ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 1, June 2025 E-ISSN: 2580-4537
Pages: 326-338 DOI: 10.13057/asianjagric/g090134

Nematicidal activity of *Trichoderma harzianum*-derived secondary metabolites against *Meloidogyne incognita* and metabolomic profiling of selected potent isolates

ANKARDIANSYAH PANDU PRADANA[1,♥], SOFIATUS SHOLEHAH[1], DWI ROSIDAH ANDRIYANI[1], MOHAMMAD HOESAIN[1], WIDI ASTUTI[2], RAHMI UTAMI HADIANI[2], RACHMI MASNILAH[1], MUH ADIWENA[3], DIANA PUTRI[4]

[1]Department of Plant Protection, Faculty of Agriculture, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia. Tel: +62-331-334054, ♥email: pandu@unej.ac.id

[2]Laboratory for Pest and Disease Observation of Food Crops & Horticulture, Department of Agriculture and Food Security, East Java Province. Jl. PB. Sudirman No. 93, Tanggul, Jember 68155, East Java, Indonesia

[3]Department of Agrotechnology, Faculty of Agriculture, Universitas Borneo Tarakan. Jl. Amal Lama No. 1, Tarakan 77123, North Kalimantan, Indonesia

[4]Department of Agroecotehnology, Faculty of Agriculture, Universitas Andalas. Kampus III Dharmasraya Pulau Punjung, Padang 25163, West Sumatra, Indonesia

Manuscript received: 27 March 2025. Revision accepted: 2 June 2025.

Abstract. *Pradana AP, Sholehah S, Andriyani DR, Hoesain M, Astuti W, Hadiani RU, Masnilah R, Adiwena M, Putri D. 2025. Nematicidal activity of* Trichoderma harzianum*-derived secondary metabolites against* Meloidogyne incognita *and metabolomic profiling of selected potent isolates. Asian J Agric 9: 326-338.* Root-knot nematodes, especially *Meloidogyne incognita*, reduce global crop yields, necessitating the development of safe and effective management approaches. While chemical nematicides can mitigate infestations, they often present environmental concerns and potentially foster resistance in target nematodes. Consequently, using antagonistic fungi, particularly *Trichoderma harzianum*, has gained traction as an eco-friendly solution. Nonetheless, the viability of *T. harzianum* under field conditions is often compromised by abiotic and biotic factors. This study investigated the nematicidal efficacy and secondary metabolite composition of four *T. harzianum* isolates (AJG, TGL, SBS, and SKS) collected from distinct geographical regions. In vitro assays revealed that the AJG and TGL isolates elicited the most potent effects, achieving >75% egg-hatching inhibition and >93% juvenile mortality at 168 hours post-treatment. Gas chromatography-mass spectrometry indicated that fatty acid esters, including methyl palmitate and methyl oleate, were the major contributors to nematicidal effects through membrane disruption. Notable differences in metabolite profiles between isolates highlight isolate-specific biochemical pathways impacting suppressive capacity. The predominance of fatty acids underscores their critical function in controlling nematodes, offering prospects for developing stable, environmentally friendly formulations. These results underscore the potential of *T. harzianum*-derived metabolites for integrated nematode management. They also emphasize the importance of isolate selection and metabolite profiling in designing targeted, sustainable strategies to combat root-knot nematodes, empowering the audience with this crucial knowledge.

Keywords: Biological control, fatty acid esters, GC-MS analysis, ovicidal activity, root-knot nematode

INTRODUCTION

Meloidogyne incognita poses a major threat to global agriculture, causing 15-20% yield losses in key crops and affecting over 2,000 plant species (Subedi et al. 2020; Tapia-Vázquez et al. 2022). Economic losses attributed to root-knot nematode infestations have been estimated at over $100 billion annually, highlighting the critical need for effective management strategies to sustain agricultural productivity and food security (Khan et al. 2021).

Chemical nematicides have traditionally dominated the management strategies employed against nematode infestations. While effective, these chemicals often have adverse environmental impacts, contribute to soil degradation, and potentially lead to the emergence of resistant nematode populations (Tudi et al. 2021; Pathak et al. 2022). Consequently, research has shifted toward environmentally friendly alternatives, including biological control strategies utilizing antagonistic fungi such as *Trichoderma* spp. (Azlay et al. 2023). These fungi have been widely adopted as biocontrol agents due to their ability to colonize root systems, inhibit pathogen growth, and stimulate plant defense mechanisms (Forghani and Hajihassani 2020; Khan et al. 2020).

Among the *Trichoderma* species, *Trichoderma harzianum* Rifai (1969) has garnered substantial attention for its effectiveness in suppressing nematode populations through mechanisms such as parasitism, antibiosis, and competition for nutrients and space (Ghazanfar et al. 2018; Sood et al. 2020). Field applications involving direct inoculation of *T. harzianum* spores or mycelium have significantly reduced nematode populations, sometimes achieving >60% suppression under favorable conditions (Feyisa et al. 2016). While concerns have been raised regarding the comparative effectiveness of *T. harzianum* versus chemical nematicides under variable environmental conditions, integrating *T. harzianum* within comprehensive Integrated Pest Management (IPM) strategies can significantly enhance its reliability and effectiveness. Successful deployment of *T. harzianum*-based strategies depends on integrating them within comprehensive IPM frameworks, supported by targeted farmer education, precise application timing, and optimized techniques. Investment in outreach programs and extension services can significantly enhance practitioner awareness, adoption, and consistent performance of biological control agents (Azlay et al. 2023).

However, the practical application of living *T. harzianum* preparations faces substantial challenges in agricultural fields. The survival and effectiveness of fungal inoculants in situ are often compromised by environmental stresses such as drought, soil pH fluctuations, UV radiation exposure, and predation by soil fauna (Soesanto et al. 2019; Khan et al. 2020). Among abiotic and biotic environmental factors, drought stress, fluctuations in soil pH, UV radiation exposure, and predation by soil fauna significantly compromise the viability and persistence of *T. harzianum* inoculants under field conditions.

Empirical studies have shown that approximately 30-40% of applied fungal inoculants experience significant viability losses within the first few weeks post-application due to abiotic stressors. Microarthropod predation and competing microorganisms also limit fungal persistence and activity, ultimately reducing efficacy to <50% of laboratory-tested levels under actual field conditions. These factors substantially reduce the practical reliability and predictability of biological control outcomes (Shahriar et al. 2022; Ayaz et al. 2023).

Recent studies have highlighted secondary metabolites from *T. harzianum* as promising alternatives to fungal biomass for nematode management. These metabolites, including volatile organic compounds, antibiotics, and enzymatic secretions, offer improved environmental stability, shelf-life, and consistent efficacy (Wang et al. 2021; Yan and Khan 2021). Laboratory assays show mortality rates of 70-80% against *M. incognita* juveniles (Khan et al. 2020; Lopes et al. 2024). Identified compounds such as 6-pentyl-α-pyrone, harzianic acid, gliotoxin, and peptaibols also exhibit antifungal, antimicrobial, and plant growth-promoting properties (Wang et al. 2021; Yan and Khan 2021). However, detailed profiling and specific mechanisms underlying their nematicidal efficacy remain limited, necessitating advancements in formulation technologies for practical agricultural use.

Initial findings on *T. harzianum* metabolites are promising, yet knowledge gaps remain regarding isolate-specific variability and nematicidal efficacy. Although regional factors might influence metabolite profiles, this study focuses on inherent metabolite diversity among isolates regardless of geographic origin. Characterizing these profiles is essential for selecting effective strains and optimizing biocontrol consistency. Secondary metabolites can be formulated as stable biopesticides or integrated into controlled-release systems, improving their practicality. Assessing their long-term ecological impacts is also necessary, despite preliminary indications of minimal negative effects (Soesanto et al. 2019; Khan et al. 2020). Therefore, this study evaluates nematicidal activity and metabolite composition from four geographically distinct *T. harzianum* isolates against *M. incognita*, identifying potent bioactive compounds for sustainable nematode management.

MATERIALS AND METHODS

Research location and experimental duration

This study was conducted at the Laboratory of Plant Protection Technology, Faculty of Agriculture, Universitas Jember, East Java, Indonesia, from June to December 2024.

Isolation and culture of *Trichoderma harzianum* strains

This study examined four *T. harzianum* isolates. Isolates TGL and AJG were provided by the Laboratory of Pest and Disease Observation for Food Crops and Horticulture-Tanggul, Department of Agriculture and Food Security, East Java Province, Indonesia. They originated from the rhizosphere soils of soybean plants in the Tanggul and Ajung Sub-districts in Jember District, East Java, Indonesia. Isolates SBS and SKS were obtained from the culture collection at the Laboratory of Plant Protection Technology, Faculty of Agriculture, Universitas Jember. These isolates were also originally isolated from soybean rhizosphere soils in the Sumbersari and Sukorambi Sub-districts, respectively, in Jember District, Indonesia. Pure cultures of all isolates were maintained on potato dextrose agar (HiMedia, India) in 9 cm diameter Petri dishes under controlled laboratory conditions.

Source and preparation of *Meloidogyne incognita* eggs and second-stage juveniles

This study utilized an *M. incognita* isolate obtained from the nematode culture collection maintained at the Laboratory of Plant Protection Technology, Faculty of Agriculture, Universitas Jember. It was propagated on tomato plants (variety Servo F1) to preserve its purity. Nematode eggs were collected by carefully harvesting symptomatic tomato roots, gently washing them, and segmenting them into 1-2 cm pieces. Next, the root segments were vigorously agitated in a 1% sodium hypochlorite solution to release the eggs, and the resulting suspension was filtered through a 600-mesh sieve to collect them. Then, the eggs were thoroughly rinsed with distilled water to remove residual sodium hypochlorite. Second-stage juveniles (J2) were prepared by incubating the clean eggs in darkness at room temperature for 7 days, facilitating the hatching of J2 nematodes, which were then used in the experimental assays (Asyiah et al. 2025).

Production and collection of *Trichoderma harzianum* secondary metabolites

Each *T. harzianum* isolate was cultured individually in 100 mL of potato dextrose broth (PDB; HiMedia, India), adjusted to pH 6.0. The cultures were incubated at 30±2°C for 7 days on a rotary shaker set at 80 rpm. After incubation, the cultures were centrifuged aseptically at 6000 rpm, and the supernatants obtained were filtered through sterile membranes with a pore size of 0.22 µm. The filtered supernatants containing the extracellular secondary metabolites of *T. harzianum* were stored aseptically at 4°C until used in the biological assays (Moo-Koh et al. 2022).

In vitro bioassay of ovicidal and nematicidal activities

In vitro bioassays were performed to evaluate the ovicidal and nematicidal potential of *T. harzianum* secondary metabolites against *M. incognita*. Five treatments were examined: secondary metabolites from four distinct *T. harzianum* isolates and a PDB control. Each treatment comprised six replicates and followed a completely randomized design.

For the ovicidal assays, 100 *M. incognita* eggs were transferred to Petri dishes containing 5 mL of 5% (v/v) secondary metabolite solutions from respective isolates or 5% PDB as a control. The eggs were incubated in the dark, with unhatched eggs counted at 24-hour intervals for up to 168 hours. In the nematicidal assays, 100 J2 *M. incognita* were introduced into test dishes containing 5 mL of the same solutions. The mortality rates of J2 nematodes were observed and recorded at 24-hour intervals up to 168 hours.

The resulting data underwent arcsine transformation before statistical analyses. The transformed data was compared between groups using analysis of variance, followed by post-hoc Tukey's honestly significant difference (HSD) test, considering a 5% significance level (Khan et al. 2021).

Metabolomic profiling of *T. harzianum* secondary metabolites by gas chromatography-mass spectrometry

Untargeted metabolomic profiling was conducted using gas chromatography-mass spectrometry (GC-MS) on a GC-MS-QP2010 Plus system (Shimadzu, Japan). The samples were analyzed in split injection mode, with an injector temperature of 250°C. The oven temperature program began at 80°C for 2 minutes, then increased at 5°C/min up to 280°C, which was maintained for 3 minutes. The carrier gas was helium, operating at a linear velocity of 36.6 cm/sec, column flow rate of 0.99 mL/min, total flow of 4.9 mL/min, and an internal column pressure of 64.1 kPa. The split ratio was adjusted to 1:1, with a carrier gas saver-to-split ratio of 5.0 after 2 minutes.

The mass spectra were recorded in electron ionization mode with the ion source and interface temperatures both set to 280°C. The mass spectral data were collected in the full scan range of m/z 40-600 at a scan speed of 1666 scans/sec and event time of 0.40 sec. The total runtime per sample was 45 minutes. Compounds in the metabolite extracts were identified by comparing their mass spectra against the Wiley9.LIB reference mass spectral database (Asyiah et al. 2025).

RESULTS AND DISCUSSION

Effect of *T. harzianum* secondary metabolites on root-knot nematode eggs and juveniles

The secondary metabolites derived from various *T. harzianum* isolates demonstrated significant efficacy in inhibiting the hatching of *M. incognita* eggs. Notable inhibition was observed starting at 48 hours of treatment. The mean percentage of unhatched eggs was 93.33% with PDB (control), compared to 99.67% and 98.50% with secondary metabolites from *T. harzianum* isolates AJG and TGL, respectively. Statistical analysis indicated significant differences between these two isolates and the control. Conversely, the mean percentage of unhatched eggs did not differ significantly between PDB (control) and the secondary metabolites from *T. harzianum* isolates SBS and SKS at this time point.

After 96 hours of treatment, the mean percentage of unhatched eggs was significantly lower with PDB (control: 75.00%) than with the secondary metabolites from *T. harzianum* isolates AJG (84.67%) and TGL (83.67%). In contrast, the mean percentage of unhatched eggs did not differ significantly between PDB (control) and the secondary metabolites from *T. harzianum* isolates SBS (76.17%) and SKS (76.33%).

A clear change in the pattern emerged after 168 hours of treatment, with all tested *T. harzianum* isolates significantly outperforming the control. At this final observation, the mean percentage of unhatched eggs with PDB (control) had decreased drastically to 17.00% and was significantly lower than with the secondary metabolites from *T. harzianum* isolates AJG (76.17%), TGL (75.33%), SBS (63.67%), and SKS (59.50%). *Trichoderma harzianum* isolates AJG and TGL consistently exhibited the greatest efficacy, with *T. harzianum* isolate AJG exhibiting the greatest overall ovicidal activity. Further data on the percentage of unhatched *M. incognita* eggs treated with *T. harzianum* secondary metabolites is presented in Figure 1.

The secondary metabolites from *T. harzianum* isolates were also effective in inducing mortality in *M. incognita* J2. Mortality effects were evident as early as 24 hours of treatment. The mean mortality was only 0.33% with PDB (control), significantly lower than with the secondary metabolites from *T. harzianum* isolates AJG (11.50%), TGL (10.83%), and SBS (3.67%). However, the mortality rate did not differ significantly between PDB (control) and the secondary metabolites of *T. harzianum* isolate SKS (1.83%) at this early time point.

After 96 hours of treatment, the mortality rates remained significantly higher with the secondary metabolites from *T. harzianum* isolates AJG (42.67%), TGL (40.17%), SBS (22.83%), and SKS (23.17%) than with PDB (control: 1.33%). This consistent pattern persisted through to the final observation at 168 hours of treatment, with the mortality rate significantly lower with PDB (control: 3.33%) than with the secondary metabolites from *T. harzianum* isolates AJG (95.50%), TGL (93.83%), SBS (52.00%), and SKS (46.00%).

These results clearly indicate the potential of *T. harzianum*-derived secondary metabolites to control both *M. incognita* eggs and J2 effectively. Among all examined *T. harzianum* isolates, the secondary metabolites from AJG consistently demonstrated superior ovicidal and nematicidal activity, suggesting isolate-specific bioactivity and potential for practical applications in nematode management strategies. Figure 2 presents the mortality rates of *M. incognita* J2 exposed to *T. harzianum* secondary metabolites.

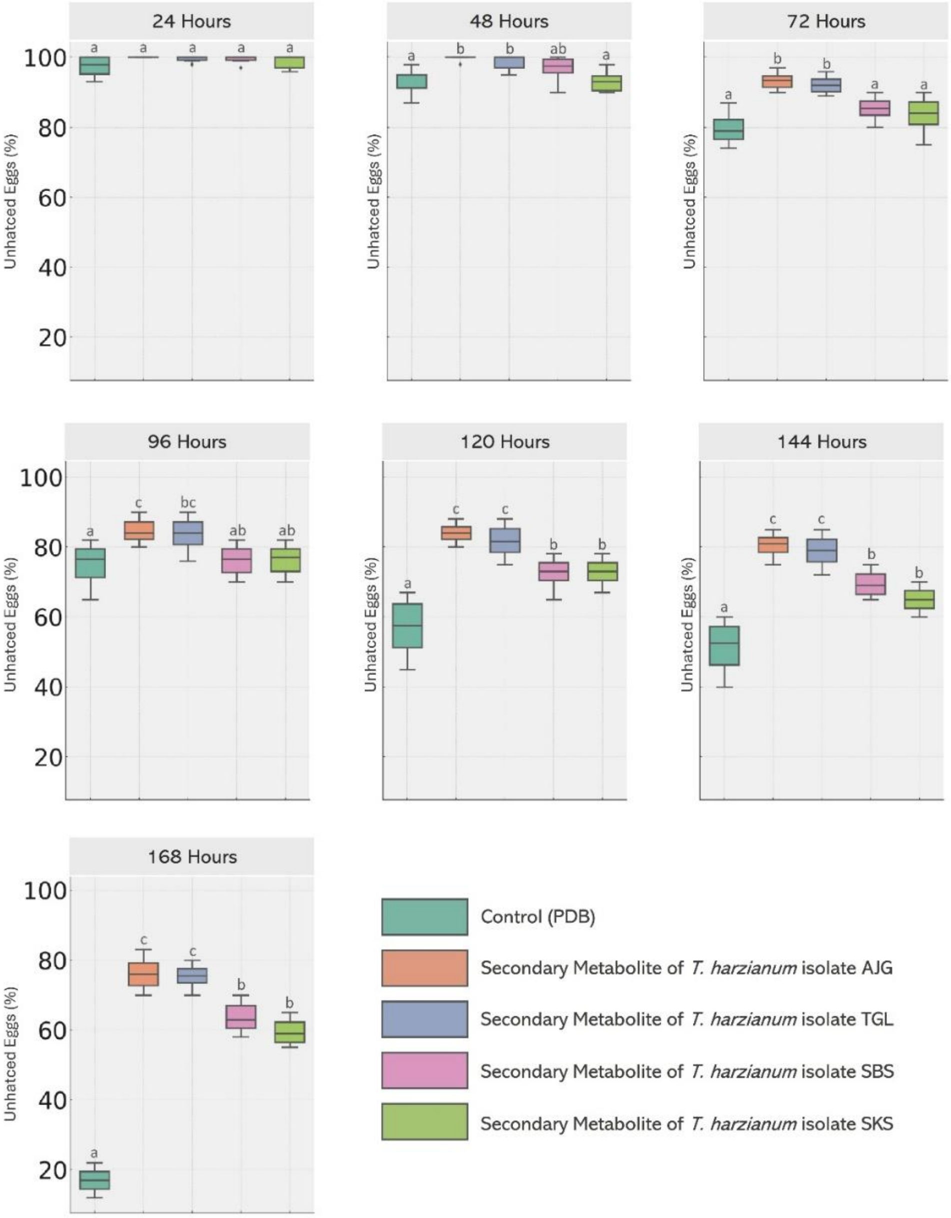


Figure 1. The ovicidal effect of *Trichoderma harzianum* secondary metabolites on *Meloidogyne incognita* eggs. Note: Groups that share at least one letter above the boxplots at the same time point are not significantly different, as determined by post-hoc Tukey's HSD tests at the 5% significance level. Shared letters indicate statistical similarity

The differential ovicidal and nematicidal activities observed among the secondary metabolites derived from different *T. harzianum* isolates could be attributed to their distinct metabolomic profiles and bioactive compound variations (Moo-Koh et al. 2022). The superior performance of *T. harzianum* isolates AJG and TGL likely results from their ability to synthesize higher concentrations of specific metabolites such as gliotoxin, harzianic acid, and other volatile organic compounds previously documented to have nematicidal properties (Rangel et al. 2021; Zaid et al. 2022). Previous research has shown that gliotoxin significantly interferes with nematode cellular metabolism, disrupting vital physiological processes such as mitochondrial function and enzyme regulation, eventually leading to high nematode mortality (Chen et al. 2020; Hamrouni et al. 2025). This research opens up exciting possibilities for further investigation into the mechanisms of action of these metabolites and their potential applications in agriculture.

In contrast, *T. harzianum* isolates SBS and SKS exhibited comparatively lower efficacy, possibly due to a lower diversity or concentration of bioactive metabolites. According to Zeilinger et al. (2016), differences in nematicidal activity among fungal isolates commonly correlate with variability in secondary metabolite synthesis, influenced by isolate-specific genetic and environmental factors during growth and metabolite production. The observed differences in bioactivity might be explained by variations in the metabolic pathways activated under similar cultivation conditions, emphasizing the importance of metabolomic characterization in selecting effective biocontrol strains (Keswani et al. 2019; Rodrigo et al. 2021).

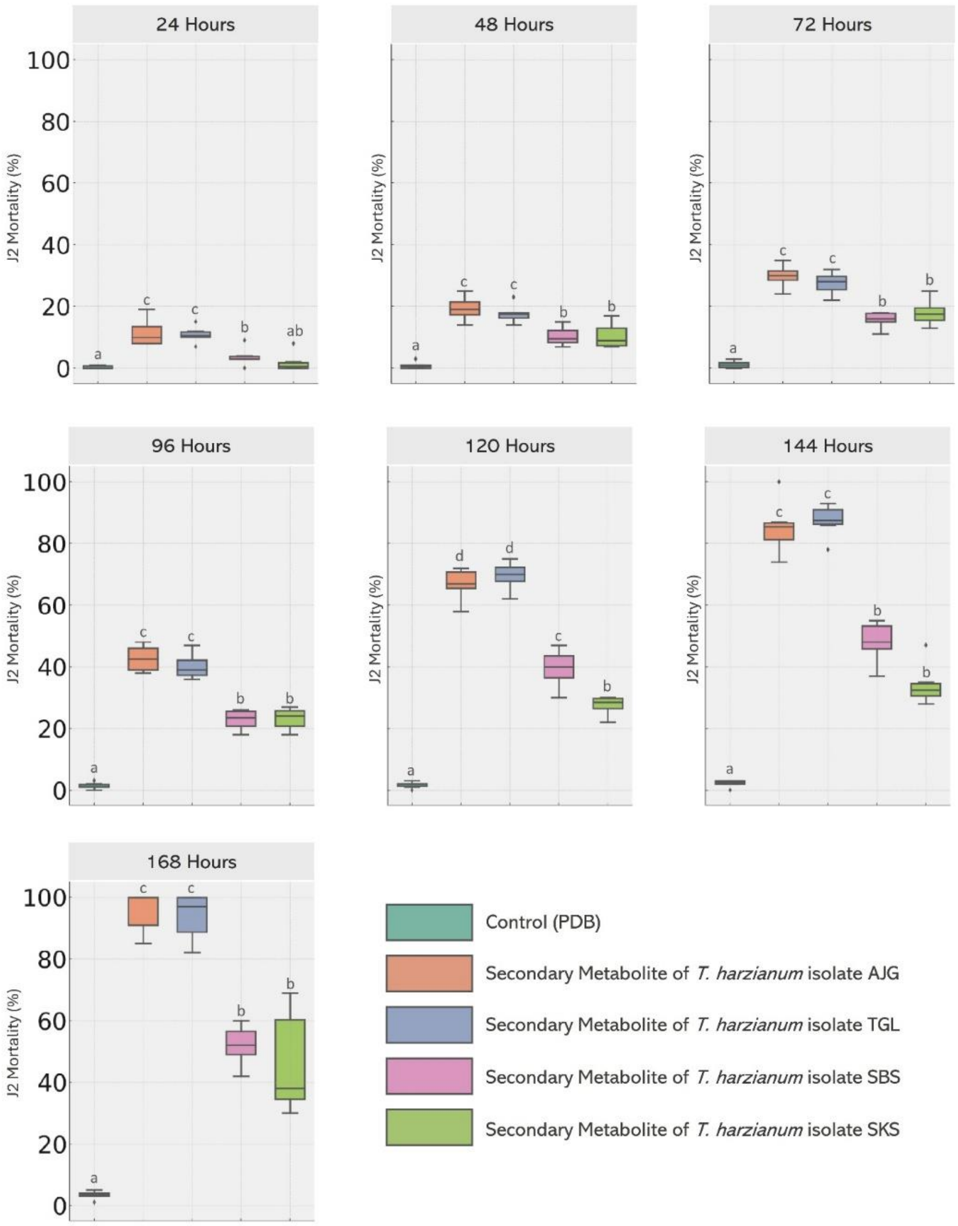


Figure 2. The nematicidal effect of *Trichoderma harzianum* secondary metabolites on *Meloidogyne incognita* J2. Note: Groups that share at least one letter above the boxplots at the same time point are not significantly different, as determined by post-hoc Tukey's HSD tests at the 5% significance level. Shared letters indicate statistical similarity

The reduced egg hatching observed with effective *T. harzianum* isolates likely involves specific fungal metabolites disrupting nematode embryogenesis and developmental signaling pathways. Previous studies indicated that metabolites such as 6-pentyl-alpha-pyrone produced by *T. harzianum* can directly interfere with the permeability and integrity of nematode eggshells, resulting in impaired embryonic development (Baazeem et al. 2021; Lopes et al. 2024). These metabolites could also indirectly affect hatching by altering the egg's surrounding chemical environment, inhibiting hatching stimulants, or enhancing the activity of natural egg inhibitors (Devi 2018).

Similarly, the high J2 mortality may reflect the effects of secondary metabolites with neurotoxic and enzyme-inhibitory properties. Some metabolites have been reported to exert direct nematicidal effects by causing irreversible damage to the nematode nervous system, resulting in paralysis and subsequent death (Yao et al. 2023). The observed variation in mortality rates among the different *T. harzianum* isolates may reflect isolate-specific differences in metabolite profiles, with highly effective isolates producing metabolites that are more potent or more readily absorbed by nematodes.

In addition to direct nematicidal and ovicidal activities, *Trichoderma*-derived secondary metabolites have been recognized for their multifaceted roles in managing plant-parasitic nematodes through induced systemic resistance (ISR) in host plants. Compounds such as harzianic acid and

6-pentyl-alpha-pyrone (6-PP), which are abundant in certain isolates of *T. harzianum*, not only exhibit direct toxicity to nematodes but also enhance plant defense responses, thereby providing integrated nematode management strategies. For instance, harzianic acid and 6-PP have been shown to induce the expression of plant defense-related genes, such as those involved in phenylpropanoid pathways, jasmonic acid signaling, and salicylic acid-dependent defenses. This systemic protection complements the direct toxicity mechanisms, reinforcing the efficacy of secondary metabolite-based biocontrol applications in agricultural practices (Rangel et al. 2021; Zaid et al. 2022).

Furthermore, the practical potential of *T. harzianum* metabolites as nematode management agents can be significantly enhanced by advancements in formulation and delivery methods. Recent research has demonstrated that formulating metabolites into nano-emulsions, encapsulated slow-release formulations, or incorporating them into bioactive carrier materials markedly improves their environmental stability, longevity, and efficacy under realistic agricultural conditions (Wang et al. 2021; Yan and Khan 2021). For example, encapsulation of gliotoxin and volatile organic compounds into biodegradable polymeric nanoparticles has shown sustained release and prolonged nematicidal activity, leading to substantial reductions in nematode populations even under challenging field conditions (Chen et al. 2020; Hamrouni et al. 2025). These advancements validate the practical use of secondary metabolites derived from *T. harzianum* and address the viability and consistency concerns associated with traditional biological control strategies.

Compound profiling of *T. harzianum* secondary metabolites via GC-MS

The secondary metabolite analysis of *T. harzianum* isolate AJG using GC-MS identified 35 distinct compounds representing diverse chemical groups such as fatty acids, alcohols, ketones, aldehydes, and hydrocarbons. Among the identified metabolites, the most abundant included 9-octadecenoic acid (Z)-, methyl ester (22.91%); hexadecanoic acid, methyl ester (22.64%); and octadecanoic acid, ethyl ester (8.78%). These compounds collectively accounted for more than half of the total peak area detected in the sample. The GC-MS chromatogram of secondary metabolites produced by *T. harzianum* isolate AJG is shown in Figure 3.

Other notable metabolites present in moderate abundances were hexadecane, 2,6,10,14-tetramethyl- (6.25%); cyclopentadecanone, 2-hydroxy- (5.35%); pentacosane (3.87%); triacontane (3.38%); and tetradecanoic acid, methyl ester (2.33%). The analysis also revealed several minor constituents, such as acetic acid, various alcohols, aldehydes, and ketones, reflecting the metabolic complexity and biochemical diversity of *T. harzianum* isolate AJG. The metabolite profile of *T. harzianum* isolate AJG is summarized in Table 1.

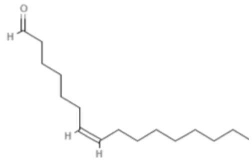
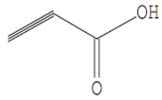
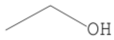
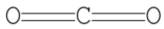
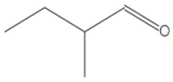
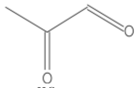
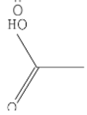
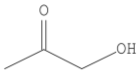
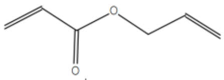
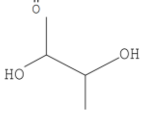
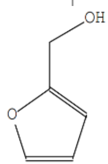
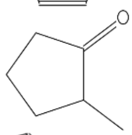
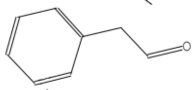
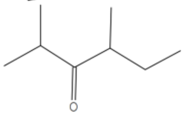
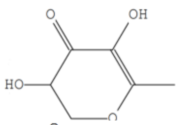
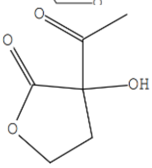
The GC-MS analysis of secondary metabolites derived from *T. harzianum* isolate TGL identified 28 distinct chemical constituents, and the GC-MS chromatogram is shown in Figure 4. Like *T. harzianum* isolate AJG, the *T. harzianum* isolate TGL produced diverse compounds, including fatty acids, esters, alcohols, ketones, aldehydes, and hydrocarbons. Among these secondary metabolites, the most abundant were 9-octadecenoic acid (Z)-, methyl ester (20.21%), and hexadecanoic acid, methyl ester (18.96%), collectively accounting for nearly 40% of the total peak area.

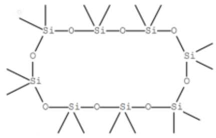
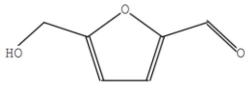
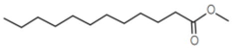
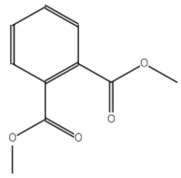
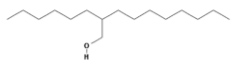
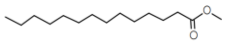
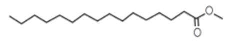
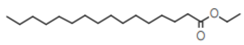
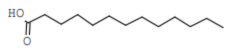
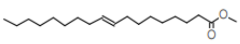
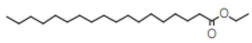
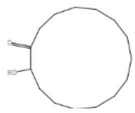
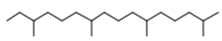
Trichoderma harzianum isolate TGL also showed notable concentrations of metabolites such as 2-furancarboxaldehyde, 5-(hydroxymethyl)- (10.47%); octadecanoic acid, ethyl ester (5.95%); acetic acid (5.85%); and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (5.29%). Several other compounds, such as 2,5-dimethyl-4-hydroxy-3(2H)-furanone and hexadecane, 2,6,10,14-tetramethyl-, were present at moderate concentrations, highlighting the biochemical complexity of this isolate.

Comparatively, *T. harzianum* isolates TGL and AJG exhibited significant similarities in their major secondary metabolites, particularly in the high abundance of 9-octadecenoic acid (Z)-, methyl ester, and hexadecanoic acid, methyl ester. However, notable quantitative differences were observed. *Trichoderma harzianum* isolate AJG had slightly higher abundances of these fatty acid esters (22.91 and 22.64%, respectively) than *T. harzianum* isolate TGL. In addition, *T. harzianum* isolate AJG showed relatively higher amounts of certain hydrocarbons and ketones, such as hexadecane, 2,6,10,14-tetramethyl-, and cyclopentadecanone, 2-hydroxy-, while *T. harzianum* isolate TGL had higher relative amounts of oxygenated aromatic compounds such as 2-furancarboxaldehyde, 5-(hydroxymethyl)-, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. A comprehensive list of the identified compounds in the metabolomic profile of *T. harzianum* isolate TGL is presented in Table 2.

The metabolite profiling of *T. harzianum* isolate AJG revealed several bioactive compounds that are likely the key players in its nematicidal and ovicidal activities against *M. incognita*. Notably, the detection of hexadecanoic acid methyl ester (methyl palmitate) and 9-octadecenoic acid (Z)-, methyl ester (methyl oleate) is particularly promising, as it indicates significant potential mechanisms underlying nematode control (Lu et al. 2020; Sall et al. 2024). Previous studies have demonstrated that methyl palmitate exhibits potent nematicidal properties through mechanisms such as disruption of lipid membranes and modulation of nematode gene expression (Elbanhawy et al. 2019). Lu et al. (2020) reported that increasing concentrations of methyl palmitate significantly inhibited *M. incognita* egg hatching by up to 95.5%, supporting its crucial role in the observed ovicidal activity of *T. harzianum* isolate AJG in this study.

Table 1. The secondary metabolites identified in *Trichoderma harzianum* isolate AJG by GC-MS

Peak #	Retention time	Area percentage (%)	Molecular weight (g mol^{-1})	Compound name	Formula	CAS registry number	Chemical structure
1	0.171	0.09	238	Z-7-Hexadecenal	$C_{16}H_{30}O$	56797-40-1	
2	1.529	0.43	70	Propiolic acid	$C_3H_2O_2$	471-25-0	
3	1.636	0.06	46	Ethanol	C_2H_6O	64-17-5	
4	1.773	0.21	118	Carbon dioxide (CAS)	CO_2	124-38-9	
5	2.445	0.04	86	Butanal, 2-methyl- (CAS)	$C_5H_{10}O$	96-17-3	
6	4.772	0.62	72	Propanal, 2-oxo- (CAS)	$C_3H_4O_2$	78-98-8	
7	5.093	2.80	60	Acetic acid (CAS)	$C_2H_4O_2$	64-19-7	
8	5.356	2.07	74	2-Propanone, 1-hydroxy- (CAS)	$C_3H_6O_2$	116-09-6	
9	5.985	0.59	112	2-Propenoic acid, 2-propenyl ester (CAS)	$C_6H_8O_2$	999-55-3	
10	6.512	1.21	90	2,3-Butanediol (CAS)	$C_4H_{10}O_2$	513-85-9	
11	7.376	0.14	98	2-Furanmethanol (CAS)	$C_5H_6O_2$	98-00-0	
12	9.735	0.46	98	Cyclopentanone, 2-methyl-	$C_6H_{10}O$	1120-72-5	
13	11.464	0.21	120	Benzeneacetaldehyde (CAS)	C8H8O	122-78-1	
14	13.624	0.56	128	3-Hexanone, 2,4-dimethyl- (CAS)	$C_8H_{16}O$	18641-70-8	
15	15.464	0.17	132	anhydro - sugar	$C_5H_8O_4$	0-00-0	-
16	16.286	1.35	144	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	28564-83-2	
17	17.847	0.12	144	2-acetyl-2-hydroxy-.gamma.-butyrolactone	$C_6H_8O_4$	135366-64-2	

18	18.607	0.20	592	Hexadecamethylcyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	556-68-3	
19	19.723	0.44	214	2-Ethyl-1-dodecanol	$C_{14}H_{30}O$	19780-33-7	-
20	20.470	4.13	126	2-furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	67-47-0	
21	21.778	0.99	214	Dodecanoic acid, methyl ester (CAS)	$C_{13}H_{26}O_2$	111-82-0	
22	22.252	0.97	214	2-Ethyl-1-dodecanol	$C_{14}H_{30}O$	19780-33-7	-
23	23.707	0.28	194	1,2-benzenedicarboxylic acid, dimethyl	$C_{10}H_{10}O_4$	131-11-3	
24	24.723	0.68	242	2-Hexyl-1-decanol	$C_{16}H_{34}O$	2425-77-6	
25	26.589	2.33	242	Tetradecanoic acid, methyl ester (CAS)	$C_{15}H_{30}O_2$	124-10-7	
26	29.203	2.06	268	Nonadecane (CAS)	$C_{19}H_{40}$	629-92-5	
27	31.100	22.64	270	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	112-39-0	
28	32.236	1.08	284	Hexadecanoic acid, ethyl ester (CAS)	$C_{18}H_{36}O_2$	628-97-7	
29	33.369	3.87	352	Pentacosane	$C_{25}H_{52}$	629-99-2	
30	34.189	2.52	214	Tridecanoic acid (CAS)	$C_{13}H_{26}O_2$	638-53-9	
31	34.987	22.91	296	9-Octadecenoic acid (Z)-, methyl ester (CAS)	$C_{19}H_{36}O_2$	112-62-9	
32	36.174	8.78	312	Octadecanoic acid, ethyl ester (CAS)	$C_{20}H_{40}O_2$	111-61-5	
33	37.173	3.38	422	Triacontane	$C_{30}H_{62}$	638-68-6	
34	37.745	5.35	240	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	4727-18-8	
35	38.944	6.25	282	Hexadecane, 2,6,10,14-tetramethyl- (CAS)	$C_{20}H_{42}$	638-36-8	
Total		100					

Note: The compounds were identified by comparing their mass spectra from the chromatogram with the Wiley9.LIB mass spectral database

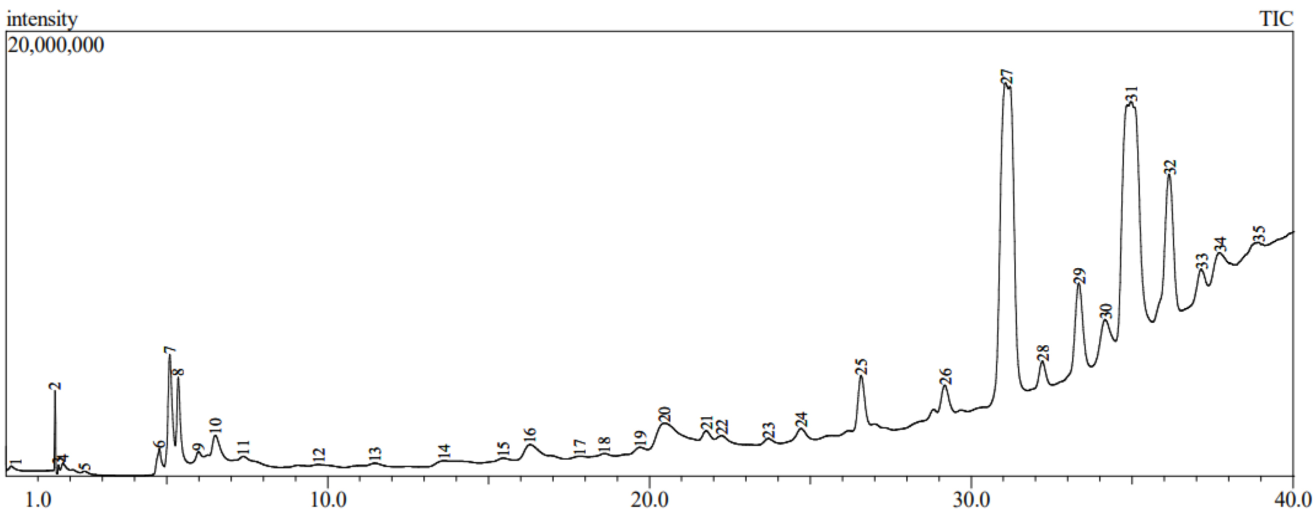
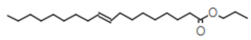
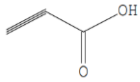
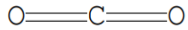
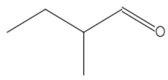
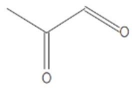
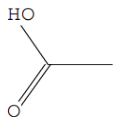
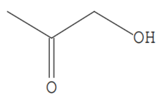
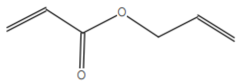
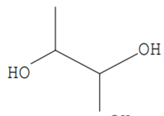
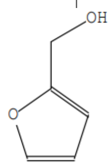
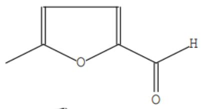
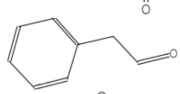
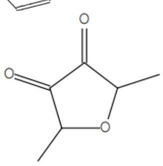
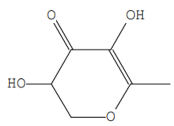
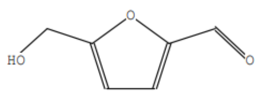
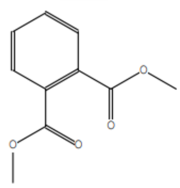
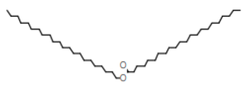
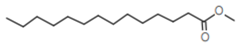
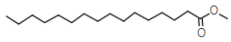
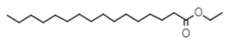
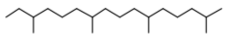
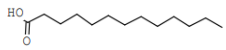
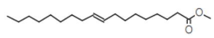
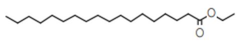
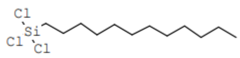
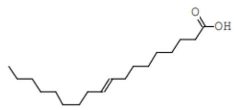


Figure 3. The GC-MS chromatogram of the secondary metabolites produced by *Trichoderma harzianum* isolate AJG

Table 2. Metabolites identified in *Trichoderma harzianum* isolate TGL by GC-MS

Peak #	Retention Time	Area Percentage (%)	Molecular Weight (g mol^{-1})	Compound Name	Formula	CAS Registry Number	Chemical structure
1	0.175	0.15	324	Oleic acid, propyl ester	$C_{21}H_{40}O_2$	111-59-1	
2	1.533	0.44	70	Propiolic acid	$C_3H_2O_2$	471-25-0	
3	1.800	0.64	118	Carbon dioxide (CAS)	CO_2	124-38-9	
4	2.466	0.25	86	Butanal, 2-methyl- (CAS)	$C_5H_{10}O$	96-17-3	
5	4.695	0.82	72	Propanal, 2-oxo- (CAS)	$C_3H_4O_2$	78-98-8	
6	5.021	5.85	60	Acetic acid (CAS)	$C_2H_4O_2$	64-19-7	
7	5.290	3.12	74	2-Propanone, 1-hydroxy- (CAS)	$C_3H_6O_2$	116-09-6	
8	5.934	1.86	112	2-Propenoic acid, 2-propenyl ester (CAS)	$C_6H_8O_2$	999-55-3	
9	6.439	3.38	90	2,3-Butanediol (CAS)	$C_4H_{10}O_2$	513-85-9	
10	7.353	2.59	98	2-Furanmethanol (CAS)	$C_5H_6O_2$	98-00-0	
11	9.500	1.13	110	5-Methyl furfural	$C_6H_6O_2$	620-02-0	
12	11.491	0.33	120	Benzeneacetaldehyde (CAS)	C_8H_8O	122-78-1	
13	13.609	3.89	128	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	3658-77-3	
14	15.463	0.75	132	Anhydro-sugar	$C_5H_8O_4$	C5H8O4	-
15	16.286	5.29	144	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	28564-83-2	
16	20.378	10.47	126	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	67-47-0	
17	23.707	0.30	194	1,2-Benzenedicarboxylic acid, dimethyl ester (CAS)	$C_{10}H_{10}O_4$	131-11-3	

18	24.760	0.15	648S	Docosanoic acid, docosyl ester (CAS)	$C_{44}H_{88}O_2$	17671-27-1	
19	26.608	0.89	242	Tetradecanoic acid, methyl ester (CAS)	$C_{15}H_{30}O_2$	124-10-7	
20	29.211	0.93	268	Nonadecane	$C_{19}H_{40}$	629-92-5	
21	31.099	18.96	270	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	112-39-0	
22	32.216	1.11	284	Hexadecanoic acid, ethyl ester (CAS)	$C_{18}H_{36}O_2$	628-97-7	
23	33.388	1.89	282	Hexadecane, 2,6,10,14-tetramethyl- (CAS)	$C_{20}H_{42}$	638-36-8	
24	34.162	2.97	214	Tridecanoic acid (CAS)	$C_{13}H_{26}O_2$	638-53-9	
25	34.869	20.21	296	9-Octadecenoic acid (Z)-, methyl ester (CAS)	$C_{19}H_{36}O_2$	112-62-9	
26	36.133	5.95	312	Octadecanoic acid, ethyl ester (CAS)	$C_{20}H_{40}O_2$	111-61-5	
27	37.493	2.75	302	Silane, trichloro-dodecyl- (CAS)	$C_{12}H_{25}Cl_3Si$	4484-72-4	
28	37.741	2.92	282	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	0-00-0	
	Total	100					

Note: The compounds were identified by comparing their mass spectra from the chromatogram with the Wiley9.LIB mass spectral database

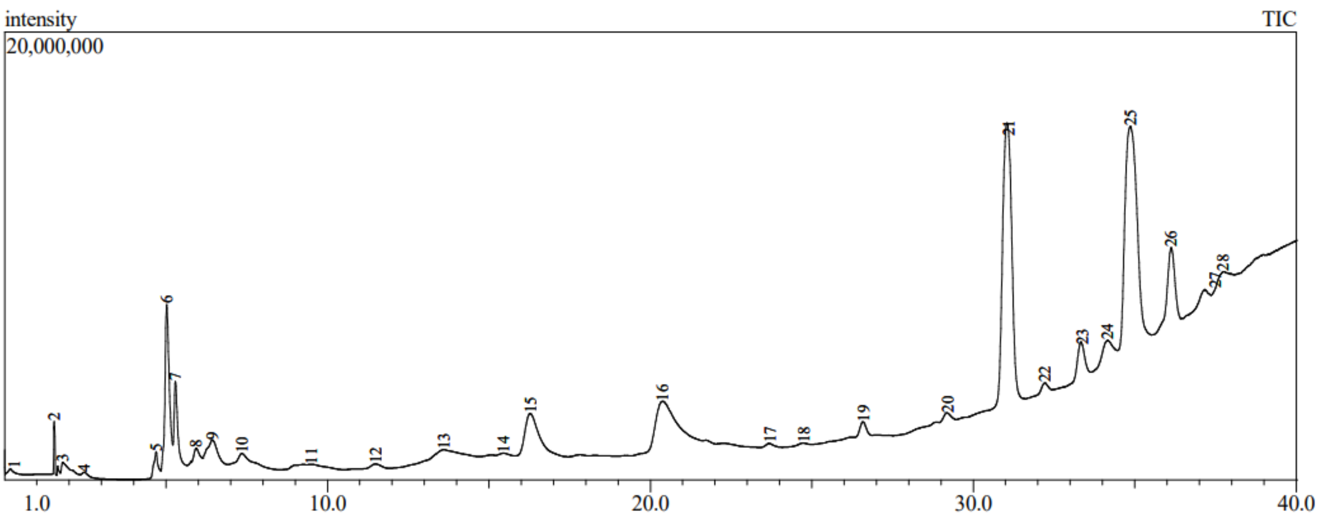


Figure 4. The GC-MS chromatogram of the secondary metabolites produced by *Trichoderma harzianum* isolate TGL

Similarly, methyl oleate, another abundant metabolite identified in *T. harzianum* isolate AJG, has been documented to interact directly with lipid membranes, increasing their permeability, which then leads to cellular disruption and nematode mortality (Asyiah et al. 2025). The substantial presence of methyl oleate (22.91%) in the metabolite profile suggests it likely contributes significantly to the high mortality rates observed in J2 nematodes treated with secondary metabolites from *T. harzianum* isolate AJG (Vieira et al. 2023). Additionally, the detection of cyclopentadecanone, 2-hydroxy-, and other cyclic ketones might further enhance the nematicidal potential due to their known antimicrobial and insecticidal activities, possibly acting synergistically with the fatty acid esters to enhance bioactivity (Helal et al. 2019; Mansour et al. 2023).

The metabolite profiling of *T. harzianum* isolate TGL also identified similar fatty acid methyl esters such as methyl palmitate and methyl oleate. However, at slightly lower abundances than for *T. harzianum* isolate AJG.

Nonetheless, *T. harzianum* isolate TGL uniquely exhibited a higher relative abundance of aromatic compounds, particularly 2-furancarboxaldehyde, 5-(hydroxymethyl)- (known as hydroxymethylfurfural), and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. These aromatic metabolites have previously been shown to exhibit antimicrobial and antifungal properties by inducing oxidative stress and disrupting membranes (Saravanan et al. 2022; Sergany 2024). While these aromatic compounds might indirectly influence nematode viability by altering their microbial environment, their direct nematicidal efficacy is comparatively less documented, potentially explaining the slightly reduced performance of *T. harzianum* isolate TGL.

Furthermore, isolate-specific differences in metabolite profiles highlight the potential influence of environmental and genetic factors on metabolite synthesis. Variations in cultivation conditions and genetic background are known to significantly affect fungal secondary metabolite production, thereby influencing their biocontrol efficacy (Goyal et al. 2017; Adiwena et al. 2023). It is plausible that environmental adaptations of *T. harzianum* isolates AJG and TGL, derived from different geographical locations, contribute to their distinct metabolite compositions and resultant bioactivity. The categorization of secondary metabolites from *T. harzianum* isolates AJG and TGL into broader chemical groups provides essential insights into their potential roles in plant pest and pathogen management (Figure 5). Grouping metabolites into chemical classes facilitates understanding their potential bioactivities and possible mechanisms of action against plant pathogens, including nematodes.

The secondary metabolite profile of *T. harzianum* isolate AJG was dominated by fatty acids and their derivatives, accounting for 61.76% of the total metabolite composition. Aldehydes represented the second-largest group (13.00%), followed by ketones (12.30%), alcohols (5.97%), and hydrocarbons (5.57%). This composition highlights the prominence of lipid-derived metabolites, particularly fatty acid methyl esters, which are widely recognized for their antimicrobial, antifungal, and nematicidal properties.

Comparatively, the secondary metabolite profile of *T. harzianum* isolate TGL was also dominated by fatty acids and their derivatives (54.39%). However, this proportion was slightly lower than in *T. harzianum* isolate AJG. Additionally, *T. harzianum* isolate TGL exhibited notable differences in the relative abundance of other metabolite groups. For example, aldehydes constituted a significant portion (13.96%), whereas furans and related aromatic compounds were substantially more abundant in *T. harzianum* isolate TGL, reflecting distinct biochemical pathways active in this isolate. Alcohols and ketones were present in moderate amounts, further underscoring differences in secondary metabolite synthesis between these isolates.

Overall, these findings indicate substantial biochemical diversity between *T. harzianum* isolates AJG and TGL, which may underlie observed differences in their biological activities against *M. incognita*. The notable dominance of fatty acids and their derivatives in both isolates emphasizes their potential utility in biological control applications. However, isolate-specific variations in metabolite profiles suggest differences in their mechanisms of action and potency, supporting the need for targeted selection and optimization of isolates for effective pest management strategies.

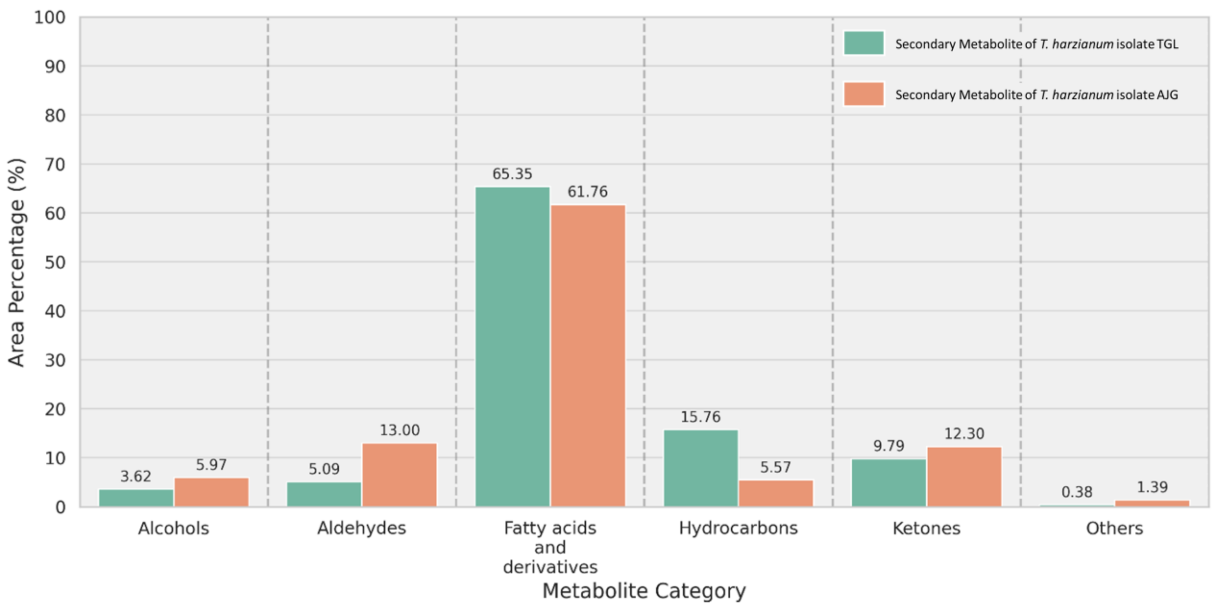


Figure 5. The classification of secondary metabolites from *Trichoderma harzianum* isolates AJG and TGL into major chemical groups

The substantial abundance of fatty acids and their derivatives in both *T. harzianum* isolates AJG and TGL suggests a strong nematicidal potential, given that fatty acid esters and related compounds have been extensively reported to disrupt nematode membranes and metabolic functions (Eloh et al. 2015). For example, hexadecanoic acid, methyl ester, and 9-octadecenoic acid, methyl ester, have previously been demonstrated to severely disrupt nematode membrane integrity, impair motility, and inhibit egg hatching (Lu et al. 2020; Fabiyi et al. 2022). Therefore, the high abundance of these fatty acid derivatives in both isolates likely contributes significantly to their overall nematicidal and ovicidal efficacy.

A notable difference between the isolates was the greater abundance of furans and aromatic aldehydes identified in *T. harzianum* isolate TGL than in *T. harzianum* isolate AJG. Aromatic compounds such as hydroxymethylfurfural and related furan derivatives found in *T. harzianum* isolate TGL have been reported to exhibit antimicrobial properties, primarily by inducing oxidative stress and disrupting membranes (Saravanan et al. 2022). While less extensively studied against nematodes, their presence in significant quantities could imply additional indirect mechanisms of pest suppression or modulation of microbial communities associated with nematodes.

Finally, the differences observed in ketone and aldehyde abundances between *T. harzianum* isolates AJG and TGL suggest that isolate-specific enzymatic and metabolic pathways influence secondary metabolite synthesis. Environmental adaptations, genetic factors, and cultivation conditions likely help shape these biochemical profiles, resulting in different levels of biological activity (Calvo et al. 2002; Keller 2019). Further studies involving more detailed metabolomic characterization and bioactivity assays could provide valuable insights into optimizing the use of these isolates for developing sustainable nematicidal formulations tailored to specific agricultural contexts.

In conclusion, this study has demonstrated the significant nematicidal activity of secondary metabolites derived from *T. harzianum* isolates against *M. incognita*, with *T. harzianum* isolates AJG and TGL exhibiting superior efficacy. Fatty acid esters, particularly methyl palmitate and methyl oleate, were identified as key bioactive compounds responsible for disrupting nematode membranes and inhibiting egg hatching. The isolate-specific differences in metabolite profiles emphasize the importance of detailed metabolomic characterization for selecting the most potent strains. These findings highlight the potential for developing effective, sustainable biological nematicides tailored to agricultural pest management.

ACKNOWLEDGEMENTS

The author sincerely thanks the University of Jember, Indonesia for financially supporting this research and publication through the MBKM-Based Research Grant (*Hibah Penelitian Berbasis Merdeka Belajar Kampus Merdeka*). This funding was awarded according to the Rector's Decree of the University of Jember No. 7554/UN25/KP/2024, dated March 20, 2024, and the Assignment Agreement No. 3202/UN25.3.1/LT/2024, dated March 21, 2024.

REFERENCES

Adiwena M, Murtilaksono A, Egra S, Hoesain M, Asyiah IN, Pradana AP, Izatika ZN. 2023. The effects of micronutrient-enriched media on the efficacy of *Bacillus subtilis* as biological control agent against *Meloidogyne incognita*. Biodiversitas 24 (1): 33-39. DOI: 10.13057/biodiv/d240105.

Asyiah IN, Mudakir I, Budiman A, Pradana AP. 2025. Media optimization for nematode-trapping fungus *Orbilia jesu-laurae* and its effect in managing *Meloidogyne incognita*. Cogent Food Agric 11 (1): 2442667. DOI: 10.1080/23311932.2024.2442667.

Ayaz M, Li CH, Ali Q, Zhao W, Chi YK, Shafiq M, Ali F, Yu XY, Yu Q, Zhao JT. 2023. Bacterial and fungal biocontrol agents for plant disease protection: Journey from lab to field, current status, challenges, and global perspectives. Molecules 28 (18): 6735. DOI: 10.3390/molecules28186735.

Azlay L, El Boukhari MEM, Mayad EH, Barakate M. 2023. Biological management of root-knot nematodes (*Meloidogyne* spp.): A review. Org Agric 13 (1): 99-117. DOI: 10.1007/s13165-022-00417-y.

Baazeem A, Almanea A, Manikandan P, Alorabi M, Vijayaraghavan P, Abdel-Hadi A. 2021. In vitro antibacterial, antifungal, nematocidal and growth promoting activities of *Trichoderma hamatum* FB10 and its secondary metabolites. J Fungi 7 (5): 331. DOI: 10.3390/jof7050331.

Calvo AM, Wilson RA, Bok JW, Keller NP. 2002. Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66 (3): 447-459. DOI: 10.1128/MMBR.66.3.447-459.2002.

Chen Y-C, Seyedsayamdost MR, Ringstad N. 2020. A microbial metabolite synergizes with endogenous serotonin to trigger *C. elegans* reproductive behavior. Proc Natl Acad Sci 117 (48): 30589-30598. DOI: 10.1073/pnas.2017918117.

Devi G. 2018. Utilization of nematode destroying fungi for management of plant-parasitic nematodes-a review. Biosci Biotechnol Res Asia 15 (2): 377-396. DOI: 10.13005/bbra/2642.

Elbanhawy AA, Elsherbiny EA, Abd El-Mageed AE, Abdel-Fattah GM. 2019. Potential of fungal metabolites as a biocontrol agent against cotton aphid, *Aphis gossypii* Glover and the possible mechanisms of action. Pestic Biochem Physiol 159: 34-40. DOI: 10.1016/j.pestbp.2019.05.013.

Eloh K, Demurtas M, Deplano A, Ngoutane Mfopa A, Murgia A, Maxia A, Onnis V, Caboni P. 2015. In vitro nematicidal activity of aryl hydrazones and comparative GC-MS metabolomics analysis. J Agric Food Chem 63 (45): 9970-9976. DOI: 10.1021/acs.jafc.5b04815.

Fabiyi OA, Baker MT, Olatunji GA. 2022. Application of fatty acid esters on *Meloidogyne incognita* infected jew's mallow plants. Pak J Nematol 40 (2): 127-137. DOI: 10.17582/journal.pjn/2022/40.2.127.137.

Feyisa B, Lencho A, Selvaraj T, Amb P, Getaneh G. 2016. Evaluation of some botanicals and *Trichoderma harzianum* for the management of tomato root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chit Wood). Adv Crop Sci Technol 4: 201. DOI: 10.5897/JEN2015.0145.

Forghani F, Hajihassani A. 2020. Recent advances in the development of environmentally benign treatments to control root-knot nematodes. Front Plant Sci 11: 1125. DOI: 10.3389/fpls.2020.01125.

Ghazanfar MU, Raza M, Raza W, Qamar MI. 2018. *Trichoderma* as potential biocontrol agent, its exploitation in agriculture: A review. Plant Prot 2 (3): 109-135.

Goyal S, Ramawat KG, Mérillon J-M. 2017. Different shades of fungal metabolites: an overview. In: Mérillon JM, Ramawat KG (eds.). Fungal Metabolites. Springer, Cham. DOI: 10.1007/978-3-319-25001-4_34.

Hamrouni R, Regus F, Farnet Da Silva A-M, Orsiere T, Boudenne J-L, Laffont-Schwob I, Christen P, Dupuy N. 2025. Current status and future trends of microbial and nematode-based biopesticides for biocontrol of crop pathogens. Crit Rev Biotechnol 45 (2): 333-352. DOI: 10.1080/07388551.2024.2370370.

Helal NM, Ibrahim N, Khattab H. 2019. Phytochemical analysis and antifungal bioactivity of *Pulicaria undulata* (L.) methanolic extract

and essential oil. Egypt J Bot 59 (3): 827-844. DOI: 10.21608/ejbo.2019.12259.1308.
Keller NP. 2019. Fungal secondary metabolism: regulation, function and drug discovery. Nat Rev Microbiol 17 (3): 167-180. DOI: 10.1038/s41579-018-0121-1.
Keswani C, Singh HB, Hermosa R, García-Estrada C, Caradus J, He Y-W, Mezaache-Aichour S, Glare TR, Borriss R, Vinale F. 2019. Antimicrobial secondary metabolites from agriculturally important fungi as next biocontrol agents. Appl Microbiol Biotechnol 103: 9287-9303. DOI: 10.1007/s00253-019-10209-2.
Khan A, Aiman SI, Baber Y, Hassan F, Usman HM, Sohail MA, Abbas A. 2021. An overview of root-knot nematodes and their management. J Entomol Zool Stud 9: 35-40.
Khan RAA, Najeeb S, Mao Z, Ling J, Yang Y, Li Y, Xie B. 2020. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic bacteria and root-knot nematode. Microorganisms 8 (3): 401. DOI: 10.3390/microorganisms8030401.
Lopes A, Rivadavea W, Silva G. 2024. *Trichoderma* secondary metabolites for effective plant pathogen control. In: Abd-Elsalam KA (eds.). Nanohybrid Fungicides. Elsevier, Amsterdam. DOI: 10.1016/B978-0-443-23950-2.00008-4.
Lu Q, Liu T, Wang N, Dou Z, Wang K, Zuo Y. 2020. Nematicidal effect of methyl palmitate and methyl stearate against *Meloidogyne incognita* in bananas. J Agric Food Chem 68 (24): 6502-6510. DOI: 10.1021/acs.jafc.0c00218.
Mansour T, Radwan WH, Mansour M, Gomaa M, Farouk F, Shepl M, Soliman AG, Abd-Elhalim BT, El-Senosy MM, Bakry A, Ebeed NM, Alsenosy NK, Elhariry H, Galal A, El-Sayed SM, Adly E, Abu-Hussien SH. 2023. Larvicidal potential, toxicological assessment, and molecular docking studies of four Egyptian bacterial strains against *Culex pipiens* L.(Diptera: Culicidae). Sci Rep 13 (1): 17230. DOI: 10.1038/s41598-023-44279-0.
Moo-Koh FA, Cristóbal-Alejo J, Andrés MF, Martín J, Reyes F, Tun-Suárez JM, Gamboa-Angulo M. 2022. In vitro assessment of organic and residual fractions of nematicidal culture filtrates from thirteen tropical *Trichoderma* strains and metabolic profiles of most-active. J Fungi 8 (1): 82. DOI: 10.3390/jof8010082.
Pathak VM, Verma VK, Rawat BS, Kaur B, Babu N, Sharma A, Dewali S, Yadav M, Kumari R, Singh S. 2022. Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. Front Microbiol 13: 962619. DOI: 10.3389/fmicb.2022.962619.
Rangel LI, Hamilton O, de Jonge R, Bolton MD. 2021. Fungal social influencers: secondary metabolites as a platform for shaping the plant-associated community. Plant J 108 (3): 632-645. DOI: 10.1111/tpj.15490.
Rodrigo S, García-Latorre C, Santamaria O. 2021. Metabolites produced by fungi against fungal phytopathogens: Review, implementation and perspectives. Plants 11 (1): 81. DOI: 10.3390/plants11010081.
Sall C, Djigal D, Faye O, Soumboundou M, Ndong A, Ndao M, Sylla SM, Sarr D. 2024. Chemical composition and evaluation of the nematicidal activity of *Datura metel* seed oil against *Meloidogyne javanica*. J Agric Chem Environ 14 (1): 90-101. DOI: 10.4236/jacen.2025.141006.
Saravanan R, Saranya N, Ragapriya V, Rajaswaminathan V, Kavino M, Krishnamoorthy A, Nakkeeran S. 2022. Nematicidal property of clindamycin and 5-hydroxy-2-methyl furfural (HMF) from the banana endophyte *Bacillus velezensis* (YEBBR6) against banana burrowing nematode *Radopholus similis*. Indian J Microbiol 62 (3): 364-373. DOI: 10.1007/s12088-022-01011-2.
Sergany M. 2024. Efficacy of certain organic extracts at three concentrations on tomato plants infected with the root-knot nematode with reference to GC-MS analysis. Egypt J Plant Prot Res Inst 7 (4): 448-463. DOI: 10.4314/ejppri.v7i4.2.
Shahriar SA, Islam MN, Chun CNW, Kaur P, Rahim MA, Islam MM, Uddain J, Siddiquee S. 2022. Microbial metabolomics interaction and ecological challenges of *Trichoderma* species as biocontrol inoculant in crop rhizosphere. Agronomy 12 (4): 900. DOI: 10.3390/agronomy12040900.
Soesanto L, Mugiastuti E, Manan A. 2019. Raw secondary metabolites application of two *Trichoderma harzianum* isolates towards vascular streak dieback on cocoa seedlings. Pelita Perkebunan 35 (1): 22-32. DOI: 10.22302/iccri.jur.pelitaperkebunan.v35i1.346.
Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M, Araniti F, Sharma A. 2020. *Trichoderma*: The "secrets" of a multitalented biocontrol agent. Plants 9 (6): 762. DOI: 10.3390/plants9060762.
Subedi S, Thapa B, Shrestha J. 2020. Root-knot nematode (*Meloidogyne incognita*) and its management: a review. J Agric Nat Resour 3 (2): 21-31. DOI: 10.3126/janr.v3i2.32298.
Tapia-Vázquez I, Montoya-Martínez AC, De los Santos-Villalobos S, Ek-Ramos MJ, Montesinos-Matías R, Martínez-Anaya C. 2022. Root-knot nematodes (*Meloidogyne* spp.) a threat to agriculture in Mexico: Biology, current control strategies, and perspectives. World J Microbiol Biotechnol 38 (2): 26. DOI: 10.1007/s11274-021-03211-2.
Tudi M, Daniel Ruan H, Wang L, Lyu J, Sadler R, Connell D, Chu C, Phung DT. 2021. Agriculture development, pesticide application and its impact on the environment. Intl J Environ Res Public Health 18 (3): 1112. DOI: 10.3390/ijerph18031112.
Vieira AF, Xatse MA, Murray SY, Olsen CP. 2023. Oleic acid metabolism in response to glucose in *C. elegans*. Metabolites 13 (12): 1185. DOI: 10.3390/metabo13121185.
Wang X-Y, Xu T-T, Sun L-J, Cen R-H, Su S, Yang X-Q, Yang Y-B, Ding Z-T. 2021. The chemical diversity, the attractant, anti-acetylcholinesterase, and antifungal activities of metabolites from biocontrol *Trichoderma harzianum* uncovered by OSMAC strategy. Bioorg Chem 114: 105148. DOI: 10.1016/j.bioorg.2021.105148.
Yan L, Khan RAA. 2021. Biological control of bacterial wilt in tomato through the metabolites produced by the biocontrol fungus, *Trichoderma harzianum*. Egypt J Biol Pest Control 31: 5. DOI: 10.1186/s41938-020-00351-9.
Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J. 2023. *Trichoderma* and its role in biological control of plant fungal and nematode disease. Front Microbiol 14: 1160551. DOI: 10.3389/fmicb.2023.1160551.
Zaid R, Koren R, Kligun E, Gupta R, Leibman-Markus M, Mukherjee PK, Kenerley CM, Bar M, Horwitz BA. 2022. Gliotoxin, an immunosuppressive fungal metabolite, primes plant immunity: evidence from *Trichoderma virens*-tomato interaction. MBio 13 (4): e00389-22. DOI: 10.1128/mbio.00389-22.
Zeilinger S, Gruber S, Bansal R, Mukherjee PK. 2016. Secondary metabolism in *Trichoderma*-chemistry meets genomics. Fungal Biol Rev 30 (2): 74-90. DOI: 10.1016/j.fbr.2016.05.001.