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Assessment of rhizosphere soil fungi *Alternaria longipes* and *Fusarium incarnatum* for bioactive compounds with respect to pharmaceutical potential

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The present investigation focused on studies on bioactive compounds of rhizosphere soil fungi *Alternaria longipes* Ellis and Everhartand *Fusarium incarnatum* Adriaana Jacobs and Lydiaof *Cicer arietinum* L (chickpea). These fungi were isolated from rhizosphere soil of *Cicer arietinum* and identified and confirmed based on morphological characteristics. These fungal species pure cultures were used for Sanger Sequencing (ITS Marker). Further homology searches were performed using the BLAST against the NCBI GenBank database. GC-MS analysis of *A. longipes* (MT635195) and *F. incarnatum (MT889972)* was carried out by using fresh mycelium and recorded 73 and 78 secondary metabolites respectively.

Keywords: *Cicer arietinum* L., GC-MS, rhizosphere soil fungi

INTRODUCTION

Rhizosphere soil fungi play crucial roles in plant-microbe interactions, soil ecology and produce various bioactive compounds. These compounds can significantly influence plant growth, health and microbial community structure in the rhizosphere. Rhizosphere soil fungi associated with chickpea have been shown to produce various bioactive compounds with potential applications of medicine. Rhizosphere soil fungi *viz*. *Trichoderma* sp*., Aspergillus* sp*., Penicillum* sp. and *Alternaria* sp. produces various bioactive compounds with their pharmaceutical applications (Tripathi *et al.* 2015). Bioactive compounds released from rhizosphere soil fungi perform both positive and negative effects on plant growth and development. Some rhizosphere soil fungi produces phytotoxic compounds that release growth promoting substances while others inhibit seedling growth and cause root-tip necrosis (Manici *et al.* 2016). Plant roots released bioactive compounds into the rhizosphere soil interact significantly with growth of fungi, influencing plant health and soil microbial communities. Root exudates containing organic acids, growth hormones and sugars can alter the rhizosphere community structure and affect fungal metabolite production (Manici *et al.* 2016).

These interactions play a crucial role in plant-soil-microbial dynamics, especially under stressful environmental conditions. Chatterjee *et al.* (2022) studied the *Alternaria alternata* isolated from rhizosphere soil of chickpea and found that a wide range of secondary metabolites were produced by *A. alternata* having antimicrobial properties. *A.alternata* has shown promise as producers of bioactive compounds.Rhizosphere fungi like *Trichoderma* sp. are more commonly studied for their beneficial effects on plant growth and health. *Trichoderma* species produce secondary metabolites such as non-ribosomal peptides, terpenoids and indole-derived compounds that can induce systemic resistance in plants and improve nutrient uptake (Contreras-Cornejo *et al.* 2016). *Trichoderma reesei* was found dominant in rhizosphere soil of chickpea to modulate arsenic speciation and improve growth in arsenic-contaminated soil. The production of compounds

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that can mitigate heavy metal stress, which may have pharmaceutical relevance. *Fusarium oxysporum*, *Rhizoctonia bataticola* and *Pythium* sp. isolated from rhizosphere soil of chickpea demonstrated biocontrol activity against fungal pathogens (Chen and Liu, 2024). They produce various mycotoxins like equisetin, enniatin B and D and fusaric acid, which are associated with plant growth inhibition and root damage (Duponnois *et al.* 2008). Additionally, rhizosphere soil fungi release other biologically active compounds such as methoxyconidiol, paecilaminol and integrastatin B (Rieusset *et al.* 2020).

Rhizosphere soil fungi produce various types of bioactive compounds associated with chickpea plants or soil fungi, play a vital role in shaping the microbial community and promoting plant growth. The interactions between plants and rhizosphere microbiomes create a complex, multitrophic system that is crucial for sustainable agriculture. In this connection present investigation is focused on bioactive compounds of rhizosphere soil fungi *viz*. *A. longipes* and *F. incarnatum* of *Cicer arietinum* L. with respect to pharmaceutical potential.

MATERIALS AND METHODS

Cicer arietinum L. rhizosphere soil samples were collected from Pahunewadi of Baramati tahsil,during December, 2022 to February, 2023. The samples were collected during the flowering stages of crop. The soil samples were collected in sterilized polythene zip bags and brought to the laboratory to isolation of soil fungi was carried out by serial dilution techniques. Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were used for isolation of fungi (Shitole *et al.,* 2019). Pure culture of fungal medium was supplemented with Streptomycin antibiotic (to avoid bacterial growth). The inoculated plates were incubated at room temperature for 7 days. Fungal growth was regularly observed during the incubation period.

After 7 days of incubation, photographs of the plates were taken and isolated fungal colonies were used to prepare slides. Slides were prepared using a cotton blue stain and lactophenol as the mounting medium. Identification of *Alternaria longipes* and *Fusarium incarnatum* fungi were done on the basis of their morphological characteristics, such as colour and nature of mycelia, asexual spores and sexual spores using the literature of Gilman (2001) and Nagamani *et al.* (2006).

Pure cultures of *Alternaria longipes* and *Fusarium incarnatum* were used for Sanger Sequencing (ITS Marker). DNA was quantified using a NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher Scientific, Inc. Wilmington, DE, USA) and the quality was checked by 1% agarose gel electrophoresis. Gel was visualized using gel documentation system (Vilber) and fragment of ITS gene was amplified by ITS1 and ITS4 primers. A single discrete PCR amplicon band of 550-600 bp was observed when resolved on a 1.2 % agarose gel. The PCR amplicons were purified to remove contaminants. Forward and reverse DNA sequencing of the PCR amplicon was carried out with forward and reverse primers using a BDT v3.1 cycle sequencing kit on an ABI 3730xl genetic analyzer. The consensus sequence of the ITS gene was generated from forward and reverse sequence data using the aligner software. The ITS gene sequence was used to carry out BLAST using the NCBI GenBank database. Based on the maximum identity score and alignments using a multiple alignment software program (Tamura *et al.* 2021).

The resulting DNA sequences were aligned using the OMEGA software embedded in MEGA7 (Nei and Kumar, 2000), manually trimmed and edited to obtain complete sequences. Species confirmation depends on the sequence similarity score. Homology searches were performed using the BLASTn program against the NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The IDs of each fungal species showed similarity searches in the sequence alignment and their accession numbers were also obtained.

GC-MS analysis

The quantification studies of bioactive compounds were done by Gas Chromatography and Mass Spectroscopy (GC-MS) from Progenome Life Science, Sambhajinagar, Maharashtra.

Detailed chromatographic conditions showed in Table-1(a) &1 (b).

Table1 (a): Chromatographic conditions of GC.

GC-MS/MS	GCMS-TQ8040 NX
Auto-injector	AOCTM-20i + s
Column	SH-Rxi-5Sil MS (30m × 0.25mm I.D., df = 0.25µm)
Liner	Topaz Liner, Splitless Single Taper w/Wool
Injector temp.	280^{0}C
Column oven temp.	60^{0}C (1 min), 40^{0}C/min to 170^{0}C (0 min), 10^{0}C/min to 310^{0}C (7.25 min)
Run time	25min
Injection mode	Splitless (High pressure at 250kPa)
Injection volume	1µL
Carrier gas	He
Linear Velocity	36.5 cm/sec (Constant mode)
Column oven temp	60^{0}C (1 min), 40^{0}C/min to 170^{0}C (0 min), 10^{0}C/min to 310^{0}C (7.25 min)

Table1 (b): Chromatographic conditions of MS

Interface temp.	300^{0}C
Ion source temp.	230^{0}C
Ionization mode	EI
Solvent cut time	3.5min
Loop Time	0.5sec

RESULTS AND DISCUSSION

GC-MS analysis of fresh mycelium of Alternaria longipes Ellis and Everhart (MT635195)

GC-MS analysis of methanolic extracts of fresh mycelia of *Alternaria longipes* Ellis and Everhart (MT635195) showed 73 metabolites. The chromatograms of the peaks were combined and compared with the database of known component spectra in the gas chromatography-mass spectrometry (GC-MS) and NISP libraries. The results showed the presence of various secondary metabolites *viz*. Carboxylic acids, Benzeneand substitute, Indolesand derivatives, Lactams, Fatty Acyls, Organo nitrogen compounds, Fatty Acyls, Indoles, Benzothiazoles, Pteridines, Indoles, Peptidomimetics, Steroids, Macrolactams, Naphthofurans, Quinolines, Pyridines, Imidazopyrimidines, Glycerolipids, Glycerophospholipids and Sphingolipids. The detailed tabulations of the GC-MS analysis of the methanolic extract *of A. longipes* is given in Table 2.

Carboxylic acids play a significant role in defending plants against *Alternaria longipes*, a fungal pathogen causing brown spot disease of chickpea. These compounds significantly reduce disease development and enhance plant growth and yield. The protective mechanism of carboxylic acids against *Alternaria* involves the activation of both enzymatic and non-enzymatic anti-oxidant defense systems. *Alternaria longipes* is known for producing beneficial compounds such as benzene and its substitutes are crucial components contributing to both beneficial (e.g., anti-diabetic) and pathogenic (e.g., plant infection) activities. The wide spread occurrence of these aromatic structures in nature underscores their significance in biological processes and potential applications in drug discovery and plant pathology research (Nehela*et al.,* 2021).

Fatty acyls and derivatives related metabolites extracted from *A. longipes* demonstrate dual roles - as potential therapeutic agents and as factors in plant pathogenesis. The anti-diabetic properties of compounds like 2,4,6-triphenylaniline highlight the potential for developing natural alternatives to synthetic drugs. However, the pathogenic nature of *A. longipes* in plants underscores the complexity of its metabolic activities. *Alternaria longipes* produce secondary metabolites with potential pharmacological properties, including anti-diabetic activity (Ranganathan and Mahalingam, 2018).

Table 2: List of bioactive compounds, retention time, m/z ratio and their nature of *Alternaria longipes* Ellis and Everhart (MT635195)

No.	Name of Compound	RT [min]	m/z ratio	Nature of compound
1.	4-Methylaminoantipyrine	1.312	227.11359	Carboxylic acids
2.	Diosgenin	1.331	104.10705	Benzeneandsubstitute
3.	D-(+)-Pipecolinicacid	1.334	148.0605 8	Indolesand derivatives
4.	L-Pyroglutamic acid	1.354	132.07674	Lactams
5.	4-hydroxy -5,8 dimethyl - quinoline -3-carboxyl	1.366	156.07681	Indolesand derivatives
6.	L-Kynurenine	1.367	137.04593	FattyAcyls
7.	Glycerophospho-N-palmitoylethanolamine	1.413	189.15929	Organonitrogencompounds
8.	L-Isoleucine	1.43	170.0925	FattyAcyls
9.	DL-Lysine	1.47	147.07646	Organooxygencompounds
10.	Palmitoylsphingomyelin	1.505	162.11255	Carboxylic acids
11.	Guanine	2.63	182.08128	FattyAcyls
12.	Monoolein	4.754	177.10213	Carboxylic acids
13.	Betaine	4.779	166.08592	Carboxylic acids
14.	N,N-DimethyldecylamineN-oxide	4.858	136.07591	Carboxylic acids
15.	Pipecolicacid	5.066	220.1176	Indoles
16.	Dehydrocholic acid	5.102	188.0708	FattyAcyls
17.	Cinnamoylglycine	5.167	229.15424	Indoles
18.	Methylindole-3-acetate	5.178	298.09622	Organonitrogencompounds
19.	Betaine	5.216	118.06509	Benzothiazoles
20.	Cholecalciferol	5.216	205.0968	Organonitrogencompounds
21.	Glycerophospho-N-palmitoylethanolamine	5.223	192.06546	Pteridines
22.	Pipecolicacid	5.251	181.06036	Carboxylic acids
23.	Indiru bin	5.293	114.09161	Indoles
24.	Guanine	5.338	146.0602	Organonitrogencompounds
25.	Oleoylethanolamide	5.363	377.14511	Peptidomimetics
26.	D-(+)-Pipecolinic acid	5.4	168.06566	Carboxylic acids
27.	Thymine	5.502	194.08124	Carboxylic acids
28.	Phenylacetylglycine	5.502	194.08124	Carboxylic acids
29.	Cinnamoylglycine	5.715	206.0813	Carboxylic acids
30.	Indole -3-acetic acid	5.737	176.07069	Indoles
31.	Methylindole-3-acetate	5.896	190.08652	Indoles
32.	Cortisol	6.077	363.21637	Steroids
33.	N,N-DimethyldecylamineN-oxide	6.104	202.21619	Organonitrogencompounds
34.	Decanoylcarnitine	6.204	316.24829	FattyAcyls
35.	Thymine	6.225	167.99384	Organonitrogencompounds
36.	2-Mercaptobenzothiazole	6.225	167.99384	Benzothiazoles
37.	L-(-)-Methionine	6.245	777.6944	Carboxylic acids
38.	Levothyroxine	6.245	777.6944	Carboxylic acids
39.	DL-Lysine	6.354	823.4115	Carboxylic acids
40.	Rifampicin	6.354	823.4115	Macrolactams
41.	Cafestol	6.487	317.21106	Naphthofurans
42.	Indirubin	6.614	263.08115	Indoles
43.	L-(-)-Methionine	6.911	302.30515	Quinolines
44.	2-Amino -1,3-octadecanediol	6.91 1	302.30515	Organonitrogens
45.	Progesterone	7.27	315.23172	Steroids
46.	DL-Glutamine	7.334	300.28934	Steroids
47.	D-Sphingosine	7.334	300.28934	Organonitrogencompounds

48.	Decanoylcarnitine	7.732	400.34222	Pyridines
49.	Palmitoylcarnitine	7.732	400.34222	FattyAcyls
50.	1-Methyladenosine	7.976	255.23154	Macrolactams
51.	Palmitoleicacid	7.976	255.23154	FattyAcyls
52.	Oleoylethanolamide	8.148	270.31509	5'-deoxyribonucleosides
53.	Octadecanamine	8.148	270.31509	Organonitrogen
54.	L-Kynurenine	8.208	291.2312	FattyAcyls
55.	5α-Dihydr otestosterone	8.208	291.2312	Steroids
56.	2,6-Di-tert-butyl-1,4-benzoquinone	8.215	221.15324	Prenollipids
57.	Diosgenin	9.042	415.32059	Prenollipids
58.	Cholecalciferol	9.212	385.34622	Steroids
59.	Glycerophospho-N-palmitoylethanolamine	9.365	454.29254	Glycerophos pholipids
60.	Docosanamide	9.465	340.35727	FattyAcyls
61.	DL-Glutamine	9.484	303.23172	Imidazopyrimidines
62.	Eicosapentaenoic acid	9.484	303.23172	FattyAcyls
63.	Oleoylethanolamide	9.792	326.30515	Organonitrogencompounds
64.	1-Palmitoylglycerol	9.876	331.28394	Glyce rolipids
65.	PC(22:5e/18:1)	9.884	820.62268	Glycerophospholipids
66.	Monoolein	10.085	357.29962	Glycerolipids
67.	Docosapentaenoic acid	10.159	331.26291	FattyAcyls
68.	Decanoylcarnitine	10.343	331.26288	Carboxylic acids
69.	all-cis-4,7,10,13,16-Docosapentaenoicacid	10.343	331.26288	FattyAcyls
70.	Stearamide	10.509	284.29489	Carboximidic acids
71.	1-Stearoylglycerol	10.686	359.31522	Glycerolipids
72.	DL-Norvaline	10.88	118.08651	Carboxylic acids
73.	Palmitoylsphingomyelin	10.95	703.57477	Sphingolipids

*RT: Retention time and m/z: specific mass-to-charge ratio.

GC-MS analysis of fresh mycelium of Fusarium incarnatum

GC-MS analysis of methanolic extracts of fresh mycelia of *Fusarium incarnatum* Adriaana Jacobs and Lydia (MT889972) extracted total 78 metabolites. The chromatograms of the peaks were combined and compared with the database of known component spectra in the gas chromatography-mass spectrometry (GC-MS) and NISP libraries. The results showed the presence of various secondary metabolites *viz*. Carboxylicacids, Peptidomimetics, Organo-nitrogen compounds, Fatty Acyls, Imidazopyrimidines, Indoles, Benzene, Purinenucleosides, Diazines, Azoles, Indoles, 5'-deoxyribonucleosides, Quinolines, Lactams, Quinolines, Imidazopyrimidines, Pteridines, Steroids, Benzothiazoles, Macrolactams, Naphthofurans, Organonitrogen compounds, Prenollipids, Steroids and Glycerophospholipids. The detailed tabulations of the GC-MS analysis of the methanolic extract of *Fusarium incarnatum* is given in Table 3.

Fusarium incarnatum produces several alkaloids and other metabolites, some of which exhibit weak anti-proliferative and cytotoxic activities against human cell lines. The metabolism of *F. incarnatum* appears to be complex and responsive to environmental factors. For instance, light modulates secondary metabolism in *Fusarium* species, affecting pathways like carotenoid synthesis. Changes in growth conditions can alter the accumulation of polyketides and modifications in the metabolism of organic acids, amino acids and sugars in related *Fusarium* sp. (Ding *et al.* 2012).

Interestingly, in *F. graminearum*, inhibition of trichothecene mycotoxin production was associated with significant changes in both primary and secondary metabolism, including alterations in the tricarboxylic acid cycle and

Table 3: List of bioactive compounds, retention time, m/z ratio and their nature of *Fusarium incarnatum* Adriaana Jacobs and Lydia (MT889972)

No.	Name of Compound	RT [min]	m/z ratio	Nature of compound
1.	DL-Lysine	1.185	147.11285	Carboxylic acids
2.	Pipecolicacid	1.186	130.08647	Carboxylic acids
3.	Carnosine	1.312	227.11359	Peptidomimetics
4.	L-Pyroglutamic acid	1.324	130.05002	Carboxylic acids
5.	Choline	1.331	104.10705	Organonitrogencompounds
6.	L-Glutamicacid	1.334	148.06058	Carboxylic acids
7.	DL-Glutamine	1.347	147.07664	Carboxylic acids
8.	Creatinine	1.351	114.06631	Carboxylic acids
9.	Creatine	1.354	132.07674	Carboxylic acids
10.	L-Histidine	1.366	156.07681	Carboxylic acids
11.	Acetyl-L-carnitine	1.367	204.12291	FattyAcyls
12.	Hypoxanthine	1.367	137.04593	Imidazopyrimidines
13.	Acetylcholine	1.369	146.11772	Organonitrogencompounds
14.	L-(-)-Methionine	1.371	150.05832	Carboxylic acids
15.	D-(+)-Pipecolinic acid	1.373	130.08641	Carboxylic acids
16.	N6,N6,N6-Trimethyl-L-lysine	1.413	189.15929	Carboxylic acids
17.	1-Methylhistidine	1.43	170.0925	Carboxylic acids
18.	D-(-)-Glutamine	1.47	147.07646	Carboxylic acids
19.	L(-)-Carnitine	1.505	162.11255	Organonitrogencompounds
20.	Betaine	1.507	118.08617	Carboxylic acids
21.	L-Tyrosine	2.63	182.08128	Carboxylic acids
22.	L-Isoleucine	3.371	132.10187	Carboxylic acids
23.	Propionylcarnitine	3.573	218.13818	FattyAcyls
24.	Guanine	4.743	152.05698	Imidazopyrimidines
25.	Serotonin	4.754	177.10213	Indoles
26.	L-Phenylalanine	4.779	166.08592	Carboxylic acids
27.	Acetanilide	4.858	136.07591	Benzene
28.	7-Methylguanosine	4.877	298.11404	Purinenucleosides
29.	Thymine	4.894	127.05043	Diazines
30.	L-Kynurenine	4.911	209.09222	Organooxygencompounds
31.	Pantothenic acid	5.066	220.1176	Organooxygencompounds
32.	4-Methylaminoantipyrine	5.068	218.12854	Azoles
33.	Indole-3-acrylicacid	5.102	188.0708	Indoles
34.	Leucylproline	5.167	229.15424	Carboxylic acids
35.	5'-S-Methyl-5'-thioadenosine	5.178	298.09622	5'-deoxyribonucleosides
36.	DL-Tryptophan	5.216	205.0968	Indoles
37.	Indole	5.216	118.06509	Indoles
38.	5-Hydroxyindole-3-aceticacid	5.223	192.06546	Indoles
39.	Enrofloxacin	5.241	360.17166	Quinolines
40.	N-Acetylserotonin	5.241	219.11269	Indoles
41.	Nicotinuricacid	5.251	181.06036	Carboxylic acids
42.	Caprolactam	5.293	114.09161	Lactams
43.	8-Hydroxyquinoline	5.338	146.0602	Quinolines
44.	Caffeine	5.356	195.0878	Imidazopyrimidines
45.	Riboflavin	5.363	377.14511	Pteridines
46.	Pyridoxal	5.4	168.06566	Pyridines
47.	Hexanoylcarnitine	5.486	260.18579	FattyAcyls
48.	Phenylacetylglycine	5.502	194.08124	Carboxylic acids
49.	Cinnamoylglycine	5.715	206.0813	Carboxylic acids
50.	Indole-3-aceticacid	5.737	176.07069	Indoles
51.	Methylindole-3-acetate	5.896	190.08652	Indoles
52.	Cortisol	6.077	363.21637	Steroids

53.	N,N-DimethyldecylamineN-oxide	6.104	202.21619	Organonitrogen compounds
54.	Decanoylcarnitine	6.204	316.24829	FattyAcyls
55.	2-Mercaptobenzothiazole	6.225	167.99384	Benzothiazoles
56.	Levothyroxine	6.245	777.6944	Carboxylic acids
57.	Rifampicin	6.354	823.4115	Macrolactams
58.	Cafestol	6.487	317.21106	Naphthofurans
59.	Indirubin	6.614	263.08115	Indoles
60.	2-Amino-1,3-octadecanediol	6.911	302.30515	Organonitrogen compounds
61.	Progesterone	7.27	315.23172	Steroids
62.	D-Sphingosine	7.334	300.28934	Organonitrogen compounds
63.	Palmitoylcarnitine	7.732	400.34222	FattyAcyls
64.	Palmitoleicacid	7.976	255.23154	FattyAcyls
65.	Octadecanamine	8.148	270.31509	Organonitrogen compounds
66.	5α-Dihydrotestosterone	8.208	291.2312	Steroids
67.	2,6-Di-tert-butyl-1,4-benzoquinone	8.215	221.15324	Prenollipids
68.	Diosgenin	9.042	415.32059	Prenollipids
69.	Cholecalciferol	9.212	385.34622	Steroids
70.	Glycerophospho-N-palmitoylethanolamine	9.365	454.29254	Glycerophospholipids
71.	Docosanamide	9.465	340.35727	FattyAcyls
72.	Eicosapentaenoic acid	9.484	303.23172	FattyAcyls
73.	Oleoylethanolamide	9.792	326.30515	Organonitrogen compounds
74.	1-Palmitoylglycerol	9.876	331.28394	Glycerolipids
75.	Monoolein	10.085	357.29962	Glycerolipids
76.	Docosapentaenoic acid	10.159	331.26291	FattyAcyls
77.	all-cis-4,7,10,13,16-Docosapentaenoicacid	10.343	331.26288	FattyAcyls
78.	Stearamide	10.509	284.29489	Carboximidic acids

*RT: Retention time and m/z: specific mass-to-charge ratio.

metabolism of several amino acids. While not specific to carboximidic acids, this demonstrates the interconnectedness of metabolic pathways in *Fusarium* species.Indoles play a significant role in cell biology and have attracted attention for their biological activities, including potential applications in treating cancer, microbial infections and various human disorders (Heravi *et al.* 2021).

Fusarium is common soil-borne pathogen of chickpea, produce various mycotoxins and other bioactive compounds in the rhizosphere soil. These include equisetin, enniatin B, enniatin D and fusaric acid (Manici *et al.* 2016). Chickpea rhizosphere soil fungi produce antifungal compounds that contribute to the suppression of plant diseases (Sindhu and Dadarwal, 2001).

Alternaria and *Fusarium* are common soil-inhabiting fungal genera that produce a wide array of secondary metabolites with diverse biological activities. These metabolites play important roles in plant-microbe interactions and have potential applications in agriculture and medicine. *Alternaria* species are known to produce total 268 different metabolites, including nitrogen-containing compounds, steroids, terpenoids, pyranones, quinones and phenolics (Lou *et al.,* 2013)same results were obtained in our work. Many of these metabolites exhibit phytotoxic activities, with aromatic polyketides and sesquiterpenoids being the main phytotoxic compounds (Xu *et al.* 2021). Some *Alternaria* metabolites, such as alternariol methyl ether, have shown anti-microbial, anti-oxidant and anti-cancer properties (Palanichamy *et al.,* 2018). *Fusarium* species, particularly *F. oxysporum*is prolific producers of secondary metabolites. These include non-ribosomal peptides, terpenoids, pyrones and indolic-derived compounds (Contreras-Cornejo *et al.* 2016). Some *Fusarium* metabolites have identified as phytotoxins, contributing to their pathogenicity in plants (Xu *et al.* 2021).

Interestingly, while these fungi can be pathogenic, some strains of *Trichoderma* and othercommon soil fungi, produce secondary metabolites that can antagonize pathogenic fungi like *Fusarium* and induce systemic resistance in plants (Contreras-Cornejo *et al.* 2016). This highlights the complex interactions between different fungal species in the rhizosphere and their impact on plant health. The secondary metabolites produced by *Alternaria* and *Fusarium* species in the rhizosphere play crucial roles in plant-microbe interactions, ranging from pathogenicity to potential beneficial effects. Understanding these metabolites and their functions can provide insights for developing new strategies in crop protection and pharmaceutical applications.

CONCLUSION

GC-MS analysis of methanolic extracts from the fresh mycelia of *Alternaria longipes* Ellis and Everhart (MT635195) and *Fusarium incarnatum* Adriaana Jacobs and Lydia (MT889972) revealed a diverse array of secondary metabolites. A total of 73 secondary metabolites and 78 secondary metabolites were identified respectively by comparing chromatogram peaks with known component spectra in the GC-MS and NISP libraries. The analysis covered a wide range of compounds including carboxylic acids, benzene derivatives, indoles, lactams, fatty acids, organonitrogen compounds, benzothiazoles, pteridines, peptidomimetics, steroids, macrolactams, naphthofurans, quinolines, pyridines, imidazopyrimidines, glycerolipids, glycerophospholipids and sphingolipids. This comprehensive metabolite profile provides valuable insights into the secondary metabolite production capabilities of *A. longipes* and *F. incarnatum*, highlighting their potential as sources of bioactive compounds. Further investigation of the biological activities and potential applications of these metabolites could lead to the discovery of novel pharmaceutical or industrial compounds.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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