Journal of Biological Control, 36(4): 199-214, 2022, DOI: 10.18311/jbc/2023/31880

Research Article

Harnessing antifungal metabolites from macro basidiomycetes against wilt inciting *Fusarium* spp.

S. B. AKSHAYA[1*], A. S. KRISHNAMOORTHY[2], S. NAKKEERAN[2], U. SIVAKUMAR[3] and G. THIRIBHUVANAMALA[2]

[1]*College of Agricultural Sciences – SRM Institute of Science and Technology, Baburayanpettai – 603203, Chengalpattu, Tamil Nadu, India*
[2]*Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India*
[3]*Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore –641003, Tamil Nadu, India*
**Corresponding author E-mail:* akshayaagri14@gmail.com

ABSTRACT: Plant diseases especially wilt disease caused by *Fusarium* spp. pose a major threat to the cultivation of vegetables. In the present study, experiments were undertaken to explore the potential antifungal metabolites produced by macro basidiomycetes *viz., Lentinus edodes, Ganoderma lucidum* and *Schizophyllum commune* against *Fusarium oxysporum* and *F. solani* causing wilt disease of cucumber and capsicum. Among these, the ethyl acetate fraction of Cell-Free Culture Filtrate (CFC) of *L. edodes* exhibited maximum per cent inhibition of the mycelial growth of *F. oxysporum* and *F. solani* (61.11 and 57.77 %, respectively) at a concentration of 2000 ppm. Characterization of antifungal metabolites of Cell Free Condensate (CFC) of ethyl acetate fraction of *L. edodes* observed as prominent bands in Thin Layer Chromatography (TLC) indicated with an RF value of 0.25 and 0.69. Further GC-MS characterization of TLC-eluted compounds from *L. edodes* indicated the presence of 14 different compounds including 2H-pyran-2-one 6-pentyl-, possessing antifungal activity. The Fourier-transform Infrared Spectroscopy (FTIR) spectrum revealed the functional groups such as alcohol (O-H), amides (C-O), aliphatic polyes (CH_2), triazenes (N=N), silicon compounds (Si-O-Si), amines (C-N) and phosphorus (P=S). The comparison of metabolite distribution patterns by Principal Component Analysis (PCA) obtained from *L. edodes* (PC 1) showed a positive correlation between the compounds. This study infers that *L. edodes* possess antifungal activity against *F. oxysporum* and *F. solani* that can be explored for formulation and application of these antifungal compounds in plant protection.

KEYWORDS: Antifungal activity, Cell-Free Condensate (CFC), FTIR, macro basidiomycetes, mycelial inhibition

(Article chronicle: Received: 07-11-2022; Revised: 23-12-2022; Accepted: 25-12-2022)

INTRODUCTION

Macro basidiomycetes (Phylum: Basidiomycota) possess a number of biological activities such as antimicrobial, antifungal, antibacterial, antiviral, antinemic, antioxidant, anticancer, anti-hypoglycemic, antioxidant and immune-modulatory activities (Iwalokun *et al.,* 2007; Borchers *et al.,* 2008; Chowdhury *et al.,* 2015 and Rathore *et al*., 2019). At present climate change coupled with the development of fungicidal resistance of *Fusarium* spp. causes severe yield loss in capsicum and cucumber crops (Gebreyohannes *et al.,* 2019). In this context, the agrochemical industry is looking for a novel and environmentally safe biomolecule for the control of pests and diseases. The presence of numerous bioactive compounds and polysaccharides in macro basidiomycetes offers scope for application in the eco-friendly management of plant disease (Lindesquist *et al*., 2005, Thangaraj Praveen *et al.,* 2021). These bioactive compounds are secreted as secondary metabolites identified from the culture filtrates and mycelial cultures of macro basidiomycetes that possess mycelial growth inhibition of plant pathogenic fungi. Sangeetha *et al.* (2015) reported the antimicrobial activity of culture filtrates of *Ophiocordyceps sinensis* against *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *cubense*. Also, ethanolic extracts of culture filtrate of *Leucopaxillus gignatea* showed antimicrobial action against *Aspergillus niger*, *Fusarium solani*, *Collectotrichum graminicolum*, *Helminthosporium maydis*, *Xanthomonas axanopodis pv. punicae*, *Pseudomonas syringa* and *Bacillus subtilis* (Feleke and Anila Doshi, 2017). Similarly, Jeeva and Krishnamoorthy (2018) reported that the fruiting body of *Coprinopsis cinerea* (Inky Cap Mushroom) exhibited antifungal properties against *F. o.* f. sp. *cubense*, *F. o*. f. sp. *lycopersici,* and *F. brachygibbosum*. Furthermore, the cell-free culture filtrate of *Ganoderma lucidum* inhibited the spore germination and mycelial growth of *Colletotrichum capsici* (Priya *et al*., 2019). In addition, Akshaya *et al.* (2021)

stated the antifungal nature of cell-free culture filtrate of *Ophiocordyceps sinensis* in inhibition of *Fusarium* spp. Macro basidiomycetes secret numerous antifungal compounds that have been tested against human and plant pathogens. The antifungal metabolite cordycepin was extracted and purified from cell-free culture filtrate condensate and mycelial mat extract of *O. sinensis* and *O. neovolkiana* (Sangeetha *et al.,* 2017). Accordingly, the antifungal compound pleurostin from *Pleurotus osteratus* and ganodermin from *G. lucidum* inhibited the mycelial growth of *F. oxysporum*, *Botrytis cinerea* and *Peyronellaea arachidicolla* (Sivanandhan *et al.,* 2017*)*. Similarly, Gayathiri *et al*. (2021) reported that the ethyl acetate extracted culture filtrates of *G. lucidum* through Gas Chromatography-Mass Spectrometry (GC-MS) indicated the presence of novel compound papaverine which inhibited mycelial growth and conidial germination of *C. gloeosporioides*. In view of above studies, a research study was construed to evaluate the antifungal activity of *Ganoderma lucidum, Lentinus edodes and Schizophyllum commune* against *F. oxysporum* and *F. solani.*

MATERIALS AND METHODS

The *Fusarium* wilt-causing pathogen, *Fusarium oxysporum* (Accession no. MZ268142) infecting cucumber and *F. solani* (Accession no. MZ268161) infecting capsicum and macro basidiomycetes cultures including, *G. lucidum, L. edodes* and *S. commune* were obtained from the culture repository of the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Extraction of bioactive metabolites from macro basidiomycetes

Mycelial discs (9 mm) from 7 days old cultures of *G. lucidum*, *L. edodes* and *S. commune* were inoculated into 250 ml conical flasks containing 100 ml of sterilized mushroom complete broth (Glucose 20 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0 g, yeast extract 2 g, peptone 2 g, and water 1000 ml) and incubated in a rotatory shaker at 25°C, 120 rpm for 20 days. After incubation, the culture filtrate and the mycelial mat from each fungus were separated by filtration through Whatman No. 40 filter paper. The filtrate was further centrifuged at 10,000 rpm for 15 mins and the mycelium-free culture filtrate was extracted with ethyl acetate at a 1:1 ratio (v/v) sequentially. The extraction was conceded three to four times. The extracts obtained from each solvent phase were concentrated under reduced pressures using a rotary evaporator at 40°C. The condensed crude metabolites were dissolved in 1 ml HPLC-grade methanol and used for further analysis (Sangeetha *et al.,* 2015a).

Testing the ethyl acetate fraction of CFC filtrates of macro basidiomycetes against *Fusarium* spp. by agar well diffusion

The ethyl acetate fraction of CFC filtrate of macro basidiomycetes was tested at different concentrations *viz*., 1000, 1500, and 2000 ppm for mycelial growth inhibition of *F. oxysporum* and *F. solani* by agar well diffusion method (Akshaya *et al.,* 2021). Potato Dextrose Agar (PDA) medium was poured into sterile Petri plates and allowed to solidify. After solidification, 5 mm diameter wells were made on four corners giving equal distance and also by leaving one cm space from the periphery using a sterile cork borer. The different concentrations of solvent-extracted metabolites (50 µl) were added to each well. Then, a 5 mm mycelial disc of *F. oxysporum* and *F. solani* was taken from seven days old culture and placed at the centre of each Petri dish and incubated at 28 ± 2 ºC for seven days. Methanolic control and water control were maintained. Observations on the per cent inhibition of the mycelial growth of the pathogens were recorded (Vincent, 1947).

Detection of antifungal compounds through Thin Layer Chromatography (TLC), GC- MS, and FT-IR analysis

Thin Layer Chromatography (TLC)

TLC analysis was carried out to identify the antimicrobial compounds present in the ethyl acetate CFC condensate of macro basidiomycetes using the mobile phase of chloroform:ethyl acetate:acetone:methanol (30:10:2:1 v/v). The pre-coated Silica gel TLC plate (Merck, Silica gel 60 F254, Germany) were spotted with 50 µl of CFC condensate of *G. lucidum, L. edodes* and *S. commune* at equal distance and placed in tanks containing solvents. The solvent systems were poured into TLC tanks and the plates were immersed in the solvent tank at an approximate height of 0.5 cm. The tanks were closed with a glass lid to trap the solvent vapour. The solvent reaches the TLC plates within 30 min. Later the plates were air-dried at room temperature and observed in a UV trans-illuminator at a wavelength of 250 nm and 330 nm. The retention factor (Rf value) was calculated and recorded using the below formula.

$$\text{Rf value} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

Specific bands detected from the TLC plate were scrapped using the sterile blade along with the silica gel and dissolved in 1 ml HPLC grade methanol and vortexed for 15 minutes. Centrifugation was done at 10,000 rpm for 10 minutes to separate the compound and silica gel, filtered through a membrane filter (0.2 µm) and stored at 4ºC, and used for further studies (Gayathiri *et al.,* 2021).

Gas Chromatography and Mass Spectrometry (GC-MS*)*

GC-MS (Thermo Fisher Scientific Limited's Trace GC Ultra and DSQII model) was employed to detect the compounds eluted from the TLC surface. The instrument

was set as follows: injector port temperature set at 250ºC, interface temperature set at 250ºC, and the source maintained at 200ºC. The analysis includes: initial oven temperature was at 70ºC for 2 min and increased to 150ºC @ 8º C/min; the final set temperature was at 260ºC 10ºC/min. The spectral bins obtained in GC-MS were analyzed and the compounds were identified against NIST mass spectral library (2014) based on the most probable hits (Jeeva and Krishnamoorthy *et al.,* 2018).

Fourier Transform Infrared Spectrophotometer (FT-IR) analysis

Due to the expression of maximum inhibition of mycelial growth of *Fusarium* spp. and the presence of maximum antimicrobial activity in the ethyl acetate fraction of CFC of *L. edodes*, the antimicrobial metabolites were subjected to FT-IR analysis for identification of functional groups. The antimicrobial metabolites of *L. edodes* (10 µl) were analyzed through FT-IR (Jasco FTIR 6800, Japan) equipped with an Attenuated Total Reflectance (ATR) unit to identify the molecular vibration of functional groups. The infrared scanning array between 650 and 4000 cm^{-1} was collected, averaging 64 scans at a resolution of 4 cm^{-1}. The spectrum obtained was analyzed using Bio-Rad KnowItAll® 2017 software (Sangeetha *et al.,* 2020).

Conformation of the antifungal activity of a standard sample of 2H-Pyran-2-one against *Fusarium* spp.

The compound 2H-Pyran-2-one from soluble metabolites of *L. edodes* with a high peak area percentage (26.45) was identified as an antifungal compound from GC-MS. Later, the standard compound was purchased from Sigma Aldrich and dissolved in water to make different concentrations *viz.,* 100, 250, 500, 750, and 1000 ppm. These concentrations were tested for antifungal activity against *Fusarium* spp. by spore germination and agar well diffusion assay. In a separate experiment, *Fusarium* conidial suspension was prepared (5 x 10^{-5} conidia/ml) and tested for spore germination assay (Gayathiri *et al.,* 2021).

Statistical analysis

All the experiments were performed in triplicate and the treatment means differences were evaluated with Duncan's Multiple Range-Test (DMRT) at 5% significance and all the data were statistically analyzed and interpreted using software package SPSS 16.0. The correlation networks were viewed in Cytoscape version 3.4.0. The prediction of metabolic pathways was analyzed using Metaboanalyst 5.0. Principal Component Analysis (PCA) was carried out through XLSTAT (2016).

RESULTS AND DISCUSSION

Antifungal activity of macro basidiomycetes against *Fusarium* spp.

Among the macro basidiomycetes tested, the ethyl acetate fraction of Cell-Free Culture (CFC) filtrate of *Lentinus edodes* at 2000 ppm exhibited maximum per cent reduction of mycelial growth of *Fusarium oxysporum* and *F. solani* with 61.11 and 57.77 % inhibition, respectively. The ethyl acetate fraction of CFC of *Ganoderma lucidum* and *Schizophyllum commune* at 2000 ppm exhibited 52.33 and 46.66 % inhibition of *F. oxysporum* respectively and against *F. solani* per cent inhibition observed was 53.33 and 44.44 respectively. (Table 1 and Table 2). Least inhibition was observed at 1000 ppm (Figure 1 and Figure 2). This study showed that the ethyl acetate fraction of CFC of *L. edodes* possessed significant higher inhibitory activity against *Fusarium* spp.

Table 1. Effect of ethyl acetate fraction of Cell Free Condensate (CFC) of macro basidiomycetes against *Fusarium oxysporum*

Conc. (ppm)	Source of ethyl acetate fraction of Cell Free Condensate against *Fusarium oxysporum*					
	Lentinus edodes		*Ganoderma lucidum*		*Schizophyllum commune*	
	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition
1000	50.00^{b} (45.00)	44.44	57.00^{b} (49.22)	36.66	64.00^{b} (54.94)	28.88
1500	46.00^{c} (41.54)	48.88	48.00^{bc} (44.81)	46.66	55.00^{c} (47.88)	38.81
2000	35.00^{d} (35.03)	61.11	42.00^{c} (40.20)	53.33	48.00^{d} (44.04)	46.66
Control	90.00^{a} (71.56)	0.0	90.00^{a} (71.56)	0.0	90.00^{a} (71.56)	0.0
SED	1.02	-	0.99	-	1.60	-
CD (0.05)	2.35	-	2.30	-	3.70	-

Table 2. Effect of ethyl acetate fraction of Cell Free Condensate (CFC) of macro basidiomycetes against *Fusarium solani*

Conc. (ppm)	Source of ethyl acetate fraction of Cell Free Condensate against *Fusarium solani*					
	Lentinus edodes		*Ganoderma lucidum*		*Schizophyllum commune*	
	Mycelial Growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition
1000	52.00[b] (46.53)	42.22	57.00[b] (49.42)	36.66	67.00[b] (55.16)	25.55
1500	45.00[c] (41.74)	50.00	49.00[bc] (37.89)	45.55	57.00[c] (49.22)	36.66
2000	38.00[d] (38.44)	57.77	43.00[c] (41.55)	52.22	50.00[d] (43.47)	44.44
Control	90.00[a] (71.56)	0.0	90.00[a] (71.56)	0.0	90.00[a] (71.56)	0.0
SED	1.37	-	4.26	-	1.26	-
CD (0.05)	3.17	-	9.83	-	2.92	-

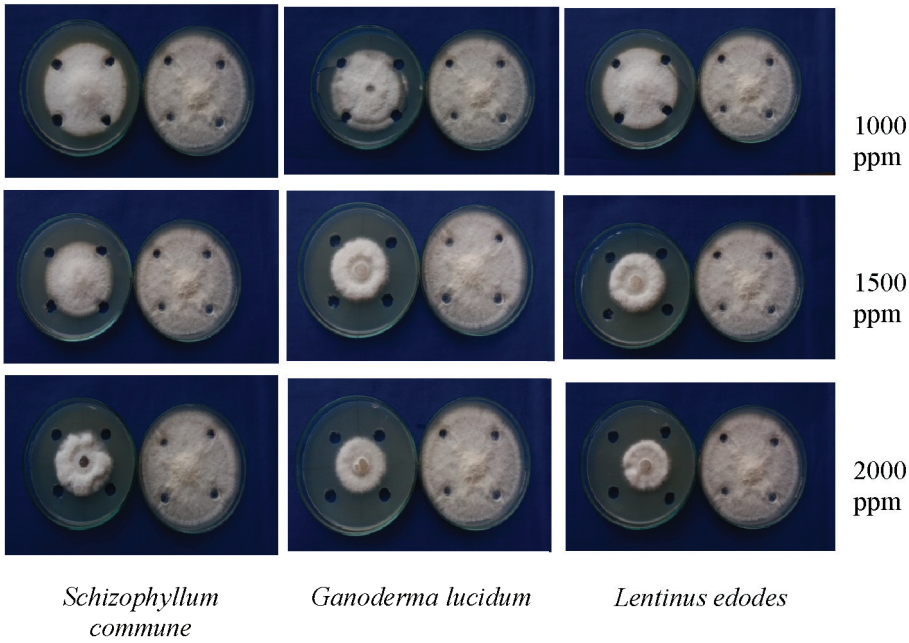

Figure 1a. Antifungal activity of ethyl acetate fraction of CFC condensate of macro basidiomycetes at different concentrations against *Fusarium oxysporum.*

Detection of antifungal metabolites by Thin Layer Chromatography (TLC)

The ethyl acetate fraction of CFC produced by *L. edodes, G. lucidum* and *S. commune* was characterized using Thin Layer Chromatography (TLC). All the three macro basidiomycetes tested produced several similar bands in methanol fraction with Rf values ranging from 0.25 to 0.75 with identifiable three discrete bands at Rf values of 0.25, 0.69, and 0.75 comprising different secondary metabolite compounds. (Figure 2). The bands obtained from the TLC plate were eluted and the compounds were identified through GC-MS analysis.

Profiling of bioactive antifungal metabolites by GC-MS

The antifungal compounds of CFC of macro basidiomycetes *L. edodes, G. lucidum* and *S. commune* characterized through GC- MS studies are presented in Figures 4a-c. Among them, GC–MS analysis of antifungal metabolite of *L. edodes* comprised 14 different compounds with retention time (RT) *viz.,* Riluzole (9.36 RT), 2H-Pyran-2-one, 6-pentyl-(10.57 RT), Tropidine (11.54 RT), Formamide (13.42 RT), Furfural (13.08 RT), Benzene, (2-methyl-butyl)-(15.53 RT), Sesquicineole (16.41 RT), Heneicosane (17.63 RT), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro (17.64 RT), n-Hexadecanoic acid (21.61 RT), 4-Octadecenal (23.15 RT),

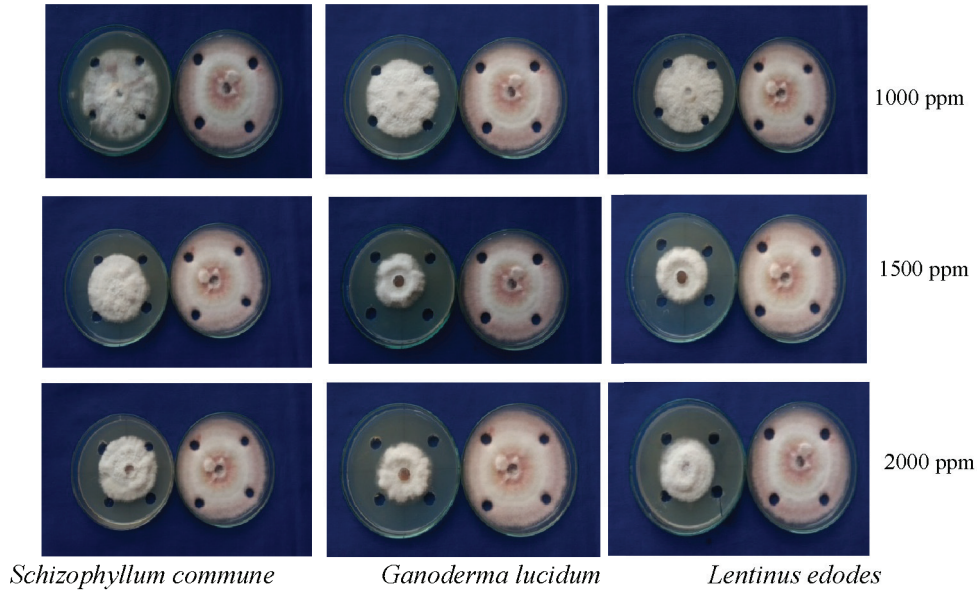

Figure 1b. Antifungal activity of ethyl acetate fraction of CFC condensate of macro basidiomycetes at different concentrations against *Fusarium solani.*

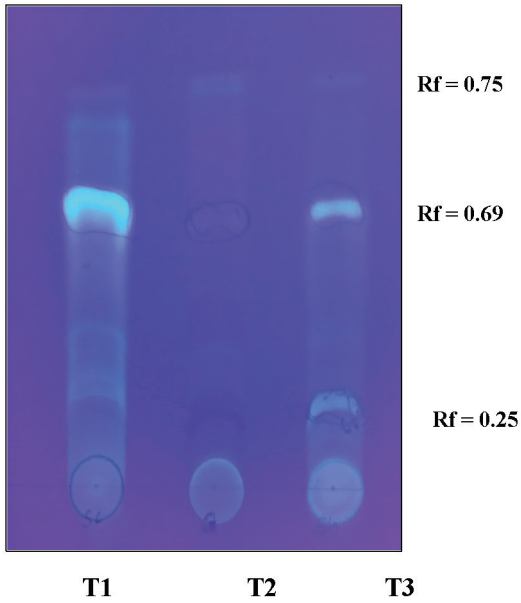

Figure 2. TLC showing the spots with different RF values (Mobile Phase: (chloroform, ethyl acetate, acetone and methanol); T1 - *Schizophyllum commune*; T2 - *Ganoderma lucidum;* T3 - *Lentinus edodes.*

17-Pentatriacontene (24.13 RT), Octadecanoic acid (24.53 RT) and Calconcarboxylic acid (28.76 RT) belonging to 18 classes including Organoheterocyclic compounds (30%), Fatty acyls (25%), Benzene and substituted derivatives (15%), Carboximidic acids and derivatives (10%), Lipids and lipid-like molecules (10%), Alkanes (5%) and Alkaloids and derivatives (5%) (Figure 4a). Likewise, the prominent compounds of *G. lucidum* indicated the presence of 12 compounds *viz.*, Phenyl Ethyl Alcohol (5.62 RT), 1-But-3-enyl-2-methoxy-benzene (9.27 RT), Benzeneethanol (10.04 RT), 2,4-Di-tert-butylphenol (11.44 RT), Benzoic acid (11.82 RT), Hexadecane (13.26 RT), Pyrrolo (1,2-a] pyrazine-1,4-dione, hexahydro-3-(2methylpropyl) (16.06 RT), Hexadecanoic acid (20.14 RT), dl-Alanine-beta-naphthylamide (22.07 RT), Methyl stearate (23.94 RT), trans-13-Octadecenoic acid (24.11 RT), and Prazosin (25.82 RT) belonging to the class Benzene and substituted derivatives (34%), Fatty acyls (33%), Organoheterocyclic compounds (17%), Carboxylic acids and derivatives (8%) and phenols (8%) (Figure 4b). Accordingly, the 9 unique compounds of *S. commune* were Dodecane (6.90 RT), 2-Coumaranone (7.44 RT), Tetradecane (9.67 RT), 4H-Pyran-4-one (10.34 RT), Hexadecane (13.42 RT), Cyclohexanone (16.11 RT), Octadecane (17.63 RT), n-Hexadecanoic acid (20.81 RT) and Octadecanoic acid (24.54 RT) that are associated with Fatty Acyls (37%), Benzene and substituted derivatives (18%), Organoheterocyclic compounds (18%), Organooxygen compounds (9%), unsaturated hydrocarbons (9%) and alkanes (9%) (Figure 4c). A 2-pyranones compound, 2H-Pyran-2-one, 6-pentyl-secreted by *L. edodes* recorded the highest peak area of 26.45% expressed at 10.57 RT could be playing important role in fungal inhibition apart from several other antimicrobial compounds expressed (Figure 3 and Table 3).

Comparison of metabolite distribution pattern by Principal Component Analysis (PCA)

The PCA analysis was done for 34 compounds identified through GC-MS obtained from 3 treatments (*G. lucidum*, *L. edodes* and *S. commune*). The results revealed that treatment and variables were correlated with 100% of total variance [principal component 1 (F1) value of 57.08% and principal component 2 (F2) value of 42.92]. The variables obtained from *L. edodes* (PC 1) were perceived at the right end of the scoring plot, which showed a positive correlation among the

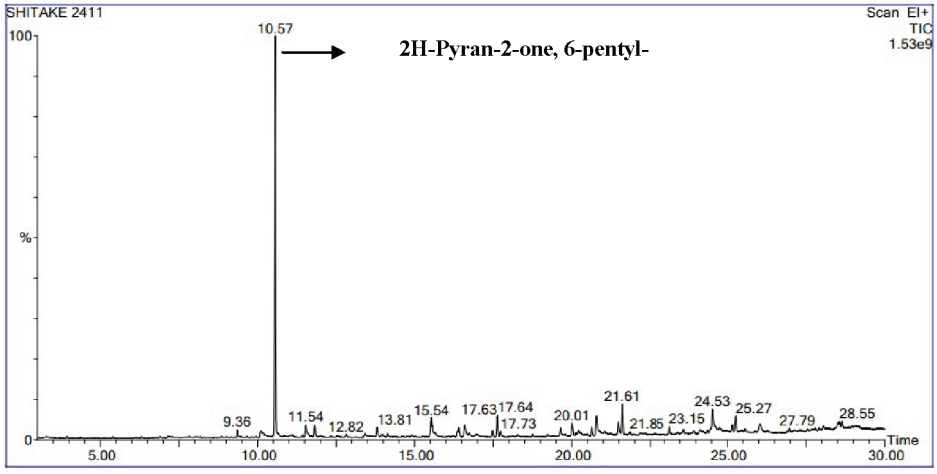

Figure 3. GC-MS Chromatogram of *L. edodes.*

Table 3. Metabolic profiling of *L. edodes* by GC-MS analysis

Retention Time	Compounds	Area Percentage	Chemical Formulae	Molecular Weight (g/mol)	Function	Reference
9.36	Riluzole	0.609	$C_8H_5F_3N_2OS$	234.19	Neuroprotective drug, antimicrobial and antitumor	Powell, 201
10.57	2H-Pyran-2-one, 6-pentyl-	26.45	$C_{10}H_{14}O_2$	166.21	Antifungal activity	Stoppacher *et al.,* 2010
11.54	Tropidine	1.655	$C_8H_{13}N$	123.19	Anatoxin	Hussain *et al.,* 2014
13.42	Formamide	0.607	CH_3NO	45.04	Antifungal activity	Han *et al.,*2017
13.80	Furfural	0.902	$C_5H_4O_2$	96.08	Antinemic activity	Kundu *et al.,* 2009
15.53	Benzene	2.169	$C_{11}H_{16}$	148.24	Antifungal activity	Guo *et al*., 2014
16.41	Sesquicineol	0.781	$C_{15}H_{26}O$	222.36	Antibacterial activity	Guerrini, A. and Sacchetti, G., 2014
17.63	Heneicosane	1.487	$C_{21}H_{44}$	296.57	Antimicrobial and antibacterial	Kundu *et al.,* 2009
17.64	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro	0.939	$C_{11}H_{18}N_2O_2$	210.27	Antifungal activity	Jeeva and Krishnamoorthy, 2015
21.61	n-Hexadecanoic acid	3.164	$C_{16}H_{32}O_2$	256.42	Antimicrobial activity	Arora, S. and Kumar, G, 2017
23.15	4-Octadecenal	0.728	$C_{18}H_{34}O$	266.46	Antifungal and antimicrobial activity	Deepa *et al*., 2016
24.13	17-Pentatriacontene	0.662	$C_{35}H_{70}$	490.93	Antimicrobial activity	Arora, S. and Kumar, G, 2017
24.53	Octadecanoic acid	3.662	$C_{18}H_{36}O_2$	284.47	Antifungal and antimicrobial activity	Nakkeeran *et al*., 2012
28.76	Calconcarboxylic acid	0.474	$C_{21}H_{14}N_2O_7S$	438.41		-

compounds. On the contrary, the variables obtained from *G. lucidum* (PC 2) were located at the left end of the scoring plot also exhibited a positive correlation. However, the variables obtained from *S. commune* (F2) were observed at the left end of the scoring plot, which showed the negative correlation of the compounds (Figure 5).

Network association of macro basidiomycetes

The Cytoscape metabolic pathway classification network sights the antifungal metabolites produced by *L. edodes, G. lucidum* and *S. commune* as represented in Figure 6. Edges in the network connections were distributed among 24 nodes and the node properties such as degree, closeness,

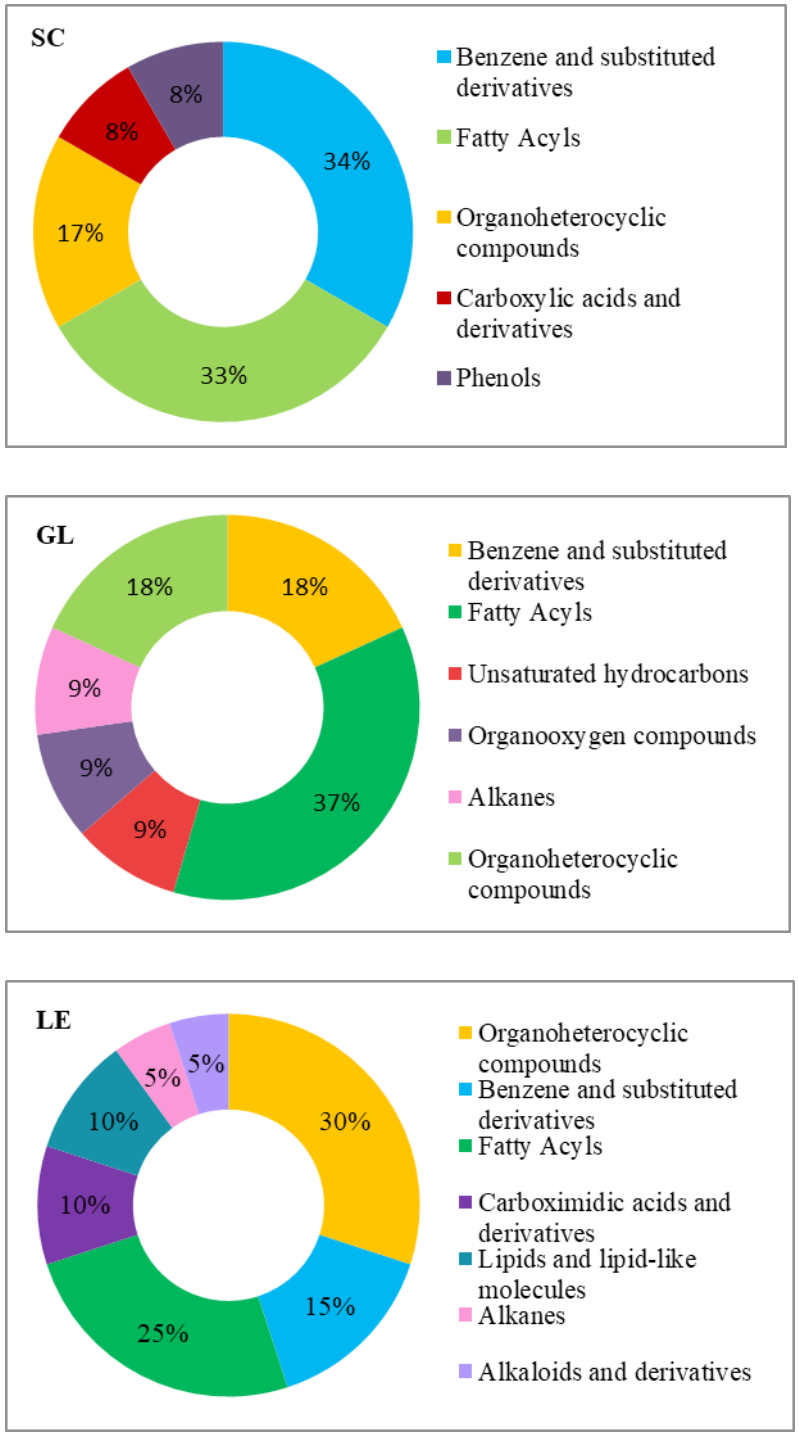

Figure 4a, 4b and 4c. Pie chart representing the metabolic diversity of *S. commune, G. lucidum* and *L. edodes* in submerged condition.

eccentricity, betweenness, eigen centrality, and coreness were investigated. The edge to node ratio is 11.5 to 1and the degree of closeness is represented as a blue line and small for differential metabolites includes hexadecane, sesquicineole, 2H-Pyran-2-one, tropidine, furfural, and heneicosane. However, the correlation (pcor value <0.5) is strongly positive for the above-stated compounds. In this, hexadecane is strongly correlated with sesquicineole, 2H-Pyran-2-one, tropidine, furfural, and heneicosane. Fascinatingly, the correlation (pcor value >0.5) is strongly negative and represented in the red line also indicates that compounds such as Tetradecane, 4H-pyran 2-one, Cyclohexanone, Octadecanoic acid, 2-Coumaranone, 2,4-Di-tert-butyl-phenol, and dodecane. Tetradecane is strongly correlated with 2-Coumaranone and 4H-pyran 2-one and cyclohexane with octadecanoic acid. Further, octadecanoic acid is also

highly correlated with 2-Coumaranone and 2, 4-Di-tertbutyl-phenol with dodecane. Thus, network analysis revealed the identified metabolite in significant metabolic pathways such as fatty acid biosynthesis, purine metabolism, glycolipid metabolism, and sphingolipid metabolism.

Red line represents negative correlation with the level of significance of 0.05, and blue indicates positive correlated antifungal metabolites in the study. The pathway showed 276 edges and 24 nodes. The edge to node ratio of 11.5 to 1. The pathways involved are fatty acid biosynthesis, purine metabolism, glycerol lipids and sphingolipid metabolism. The highlighted blocks were identified in KEGG database whereas the white blocks signify metabolites that are involved in plant hormones signal transduction pathways and defence response as revealed by KEGG pathways.

Metabolite set enrichment and key pathways

To identify the biologically meaningful patterns of antifungal metabolites, the quantitative metabolomics data were enriched using Metabolite Set Enrichment Analysis (MSEA). At this juncture, a set of functionally related preselected metabolites with no arbitrary cut-off threshold potential was used to identify delicate but constant changes among them. Hence, the Over-Representation Analysis (ORA) was performed in Metabo analyst 5.0 and the summaries of metabolic pathways involved in *L. edodes* that were mentioned above are elucidated in

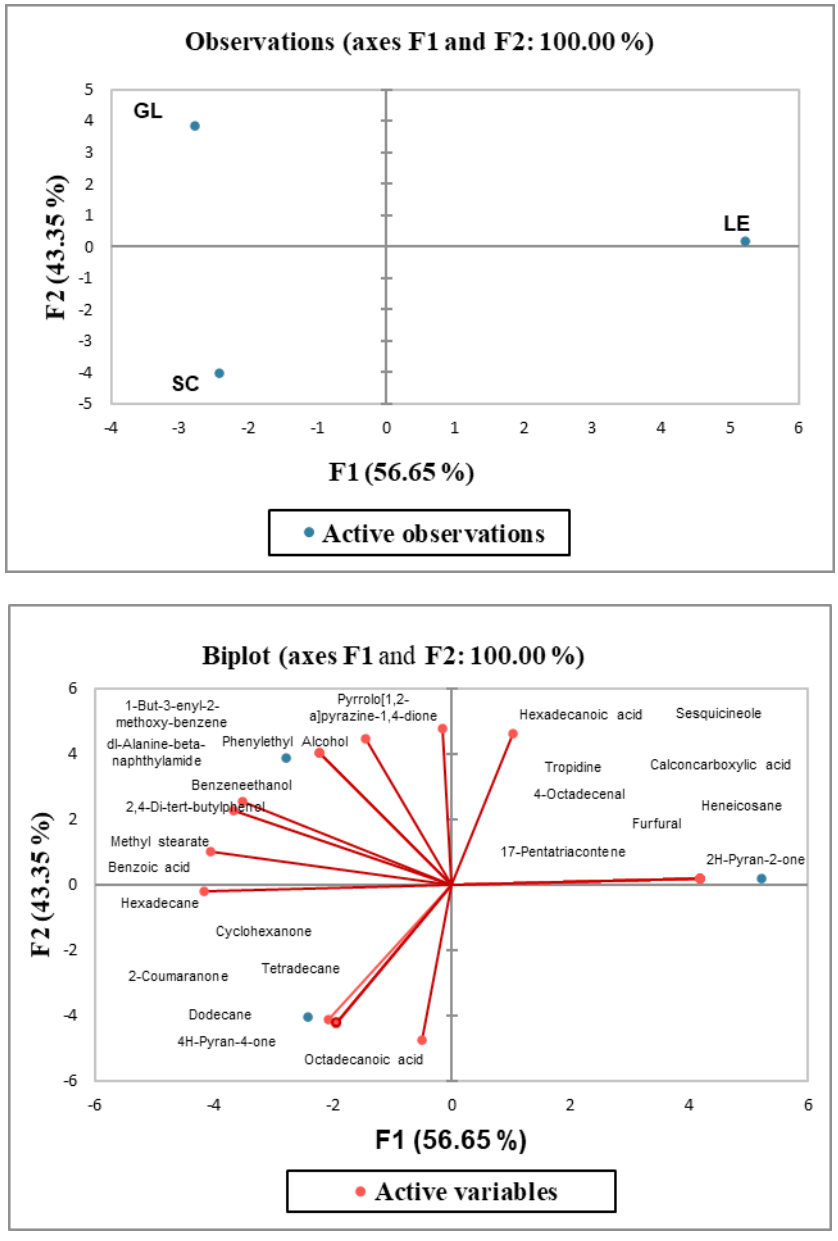

Figure 5. PCA biplot of antifungal compounds showing the spatial distribution among the treatments. LE - *Lentinus edodes*; GL - *Ganoderma lucidum*; SC - *Schizophyllum commune.*

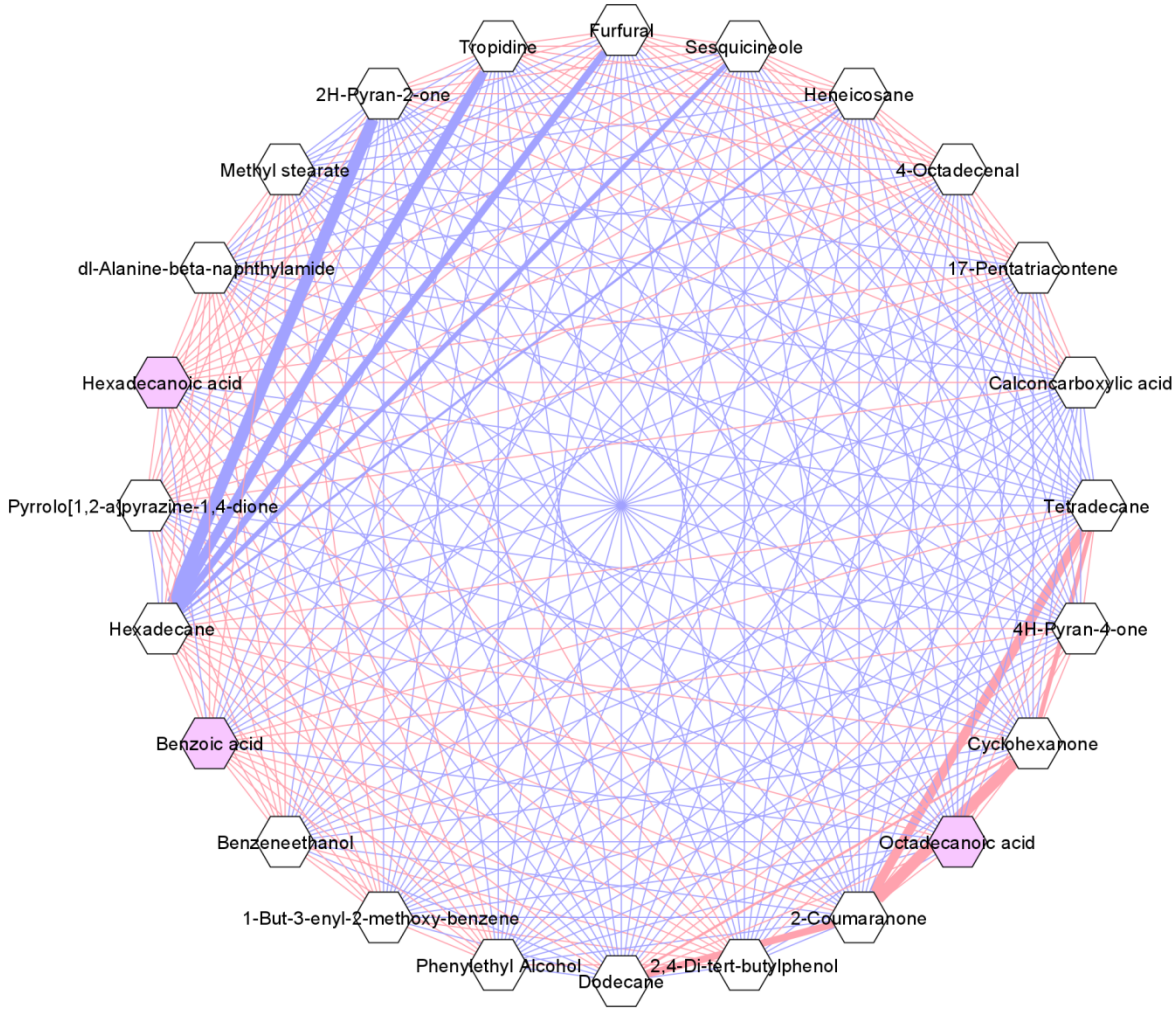

Figure 6. Correlation based net pathway network of antifungal metabolites conferring defense response constructed using Metscape app and visualized in Cytoscape version 3.6.1.

Table 4. ORA was implemented using the hypergeometric test to evaluate whether a particular metabolite set was represented more than expected time by chance within the given compound list. Based on one-tailed *p* values (>0.5) the majority of the biological pathway produced by *L. edodes viz.*, fatty acid biosynthesis, ethanol degradation, glycolipid metabolism, plasmalogen synthesis, mitochondrial beta-oxidation of long-chain saturated fatty acids, pentose phosphate pathway, amino sugar metabolism, fatty acid elongation in Mitochondria, aspartate metabolism, purine metabolism, fatty acid metabolism, Steroid biosynthesis, pyruvate metabolism, and bile acid biosynthesis. Most of the identified pathways were associated with cytosol and mitochondria (Figure 7).

Fourier Transmission Infrared Spectroscopy (FTIR) analysis

The ethyl acetate fraction of cell-free culture filtrate of *Lentinus edodes* that was analysed for the functional groups by FT-IR revealed that the spectrum showed the leading bands in the regions between 626.75 cm^{-1} and 3844.44 cm^{-1}. The peak in the spectrum revealed the presence of alcohol, aliphatic polyester, amides, phosphorus, silicon compounds, halogens, alkanes, triazanes, and Azo compounds functional groups. The wide absorption peak at 3308.2 cm^{-1} -3844.4 cm^{-1} confirms the presence of alcohol OH deformation and amides C-O stretch. The band around 2830.99 cm^{-1} - 2941.88 cm^{-1} reveals the presence of aldehydes C–H stretching. The band at 1448.28 cm^{-1} - 1660.41 cm^{-1} is due to aliphatic polyes CH_2 antisymmetric stretch and Triazanes (N=N) medium stretch. The band near 1019 cm^{-1} - 1114.65 cm^{-1} represents the silicon compounds O-H stretching and amines C-N stretching. The peak of 623.8 cm^{-1} was corresponding to Phosphorus compound P-O-P stretching. The presence of different functional groups indicated the existence of a variety of potential biomolecules produced by *L. edodes* (Figure 8).

Antifungal activity of 2H-Pyran-2-one

Among the different concentrations of the pure compound, 2H-Pyran-2-one tested at 1000 ppm concentration completely suppressed the mycelial growth of *F. oxysporum* and *F. solani* (100% inhibition). The least inhibition was

Table 4. Analysis of metabolites produced by *Lentinus edodes* based on key metabolic pathways

Pathways	Total	Expected	Raw p	FDR
Fatty Acid Biosynthesis	35	0.752	3.171E-02	1.00E+00
Ethanol Degradation	19	0.408	2.423E-01	1.00E+00
Glycerolipid Metabolism	25	0.537	2.435E-01	1.00E+00
Plasmalogen Synthesis	26	0.559	2.46E-01	1.00E+00
Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids	28	0.602	2.517E-01	1.00E+00
Amino Sugar Metabolism	33	0.709	2.538E-01	1.00E+00
Fatty Acid Elongation In Mitochondria	35	0.752	2.538E-01	1.00E+00
Aspartate Metabolism	35	0.752	2.588E-01	1.00E+00
Sphingolipid Metabolism	40	0.859	2.615E-01	1.00E+00
Fatty acid Metabolism	43	0.924	3.539E-01	1.00E+00
Steroid Biosynthesis	48	1.03	3.656E-01	1.00E+00
Pyruvate Metabolism	48	1.03	2.767E-01	1.00E+00
Bile Acid Biosynthesis	65	1.4	4.167E-01	1.00E+00

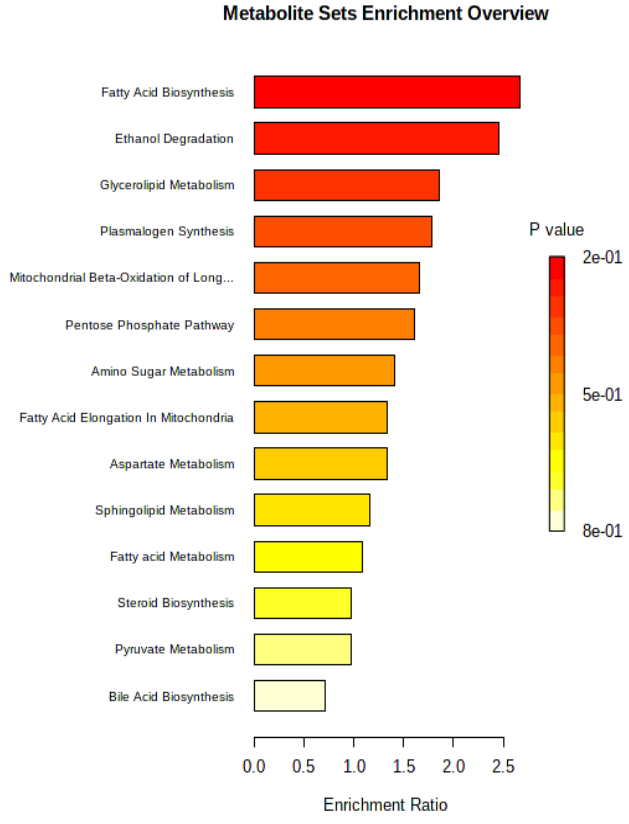

Figure 7. Analysis of antifungal metabolites of *L. edodes* pathways based on one tailed *t*-test performed in Metabo Analyst 5.0.

observed at 100 ppm (Table 5). Therefore, 2H-Pyran-2-one @ 1000 ppm concentration is highly significant in suppressing the mycelial growth of the pathogens when compared with other concentrations. The distortion in mycelial was observed near the agar well zone whereas the control showed normal mycelial growth (Figure 9 and Figure 10).

In addition, 2H-Pyran-2-one when tested against conidial germination of *Fusarium* spp. with 1000 ppm concentration there was complete inhibition of the conidial germination at 48 hrs. However, the conidial germination was 13.33 in numbers at 12 and 24 h and 20.00 in numbers at 48 hrs at 750 ppm concentration. But, the maximum conidial germination (50.00 numbers) was observed at 100 ppm concentration. Hence at 1000 ppm concentration 2H-Pyran-2-one is highly effective against *Fusarium* spp. (Table 6).

The secondary metabolites from macro basidiomycetes are known to exhibit antifungal, antibacterial, and antiviral properties active against plant pathogens (Vamanu *et al.*, 2018 and Elisashvili, 2012) and human pathogens (Gao *et al.*, 2003 and Keypour *et al.*, 2008). Radhajeyalakshmi *et al.* (2011) found that the ethyl acetate fraction of *L. edodes*, *Auricularia polytricha* and *Volvareilla volvacea* inhibited the mycelial growth of *Alternaria solani, Colletotrichum capsici, Phytophthora* and *Rhizoctonia solani.* In the present study, the ethyl acetate fraction of CFC of *L. edodes*, *G. lucidum* and *S. commune* showed antifungal activity against *Fusarium* spp. However, the ethyl acetate extracted CFC of *L. edodes* exhibited a significant reduction in mycelial growth of *F. oxysporum* and *F. solani.* The results are in line with the findings of Gayathiri *et al*. (2021) wherein they stated that the ethyl acetate fraction of *G. lucidum* inhibited mycelial growth of *C. gloeosporioides* by 68.55%. Characterization of antifungal compounds from ethyl acetate fraction of *L. edodes*, *G. lucidum* and *S. commune* through TLC revealed the presence of prominent bands with different Rf values such as 0.25, 0.69 and 0.75. Uma Gowrie *et al*. (2014) identified terpenoids in *G. lucidum* through TLC with an Rf value of 0.27. The reason behind the TLC eluted bands possessing antifungal activity could be due to their intrinsic nature and the presence of toxic metabolites such as phenols, terpenoids, flavonoids, tannins, alkaloids, alkanes, esters, and fatty acid which has been confirmed through GC-MS analysis. Pyrazines, furan, and pyrroles with a distinct popcorn and nutty aroma to foods are also found in mushrooms. The compounds 1-isoamyl-2-formyl pyrrole and 1-(2-methyl butyl)-2-formyl pyrrole was determined with high concentration (1.99%–4.94%) in *Boletus edulis* (Zhou *et al*., 2015). In our study the TLC eluted bands from CFC of *L. edodes* revealed the presence of major antimicrobial compounds *viz.,* 2H-pyran-2-one 6-pentyl-, pyrrolo [1,2-a] pyrazine-1,4-dione, n-hexadecanoic acid. Vinodkumar *et al*. (2017) and Sangeetha *et al.* (2018) also reported that Pyrrolo[1,2-a] pyrazine-1,4-dione a heterocyclic aromatic organic compound has antifungal activity against *Sclerotinia sclerotiorum* and *Fusarium* spp. Notably, the above-stated findings confirm with our study suggesting that the Pyrrolo[1,2-a] pyrazine-1,4-dione, belonging to the class 1,4 thiazines and 2H-Pyran-2-one, 6-pentyl-, belonging to

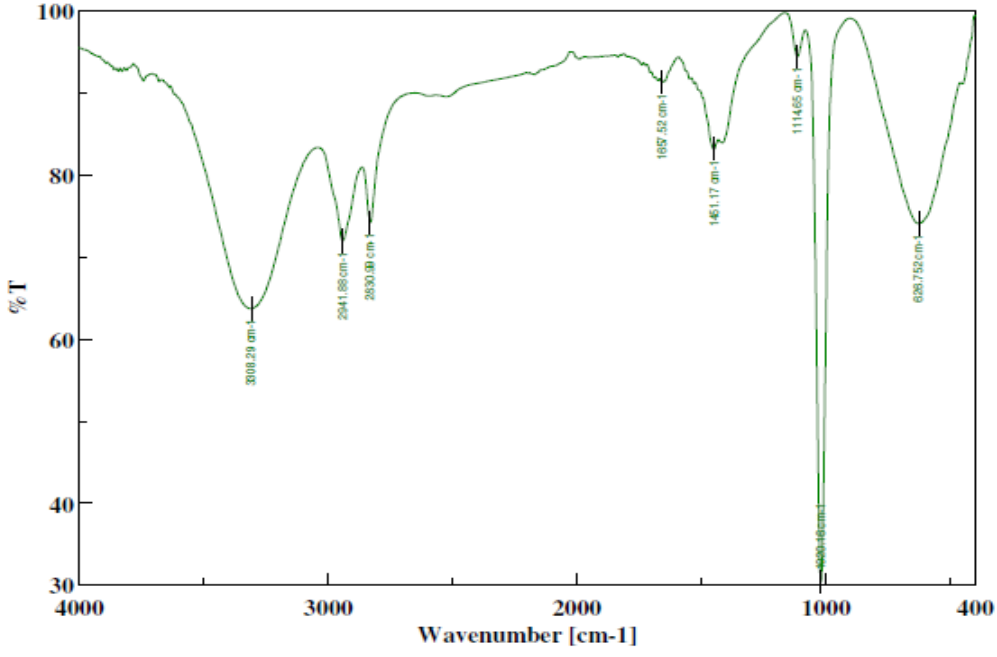

Figure 8. FTIR spectral analysis of *L. edodes.*

the class pyranones and derivatives produced by *L. edodes* possess antifungal activity. Additionally, 2H pyran 2-one, belonging to the polyketide group possessed characteristics of coconut-like aroma which has antifungal and antimicrobial activity (Akshaya *et al.,* 2021 and Sridharan *et al*., 2021).

More fascinatingly, the compound 2H pyran 2-one (RT 10.57) identified in *L. edodes* through GC-MS with the highest peak area percentage (26. 45) @ 1000 ppm concentration completely suppressed the mycelial growth and conidial germination of *F. oxysporum* and *F. solani.* The antifungal nature of 2H pyran 2-one, 6 pentyl for further confirmed by findings of Jin *et al*. (2020) who reported that the important active metabolite 6-Pentyl-2H-pyran-2-one (6PP) was identified in the fermentation broth of *Trichoderma atroviridae* (T2) possessed antifungal activity against *Cylindrocarpon destructans*. Interestingly, the pure form of 6-n-pentyl-2H-pyran-2-one (6-PAP) having antagonistic and antifungal activity against *F. culmorum*, *F. graminearum*, and *F. avenaceum* have been confirmed (Jelen *et al.,* 2014).

Additionally, pathway topology was analyzed for the compounds produced by these macro basidiomycetes. Thus, soluble metabolites played a vital role in the survival and competition against plant pathogens. Though, understanding of the metabolic pathway remains doubtful. Our study revealed that fatty acid biosynthesis plays a major role In Over-Representation Analysis (ORA). Fatty acids are

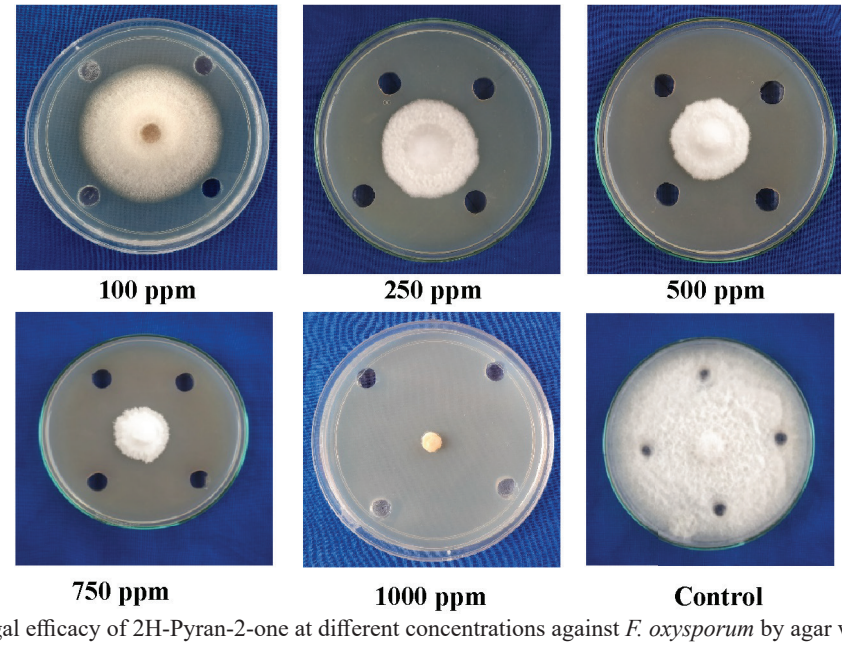

Figure 9. Antifungal efficacy of 2H-Pyran-2-one at different concentrations against *F. oxysporum* by agar well diffusion assay.

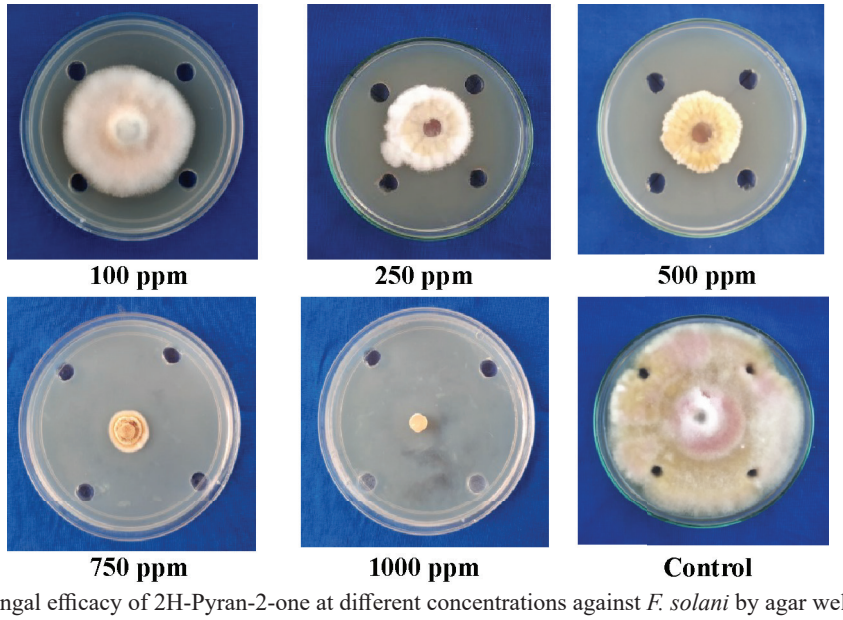

Figure 10. Antifungal efficacy of 2H-Pyran-2-one at different concentrations against *F. solani* by agar well diffusion assay.

Table 5. Effect of standard compound 2H-Pyran-2-one inhibited the mycelial growth of *Fusarium oxysporum* and *F. solani* at different concentrations

Concentrations (PPM)	*Fusarium oxysporum*		*Fusarium solani*	
	Mycelial growth (mm)	Per cent inhibition (PI)	Mycelial growth (mm)	Per cent inhibition (PI)
100	49.33[d] (44.62)	35.19	45.33[d] (42.32)	39.63
250	45.00[d] (42.13)	40.00	38.67[d] (38.45)	47.04
500	34.33[c] (35.87)	51.85	30.33[c] (33.42)	56.30
750	15.00[b] (22.79)	73.33	12.33[b] (20.56)	76.30
1000	0.00[a] (0.28)	100.00	0.00[a] (0.28)	100.00
Control	90.00[e] (71.57)	0.00	90.00[e] (71.57)	0.00
SED	2.82	-	3.59	-
CD	6.14	-	7.83	-

Table 6. Effect of standard compound 2H-Pyran-2-one on spore germination of *Fusarium oxysporum* and *F. solani* at different concentrations

Concentrations (PPM)	Different Hours					
	12 h		24 h		48 h	
	SG	PI	SG	PI	SG	PI
100	43.33[d] (41.68)	13.4	46.67[e] (43.08)	6.66	50.00[d] (45.00)	0.00
250	33.33[c] (35.26)	34.4	36.00[d] (37.26)	28.2	36.67[c] (37.26)	28.8
500	26.66[c] (31.09)	48.1	26.67[c] (31.09)	48.6	30.00[bc] (33.21)	40.0
750	13.33[b] (21.41)	73.4	13.33[b] (21.41)	74.1	20.00[b] (28.88)	60.12
1000	0.00[a] (0.28)	100.00	0.00[a] (0.28)	100.00	0.00[a] (0.28)	100.00
Control	50.00[d] (45.00)	0.00	50.00[e] (45.00)	0.00	50.00[d] (45.00)	0.00
SED	3.84	-	3.84		4.30	-
CD	8.38	-	8.38		9.37	-

Whereas, SG - No of conidia germinated, PI- Percent inhibition of germinated spore.
Means followed by a common letter are not significantly different at the 5% level by DMRT
Values in parenthesis are arcsine transformed values.

ubiquitous and involved in cell energy storage, membrane structure, and various signalling pathways in plants and fungi. Although, several researchers found that many fatty acids have antimicrobial and antifungal activity (Liu *et al.,* 2007; Lim *et al*., 2017). Similarly, enhanced level of palmoleotic acid exhibited increased resistance to *Verticillium dahlia,* and this supporting evidence correlated with our results that fatty aclyls produced in *L. edodes* such as hexadecanoic acid, phthalic acid, and octadecanoic acid might have antifungal activity against *Fusarium* sp. (Walley *et al.,* 2013). Thus, fatty acids should be further explored for alternative approaches in the biocontrol of phytopathogens.

FTIR revealed the presence of esters, alcohol, and halide groups which supports our results. Apart from the major compounds, triterpenes and polysaccharides other compounds *viz*., ketones, esters, lactones, alcohols, ethers, and hydroxy benzenes were also detected (Choonga *et al.,* 2011). However, Silicon compound Si-O-Si (1020-1010 cm^{-1}) and Alcohol O-H (3400-3200 cm^{-1}) variably strong were found in the secondary metabolites of *G. lucidum* (Sangeetha *et al.,* 2020). Also, in our results, we found silicon compounds with the same wavelength. Although alcohols O-H stretch (3400-3200 cm^{-1}) variably strong and aldehydes medium to weak C-H Stretch (2830-2650 cm^{-1}) were also found in metabolites

produced by macrobasidiomyces which might be responsible for antifungal activity (Ren *et al.,* 2014). Likewise, the *Termitomyces* strain revealed four dominant spectral peaks with fatty acid region conquered by C– H (3450–2850 cm^{-1}); amide region dominated by C=O amide I and N–H amide II bands of proteins and peptides (1800–1500 cm^{1}) (D'Souza and Kamat, 2017). Though FT-IR revealed the presence of different spectral windows which produced specific functional groups such as amines, alcohols, aldehydes, and alkanes mainly present in plants and microorganisms which might be responsible for plant developmental processes, environmental stress responses, plant-microbe interactions and plant response to infection (Aygun *et al.,* 2020). Finally, the results of the present investigations revealed that the bioactive antimicrobial compound 2H pyran 2-one of *L. edodes* could be potentially explored for the management of soil-borne plant pathogens that induce wilt diseases in crop plants.

CONCLUSION

Among the macro basidiomycetes tested, *L. edodes* possessing 2H pyran 2-one, 6 pentyl with antifungal activity proved effective against complete suppression of mycelial growth and conidial germination of *Fusarium oxysporum* and *F. solani*. Hence, the findings of the present study offer further scope for structural identification and development of commercial formulations that would find application at the field level.

ACKNOWLEDGMENTS

The authors thank the Department of Plant Pathology, Tamil Nadu Agricultural University for providing the facilities through the fund support obtained from UGC-SAP-DRS II on Mushroom Biotechnology and ICAR-AICRP scheme on Mushroom, to carry out the research work.

REFERENCES

Ahmad, B., Khan, I., Bashir, S. and Azam, S. 2012. Chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal and antibacterial activities of the essential oils of *Acacia modesta*. *J. Med. Plants Res*., **6**(31): 4653–4659. https://doi.org/10.5897/JMPR12.016

Akshaya, S. B., Krishnamoorthy, A. S., Sangeetha, C., Nakkeeran, S. and Thiribhuvanamala, G. 2021. Investigation on antifungal metabolites of Chinese caterpillar fungus *Ophiocordyceps sinensis* (Berk.) against wilt causing pathogen, *Fusarium* spp. *Ann. phytomedicine*, **10**(1): 195–201. https://doi.org/10.21276/ap.2021.10.1.20

Arora, S, and Kumar G. 2017. Gas Chromatography-Mass Spectrometry (GC-MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of *Cenchrus setigerus* Vahl (Poaceae). *Antiseptic*, **2**: 0–31.

Aygun, A., Ozdemir, S., Gulcan, M., Cellat, K. and Şen, F. 2020. Synthesis and characterization of Reishi mushroom-mediated green synthesis of silver nanoparticles for the biochemical applications. *J Pharm. Biomed. Anal,* **178**: 112970. PMid: 31722822. https://doi.org/10.1016/j.jpba.2019.112970

Borchers, A. T., Krishnamurthy, A., Keen, C. L., Meyers, F. J. and Gershwin, M. E. 2008. The immunobiology of mushrooms. *Exp. Biol. Med.*, 233(3): 259–276. PMid: 18296732. https://doi.org/10.3181/0708-MR-227

Chinnusamy, S. and Krishnamoorthy, A. S. 2017. Identification of 3'deoxyadenosine (Cordycepin) from the medicinal mushrooms, *Ophiocordyceps* spp. *Int. J. Chem. Studies*, **5**(3): 788–792.

Choong, Y. K., Sun S. Q., Zhou, Q., Ismail, Z., Rashid, B. A, Tao, J. X. 2011. Determination of storage stability of the crude extracts of *Ganoderma lucidum* using FTIR and 2D-IR spectroscopy. *Vib Spectrosc*, **57**(1): 87–96. https://doi.org/10.1016/j.vibspec.2011.05.008

Chowdhury, M. M., Kubra, K., Ahmed, S. R. 2015. Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh. *Ann. Clin. Microbiol. Antimicrob*, **4**(1): 1–6. PMid: 25858107 PMCid: PMC4328533. https://doi.org/10.1186/s12941-015-0067-3

D'Souza, R. A. and Kamat, N. M. 2017. Potential of FT-IR spectroscopy in chemical characterization of *Termitomyces* Pellets. *J. Appl. Biol. Biotechnol.*, **5**(4): 080–084.

Elisashvili, V. I. 2012. Submerged cultivation of medicinal mushrooms: bioprocesses and products. *Int. J. Med. Mushrooms*, **14**(3). PMid: 22577974. https://doi.org/10.1615/IntJMedMushr.v14.i3.10

Gayathiri, M., Thiribhuvanamala, G., Krishnamoorthy, A. S., Haripriya, S., Akshaya, S. B., and Pravin, A. I. 2021. Characterization of antimicrobial metabolites from medicinal mushrooms against *Mango anthracnose* pathogen *Colletotrichum gloeosporioides* (Penz.) Sacc. *Ann Phytomed*, **1**: 185–94. https://doi.org/10.21276/ap.2021.10.1.19

Guerrini, A. and Sacchetti, G. 2014. Chemical fingerprinting of medicinal and aromatic plant extracts: HPTLC bioautographic assays as preliminary research tool to match chemical and biological properties. *J. Med. Aromat. Plants*, **3**(3): 2167–0412. https://doi.org/10.4172/2167-0412.1000e152

Guo, Z., Li, Q., Wang, G., Dong, F., Zhou, H. and Zhang, J. 2014. Synthesis, characterization, and antifungal activity of novel inulin derivatives with chlorinated benzene. *Carbohydr. Polym*, **99**: 469–473. PMid: 24274532. https://doi.org/10.1016/j.carbpol.2013.08.044

Han, X. Y., Li, S. B., Liang, G. C., Zhou, G., Zhong, Y. F., Qi, H., Song, Y. L. and Qiao, X. Q. 2017. Synthesis and antifungal activities of N-1,3,4-thiadiazol-2-yl-4-oxo-thiochroman-2-yl-formamide derivatives. Yao xue xue bao = *Acta Pharm. Sin.* , **52**(1): 113–119.

Iwalokun, B. A., Usen, U. A., Otunba, A. A. and Olukoya, D. K. 2007. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *Afr. J. Biotechnol.*, **6**(15). https://doi.org/10.5897/AJB2007.000-2254

Jeeva, S. and Krishnamoorthy, A. S. 2018. Antifungal Potential of Myco-molecules of *Coprinopsis cinerea* (Schaeff) S. Gray s. lat. against *Fusarium* spp. *Madras Agric. J.*, **105**:1. https://doi.org/10.29321/MAJ.2018.

JeleŞ H., Błaszczyk, L., Chełkowski, J., Rogowicz, K. and Strakowska, J. 2014. Formation of 6-n-pentyl-2H-pyran-2-one (6-PAP) and other volatiles by different *Trichoderma* species. *Mycol. Prog,* **13**: 589–600. https://doi.org/10.1007/s11557-013-0942-2

Keypour, S., Riahi, H., Moradali, M. F. and Rafati, H. 2008. Investigation of the antibacterial activity of a chloroform extract of Ling Zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae), from Iran. *Int J Med Mushrooms,* **10**(4). https://doi.org/10.1615/IntJMedMushr.v10.i4.70

Kundu, A., Shakil, N. A., Saxena, D. B., Pankaj, Kumar, J. and Walia, S. 2009. Microwave assisted solvent-free synthesis and biological activities of novel imines (Schiff bases). *J. Environ. Sci*. *Health*, Part B, **44**(5): 428–434. PMid: 20183046. https://doi.org/10.1080/03601230902934645

Lim, G. H., Singhal, R., Kachroo, A. and Kachroo, P. 2017. Fatty acid-and lipid-mediated signaling in plant defence. *Annu. Rev. Phytopathol.*, **55**: 505–536. PMid: 28777926. https://doi.org/10.1146/annurev-phyto-080516-035406

Liu, J. K. 2007. Secondary metabolites from higher fungi in China and their biological activity. *Drug Discov Ther.,* **1**(2): 94–103.

Nakkeeran, S., Surya, T. and Vinodkumar, S. 2020. Antifungal potential of plant growth promoting *Bacillus* species against blossom blight of rose. *J. Plant Growth Regul.,* **39**: 99–111. https://doi.org/10.1007/s00344-019-09966-1

Nguyen, Q. T., Ueda, K., Tamura, T., Kihara, J., Itoh, K., Yoshikiyo, K., Sakaguchi, Y. and Ueno, M. 2018. Antifungal activity of a novel compound purified from the culture filtrate of *Biscogniauxia* sp. O821 against the rice blast fungus *Magnaporthe oryzae*. *J. Gen. Plant Pathol.*, **84**: 142–147. https://doi.org/10.1007/s10327-018-0767-6

Powell, L. A. 2015. Synthesis of novel *Riluzole analogues* (Doctoral dissertation, University of Huddersfield).

Priya, K., Thiribhuvanamala, G., Kamalakannan, A. and Krishnamoorthy, A. S. 2019. Antimicrobial activity of biomolecules from mushroom fungi against *Colletotrichum capsici* (Syd.) Butler and Bisby, the Fruit Rot. Pathogen. of Chilli. *Int. J. Cur. Microbiol. Appl. Sci*., **8**(6): 1172–1186. https://doi.org/10.20546/ijcmas.2019.806.145

Rathore, H., Prasad, S., Kapri, M., Tiwari, A. and Sharma, S. 2019. Medicinal importance of mushroom mycelium: Mechanisms and applications. *J. Funct. Foods.,* **56**: 182–193. https://doi.org/10.1016/j.jff.2019.03.016

Ren, L., Hemar, Y., Perera, C. O., Lewis, G., Krissansen, G. W. and Buchanan, P. K. 2014. Antibacterial and antioxidant activities of aqueous extracts of eight edible mushrooms. *Bioact. Carbohydr. Diet. Fibre.,* **3**(2): 41–51. https://doi.org/10.1016/j.bcdf.2014.01.003

Sangeetha, B., Krishnamoorthy, A. S., Amirtham, D., Sharmila, D. J. S., Renukadevi, P. and Malathi, V. G. 2019. FT-IR spectroscopic characteristics of *Ganoderma lucidum* secondary metabolites. *J. Appl. Aci. Technol.,* 38(6): 1–8. https://doi.org/10.9734/cjast/2019/v38i630453

Sangeetha, C., Krishnamoorthy, A. and Amirtham, D. 2015. Antifungal bioactive compounds from Chinese caterpillar fungus (*Ophiocordyceps sinensis* (Berk.) GH Sung *et al*.) against plant pathogens. *Madras Agric. J.,* **102**(10/12): 353–357. https://doi.org/10.29321/MAJ.10.001133

Sangeetha, C., Krishnamoorthy, A. S., Kumar, N. K. and Pravin, I. A. 2018. Effect of headspace and trapped volatile organic compounds (vocs) of the Chinese caterpillar mushroom, *Ophiocordyceps sinensis* (ascomycetes), against soil-borne plant pathogens. *Int J Med Mushrooms,* **20**(9). PMid: 30317977. https://doi.org/10.1615/IntJMedMushrooms.2018027311

Sivananthan, S., Khusro, A., Paulraj, M. G., Ignacimuthu, S. and Al-Dhabi, N. A. 2017. Biocontrol properties of basidiomycetes: An overview. *J Fungi*, **3**(1): 2. PMid: 29371521 PMCid: PMC5715959. https://doi.org/10.3390/jof3010002

Sridharan, A. P., Sugitha, T., Karthikeyan, G., Nakkeeran, S. and Sivakumar, U. 2021. Metabolites of *Trichoderma longibrachiatum* EF5 inhibits soil borne pathogen, *Macrophomina phaseolina* by triggering amino sugar metabolism. *Microb. Pathog.,* **150**: 104714. PMid: 33383148. https://doi.org/10.1016/j.micpath.2020.104714

Stoppacher, N., Kluger, B., Zeilinger, S., Krska, R. and Schuhmacher, R. 2010. Identification and profiling of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GC-MS. *J. Microbiol. Methods.,* **81**(2): 187–193. PMid: 20302890. https://doi.org/10.1016/j.mimet.2010.03.011

Thangaraj, P., Subbiah, K. A., Uthandi, S. and Amirtham, D. 2021. Antifungal volatiles from macrobasidiomycetes inhibits *Fusarium oxysporum* f. sp. *lycopersici*. *Madras Agric. J.,* **108**(1-3): 1.

Uma Gowrie, S., Chathurdevi, G. and Rani, K. 2014. Evaluation of Bioactive Potential of Basidiocarp Extracts of *Ganoderma lucidum*. *Int. J. Pharm. Res. Allied Sci*. **3**: 36–46.

Vamanu, E., Pelinescu, D. and Avram, I. 2018. Antioxidative effects of phenolic compounds of mushroom mycelia in simulated regions of the human colon, *in vitro* study. *Polish J. Food Nutr. Sci.*, **68**(1). https://doi.org/10.1515/pjfns-2017-0010

Vinodkumar, S., Nakkeeran, S., Renukadevi, P. and Malathi, V. G. 2017. Biocontrol potentials of antimicrobial peptide producing *Bacillus* species: multifaceted antagonists for the management of stem rot of carnation caused by *Sclerotinia sclerotiorum*. *Front Microbiol.,* **8**: 446. PMid: 28392780 PMCid: PMC5364326. https://doi.org/10.3389/fmicb.2017.00446

Walley, J. W., Kliebenstein, D. J., Bostock, R. M. and Dehesh, K. 2013. Fatty acids and early detection of pathogens. *Curr. Opin. Plant Biol.,* **16**(4): 520–526. PMid: 23845737. https://doi.org/10.1016/j.pbi.2013.06.011

Yang, Z., Yu, Z., Lei, L., Xia, Z., Shao, L., Zhang, K. and Li, G. 2012. Nematicidal effect of volatiles produced by sp. *J. Asia Pac. Entomol.* **15**(4): 647–650. https://doi.org/10.1016/j.aspen.2012.08.002

Zhou, J., Feng, T. and Ye, R. 2015. Differentiation of eight commercial mushrooms by electronic nose and Gas Chromatography-Mass Spectrometry. *J. Sens.,* 2015. https://doi.org/10.1155/2015/374013