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Effect of PGPB Secondary Metabolites in Inducing Systemic Resistance (ISR) and Enhancing Peroxidase Activity in Tomato Plants against *Meloidogyne* spp. Nematode Attack

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ABSTRACT

Meloidogyne spp. nematode is important pathogen of tomato plants that causes a characteristic symptom, namely root knot. One alternative to controlling *Meloidogyne* nematodes is by utilizing PGPB secondary metabolites. The objective of this research is to explore the potential of secondary metabolites produced by PGPB in inducing systemic resistance (ISR) and increasing peroxidase enzyme activity in tomato plants attacked by Meloidogyne spp. This study used a completely randomized design (CRD), consisting of 11 treatments, 3 replicates. The observation parameters included Induced Systemic Resistance (ISR) Potential Test and peroxidase activity (PO) analysis. The results showed that the secondary metabolites from the Bacillus mycoides (MRSNUMBE.2.2) treatment were the best treatment in the ISR observation, which was indicated by the lowest nematode penetration rate, which was 3.66 nematodes in plant root tissue. In the observation of peroxidase activity, secondary metabolites from Bacillus waihenstephanensis (RBTLL.3.2) showed the highest peroxidase enzyme activity, which was 0.0437 µg/ml on day 7 and 0.0500 µg/ml on day 14.

INTRODUCTION

Meloidogyne spp. nematodes are one of the most destructive pathogens affecting tomato plants (Tariq-Khan *et al*., 2025). *Meloidogyne* spp. nematode attacks cause typical symptoms on the roots, namely causing gall or knot on the roots of the plant, followed by symptoms of chlorosis on the leaves and plants become stunted (Pratiwi *et al*., 2020) .*Meloidogyne* spp. nematode attack can cause damage to tomato plants with a damage level of 68.3% (Khotimah *et al*., 2020).

A commonly used control strategy against *Meloidogyne* spp. is the use of synthetic nematicides with the active ingredient carbofuran to control *Meloidogyne* spp. (Greco *et al*., 2024). Excessive use of synthetic pesticides can cause human health problems and environmental damage, so environmentally safe alternative control methods are needed (Habazar *et al*., 2021). One alternative to nematode control is by utilizing secondary metabolites produced by microorganism groups, particularly Plant Growth-Promoting Bacteria (PGPB).

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PGPB bacteria are bacteria that interact with plants and are distributed in the rhizosphere, phyllosphere, and endophytes (Ramirez-Lopez *et al*., 2025). PGPB have been reported to produce secondary metabolites capable of suppressing pathogen development and enhancing plant growth (Efthimiadou *et al*., 2020; Yanti *et al*., 2022). In addition, PGPB secondary metabolites can also induce plant resistance, thereby inhibiting the development of plant pathogens. (Kaleh *et al*., 2024).

PGPB secondary metabolites are also known to stimulate peroxidase enzyme activity in plants (Hafsah, 2022). In plant defense systems, peroxidase enzymes function as one of the components involved in the response to attacks by pests by inhibiting the development and penetration of pathogens into root tissues (Nadarajah, 2024). Peroxidase contributes to cell wall lignification and the formation of phenolic compounds in plants. Strong cell walls inhibit the entry of pathogens during infection. (Zondo & Mafa, 2025).

The objective of this research is to explore the potential of secondary metabolites produced by *Plant* Growth-*Promoting Bacteria* (PGPB) in inducing systemic resistance (ISR) and increasing peroxidase enzyme activity in tomato plants infested with *Meloidogyne* spp. nematodes.

MATERIALS AND METHOD

Experimental site and treatment

This study was conducted at the Microbiology Laboratory of the Department of Plant Protection and Experimental Fields, Faculty of Agriculture, Andalas University, from May to September 2024. This study used a completely randomized design (CRD), consisting of 11 treatments and 3 replicates. The treatments used included:

1. *Bacillus thuringiensis* (MRSNRZ.3.1),
2. *B. subtilis* (MRTDUMBE.3.2.1),
3. *B. mycoides* (MRSNUMBE.2.2),
4. *B. waihenstephanensis* (RBTLL.3.2),
5. *B. cereus* (MRPLUMBE.1.3),
6. *Bacillus* sp (MRSPRZ.1.)
7. *Pseudomonas hibiscicola* (MRTLDRZ .2.2)
8. *Achromobakter insolitus* (MRBPUMBE.1.3)
9. Carbofuran pesticide
10. Negative Control (Inoculated with *Meloidogyne* spp. and without PGPB introduction),
11. Positive Control (Without *Meloidogyne* spp. and without PGPB introduction)

Filtrate Culture of PGPB Bacteria

PGPB isolates were made filtrate cultures by growing PGPB bacteria on Tryptic Soy Agar (TSA) and Nutrient Agar (NA0 media for 48 hours at room temperature. Single colonies of bacteria were transferred into 100 ml of Tryptic Soy Broth (TSB) and Nutrient Broth (NB) media and Incubated at 25˚C for 2 days at a speed of 150 rpm in a rotary shaker. Then the bacterial culture was centrifuged at 7000 rpm for 15 minutes, filtered with Whatmann filter paper and 0.22µm diameter millipore, put into 250% ml cultured bottles and stored at 4 ° C before application. The bacterial filtrate used for each treatment has a concentration of 25% PGPB.

Induced Systemic Resistance (ISR) Potential Test

The Induced Systemic Resistance (ISR) potential of PGPB bacteria against *Meloidogyne* spp. was tested using *the split-root system* method on 1-month-old tomato plants (Hasky-Günther *et al*., 1998). Plants were split-root and planted in separate pots, then one of the pots was watered with PGPB filtrate. Six days after treatment, the other pot was inoculated with nematodes. Observations were made two weeks after inoculation to count the population of root-infecting nematodes using acid fuchsin staining and microscopy.

Peroxidase activity analysis

Peroxidase activity was measured based on the direct absorbance measurement method using a spectrophotometer. One gram of root sample was weighed and crushed with a mortar in a 0.01 M phosphate buffer solution, pH 6, at a ratio of 1:4. The root extract was centrifuged

at 5,000 rpm for 30 minutes at 4 °C, then filtered with Whatman filter paper. The supernatant obtained was used as the enzyme preparation. Enzyme activity was monitored by pouring 0.2 ml of Enzyme Preparation diluted 1:3 with 0.01 M phosphate buffer solution, pH 6, into a 1 cm diameter test tube containing 5 ml of 0.5 M pyrogallol solution and 0.5 ml of 1% H_2O_2. The solution was then homogenized for 5-10 seconds, and the absorbance was measured at a wavelength of 420 nm at 30-second intervals for 150 seconds. If the absorbance value was too high, the enzyme solution could be diluted with phosphate buffer. Peroxidase activity was measured by observing the change in absorbance, before calculation, the absorbance value obtained was first reduced by the blank. The average absorbance value (AOD = b) of one observation was sought using the regression equation (Y = a + bx). Enzyme activity unit (UAE) was calculated using the formula: UAE = AOD x enzyme preparation (ml)/control wet weight (g).

Data Analysis

The data were analyzed using analysis of variance. If there were significant differences, the analysis was continued with Duncan's Multiple Range Test (DNMRT) at a significance level of 5% using SPSS software.

RESULTS AND DISCUSSION

Induced Systemic Resistance (ISR) Potential Test

The application of PGPB secondary metabolites in all observations of plant resistance induction (ISR) showed significant differences between treatments with Negative Control (Table 1). The results showed that induced resistance occurred in all PGPB secondary metabolite treatments. All treatments resulted in lower nematode populations than the negative control, and their effects were comparable to those of synthetic pesticides. *Bacillus mycoides* (MRSNUMBE.2.2) showed the best results with the lowest number of nematodes in the roots, namely 3.66 heads. Nematodes that successfully enter the plant tissue.

Table 1. Effect of filtrate culture on Meloidogyne spp. nematode population in tomato roots using split-root system method

Culture filtrate of PGPB	Nematodes in the root tissue/100 g sample	
*B. thuringiensis (*MRSNRZ.3.1)	10.00	bc
B. subtilis (MRTDUMBE.3.2.1)	6.00	bc
B. mycoides (MRSNUMBE.2.2)	3.66	c
B. waihenstephanensis (RBTLL.3.2)	5.33	bc
B. cereus (MRPLUMBE.1.3)	8.00	bc
Bacillus sp (MRSPRZ.1.1)	10.66	ab
Pseudomonas hibiscicola (MRTLDRZ .2.2)	9.00	bc
Achromobakter insolitus (MRBPUMBE.1.3)	7.66	bc
Carbofuran pesticide	6.33	bc
Negative control	16.66	a

Note: Means in the same column followed by the same letters are not significantly different according to DNMRT at α=5%.

The induction of systemic resistance by PGPB culture filtrates indicates that bacterial secondary metabolites function as effective elicitors of plant defense responses. The ability of cell-free filtrates to trigger resistance suggests that ISR is mediated primarily by bioactive compounds released by the bacteria rather than by direct bacterial colonization of plant tissues. Among the secondary metabolites produced by plant growth-promoting bacteria, antibiotic lipopeptides synthesized by members of the genus *Bacillus*, such as surfactin and fengycin, are widely recognized as key ISR inducers. These compounds act not only as antimicrobial agents but also as signaling molecules that activate endogenous plant defense pathways (Saiyam *et al.,* 2024; Mahapatra *et al.,* 2024). Their role as elicitors enables plants to perceive microbial-derived signals and initiate a defense response without pathogen challenge.

Lipopeptides interact with the plant plasma membrane and induce transient alterations in membrane integrity, which serve as early defense signals. According to Schellenberger *et al.* (2019), this interaction triggers rapid cellular responses, including calcium ion

(Ca^{2+}) influx and the generation of reactive oxygen species (ROS). These early signaling events activate downstream defense signaling cascades, predominantly mediated by jasmonic acid and ethylene, which are hallmark pathways of ISR. Activation of JA- and ET-dependent signaling pathways leads to increased expression of gene products involved in cell wall strengthening, lignification, and phenolic compound accumulation. These structural and biochemical modifications enhance the resistance of root tissues to nematode penetration and establishment, thereby limiting successful invasion and development within the plant.

Furthermore, ISR induced by PGPB metabolites is typically associated with a priming effect, whereby plants remain in a heightened state of defense readiness. This primed state allows plants to respond more rapidly and effectively upon nematode attack, resulting in reduced infection success. Such systemic and durable resistance provides a plausible explanation for the strong suppression of nematode infection observed following treatment with PGPB culture filtrates.

Peroxidase activity analysis

The application of PGPB filtrate culture on tomato plants shows an effect on peroxidase enzyme activity. Plants applied with PGPB filtrate culture have higher peroxidase activity than control plants and pesticides (Table 2). On day 7 after the application of filtrate culture, the treatment of *Bacillus waihenstephanensis* (RBTLL.3.2) was the treatment with the highest peroxidase activity of 0.0437 µg/ml. Furthermore, on day 14 there was an increase in peroxidase activity in all treatments applied with PGPB filtrate culture, the treatment of *B. waihenstephanensis* (RBTLL.3.2) showed the highest peroxidase activity on day 14 which was 0.0500 µg/ml. On day 14, *Bacillus mycoides* (MRSNUMBE.2.2) also showed a significant increase in peroxidase activity of 0.0497 µg/ml. The higher peroxidase activity in the PGPB secondary metabolite treatment indicates that the secondary metabolites produced by PGPB can enhance plant defense responses against attacks by *Meloidogyne* spp. nematodes.

Peroxidase activity is closely associated with plant defense responses against plant-parasitic nematodes, including *Meloidogyne* spp. Elevated peroxidase activity reflects the activation of enzymatic defense mechanisms that contribute to the restriction of nematode penetration, establishment, and development within plant roots. The enhancement of peroxidase activity following treatment with PGPB culture filtrates indicates that bacterial secondary metabolites play a critical role in stimulating host resistance responses.

Peroxidases are key components in the oxidative defense system of plants. These enzymes catalyze the formation of ROS, particularly H_2O_2, which functions as a direct antimicrobial agent and as a signaling molecule in plant defense pathways. The accumulation of H_2O_2 at the site of infection

Table 2. Effect of PGPB secondary metabolites on peroxidase activity in tomato plants

Treatment	7 days(µg/ml)	14 days (µg/ml)
*B. thuringiensis (*MRSNRZ.3.1)	0.0343 a	0.0383 ab
B. subtilis (MRTDUMBE.3.2.1)	0.0373 a	0.0490 a
B. mycoides (MRSNUMBE.2.2)	0.0403 a	0.0497 a
B. waihenstephanensis (RBTLL.3.2)	0.0437 a	0.0500 a
B. cereus (MRPLUMBE.1.3)	0.0403 a	0.0467 a
Bacillus sp (MRSPRZ.1.1)	0.0397 a	0.0463 a
Pseudomonas hibiscicola (MRTLDRZ .2.2)	0.0433 a	0.0407 ab
Achromobakter insolitus (MRBPUMBE.1.3)	0.0403 a	0.0463 a
Carbofuran pesticide	0.0343 a	0.0380 ab
Positive control	0.0360 a	0.0393 ab
Negative control	0.0323 a	0.0317 b

Note: Means in the same column followed by the same letters are not significantly different according to DNMRT at α=5%

has been shown to cause toxic effects on nematodes while simultaneously stimulating defense responses, such as cell wall strengthening and the expression of defense genes (Molinari & Leonetti, 2023).

In addition to its direct toxic effects, peroxidase also plays a role in inducing a hypersensitive response (HR), characterized by localized cell death at the site of infection. This causes nematodes to lose the living host tissue and nutrients necessary for their survival, thereby limiting their ability to successfully colonize roots. The involvement of peroxidase-mediated HR has been widely reported as an effective defense strategy against sedentary endoparasitic nematodes such as *Meloidogyne* spp. (Afifah *et al.,* 2019).

Peroxidase activity is also strongly linked to lignification processes in plant cell walls. These enzymes catalyze the polymerization of cinnamyl alcohols during lignin biosynthesis, resulting in the strengthening of cell wall structures. Enhanced lignification creates a robust physical barrier that restricts nematode penetration and migration within root tissues. The accumulation of lignin and phenolic compounds further contributes to the formation of unfavorable conditions for nematode development (Ismawanti *et al.,* 2022; Yang *et al.,* 2023).

The induction of peroxidase activity by PGPB culture filtrates suggests that secondary metabolites act as elicitors of ISR. Through ISR, plants enter a primed defensive state, enabling faster and stronger activation of enzymatic and structural defenses upon nematode challenge. This priming effect enhances the effectiveness of peroxidase-mediated responses, thereby improving plant resistance to *Meloidogyne* spp.

Overall, increased peroxidase activity represents a crucial biochemical marker of plant defense activation mediated by PGPB-derived secondary metabolites. The stimulation of oxidative responses, hypersensitive cell death, and cell wall reinforcement collectively contributes to enhanced resistance against root-knot nematodes, supporting the potential of PGPB metabolites as sustainable alternatives for nematode management.

CONCLUSION

The results showed that PGPB secondary metabolites were able to induce plant resistance (ISR) of tomato plants, the treatment of *B. mycoides* (MRSNUMBE.2.2) was the best treatment in ISR observations, indicated by the lowest nematode penetration rate of 3.66 nematodes in plant root tissue. The results also showed an increase in peroxidase enzyme activity in tomato plants treated with PGPB secondary metabolites compared to the control group. Secondary metabolite *B. waihenstephanensis* (RBTLL.3.2) is the treatment with the highest peroxidase enzyme activity of 0.0437 µg/ml on day 7 and 0.0500 µg/ml on day 14.

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REFERENCES

Afifah, E.N., R.H. Murti, and T.R. Nuringtyas. 2019. Metabolomics approach for the analysis of resistance of four tomato genotypes (*Solanum lycopersicum* L.) to root-knot nematodes (*Meloidogyne incognita*). Open Life Sciences 14(1): 141–149. https://doi.org/10.1515/biol-2019-0016.

Efthimiadou, A., N. Katsenios, S. Chanioti, M. Giannoglou, N. Djordjevic, and G. Katsaros. 2020. Effect of foliar and soil application of plant growth promoting bacteria on growth, physiology, yield and seed quality of maize under Mediterranean conditions. Scientific Reports 10(1): 1–11. https:// doi.org/10.1038/s41598-020-78034-6.

Greco, N., Minuto, A., d'ericco, F. P., & d'Ericco, G. I. A. D. A. (2024). Synthetic nematicides in italy: past and present role in the management of plant parasitic nematodes and future perspectives of control. Redia: Journal of Zoology/Giornale di Zoologia, 107.

Habazar, T., Winarto, Y. Obel, Y. Yanti, M.R. Dani, and D. Monica. 2021. Biocontrol of

Meloidogyne sp. on tomato plants by selected *Bacillus* spp. IOP Conference Series: Earth and Environmental Science 757(1). https://doi.org/10.1088/1755-1315/757/1/012019.

Hafsah, S. 2022. Biokontrol rizobakteri indigenous terhadap *Synchytrium pogostemonis* penyebab penyakit budok pada nilam Aceh (*Pogostemon cablin* Benth.). Kultivasi 21(1). https://doi.org/10.24198/kultivasi.v21i1.36316.

Hasky-Günther, K., S. Hoffmann-Hergarten, and R.A. Sikora. 1998. Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43). Fundamental and Applied Nematology 21(5): 511–517.

Ismawanti, A., E. Nurcahyani, and S. Farizi. 2022. Effect of indole acetic acid (IAA) by *Serratia marcescens* strain MBC1 on soybean (*Glycine max* L.) germination. Indonesian Journal of Biotechnology and Biodiversity 6(1).

Kaleh, A.M., P. Singh, K. Ooi Chua, and J.A. Harikrishna. 2024. Modulation of plant transcription factors and priming of stress tolerance by plant growth-promoting bacteria: A systematic review. Annals of Botany: 1–15. https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcae166/7758362.

Khotimah, N., N. Wijaya, and M. Sritamin. 2020. Perkembangan populasi nematoda puru akar (*Meloidogyne* spp.) dan tingkat kerusakan pada beberapa tanaman familia Solanaceae. Jurnal Agroekoteknologi Tropika 9(1). https://ojs.unud.ac.id/index.php/JAT.

Mahapatra, S., Chakraborty, S., Samanta, M., Das, S., & Islam, T. (2022). Current understanding and future directions of biocontrol of plant diseases by *Bacillus* spp., with special reference to induced systemic resistance. In *Bacilli in Agrobiotechnology: plant stress tolerance, bioremediation, and bioprospecting* (pp. 127-150). Cham: Springer International Publishing.

Molinari, S., & Leonetti, P. (2023). Inhibition of ROS-scavenging enzyme system is a key event in tomato genetic resistance against root-knot nematodes. International journal of molecular sciences, 24(8), 7324.

Nadarajah, K. K. (2024). Defensive strategies of ROS in plant–pathogen interactions. In Plant Pathogen Interaction (pp. 163-183). Singapore: Springer Nature Singapore.

Pratiwi, N.W.K., R. Amrulloh, F. El Auly, and F. Kurniawati. 2020. Deteksi dan identifikasi nematoda puru akar (*Meloidogyne* spp.) pada tanaman bit menggunakan metode DNA barcoding. Jurnal Fitopatologi Indonesia 16(1): 1–8. https://doi.org/10.14692/jfi.16.1.1-8.

Ramírez-López, M., Bautista-Cruz, A., Toledo -López, A., & Aquino-Bolaños, T. (2025). Rhizospheric and endophytic plant growth-promoting bacteria associated with *Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner: A review of their agronomic potential. Microorganisms, 13(11), 2567.

Saiyam, D., Dubey, A., Malla, M. A., & Kumar, A. (2024). Lipopeptides from Bacillus: Unveiling biotechnological prospects—Sources, properties, and diverse applications. *Brazilian Journal of Microbiology*, *55*(1), 281-295.

Schellenberger, R., M. Touchard, C. Clément, F. Baillieul, S. Cordelier, J. Crouzet, and S. Dorey. 2019. Apoplastic invasion patterns triggering plant immunity: Plasma membrane sensing at the frontline. Molecular Plant Pathology 20(11): 1602–1616. https://doi.org/10.1111/mpp.12857.

Tariq-Khan, M., Rehman, T. U., Mukhtar, T., Mahmood, B., & Ahmed, R. (2025). Population dynamics and virulence patterns of root-knot nematodes (*Meloidogyne* spp.) on tomato in Poonch Highlands, Azad Jammu and Kashmir, Pakistan. Journal of Phytopathology, 173(2), e70060.

Yang, J.W., S.U. Park, H.U. Lee, K.J. Nam, K.L. Lee, J.J. Lee, J.H. Kim, S.S. Kwak, H.S. Kim, and Y.H. Kim. 2023. Differential responses of antioxidant enzymes and lignin metabolism in susceptible and resistant sweetpotato cultivars during root-knot nematode infection. Antioxidants 12(6). https://doi.org/10.3390/antiox12061164.

Yanti, Y., H. Hamid, N. Nurbailis, and N.L. Suriani. 2022. Biological activity of indigenous selected plant growth promoting rhizobacteria isolates and their ability to improve the growth traits of shallot (*Allium ascalonicum* L.). Philippine Journal of Science 151(6): 2327–2340. https://doi.org/10.56899/151.6B.03.

Zondo, S. N., & Mafa, M. S. (2025). Peroxidase and β-1, 3-glucanase synergistic functions strengthen plant cell wall and protect wheat against Diuraphis noxia infestation. *Planta*, *262*(2), 41.