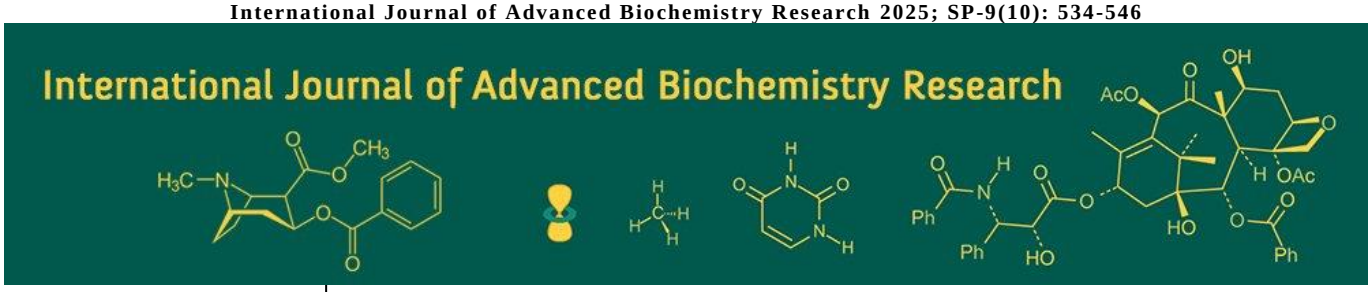


ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(10): 534-546
www.biochemjournal.com
Received: 15-07-2025
Accepted: 18-08-2025

Resmi AR
Regional Agricultural Research Station, Kerala Agricultural University, Pilicode, Kasaragod, Kerala, India

Reshmy Vijayaraghavan
Department of Plant Pathology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India

Deepa James
Department of Plant Pathology, Cocoa Research Centre, Vellanikkara, Kerala, India

Berin Pathrose
Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India

Beena VI
Department of Soil Science and Agricultural Chemistry, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India

Vimala Jose
Department of Botany, St. Thomas College, University of Calicut, Thrissur, Kerala, India

Corresponding Author:
Resmi AR
Regional Agricultural Research Station, Kerala Agricultural University, Pilicode, Kasaragod, Kerala, India

Metabolomic profiling of *Trichoderma* spp. using gas chromatography-tandem mass spectrometry (GC-MS/MS)

Resmi AR, Reshmy Vijayaraghavan, Deepa James, Berin Pathrose, Beena VI and Vimala Jose

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10Sg.5884

Abstract
Fungi are known to produce a wide array of bioactive secondary metabolites encompassing diverse chemical classes, including monoterpenes, sesquiterpenes, alcohols, aldehydes, aromatic compounds, esters, furans, ketones, and sulfur-and nitrogen-containing compounds. Among them, *Trichoderma* have emerged as the most extensively studied fungal biocontrol agent, widely employed as biofungicides and biofertilizers in both greenhouse and field applications. The secondary metabolites produced by *Trichoderma* spp. have attracted considerable scientific interest due to their unique chemical structures and significant biological activities. In recent years, a substantial number of these metabolites have been isolated and characterized. These compounds play a crucial role not only in the mycoparasitic activity of *Trichoderma* spp. but also in mediating their interactions with plants and other organisms in the surrounding environment. In the present study, we investigated the *in vitro* potential of *Trichoderma* spp. to produce bioactive secondary metabolites. The study focused on analyzing extracts of 10 different isolates of *Trichoderma* spp. using ethyl acetate as solvent and the compounds have been identified by gas chromatography-tandem mass spectrometry (GC-MS/MS). All *Trichoderma* isolates were found to produce secondary metabolites, with the highest production observed in isolate T_7. GC-MS analysis identified 22 potential compounds with numerous benefits that could be used in agriculture. Most of the detected compounds were related to aliphatic (alkanes and ketones) hydrocarbons and aromatic compounds (diterpenoids), which include phenol, 2,4-bis (1,1-dimethylethyl)-, Asarone, myristic acid, retinoic acid, stearic acid, 8-pentadecanone, 10-nonadecanone, etc. The antifungal properties of these compounds suggest that the biological control efficacy of *Trichoderma* species may be closely associated with their production. These compounds hold promise as natural fungicidal agents for crop protection, offering effective control of fungal pathogens while minimizing environmental impact.

Keywords: *Trichoderma*, secondary metabolites, gas chromatography-tandem mass spectrometry

Introduction
Fungi are known to produce a diverse array of volatile organic compounds (VOCs), which, due to their small molecular size and high vapor pressure, readily diffuse through the atmosphere and soil under ambient temperature and pressure conditions. These VOCs typically exhibit low to moderate water solubility and are often characterized by distinctive odors (Hung *et al*., 2015) [11]. To date, approximately 500 fungal VOCs have been identified, although only about 100 fungal species out of the estimated 100, 000 described have been studied for their VOC production (Korpi *et al*., 2009; Hung *et al*., 2015) [14, 11]. Fungal VOCs serve important ecological and signaling functions in natural environments. They mediate complex interactions not only among fungi themselves but also between fungi, plants, and bacteria (Morath *et al*., 2012) [19]. These compounds arise as both intermediate and final products of various metabolic pathways and predominantly include mono and sesquiterpenes, alcohols, ketones, lactones, esters, fatty acids, sulfur containing compounds, simple pyrans, and benzene derivatives (Korpi *et al*., 2009) [14]. Functionally, fungal VOCs are involved in a wide range of biological processes, including microbial communication, environmental adaptation, and biocontrol (Bitas *et al*., 2013) [3]. They can play roles in defense mechanisms against predators, parasites, and pathogens, and may contribute to competitive interactions among microbial species (Stoppacher *et al*., 2010) [33].

Trichoderma is a well-known genus of soilborne fungi recognized for its antagonistic activity against a wide range of plant pathogenic microorganisms (Cook and Baker, 1983; Nurbailis, 1992; Harman, 2000; Pushpavathi *et al*., 2016) [6, 28, 13, 31]. Its antagonism is mediated through multiple mechanisms, including competition for nutrients and space, mycoparasitism, and antibiosis (Cook and Baker, 1983; Howell, 2003; Nurbailis, 2008) [5, 10, 25]. Nurbailis *et al*. (2006) [26] demonstrated that isolates of *T. viride* and *T. harzianum* obtained from the banana rhizosphere were capable of inhibiting the growth of *Fusarium oxysporum* f. sp. *cubense via* an antibiosis-based mechanism. Species of *Trichoderma* are also prolific producers of bioactive secondary metabolites with antifungal and antibacterial properties. These include a wide range of chemical classes such as polyketides, pyrones, and terpenes (Naher *et al*., 2014) [21]. Furthermore, Leelavathy *et al*. (2014) [18] reported that crude extracts of *T. harzianum*, when applied at varying concentrations, effectively suppressed the growth of several pathogenic bacterial species, highlighting its potential as a biocontrol agent.

Trichoderma species have been extensively studied and widely utilized in biological control strategies against phytopathogenic fungi, primarily due to their remarkable capacity to produce a diverse array of enzymes and secondary metabolites (Guo *et al*., 2022 and Khan *et al*., 2020) [8, 13]. As a filamentous fungus, *Trichoderma* exhibits broad applicability across industrial, agricultural, and environmental sectors (Mukherjee *et al*., 2013) [20]. One of its major advantages lies in its ability to synthesize a wide spectrum of bioactive compounds, many of which are considered potential leads for drug development. In agriculture, *Trichoderma* is commonly employed as a biofungicide and bioremediation agent, offering protective benefits to host plants throughout their life cycle and thereby functioning as an effective biocontrol agent (Segaran and Sathiavelu, 2019., Adeleke and Babalola, 2021 and Sheeba *et al*., 2019) [29, 1, 31]. To date, approximately 200 secondary metabolites have been identified from *Trichoderma* species, encompassing chemical classes such as terpenoids, polyketides, peptides, alkaloids, and lactones (Khan *et al*., 2020) [13] Among these, sesquiterpenes and diterpenes isolated from *Trichoderma* have demonstrated a wide range of bioactivities, including antimicrobial, antialgal, anticancer, and phytotoxic effects (Zhang *et al*., 2021) [36], underscoring their potential for pharmaceutical and agricultural applications.

Fungal strains belonging to the genus *Trichoderma* are well recognized producers of a wide variety of volatile organic compounds (VOCs). The VOC profile of a given *Trichoderma* species or strain is highly dynamic and influenced by several factors, including the substrate composition, incubation duration, nutrient availability, temperature, and other environmental conditions (Tait *et al*., 2013) [34]. VOCs produced by filamentous biocontrol fungi such as *Trichoderma* spp. exhibit potent antibiotic activity against a broad spectrum of phytopathogenic molds. In addition to their antagonistic effects, these volatile metabolites have been shown to promote plant growth and induce systemic resistance, thereby enhancing the host plant's resilience to fungal infections (Vinale *et al*., 2008) [35]. Recent reviews have highlighted capacity of *Trichoderma* spp. to synthesize a diverse array of both volatile metabolites such as pyrones and sesquiterpenes and non-volatile secondary metabolites, including peptaibols, which collectively contribute to its effectiveness as a biocontrol agent and plant growth promoter.

The analysis of volatile fungal metabolites is commonly conducted using gas chromatography (GC) techniques, which have been applied to various fungal genera, including *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, and *Trichoderma*. After inoculating fungi in liquid or on solid growth medium (Nemcovic *et al*. 2008) [22], volatiles can be extracted in different ways, such as with organic solvents (Reithner *et al*. 2005) [28], solid phase extraction using C18 or silica gel columns (Keszler *et al*. 2000) [15], online gas enrichment on adsorption tubes or various headspace (HS) techniques and solid phase microextraction (Stoppcher *et al*. 2010) [33]. MS detection enables the selective identification of individual volatiles, and structural elucidation is typically confirmed by comparing acquired mass spectra with reference libraries (Jelen, 2003; Stoppacher *et al*., 2010) [12, 33].

Evaluating the secondary metabolite production potential of *Trichoderma* isolates is crucial for understanding their functional roles and biocontrol capabilities. The present study aims to assess the ability of 10 distinct *Trichoderma* isolates, collected from rhizosphere soils of Kerala, to produce bioactive secondary metabolites. Metabolites were extracted from the culture filtrates using ethyl acetate, and the resulting compounds were subsequently identified and characterized using gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis.

Materials and Methods

Collection and maintenance of *Trichoderma* spp.

The study was conducted with 10 different isolates of *Trichoderma* spp. (T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10}) isolated from the rhizosphere soil samples collected from different locations of Kerala and maintained at the Department of Plant Pathology, College of Agriculture, Vellanikkara, Thrissur, Kerala Agricultural University. The cultures were maintained in the Potato Dextrose Agar (PDA) medium. Molecular characterization of all the 10 isolates was carried out, leading to their precise identification at the species level. Among these, isolate T_1 was identified as *Trichoderma afroharzianum*. Isolates T_2, T_3, T_4, T_5, T_6, T_7, T_8 and T_{10}were characterized as *Trichoderma asperellum*, while isolate T_9 was confirmed as *Trichoderma harzianum*.

Production and extraction of secondary metabolites from *Trichoderma* spp.

The agar plugs of *Trichoderma* spp., measuring 7mm in diameter, were taken from actively growing margins of all the 10 isolates grown on PDA media and were inoculated into 250 mL conical flasks containing 100 mL of potato dextrose broth medium (PDB) supplemented with chloramphenicol antibiotic, incubated in static conditions for 9 days at 25 °C. After the incubation, the cultures were filtered under vacuum through filter paper (Whatman No. 4). The final filtrate is the crude extract of secondary metabolites (Shobha *et al*., 2020) [32]. The secondary metabolites were extracted by partitioning with ethyl acetate. The solvent ethyl acetate was evaporated from the filtrate using a vacuum rotary evaporator. The extract was further dissolved in HPLC grade ethyl acetetate, filtered through PVDF syringe filter and was maintained at −20 °C in the deep freezer until further use.

Identification of Secondary Metabolites

The Mass Spectrum Gas Chromatography Tandem Mass Spectroscopy (GC-MS/MS) analysis was performed for the determination of compounds using Thermo Scientific TSQ 8000 EVO Triple Quadrupole Mass Spectrometer with Thermo Scientific TG 5MS column (length: 30m, I.D: 0.25mm, film thickness 0.5 µm) and Xcalibur software. For the separation of components, helium gas was used as a carrier gas, which maintained the constant flow of 1 mL/min. The temperature of the injector was maintained at 250 °C. The sample of 1 µL was injected into the instrument. The temperature of the oven was programmed as follows: initial temperature of 50 °C for 1 min, followed by ramping to 120 °C at the rate of 10 °C min^{-1} and 270 °C at the rate of 5 °C $min^{-1}$for 5 min. The conditions for the mass detector were a temperature of 290 °C for the transfer line, a temperature of 310 °C for the ion source, an ionization mode electron impact at 70 eV, and a mass scan range of 35-500 m/z. The comparison of spectrum of components were carried out with the database of spectrum of known components stored in the National Institute of Standard and Technology (NIST) library.

Results

The ethyl acetate extract of the *Trichoderma* spp. was analyzed by GC-MS/MS and the analysis has led to the identification of different compounds present in culture filtrate of *Trichoderma* spp. Interpretation of GC-MS/MS data was carried out using the database of the NIST. The unknown spectrum of components was compared with that of the known components contained in the NIST library. The name, molecular weight, formula, peak area percent, nature and functional properties of the secondary metabolites extracted were ascertained. The significant compounds that were found in the ethyl acetate solvent extract for T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} as follows.

GC-MS/MS analysis of T_1 isolate of *Trichoderma* revealed the presence of seven major compounds *viz*. aliphatic hydrocarbons such as cyclohexane, hexyl-and cyclohexane, octyl-and aromatic compound, phenol 2,4-bis(1,1-dimethylethyl)-, which is known for its antioxidant, antibacterial, and antifungal activities, suggesting a possible role in the biocontrol efficacy of the isolate. Another notable aromatic derivative was asarone a phenylpropanoid with documented antifungal, antibacterial, and insecticidal properties, though it can be neurotoxic at higher concentrations. The aliphatic ketones, namely 8-pentadecanone and 10-nonadecanone, are known for their antimicrobial and insect-repellent activities, which may further support the antagonistic nature of this isolate. Other major compound identified was piperonyl butoxide, a well-known synthetic synergist that inhibits insect detoxification enzymes and enhances the activity of insecticides, especially pyrethroids (Table 1).

Table 1: List of compounds of *Trichoderma afroharzianum* (T_1) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	10.2	Cyclohexane, hexyl-	$C_{12}H_{24}$	168.32	54.76	1.78	Aliphatic, Saturated hydrocarbon (alkane)	Generally inert, used as solvent
2	14.3	Cyclohexane, octyl-	$C_{14}H_{28}$	196.37	51.01	2.57	Aliphatic, Saturated hydrocarbon (alkane)	Mild hydrophobic membrane-disruptive effects
3	15.65	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	56.79	1.63	Aromatic, Substituted phenol	Antioxidant, antibacterial, and antifungal properties
4	18.03	Asarone (1,2,4-Trimethoxy-5-(1-propenyl) benzene)	$C_{12}H_{16}O_3$	208.25	65.30	6.63	Aromatic, Phenyl propene, Methoxybenzene derivative	Antifungal, antibacterial, insecticidal, and neurotoxic at high doses
5	19.07	8-Pentadecanone	$C_{15}H_{30}O$	226.40	85.54	2.74	Aliphatic, Ketone	Mild antibacterial and insect repellent activity
6	23.22	10-Nonadecanone	$C_{19}H_{38}O$	282.50	59.95	3.99	Aliphatic, Ketone	Used in chemical ecology studies; antimicrobial and insect-repellent activity
7	32.89	Piperonyl butoxide	$C_{19}H_{30}O_5$	338.44	96.44	5.58	Aromatic + Ether, Synthetic organic compoud	Synergist: inhibits detox enzymes in insects; enhances pesticide (esp. pyrethroid) effectiveness.

Trichoderma isolate T_2 produced four major compounds. These metabolites represent a mix of phenolic derivatives, phenylpropenes, fatty acids, and terpenoids, each with known biological activities relevant to plant health and microbial interactions. They include phenol 2,4-bis(1,1-dimethylethyl)-and asarone as that of T_1**.** The aliphatic fatty acid tetradecanoic acid (myristic acid) was also identified which plays important roles in membrane fluidity, lipid signaling, and protein acylation**.** In addition, it has been reported to exhibit antibacterial and antifungal effects**,** thereby contributing to *Trichoderma*'s antagonistic activity against pathogens. The most abundant metabolite identified was retinoic acid, a diterpenoid that plays fundamental roles in regulating cell differentiation, growth, immune responses, and tissue maintenance. Its presence indicates that *Trichoderma* may produce signaling molecules analogous to plant hormones, potentially influencing plant defense mechanisms and developmental processes (Table 2).

Table 2: List of compounds of *Trichoderma asperellum* (T_2) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (mins)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	15.66	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	59.80	1.66	Aromatic, Substituted phenol	Antioxidant, antibacterial, and antifungal properties
2	18.03	Asarone (1,2,4-Trimethoxy-5-(1-propenyl) benzene)	$C_{12}H_{16}O_3$	208.25	45.59	3.25	Aromatic, Phenylpropene, Methoxybenzene derivative	Antifungal, antibacterial, insecticidal, and neurotoxic at high doses
3	20.90	Tetradecanoic acid (Myristic acid)	$C_{14}H_{28}O_2$	228.37	41.89	1.34	Aliphatic saturated fatty acid	Antibacterial and antifungal activity. Role in membrane fluidity, lipid signaling, and protein acylation
4	34.37	Retinoic acid	$C_{20}H_{28}O_2$	300.44	30.74	4.40	Aromatic, Di terpenoid	Essential for cell differentiation, growth, immune function, and epithelial tissue maintenance

The metabolite profile of the *Trichoderma* T_3 isolate revealed the presence of heterocyclic derivatives, lactones, phenylpropenes, and fatty acids, many of which are known for their antimicrobial potential. Among these, 2,5-furandione, dihydro-3-methylene, a heterocyclic maleic anhydride derivative, is associated with antimicrobial, antifungal, and anticancer properties, indicating its possible role in the defensive chemistry of the isolate. A prominent metabolite, 2H-Pyran-2-one, 6-pentyl, a lactone with an aliphatic side chain, is a well-documented *Trichoderma* metabolite recognized for its strong antifungal and antibacterial activities, contributing significantly to the biocontrol efficacy of the fungus. The aromatic compound asarone and the saturated fatty acid tetradecanoic acid (myristic acid) was also identified as that of previous isolates. Another fatty acid, octadecanoic acid (stearic acid), was also present, contributing mild antimicrobial activity while playing roles in structural stability and energy metabolism. Collectively, these metabolites suggest that T_3 produces a versatile chemical arsenal that enhances its antagonistic ability and ecological adaptability (Table 3).

Table 3: List of compounds of *Trichoderma asperellum* (T_3) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (mins)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	6.86	2,5-Furandione, dihydro-3-methylene	$C_5H_4O_3$	112.08	69.74	1.30	Heterocyclic, semi-aromatic, Maleic anhydride derivative	antimicrobial, antifungal, and anticancer activities
2	14.63	2H-Pyran-2-one, 6-pentyl-	$C_{10}H_{14}O_2$	166.22	66.46	5.61	Aromatic-like with aliphatic chain	Strong antifungal and antibacterial compound
3	18.02	Asarone (1,2,4-Trimethoxy-5-(1-propenyl) benzene)	$C_{12}H_{16}O_3$	208.25	62.96	2.20	Aromatic, Phenyl propene, Methoxybenzene derivative	Antifungal, antibacterial, insecticidal, and neurotoxic at high doses
4	20.92	Tetradecanoic acid (Myristic acid)	$C_{14}H_{28}O_2$	228.37	63.41	2.4	Aliphatic saturated fatty acid	Antibacterial and antifungal activity. Role in membrane fluidity, lipid signaling, and protein acylation
5	28.61	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	284.48	62.55	1.88	Aliphatic saturated fatty acid	Mild antibacterial and antifungal activity

GC-MS/MS analysis of the *Trichoderma* isolate T_4 revealed three major metabolites, primarily consisting of lactones and long-chain aliphatic ketones, each with significant ecological and antimicrobial functions. The characteristic lactone 2H-Pyran-2-one, 6-pentyl, widely regarded as a signature metabolite of *Trichoderma*, was identified and is well known for its potent antifungal and antibacterial properties, contributing substantially to the genus's biocontrol potential. Alongside this, the aliphatic ketone 8-pentadecanone and long-chain ketone, 10-nonadecanone, was also presentas that of T_1. Together, these metabolites highlight the multifaceted ecological strategies of the T_4 isolate in promoting plant health and resilience (Table 4).

Table 4: List of compounds of *Trichoderma asperellum* (T_4) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	14.61	2H-Pyran-2-one, 6-pentyl-	$C_{10}H_{14}O_2$	166.22	60.97	4.42	Aromatic-like with aliphatic chain	Strong antifungal and antibacterial compound
2	19.08	8-Pentadecanone	$C_{15}H_{30}O$	226.4	89.00	2.03	Aliphatic, Ketone	Mild antibacterial and insect repellent activity
3	23.22	10-Nonadecanone	$C_{19}H_{38}O$	282.5	43.86	3.09	Aliphatic, Ketone	Used in chemical ecology studies; possible antimicrobial and insect-repellent activity.

The metabolite profile of the *Trichoderma* isolate T_5 revealed five major metabolites belonging to aliphatic hydrocarbons, substituted phenols, lactones, and long-chain ketones, many of which are biologically active and ecologically significant. The aliphatic saturated hydrocarbon cyclohexane, octyl-, 2H-Pyran-2-one, 6-pentyl, phenol 2,4-bis(1,1-dimethylethyl)-, 8-pentadecanone and 10-nonadecanone were detected as that of other isolates. Collectively, these metabolites demonstrate the diverse chemical arsenal of T_5 that underpins its biocontrol efficacy (Table 5).

Table 5: List of compounds of *Trichoderma asperellum* (T_5) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	14.3	Cyclohexane, octyl-	$C_{14}H_{28}$	196.37	55.46	2.40	Aliphatic, Saturated hydrocarbon (alkane)	Mild hydrophobic membrane-disruptive effects
2	14.61	2H-Pyran-2-one, 6-pentyl-	$C_{10}H_{14}O_2$	166.22	55.09	1.80	Aromatic-like with aliphatic chain	Strong antifungal and antibacterial compound
3	15.66	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	60.09	1.67	Aromatic, Substituted phenol	Antioxidant, antibacterial, and antifungal properties
4	19.09	8-Pentadecanone	$C_{15}H_{30}O$	226.4	87.13	2.88	Aliphatic, Ketone	Mild antibacterial and insect repellent activity
5	23.24	10-Nonadecanone	$C_{19}H_{38}O$	282.5	63.32	4.00	Aliphatic, Ketone	Used in chemical ecology studies; possible antimicrobial and insect-repellent activity.

The *Trichoderma* isolate T_6 showed presence of three key metabolites, namely a substituted phenol and two long-chain aliphatic ketones, each associated with antimicrobial and ecological functions. The compounds include Phenol, 2,4-bis(1,1-dimethylethyl)-,8-pentadecanone and 10-nonadecanone assimilar to ther isolates suggesting a role in safeguarding plants against both microbial pathogens and insect pests. Together, these metabolites highlight the multifaceted chemical defense strategies of the T_6 isolate in plant protection (Table 6).

Table 6: List of compounds of *Trichoderma asperellum* (T_6) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	15.65	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	57.38	1.39	Aromatic, Substituted phenol	Antioxidant, antibacterial, and antifungal properties
2	19.08	8-Pentadecanone	$C_{15}H_{30}O$	226.4	87.29	2.84	Aliphatic, Ketone	Mild antibacterial and insect repellent activity
3	23.24	10-Nonadecanone	$C_{19}H_{38}O$	282.5	64.41	4.61	Aliphatic, Ketone	Used in chemical ecology studies; possible antimicrobial and insect-repellent activity.

The metabolite profile of the *Trichoderma* isolate T_7 revealed eight major metabolites, including aromatic furans, substituted phenols, ketones, fatty acids, and aromatic glycosides, many of which are known for their antimicrobial and antioxidant activities. The aromatic furanone 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one was identified, which is associated with antioxidant and mild antimicrobial effects and is also recognized as a natural flavoring compound. Cyclotetrasiloxane, octamethyl-, a cyclic siloxane typically used as a solvent in cosmetic formulations, was also detected, exhibiting low antimicrobial activity. Another metabolite, 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-, functions as an antioxidant, flavor enhancer, and iron chelator. 5-Hydroxymethylfurfural (HMF), an aromatic furan derivative, was found and is well known for its antioxidant and antibacterial properties, as well as its role as a marker of sugar degradation. The aromatic glycoside vanillin lactoside was present, possessing both antioxidant and antibacterial activities. The compounds phenol, 2,4-bis(1,1-dimethylethyl) and myristic acid was also detected as reported in other isolates, thereby contributing to the isolate's biocontrol potential. Additionally, benzophenone, an aromatic ketone with antimicrobial, antifungal, and UV-protective properties, was identified. Collectively, these metabolites highlight the diverse chemical profile of T_7 and its strong biocontrol relevance (Table 7).

Table 7: List of compounds of *Trichoderma asperellum* (T_7) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	6.14	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.13	79.05	1.68	Aromatic furanone	Flavoring, antioxidant, mild antimicrobial
2	6.3	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296.61	76.44	5.43	Aliphatic cyclic siloxane	Cosmetic solvent, low antimicrobial activity
3	8.6	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-	$C_6H_6O_3$	126.11	83.54	1.68	Aromatic pyrone	Flavor enhancer, antioxidant, iron-chelating
4	9.97	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	93.62	11.20	Aromatic furan derivative	Antioxidant, antibacterial, sugar degradation marker
5	13.33	Vanillin lactoside	$C_{20}H_{26}O_{11}$	442.41	58.94	1.69	Aromatic glycoside	Antioxidant, antibacterial
6	15.65	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	67.18	4.38	Aromatic, Substituted phenol	Antioxidant, antibacterial, and antifungal properties
7	18.23	Benzophenone	$C_{13}H_{10}O$	182.22	79.93	2.61	Aromatic ketone	Antimicrobial, UV protector, antifungal
8	20.88	Tetradecanoic acid (Myristic acid)	$C_{14}H_{28}O_2$	228.37	53.88	3.63	Aliphatic saturated fatty acid	Antibacterial and antifungal activity. Role in membrane fluidity, lipid signaling, and protein acylation

GC-MS/MS analysis of the *Trichoderma* isolate T_8 identified four major metabolites including cyclohexane, octyl-, phenol 2,4-bis(1,1-dimethylethyl), 8-pentadecanone and 10-nonadecanone as identified in other isolates. Together, these metabolites reflect the chemical strategies employed by T_8 to strengthen its biocontrol activity (Table 8).

Table 8: List of compounds of *Trichoderma asperellum* (T_8) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	14.3	Cyclohexane, octyl-	$C_{14}H_{28}$	196.37	50.56	1.99	Aliphatic, Saturated hydrocarbon (alkane)	Mild hydrophobic membrane-disruptive effects
2	15.65	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	58.75	1.49	Aromatic, Substituted phenol	Antioxidant, antibacterial, and antifungal properties
3	19.09	8-Pentadecanone	$C_{15}H_{30}O$	226.4	87.2	3.07	Aliphatic, Ketone	Mild antibacterial and insect repellent activity
4	23.24	10-Nonadecanone	$C_{19}H_{38}O$	282.5	68.93	4.89	Aliphatic, Ketone	Used in chemical ecology studies; possible antimicrobial and insect-repellent activity.

Metabolite assay of the *Trichoderma* isolate T_9 revealed five major metabolites, primarily consisting of aliphatic cyclic diketones, aromatic furans, and pyrones, all of which are associated with antioxidant and antimicrobial activities. The aliphatic cyclic diketone 1,2-cyclopentanedione was identified, which is regarded as a biosynthetic intermediate and exhibits weak antimicrobial effects. The aromatic furanone 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one was also present, known for its antioxidant, mild antimicrobial, and flavoring properties, indicating a possible role in ecological competitiveness. Another metabolite, 2H-pyran-2, 6(3H)-dione, a pyrone/lactone, is likely involved in biosynthetic pathways and may possess antimicrobial activity. The pyrone derivative 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-, with strong antioxidant, antibacterial, and iron-chelating functions, further highlights the ecological adaptability of the isolate. The dominant metabolite was 5-hydroxymethylfurfural, an aromatic furan derivative widely recognized for its antioxidant and antibacterial properties and its role as a marker of sugar degradation, reflecting the metabolic activity of the culture. Collectively, these metabolites emphasize the biochemical versatility of T_9 and its potential role in plant protection (Table 9).

Table 9: List of compounds of *Trichoderma harzianum* (T_9) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

Peak No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	5.42	1,2-Cyclopentanedione	$C_5H_6O_2$	98.10	65.67	4.25	Aliphatic cyclic diketone	Intermediate, weak antimicrobial
2	6.17	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.13	93.85	2.55	Aromatic furanone	Flavoring, antioxidant, mild antimicrobial
3	6.38	2H-Pyran-2, 6(3H)-dione	$C_5H_4O_3$	112.08	75.30	3.68	Aromatic pyrone/lactone	Potential antimicrobial, biosynthetic role
4	8.65	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-	$C_6H_6O_3$	126.11	93.12	7.65	Aromatic pyrone derivative	Antioxidant, antibacterial, iron-chelating agent
5	10.05	5-Hydroxymethyl furfural	$C_6H_6O_3$	126.11	92.17	27.60	Aromatic furan derivative	Antioxidant, antibacterial, marker for food quality

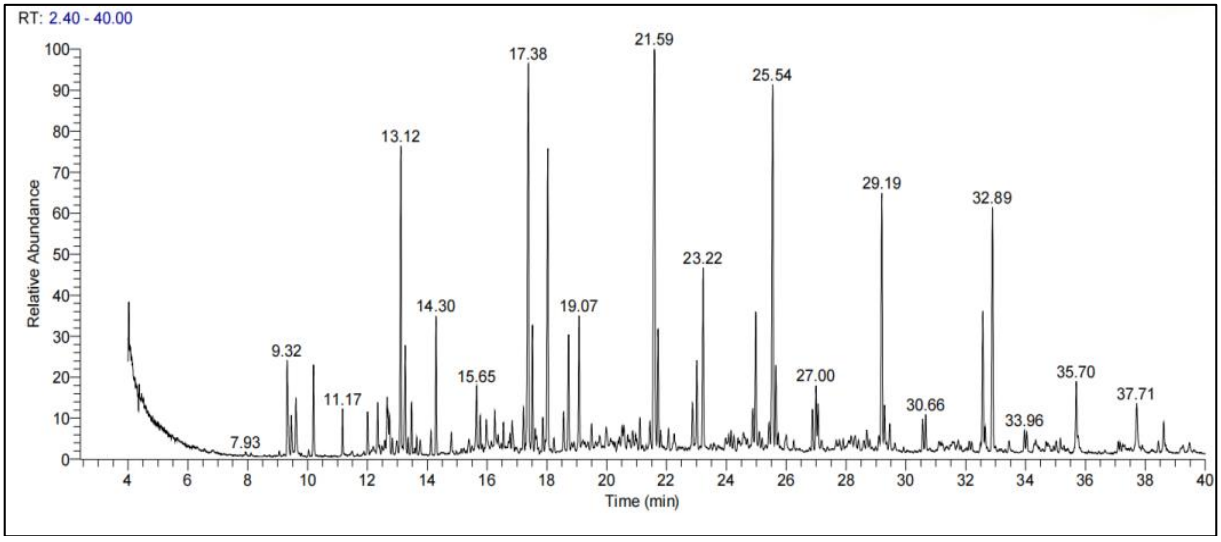
GC-MS/MS analysis of the *Trichoderma* isolate T_{10} revealed two major metabolites, the aromatic lactone 2H-Pyran-2-one, 6-pentyl-and the aliphatic ketone 8-as present in other isolates. Together, these metabolites highlight the biocontrol capacity of the T_{10} isolate (Table 10).

Table 10: List of compounds of *Trichoderma asperellum* (T_{10}) detected by Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS)

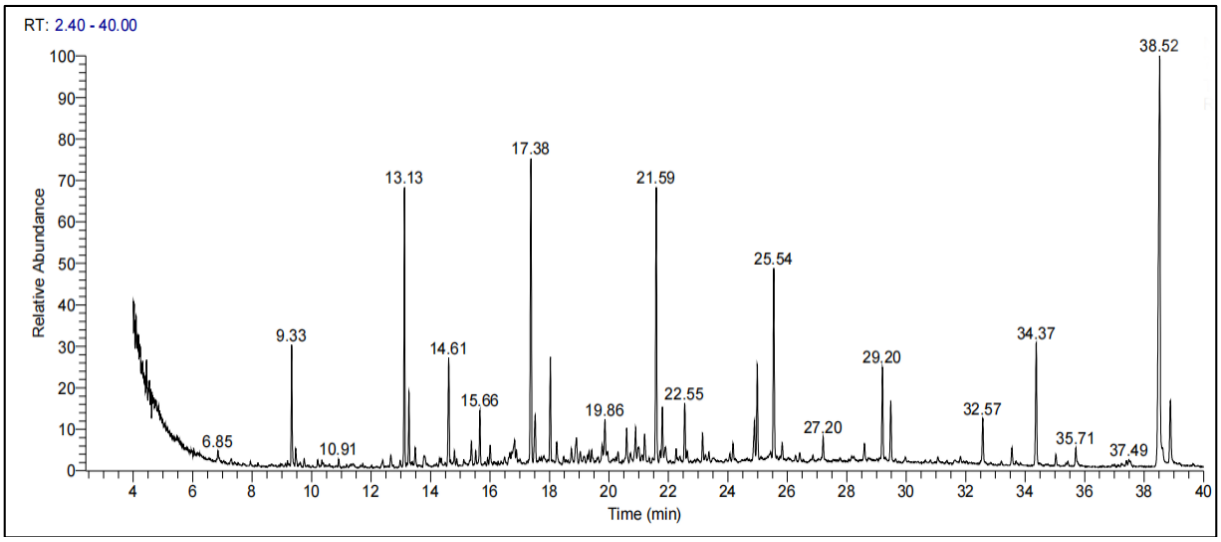
Peak No.	Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight	Library match (%)	Peak area (%)	Nature and chemical class	Function/properties
1	14.64	2H-Pyran-2-one, 6-pentyl-	$C_{10}H_{14}O_2$	166.22	63.86	8.98	Aromatic lactone (α-pyrone)	Strong antifungal, mild antibacterial
2	19.08	8-Pentadecanone	$C_{15}H_{30}O$	226.4	88.76	1.92	Aliphatic, Ketone	Mild antibacterial and insect repellent activity

Among the 10 isolates of *Trichoderma* spp., T_7 (*Trichoderma asperellum*) isolate produced maximum number of metabolites which are having antimicrobial properties, followed by T_1 (*Trichoderma afroharzianum*), T_3 (*Trichoderma asperellum*), T_5 (*Trichoderma asperellum*) and T_9 (*Trichoderma harzianum*).

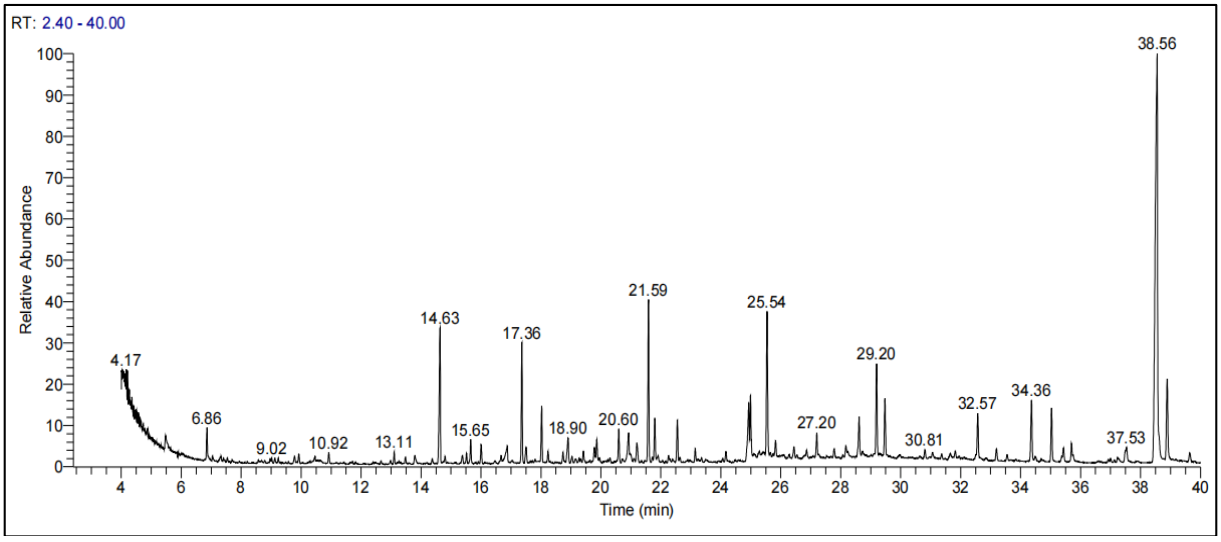
Figure 1 (a-j) presents the GC-MS/MS chromatograms of all the 10 *Trichoderma* isolates, each exhibiting distinct metabolite profiles that reflect the biochemical diversity among the isolates



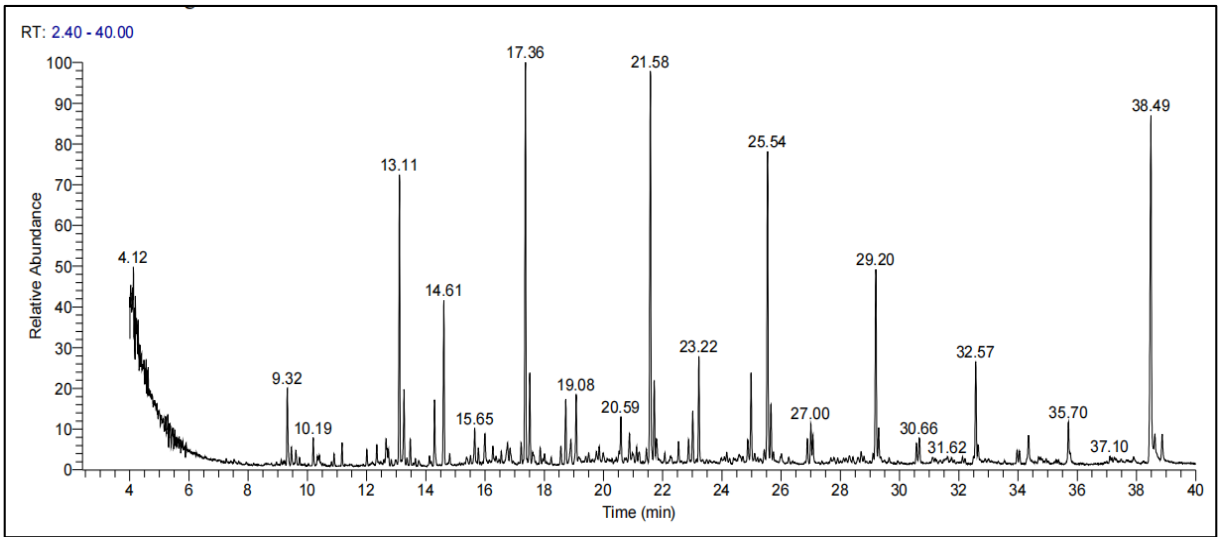
a)


RT: 2.40 - 40.00
Relative Abundance
Time (min)
38.52
17.38
13.13
21.59
25.54
9.33
14.61
34.37
29.20
15.66
22.55
19.86
32.57
27.20
35.71
6.85
10.91
37.49

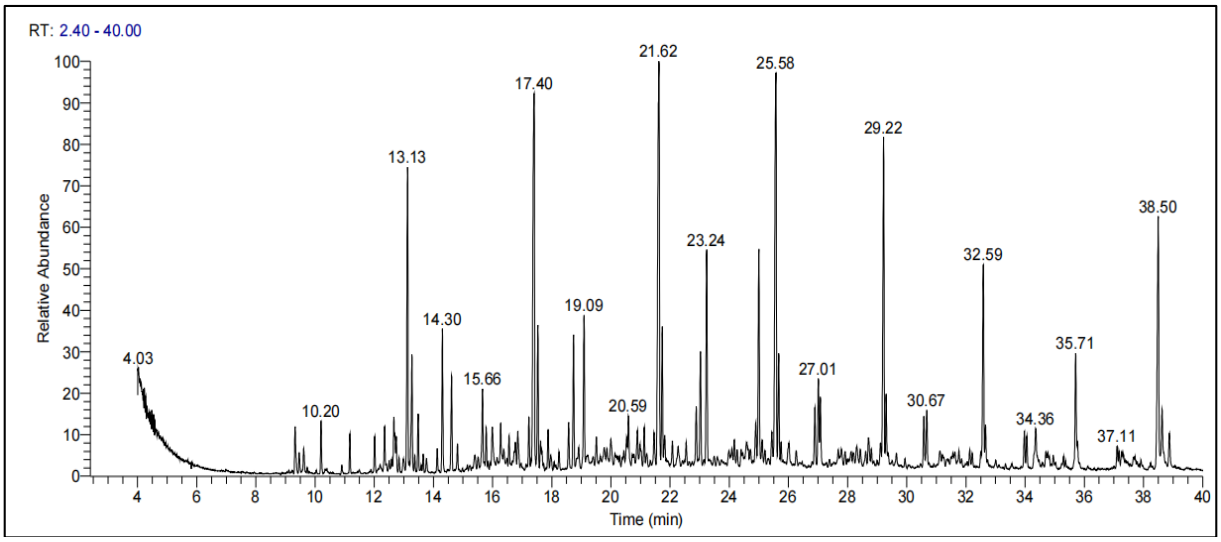
b)


RT: 2.40 - 40.00
Relative Abundance
Time (min)
38.56
21.59
14.63
25.54
17.36
4.17
29.20
34.36
32.57
6.86
20.60
18.90
27.20
37.53
9.02
10.92
13.11
15.65
30.81

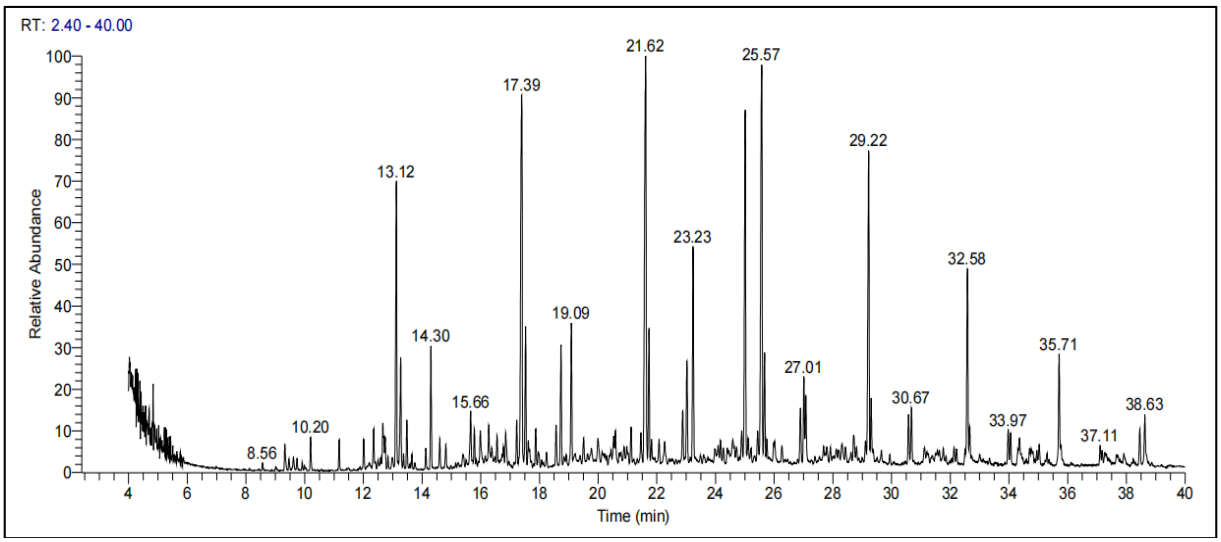
c)


RT: 2.40 - 40.00
Relative Abundance
Time (min)
17.36
21.58
38.49
25.54
13.11
29.20
4.12
14.61
23.22
32.57
9.32
19.08
20.59
35.70
15.65
27.00
30.66
31.62
37.10
10.19

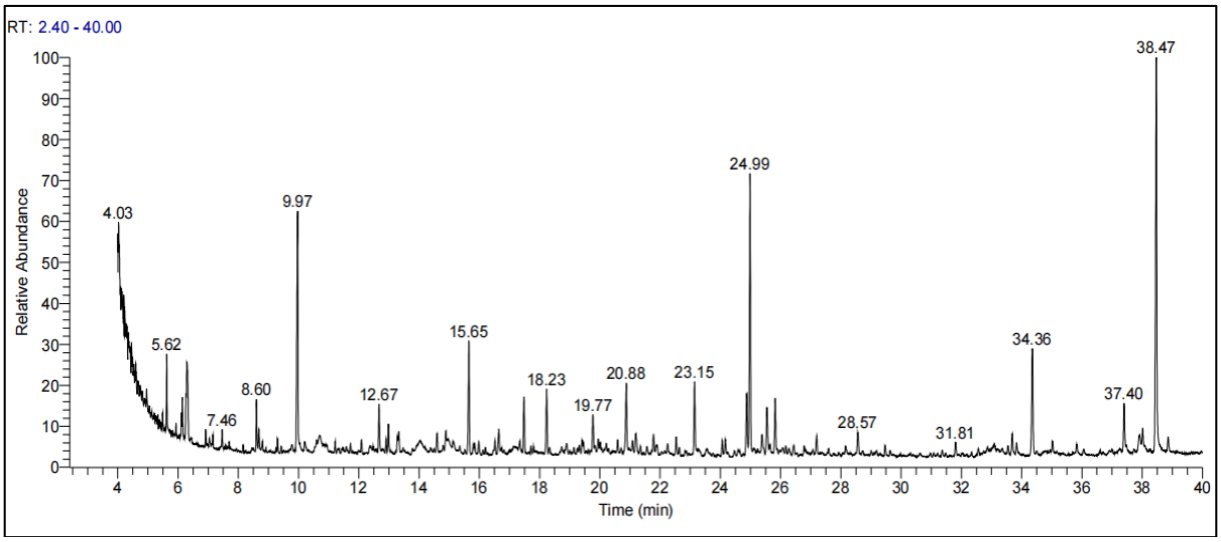
d)


RT: 2.40 - 40.00
Relative Abundance
Time (min)
4.03
10.20
13.13
14.30
15.66
17.40
19.09
20.59
21.62
23.24
25.58
27.01
29.22
30.67
32.59
34.36
35.71
37.11
38.50

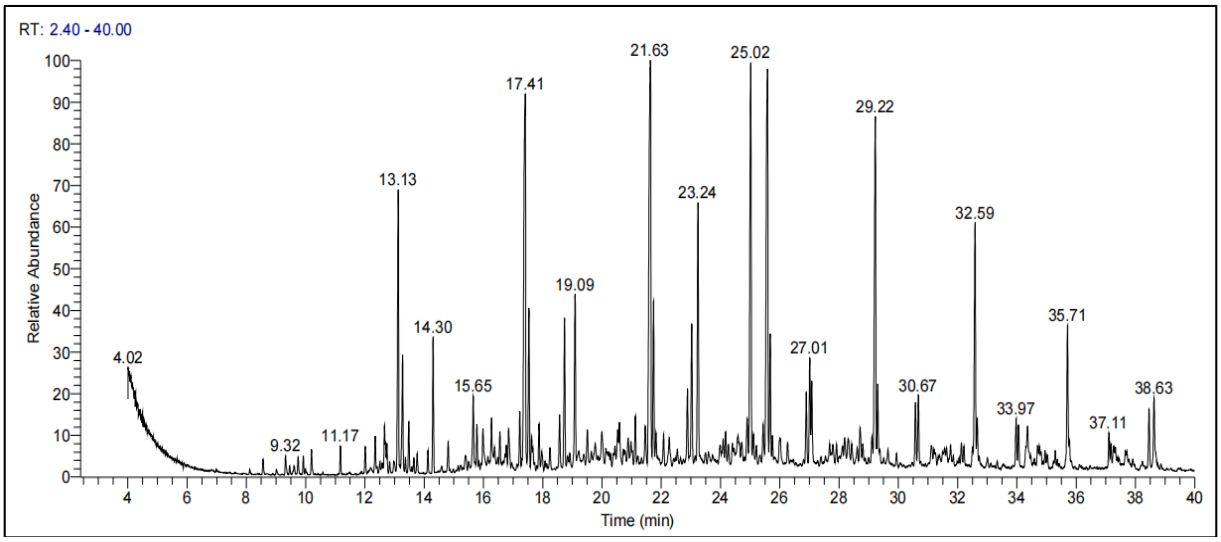
e)


RT: 2.40 - 40.00
Relative Abundance
Time (min)
8.56
10.20
13.12
14.30
15.66
17.39
19.09
21.62
23.23
25.57
27.01
29.22
30.67
32.58
33.97
35.71
37.11
38.63

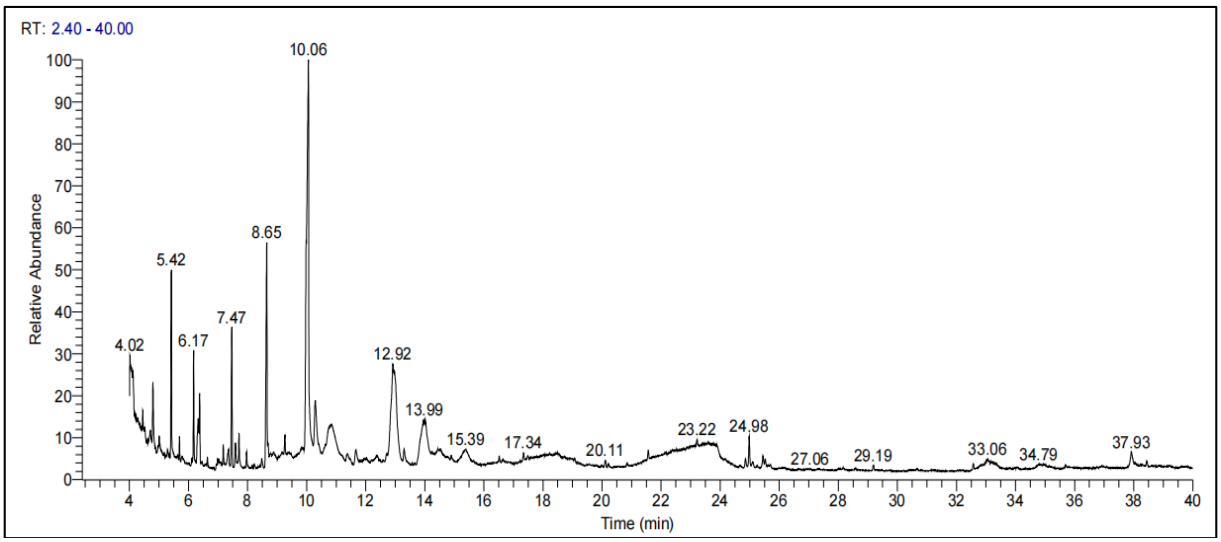
f)


RT: 2.40 - 40.00
Relative Abundance
Time (min)
4.03
5.62
7.46
8.60
9.97
12.67
15.65
18.23
19.77
20.88
23.15
24.99
28.57
31.81
34.36
37.40
38.47

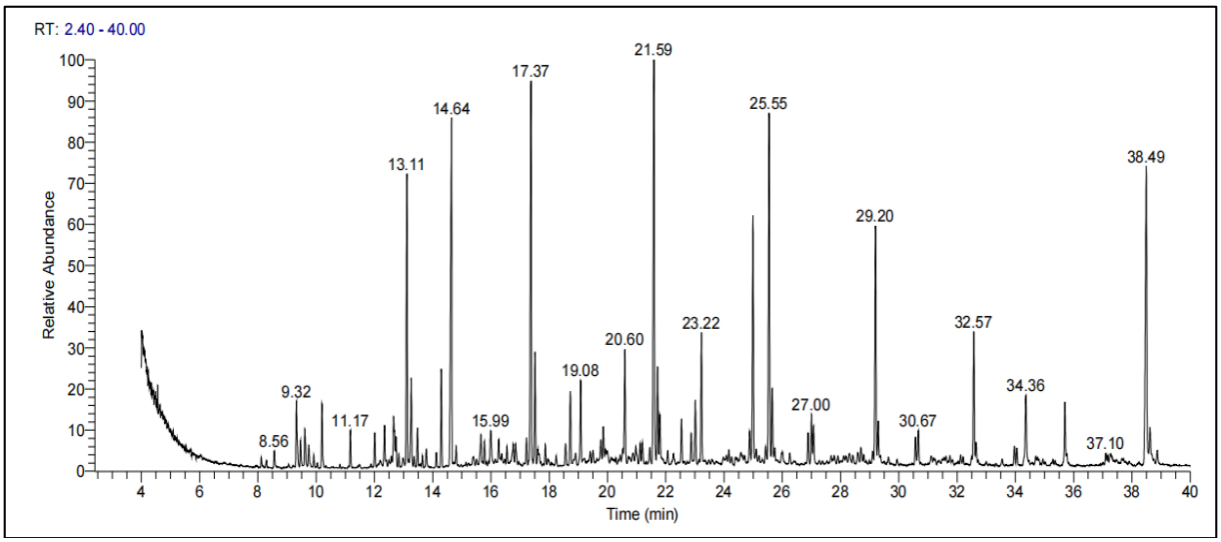
g)



h)



i)



j)

Fig 1. (a-j): Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS) of compounds identified from secondary metabolite crude extracts of *Trichoderma* spp.(a) T_1 (b) T_2 (c) T_3 (d) T_4 (e) T_5 (f) T_6 (g) T_7 (h) T_8 (i) T_9 and (j) T_{10}.

Discussion

Trichoderma species play a pivotal role in the biosynthesis of diverse secondary metabolites that contribute significantly to ecological and physiological functions such as microbial competition, symbiotic interactions, metal ion transport, cellular differentiation, signal transduction, and mycoparasitism. The bioactive compounds produced by *Trichoderma* spp. have been shown to exert positive effects on plants by enhancing growth and development, as well as activating defense mechanisms against various abiotic stresses and pathogenic invasions. The present study investigated the potential of *Trichoderma* spp. in producing bioactive secondary metabolites. As GC-MS/MS was done towards profiling the metabolites present in the culture filtrates of 10 different isolates of *Trichoderma*, the results showed the presence of seven major secondary metabolites, viz. phenol, 2,4-bis(1,1-dimethylethyl)-, asarone, myristic acid (tetra decanoic acid), retinoic acid, stearic acid, 8-pentadecanone, and 10-nonadecanone. Most of the metabolites identified in this study are widely used as antimicrobials, anti-oxidants, cosmetic solvents, flavouring agents, UV protectors and pesticides. Specifically, phenol, 2,4-bis(1,1-dimethylethyl)-is reported to have antioxidant, antibacterial and antifungal properties. The occurrence of volatile compounds in the extract has been correlated with the growth of the plant (Lee *et al*., 2016) [17]. The induction of phenolic compounds in response to *Trichoderma* has been associated with enhanced biochemical defense mechanisms in plants, contributing to resistance against various phytopathogens. Enhanced biosynthesis of phenolic compounds directly influences antioxidant activity by functioning as efficient free radical scavengers. Additionally, these metabolites contribute to cell wall reinforcement, thereby strengthening plant defense mechanisms against biotic stress factors.

Phenol, 2,4-bis(1,1-dimethylethyl) is frequently reported in GC-MS profiles of *Trichoderma* culture extracts and VOC/headspace studies by multiple *Trichoderma* strains (e.g., *T. asperellum*, *T. koningiopsis* and others) (Choez-Guaranda *et al*., 2023) [4]. It has broad antifungal and antibacterial activity (inhibits spore germination and hyphal growth) and has been purified and shown to be active against plant pathogens (*Fusarium* spp. and others). In *Trichoderma*, the presence of this metabolite contributes to antibiosis (direct pathogen inhibition) and therefore to biocontrol activity. Mechanistic work indicates membrane/β-tubulin interactions and growth/hyphae disruption in target fungi (Dharni *et al*., 2014) [6]. All the isolates except T_3, T_4, T_9 and T_{10} produced this metabolite.

The other identified metabolite, asarones, are reported to have antifungal and nematicidal properties in plant/pathogen assays; when present in *Trichoderma* metabolite blends, they can contribute to pathogen suppression (direct toxicity and interference with ergosterol biosynthesis reported in other systems). *Trichoderma* strains (including isolates of *T. longibrachiatum*/*T. harzianum*) have been shown to produce the metabolite asarone (Ahmed *et al*., 2022) [2]. This compound is produced by the isolates, T_1, T_2 and T_3.

Medium-chain and long-chain fatty acids (including myristic, stearic and retinoic acid) frequently appear in ethyl-acetate/methanolic extracts and GC-MS lists from *Trichoderma* isolates. These fatty acids may be present as free acids or esters. They exert direct antifungal effects (membrane perturbation, inhibition of spore germination) and can modulate mycotoxin production in pathogens. In *Trichoderma* extracts, myristic-type fatty acids likely act as part of a multifactorial antifungal mixture and may also participate in signaling/plant-growth modulation (some fungal fatty-acid derivatives act as plant growth regulators) (Choez-Guaranda *et al*., 2023) [4]. Long-chain fatty acids like stearic have been associated with antifungal effects (membrane disruption, inhibition of spore germination) and can contribute to the overall antagonistic activity of *Trichoderma* extracts. They also can be precursors for oxylipin signaling molecules that influence plant responses and pathogen behavior (Guimaraes and Venancio, 2022) [7]. The isolates T_2, T_3 and T_7 produced myristic acid, isolate T_2 produced retinoic acid and isolate T_3 produced stearic acid.

Moreover, two of the identified compounds, aliphatic ketones (8-pentadecanone and 10-nonadecanone) are insect repellents and they also exert direct antagonism against fungal pathogens or elicit plant responses (reduced disease severity, growth promotion) (Lee *et al*., 2016) [17]. The isolates T_1, T_4, T_5, T_6, T_8 and T_{10} produced these compounds.

Conclusion

The present study highlights the potential of selected *Trichoderma* spp. isolates to produce a diverse array of bioactive secondary metabolites. Secondary metabolites produced by the 10 isolates of the beneficial fungi *Trichoderma* spp. have been extracted and characterized. Given the significant threat posed by fungal and bacterial pathogens to crop productivity, the identification and characterization of *Trichoderma* isolates with enhanced capacities for metabolite production is of considerable importance. It is well recognized that biocontrol fungi, such as *Trichoderma* spp., are able to produce compounds with multiple activities, including direct/indirect toxic effects against plant pathogens, plant defence induction or growth promotion. These isolates exhibit antibacterial and antifungal properties, making them strong candidates for integration into soil and plant health management systems. Importantly, the study confirmed the production of key secondary metabolites, including Phenol, 2,4-bis(1,1-dimethylethyl)-, asarone, myristic acid, retinoic acid, stearic acid, 8-pentadecanone, 10-nonadecanone *etc*. These compounds are known to play critical roles in the biological control of phytopathogens, primarily through mechanisms such as disruption of microbial membranes and degradation of fungal cell walls. The ability of *Trichoderma* spp. to biosynthesize these metabolites underscores their potential application in sustainable agriculture, offering an eco-friendly alternative for enhancing plant health and protecting crops from pathogenic threats.

Acknowledgement

The authors extend heartfelt gratitude to the Department of Plant Pathology and Pesticide Residue Testing Laboratory, College of Agriculture, Vellanikkara, KAU, Thrissur for their steadfast support and invaluable encouragement throughout the course of this work.

References

1. Adeleke BS, Babalola OO. Pharmacological potential of fungal endophytes associated with medicinal plants: a review. J Fungi. 2021;7:147.

2. Ahmed M, Khan KU, Ahmad S, Aati HY, Ovatlarnporn C, Rehman MS, *et al*. Comprehensive phytochemical profiling, biological activities, and molecular docking studies of *Pleurospermum candollei*: an insight into potential for natural products development. Molecules. 2022;27(13):4113.
3. Bitas V, Kim HS, Bennett JW, Kang S. Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. Mol Plant Microbe Interact. 2013;26:835-843.
4. Choez-Guaranda I, Espinoza-Lozano F, Reyes-Araujo D, Romero C, Manzano P, Galarza L, Sosa D. Chemical characterization of *Trichoderma* spp. extracts with antifungal activity against cocoa pathogens. Molecules. 2023 Apr;28(7):3208. doi:10.3390/molecules28073208.
5. Cook RJ, Baker KF. The nature and practice of biological control of plant pathogens. St Paul, MN: American Phytopathological Society; 1983.
6. Dharni S, Sanchita, Maurya A, Samad A, Srivastava SK, Sharma A, *et al*. Purification, characterization, and *in vitro* activity of 2,4-Di-tert-butylphenol from Pseudomonas monteilii PsF84: conformational and molecular docking studies. J Agric Food Chem. 2014 Jul 2;62(26):6138-6146. doi:10.1021/jf5001138.
7. Guimaraes A, Venancio A. The potential of fatty acids and their derivatives as antifungal agents: a review. Toxins (Basel). 2022;14(3):188.
8. Guo R, Li G, Zhang Z, Peng X. Structures and biological activities of secondary metabolites from *Trichoderma harzianum*. Mar Drugs. 2022;20:701.
9. Harman GE. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis. 2000; Publication No. D-2000-0208-01F.
10. Howell CR. Mechanism employed by *Trichoderma* species in the biological control of plant disease: the history and evolution of current concepts. USDA/ARS Southern Plains Agricultural Research Center; 2003.
11. Hung R, Lee S, Bennett JW. Fungal volatile organic compounds and their role in ecosystems. Appl Microbiol Biotechnol. 2015;99:3395-3405.
12. Jelen HH. Use of solid phase microextraction (SPME) for profiling fungal volatile metabolites. Lett Appl Microbiol. 2003;36:263-267.
13. Khan RAA, Najeeb S, Hussain S, Xie B, Li Y. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms. 2020;8:817.
14. Korpi A, Jarnberg J, Pasanen AL. Microbial volatile organic compounds. Crit Rev Toxicol. 2009;39:139-193.
15. Keszler A, Forgacs E, Kotai L, Vizcaino JA, Monte E, Garcia-Acha I. Separation and identification of volatile components in the fermentation broth of *Trichoderma atroviride* by solid phase extraction and gas chromatography-mass spectrometry. J Chromatogr Sci. 2000;38:421-424.
16. Lakhdari W, Benyahia I, Bouhenna MM, Bendif H, Khelafi H, Bachir H, *et al*. Exploration and evaluation of secondary metabolites from *Trichoderma harzianum*: GC-MS analysis, phytochemical profiling, antifungal and antioxidant activity assessment. Molecules. 2023;28(13):5025.
17. Lee S, Yap M, Behringer G, Hung R, Bennett JW. Volatile organic compounds emitted by Trichoderma species mediate plant growth. Fungal Biol Biotechnol. 2016 Sep 29;3:7
18. Leelavathy MS, Vani L, Reena P. Antimicrobial activity of *Trichoderma harzianum* against bacteria and fungi. Int J Curr Microbiol Appl Sci. 2014;3(1):96-103.
19. Morath SU, Hung R, Bennett JW. Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. Fungal Biol Rev. 2012;26:73-83.
20. Mukherjee PK, Horwitz BA, Singh US, Mukherjee MM, Schmoll M. *Trichoderma* in agriculture, industry and medicine: an overview. In: *Trichoderma: Biology and Applications*. CABI; 2013. p. 1-9.
21. Naher L, Yusuf UK, Ismail A, Hossain K. *Trichoderma* spp: a biocontrol agent for sustainable management of plant disease. Pak J Bot. 2014;46(4):1489-1493.
22. Nemcovic M, Jakubikova L, Viden I, Vladimir F. Induction of conidiation by endogenous volatile compounds in *Trichoderma* spp. FEMS Microbiol Lett. 2008;284:231-236.
23. Nurbailis, Djamaan A, Rahma H, Liswarni Y. Secondary metabolite production by *Trichoderma* spp. and its potential as antibacteria. Int J Curr Microbiol App Sci. 2019;8(4):196-201.
24. Nurbailis. Pengendalian hayati *Sclerotium rolfsii* penyebab penyakit busuk batang kacang tanah (*Arachis hypogaea* L.) dengan kompos dan cendawan antagonis. Bogor: Program Pascasarjana, IPB; 1992.
25. Nurbailis, Mardinus, Nasir N, Dharma A, Habazar T. Penapisan *Trichoderma* spp. dari rizosfir pisang untuk menekan pertumbuhan *Fusarium oxysporum* f.sp. *cubense in vitro*. J Manggaro. 2008;9:16-21.
26. Nurbailis, Mardinus, Nasril N, Dharma A. Penapisan isolat *Trichoderma* dari rizosfir tanaman pisang di Sumatera Barat untuk pengendalian penyakit layu *Fusarium*. J Akta Agrosia. 2006;9(1).
27. Pushpavathi Y, Dash SN, Laxmi Madhavi NN, Sandhya Deepika D. Biological control of Fusarium wilt disease in banana with emphasis on *Trichoderma* spp. and *Pseudomonas* spp. Plant Arch. 2016;16(1):51-9.
28. Reithner B, Brunner K, Schuhmacher R, Peissl P, Seidl V, Krska R, *et al*. The G protein α subunit Tga1 of *Trichoderma atroviride* is involved in chitinase formation and differential production of antifungal metabolites. Fungal Genet Biol. 2005;42:749-760.
29. Segaran G, Sathiavelu M. Fungal endophytes: a potent biocontrol agent and a bioactive metabolites reservoir. Biocatal Agric Biotechnol. 2019;21:101284.
30. Shahiri Tabarestani M, Rahnama K, Jahanshahi M, Nasrollahnejad S, Fatemi MH. Identification of volatile organic compounds of some *Trichoderma* species using static headspace gas chromatography-mass spectrometry. Mycol Iran. 2016;3(1):47-55.
31. Sheeba H, Ali MS, Anuradha V. Bioactive compounds and antimicrobial activity of fungal crude extract from medicinal plants. J Pharm Sci Res. 2019;11:1826-1833.
32. Shobha B, Lakshmeesha TR, Ansari MA, Almatroudi A, Alzohairy MA, Basavaraju S, *et al*. Mycosynthesis of ZnO nanoparticles using *Trichoderma* spp. isolated from rhizosphere soils and its synergistic antibacterial effect against *Xanthomonas oryzae* pv. *oryzae*. J Fungi. 2020;6(3):181.
33. Stoppacher N, Kluger B, Zeilinger S, Krska R, Schuhmacher R. Identification and profiling of volatile

metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GC-MS. J Microbiol Methods. 2010;81:187-193.
34. Tait E, Perry JD, Stanforth SP, Dean JR. Identification of volatile organic compounds produced by bacteria using HS-SPME-GC-MS. J Chromatogr Sci. 2013;51(4):363-373. doi:10.1093/chromsci/bmt042
35. Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, *et al*. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiol Mol Plant Pathol. 2008;72:80-86.
36. Zhang JL, Tang WL, Huang QR, Li YZ, Wei ML, Jiang LL, *et al*. *Trichoderma*: a treasure house of structurally diverse secondary metabolites with medicinal importance. Front Microbiol. 2021;12:723828.