %
<i>V. lecanii</i>	25%	10.3±0.68 h	12.6±0.80 ef	15.4±0.42 h	19.4±0.59 h
	50%	12.8±0.64 g	14.6±0.82 ef	18.6±0.86 g	21±0.33 gh
	100%	14.3±0.65 f	15.6±0.17 e	19.3±0.86 fg	22.1±0.14 fg
melithorin®	0.25 ml/L	38.8±0.38 c	45±1.09 c	49.6±0.67 c	57±0.51 c
	0.5 ml/L	51.3±0.29 b	62.4±4.51 b	64.9±1.20 b	76±1.03 b
	1 ml/L	74.6±0.44 a	78.5±1.00 a	83.2±0.57 a	89.4±0.62 a
H <sub>2</sub> O <sub>2</sub>	50 mM	12.4±0.61 g	15.1±0.59 ef	21.4±0.34 f	23.4±0.58 f
	100 mM	16.6±0.37 e	20.9±0.33 d	26.1±0.92 e	29.3±0.41 e
	150 mM	21.7±0.48 d	25.3±0.92 d	30.6±0.74 d	33.7±0.59 d
Control		6.5±0.51 i	10.6±0.67 f	14.5±0.71 h	17.4±0.39 i
LSD 5%		1.55	4.73	2.27	1.68

Data represents mean ± SD, n=3), letters referred to as significant in static analysis). using the least significant difference (LSD) test at a 5% probability level.



**Figure 1.** Impact of *V. lecanii*, melithorin®, and H<sub>2</sub>O<sub>2</sub> on reducing MI parameters *in vivo*. Data represent mean ± SD, n = 3), letters referred to as significant in static analysis). using the least significant difference (LSD) test at a 5% probability level.

was achieved using melithorin® followed by *Verticillium lecanii* and H<sub>2</sub>O<sub>2</sub> which recorded 113%, 64.76%, and 59.37% increases, respectively, compared to infected control plants.

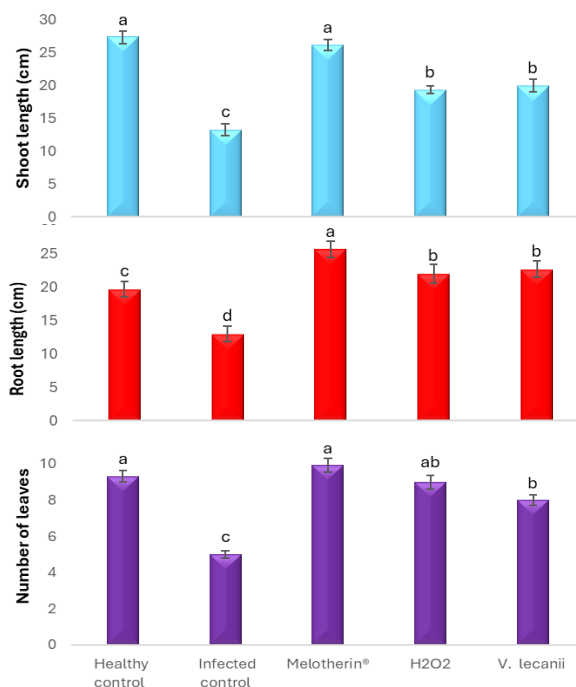
Results in Figure 4B showed that the content of free proline increased by 76.18% in MI-infected plants compared to the healthy controls. Application of all tested treatments enhanced these increases compared to the infected controls. The highest proline level was obtained using melithorin® followed by H<sub>2</sub>O<sub>2</sub> and *Verticillium lecanii* which recorded 135.14%, 29.73%, and 22.97% increases, respectively. Data in Figure 4C showed that phenol content levels increased by 119% in the nematode-infected plants compared to the healthy control, and more increases were pronounced after the application of melithorin®, *Verticillium lecanii*, and H<sub>2</sub>O<sub>2</sub>. The highest phenol level was recorded with *Verticillium lecanii* (48.1%) followed by H<sub>2</sub>O<sub>2</sub> (15.8%) and then melithorin® (10.1%).

### Stress marker

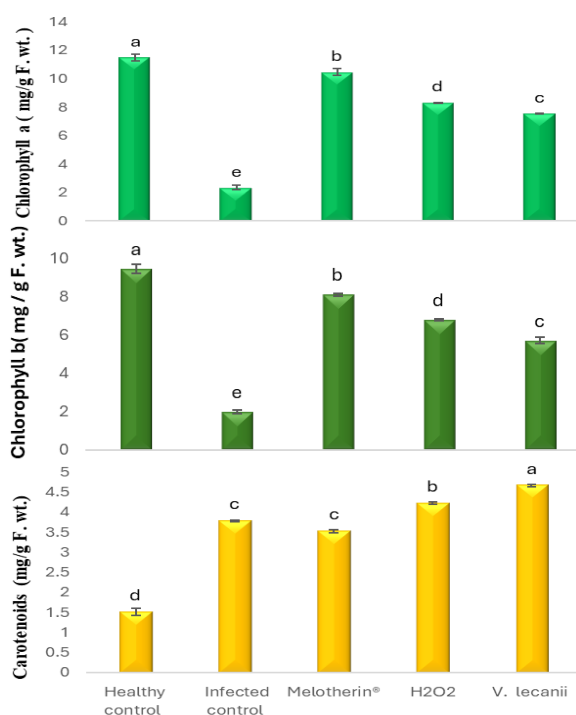
Data in Figure 5 showed that MI infection generated significant increases in MDA as well as H<sub>2</sub>O<sub>2</sub> contents of 138% and 205%, respectively, compared to healthy control plants. Moreover, all treatments alleviated these increases in stress markers. *Verticillium lecanii* was the most effective treatment alleviating increases in MDA by 16.6%, while melithorin® was the best treatment alleviating increases in H<sub>2</sub>O<sub>2</sub> by 35.4%.

### DISCUSSION

The problem of nematode infection affects many crops and has captured the attention of scientists hoping to control it. Scientists have been exploring the use of plant-growth microorganisms as alternatives to chemical nematicides for managing nematode infestations (Attia et al., 2021). In this study, we included melithorin® as a treatment due to its well-documented efficacy in controlling nematode populations in various agricultural settings.



**Figure 2.** Effect of *V. lecanii*, melithorin®, and H<sub>2</sub>O<sub>2</sub> on growth parameters of tomato plants infected with MI. Data represent SD, n=3), letters referred to as significant in static analysis). using the least significant difference (LSD) test at a 5% probability level.

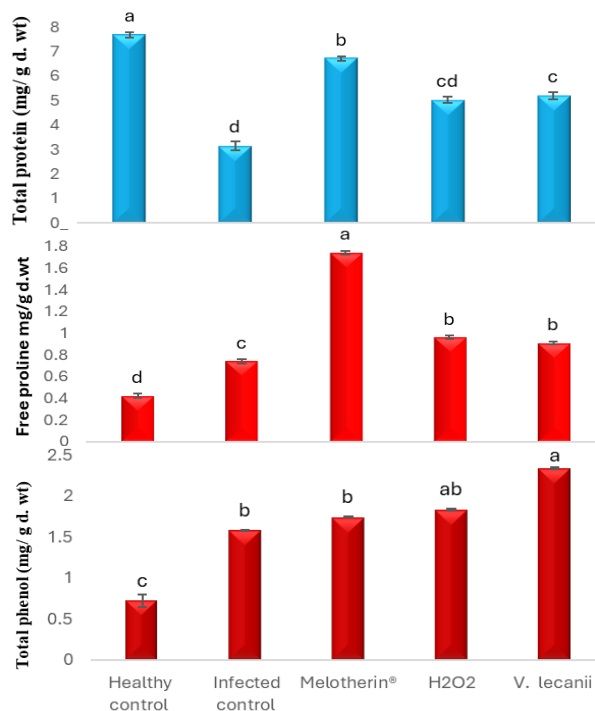


**Figure 3.** Effect of the tested elicitors on chlorophyll a, chlorophyll b, and carotene contents of tomato plants infected with MI. Data represent mean  $\pm$  SD, n=3), letters referred to as significant in static analysis).

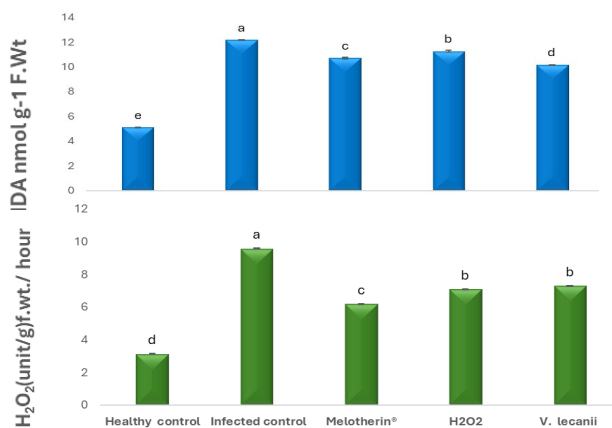
We aimed to determine if the *Verticillium lecanii* could provide a competitive or potentially greater benefit to chemical control strategies. In addition, melithorin® acts as a control to exhibit the validity of our experimental design and the reliability of our findings. The obtained results in the current study appeared at all applied concentrations (1 mL/L, 0.5 mL/L, and 0.25 mL/L). Our results also showed that the application of melithorin® (1 mL/L) was the most effective treatment raising MI mortality by 89.4% after 96 hours of application, followed by hydrogen peroxide (150 mM) and then *Verticillium lecanii* after the same time of application recording 33.7% and 22.1% mortality, respectively. Our results confirm the fact that melithorin® has a very strong effect on the death of nematodes, as it works to inhibit the nematode's nervous system (Luo et al., 2023). Melithorin® targets nematode pests by inhibiting acetylcholinesterase and blocking nerve impulse conduction. Fosthiazate (Woods et al., 1999; Huang et al., 2016; Wu et al., 2019) describes systemic phosphorous nematicides that affect the central nervous system of nematodes, with a distinction between the elimination of all stages of nematodes, wherein the current results are consistent with several studies demonstrating the effectiveness of the pesticide. This is due to the toxic effect of the substance (fosthiazate) and its effect on the nervous system of the nematode, paralyzing its movement and causing death (Yue et al., 2020). Also, the H<sub>2</sub>O<sub>2</sub> effect may be due to its toxicity on nematode cells, causing oxidative explosions and leading to rapid death (Zhou et al., 2018). The effect of *V. lecanii* is due to its production of many toxic substances that cause physiological disorders within the nematode and lead to its secret death (Bamisile et al., 2022). *Verticillium lecanii* produces several hydrolytic enzymes, particularly chitinases, proteases, and lipases, which are vital for degrading the nematode cuticle (Upadhyay et al., 2014). This enzymatic attack facilitates fungal penetration into the nematode body, leading to internal colonization and eventual death of the nematode (Rahman et al., 2023). Researchers have lately started employing bioinducers as nematode insecticides. The choice of *V. lecanii* was based on the ability to produce HCN, IAA, and siderophores in this study, which has been demonstrated by many previous studies (ul Islam et al., 2023). Due to its severe injurious effects on pathogens, HCN has been classified as a biological resistance agent (Aebi, 1984; Attia et al., 2020).

**Table 2.** Effect of the tested elicitors on antioxidant enzymes of tomato plants infected with MI. Data represent mean ± SD, n = 3), letters referred to as significant in statically analysis).

Treatment	POD (U/g F.wt./hr.)	CAT (U/g F.wt./hr.)	PPO (U/g F.wt./hr.)	SOD (U/g F.wt./hr.)
Healthy control	0.15 <sup>e</sup>	0.19 <sup>c</sup>	0.12 <sup>e</sup>	0.20 <sup>d</sup>
Infected control	0.24 <sup>d</sup>	0.23 <sup>b</sup>	0.24 <sup>d</sup>	0.67 <sup>c</sup>
melithorin®	0.55 <sup>a</sup>	0.24 <sup>b</sup>	0.39 <sup>b</sup>	1.16 <sup>a</sup>
H <sub>2</sub> O <sub>2</sub>	0.44 <sup>b</sup>	0.28 <sup>a</sup>	0.43 <sup>a</sup>	1.01 <sup>b</sup>
<i>V. lecanii</i>	0.32 <sup>c</sup>	0.27 <sup>a</sup>	0.31 <sup>c</sup>	1.00 <sup>b</sup>
LSD (5%)	0.50	0.02	0.02	0.05



**Figure 4.** Effect of tested elicitors on total soluble protein, free proline, and total phenols in tomato plants infected with MI. Data represent mean±SD, n=3), letters referred to as significant in statically analysis).



**Figure 5.** Effect of the tested elicitors on MDA and H<sub>2</sub>O<sub>2</sub> of tomato plants infected with MI. Data represents mean ± SD, n = 3), letters referred to as significant in statically analysis).

The importance of *V. lecanii*, which produces HCN in the fight against pathogens, as a safe and environmentally friendly alternative is shown in ul Islam et al. (2023). HCN is a substance that exhibits interest in a variety of natural forms and has antifungal properties in addition to its potent role in promoting plant defenses against infections (Meena et al., 2020; Costa et al., 2022; Dimkić et al., 2022). The nematodes are a threat to plant growth because the infection delays vegetative development and transfers nutrients from the soil into other parts of the plants' root systems (Hussain et al., 2023). According to data collected in the present study, there has been a significant decrease in growth parameters of nematode-infested plants compared with healthy controls. These results agree with Vega et al. (2006) and Sharaf et al. (2016). Infected tomato plants were treated with all treatments. The present data indicated that melithorin® followed by *V. lecanii* and H<sub>2</sub>O<sub>2</sub>, respectively. Melithorin® showed an improvement in shoot lengths, root lengths, and the number of leaves by 96%, 97.3%, and 98.6%, while *V. lecanii* recorded 50%, 74.2%, and 60%, followed by H<sub>2</sub>O<sub>2</sub> which recorded 45%, 69%, and 80%, respectively. Results from this study demonstrated that photosynthetic pigments, such as chlorophyll a and chlorophyll b, were significantly reduced because of the nematode infection. These findings are in line with several other investigations (Korayem et al., 2012; Bali et al., 2018; Labudda et al., 2018). The nematode's inability to stop oxidative intervals in the cells and the plant's inability to complete the photosynthetic process could be the cause of this lack of photosynthetic pigments (Ahmed et al., 2009). Regarding the impact of the studied elicitors, it was found that melithorin® recorded the highest alleviating values of photosynthetic pigments followed by H<sub>2</sub>O<sub>2</sub> and *V. lecanii*, respectively. Through response to and overcoming nematode stress, the plant indicates that the treatments *V. lecanii*, hydrogen peroxide, and melithorin® are effective in removing the nematode threat to it.

The high activity of antioxidant enzymes is the best indicator of stress tolerance and injury resistance (El-Batal et al., 2019; Attia et al., 2021). In terms of the tested elicitors' impact, it was discovered that all of them exceeded the nematode-only infected ones in terms of high antioxidant enzyme activity values. The highest values of antioxidant enzyme activities were found in hydrogen peroxide, which was followed by melithorin® and *V. lecanii*, in that order. The high activity of antioxidant enzymes reduces free radicals and improves plant immunity (Shah et al., 2001; Xie et al., 2008). The findings in the present study showed that the number of biochemical changes in tomato plants infected by nematodes was significantly higher than their number in healthy, non-infected plants. In concepts of the effectiveness of the elicitors under test, tomato plants that were infected and treated with melithorin® and *V. lecanii* showed the strongest improvements in biochemical contents, with protein, phenol, and proline in terms of enhancing biochemical response recording. Throughout comparison to both healthy and infected tomato plants, melithorin® demonstrated a highly efficacious response in terms of morphological and biochemical contents. Due to infection by root-knot nematodes, the stress markers malondialdehyde (MDA) and hydrogen peroxide observed marked increases in their activities (Ali and Ohri, 2023). It was found that *Verticillium lecanii* was the most effective treatment alleviating increases in MDA by 16.6%, while melithorin® was the best treatment alleviating increases in H<sub>2</sub>O<sub>2</sub> by 35.4%. A decrease in the level of MDA and H<sub>2</sub>O<sub>2</sub> is clear evidence that melithorin® and *V. lecanii* eliminated the nematodes and led to recovery of the plant from the damage inflicted on it (Raheel et al., 2022). Additionally, future research could delve into optimizing the formulations and dosages of melithorin® and *V. lecanii* to maximize their nematocidal effects while minimizing the environmental impact. Investigating the synergistic effects of combining these biocontrol agents with other biological or chemical treatments could further improve their efficacy. Long-term field studies are also needed to assess the durability of these treatments across different environmental conditions and agricultural systems. Lastly, a deeper investigation into the specific molecular mechanisms by which these treatments enhance plant defense, such as the role of signaling pathways involved in oxidative stress, hormonal regulation, and immune response, would provide valuable insights. Such studies could help fine-tune the use of these agents for broader

agricultural applications, contributing to sustainable nematode management strategies.

## CONCLUSION

In conclusion, the results of this study demonstrate the efficacy of melithorin®, *Verticillium lecanii*, and hydrogen peroxide in combating nematode infestations and improving plant health in both *in vitro* and *in vivo* experiments. Among these treatments, melithorin® consistently proved to be the most effective, achieving the highest nematode mortality rates (89.4%) and significant improvements in plant growth parameters, photosynthetic pigments, and biochemical markers. *Verticillium lecanii* also showed notable effects, primarily through the production of hydrolytic enzymes and hydrogen cyanide, which contributed to nematode mortality and enhanced plant defense mechanisms. The improvement in plant growth and health, especially regarding chlorophyll content, shoot and root lengths, and leaf number, underscores the potential of these treatments as eco-friendly alternatives to chemical nematicides. Melithorin® and *V. lecanii* not only reduced nematode populations but also alleviated oxidative stress, as seen in their effects on antioxidant enzyme activities and stress markers like MDA and hydrogen peroxide. Overall, these treatments offer promising solutions for managing nematode infections while promoting plant health and productivity.

## PLANT COLLECTION

The plant collection and use conform to all the relevant guidelines.

## CONSENT FOR PUBLICATION

All authors agree with publication.

## AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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