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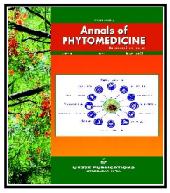

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Exploration of biomolecules from *Pisolithus tinctorius* (Pers.) against major soil-borne plant pathogens

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Abstract

Pisolithus spp. is highly distributed and form mycorrhizal associations with broad range of woody plants in the forest ecosystem. It is more interesting to note that *Pisolithus tinctorius* is known to form mycorrhizal association with eucalyptus and promotes plant growth. Soil borne plant pathogens are main threat to both agricultural and horticultural crop causing wilt, root rot and seedling blight. *P. tinctorius* fruiting bodies were collected from eucalyptus plantations at Forest College and Research Institute at Mettupalayam, the morphological characterization studies revealed the presence of deeply rooted stipe bearing yellow to brownish peridium releasing brownish-black basidiospores. As an eco-friendly approach, the present study is focused on exploring biomolecules from *P. tinctorius* against these pathogens. The bioactive compounds of *P. tinctorius* MTP 1 isolate was extracted and tested for their inhibitory activity against soil-borne plant pathogens, *viz*., *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Synder and Hansen; *Macrophomina phaseolina* (Goid); *Rhizoctonia solani* (Kuhn) and *Sclerotium rolfsii* (Sacc). The ethyl acetate fraction of whole fruiting body (inclusive of basidiospores) extract of *P. tinctorius* yielded 0.2% biomolecule composite while, that mycelial mat yielded a negligible quantity of molecules. The biomolecules composite of ethyl acetate fraction of fruiting body at a concentration of 150 µl resulted in the maximum inhibition of *R. solani* (840 mm^2) when tested by agar well diffusion study. In the case of *M. phaseolina* and *F. o*. f. sp. *lycopersici*, maximum inhibition of 790 mm^2 and 680 mm^2, respectively was observed. The GC-MS analysis of biomolecules composite of ethyl acetate fraction of sporocarps indicated the presence of compounds belonging to nature of fatty acids, aromatic alcohol, and flavonoid, terpenoids and steroids of antimicrobial nature and suggests exploitation of such molecules in the management of soil-borne plant pathogens.

1. Introduction

The mycorrhizal association is a symbiotic mutual association between soil fungi and plant root systems. There are several types of mycorrhizal associations, among which arbuscular mycorrhizae and ectomycorrhizae are the most widely distributed. In nature, 83 per cent of dicots, 79 per cent of monocots and all gymnosperms are associated with mycorrhizal fungi (Wilcox *et al*., 1991). Ectomycorrhizal fungi (ECM) are capable of infecting several arboreous species; thus, this symbiosis is widespread both in temperate and boreal forests and has been proposed to enhance significantly forest production (Smith and Read, 1997). The ECM fungi bring several advantages to plants, including increased root area for absorption, enhanced uptake of nutrients, and drought tolerance (Duddridge *et al*.,1980). ECM can also help to increase the growth and nutrient contents of plants growing in marginal soil (Jones *et al*., 1991). Water stress appears to be one of the major causes for the failure of micro-propagated plants during acclimation. The compatible mycorrhizal fungi in the substrates during the weaning process, not only improve the nutritional status of the plants but also increase their resistance to water stress *ex situ*, by increasing their weaning rate and reduce the drought stress (Sebastiana *et al*., 2018). Apart from all these benefits, ECM fungi are well known to protect their host plants from diseases by several means including induced resistance against plant pathogens (Khan *et al.*, 2010). *Pisolithus* is an ECM fungus that belongs to phylum: Basidiomycota; order: Sclerodermatales and family: Sclerodermataceae. The genus is characterized by conspicuous fruiting bodies with variable shape and size, often with well-developed rooting base. Considerable heterogeneity exists within the genus in terms of cultural characters, carpophores, and basidiospore morphology. Several *Pisolithus* spp. including *Pisolithus kisslingi* E. Fisch; *Pisolithus pusillum* Pat. and *Pisolithus aurantioscabrosus* have been described in tropical South East Asia mainly based on distinctive carpophore and basidiospore morphology (Watling *et al*., 1995).

Bioactive compounds are widely distributed in the fruiting bodies, mycelium, and culture filtrates of *P. tinctorius*. Some useful biomolecules obtained from the fruiting bodies of *P. tinctorius* include lanostane-triterpene and naphthalenoid-pulvinic acid derivatives. Zamuner *et al.* (2005) collected fruting bodies of *P. tinctorius* from eucalyptus plantation and isolated a new triterpene compound

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called pisosterol from the fruiting bodies. They have also isolated four different lanostanetriterpenoid compounds from the mycelial cultures and fruiting bodies of *P. tinctorius*. Ameri *et al.* (2011) isolated several bioactive compounds which include diterpenoids, sesquiterpenoids and fractions of polysaccharides, *viz.*, I, II, IIIA, and IIIB from the fruit bodies of *P. albus*. Moreover, reports about the antimicrobial activities of *P. tinctorius* and its role in biological control programmes of plant pathogens are scanty. Most of the literature focused on the possible usage of *P. tinctorius* isolates as ECM fungi with host plants for growth enhancement. Reports on genetic variability, taxonomic phylogeny, soil fertility management, reduction of chaemotoxis and erosion control are also sparsely available ((Zeng *et al.*, 2003). Nevertheless, Shrestha *et al.* (2005) conducted *in vitro* studies and reported the antimicrobial potentials of *Pisolithus* spp. against a spectrum of bacterial pathogens. The metabolites of *Pisolithus* spp. exhibited greater inhibition against *Salmonella typhi, Kleibsiella* sp., *Bacillus* sp., *Escherichia coli, Pseudomonas aeruginosa, Agrobacterium tumifaciens*, and a low level of inhibition against *S. aureus* and *Shigella dysenteriae*. The triterpene pisosterol has been shown to have antitumor activity against tumor cell lines, leukemia, and melanoma cells (Montenegro *et al.*, 2004). With these basic backgrounds, the current investigation had been focused mainly to explore the multifaceted molecules of *P. tinctorius* in plant disease management.

2. Material and Methods

2.1 Collection and isolation of fruiting body of *P. tinctorius*

The fruiting bodies of *P. tinctorius* were collected from eucalyptus plantations at Forest College and Research Institute, Jakkanari Range, Mettupalayam (Latitude: 770 56' E; Longitude:110 19' N; Altitude 300 m MSL), Coimbatore district, Tamil Nadu (Figure1). The collected sporophores were brought to the laboratory in paper bags. Morphological identification was done by macroscopic and microscopic examination. All the specimens were stored at 4ºC prior to the isolation and purification of the fungal culture. The small pieces of fruiting bodies cut from the gleba were surface sterilized with 70 per cent of ethanol for 2 min followed by 0.1 per cent sodium hypocloride for one min and then rinsed four times with sterile water. Sterilized pieces of *Pisolithus* were kept in a special medium under room temperature.

Figure 1: ***Pisolitus tinctorius*** **isolate MTP1 growing in Eucalyptus plantation.**

2.2 Extraction of bioactive compounds from the fruiting bodies of *P. tinctorius* MTP1

Pisolitus tinctorius MTP1 sporophore samples drawn from the preserved specimen stored at 4ºC were milled to a fine powder in a mixer blender. Samples weighing 20 g were extracted with 100 ml of different solvents, *viz.*, chloroform, ethyl acetate, methanol, and water successively with intermittent warming in a waterbath maintained at 55-60°C. The extracted samples were stored at 28 ± 2°C in darkness for 24 h. The process was repeated for the second time. The extractants obtained were pooled together and filtered through a Whatman No.1 filter paper and concentrated to dryness in a rotary evaporator. The condensate was later dissolved in DMSO, filtered through a membrane filter (0.2 µm), and stored at 4°C for further study.

2.3 Extraction of bioactive compounds from the mycelia of *P. tinctorius*

Five g of freeze-dried mycelia obtained from the submerged cultures of *P. tinctorius* maintained in MMN broth (pH: 5.5) at 30ºC for 30 d in complete darkness were powdered in liquid nitrogen; and extracted three times with diethyl ether used at the rate of one ml each time. The extracted sample was taken in the Eppendorf tubes of 2 ml capacity and was centrifuged at 10,000 *g* for 5 min in a bench centrifuge (stored at 4ºC).

2.4 Bioassay of whole fruiting body extracts

Twenty g of the samples were drawn out from the powdered sporocarps and extracted with 100 ml of organic solvents *viz.*, chloroform, ethyl acetate, methanol, and water, as described in the bioassay of different solvents fraction of secondary metabolites.

2.5 Gas chromatography - mass spectrometry (GC-MS)

Characterization of biomolecules extracted from fruiting bodies and mycelia was done by GC-MS analysis. Volatile components were identified by GC-MS using a column Elite-5MS (100% Dimethylpolysiloxane), 30 x 0.25 mm x 0.25 µm df equipped with GC Clarus 500 Perkin Elmer. The turbo mass-gold-perkin- Elmer detector was used. The carrier gas flow rate was 1 ml min-1, split 10:1, and injected volumes were 3 µl. The column temperature was maintained initially at 110ºC at the rate of 10ºC min^{-1}. No hold followed by an increase up to 280ºC at the rate of 5ºC /min^{-9} (hold). The injector temperature was 250ºC and this temperature was held constant for 36 min. The electron impact energy was 70 eV, Juliet line temperature was set at 2000ºC and the source temperature was set at 200ºC. Electron impact (EI) mass scan (m/z) was recorded in the 45-450 aMU range. Using computer searches on the NIST Version 2011 MS data library and comparing the spectrum obtained through GC/MS, the compounds present in the crude sample were identified.

2.6 Statistical analyses

Statistical analyses of all the experiments were conducted using the following methods suggested by Gomez and Gomez, (1984). The mean differences were adjusted with Duncan's Multiple Range Test (DMRT) using the statistical computer package program, IRRISTAT version- 92 developed by the International Rice Research Institute Biometrics unit, the Philippines.

3. Results

Soil-borne plant pathogens are known to infect several economically important crops and bring about huge yield loss. The impediments in using fungicides to control these pathogens include residual toxicity, health hazard, and environmental pollution. On the other hand, the use of biodegradable antifungal organic compounds of microbial origin, more specifically, those compounds obtained from macro-fungi will have a greater attraction in crop disease management. In the current investigation, some of the bioactive compounds of the macro-basidiomycete *P. tinctorius* have been separated and their efficacies were tested against soil-borne plant pathogens *viz.*, *F. o.*f. sp. *lycopersici*, *M. phaseolina*, *R. solani*, and *S. rolfsii*. The results are presented below.

3.1 Morphological characterization of *P. tinctorius* MTP1

Morphological and microscopic characters of fruiting bodies found under eucalyptus plantations at Forest College and Research Institute, Mettupalayam, Jakkanari Forest Range, Coimbatore district, Tamil Nadu were examined. The shape of the fruiting bodies varied from globose to subglobose; 4 to 7 cm in diameter. The total length of the sporocarp varied from 3.0 to 8.5 cm. Sometimes the basidiocarps were deeply rooted with yellowish fibrous stipe measuring 2 cm in diameter. On average, the stipe length was 3 cm. The peridium was thin-walled with bright yellow-to-yellowish-brown or blackish/olive green tone. The texture of peridium varied from fleshy to slightly viscid in juvenile stages. The peridium was fragile and pulvinate at maturity. The gleba and peridioles had pale white, yellowish-brown, or olivaceous powdery spore mass. Breakdown of round and smooth peridioles resulted in the release of clouds of basidiospores. The spores were spherical, 7 to 8 µm in diameter with ornamentations up to 0.5 µm. Constituent hyphae were thin and thick-walled measuring approximately 2-3 mm in diameter. Hyphae were hyaline to brown (Figure 2).

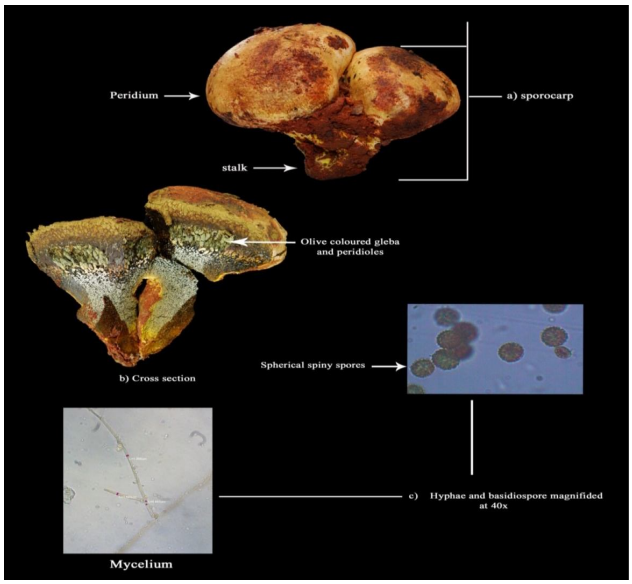


Figure 2: Morphology of *Pisolithus tinctorius* isolate MTP1.

3.2 Extraction of bioactive compounds from *P. tinctorius* isolates MTP1

Bioactive compounds were extracted from the whole fruiting bodies of *P. tinctorius* isolate MTP1 utilizing different solvents, *viz.*, ethyl acetate, methanol, chloroform, diethyl ether, and water. Per cent recovery of substance (calculated in terms of w/v) from the whole fruiting body is presented in Table 1. From 20 g of fruiting body sample, 40 mg of ethyl acetate fraction; 32 mg of methanol fraction; 28 mg of chloroform fraction, and 10 mg of water evaporated residue were obtained. A maximum level of 0.04% recovery was observed when ethyl acetate was used as the solvent. When methanol and chloroform were used, the recovery levels were 0.032 and 0.028%. But, when water alone was used to dissolve the sample, only 0.01% residue could be recovered after evaporation. Similarly, diethyl ether was used as the solvent for extracting compounds from the mycelial mat. However, the recovery percent was negligible.

3.3 Effect of ethyl acetate fraction of fruiting bodies

The antifungal nature of the bioactive compound of *P. tinctorius* in its crude was tested separately against *F. o.* f. sp. *lycopersici*, *R. solani*, *M. Phaseolina*, and *S. rolfsii* by agar well diffusion method at 50, 100, and 150 µl concentrations. The results showed that the ethyl acetate fractions of *P. tinctorius* sporocarp-based bioactive compounds were more inhibitory to *R. solani* (840 mm^2) as compared *M. phaseolina* (790mm^2) and *F. o.* f. sp. *lycopersici* (680 mm^2) when used at a concentration of 150 µl. This fraction of the bioactive compound was also not found to inhibit *S. rolfsii* (Table 2).

3.4 Characterization of bioactive compounds of CFC filtrate condensate, fruiting body, and mycelial mat through GC-MS

The ethyl acetate fraction of whole fruiting bodies (including basidiospores) and diethyl ether fraction of mycelial mat were subjected to GC-MS analysis. The results showed an array of biomolecules as tabulated in Tables 3 and 4. The biomolecules composite of sporocarps contained several volatile compounds like hexadeconic acid; 2-penta-deuterio-isopropenyl-3-hepta-deuterio-isopropyl naphthalene; tetrakis-dimethyl-silyl-carbodiimide); di-(2-ethylhexyl) phthalate and Hesperetin (1-[2,4,6-tris (trimethylsiloxy) phenyl] 3- [3-methoxy-4-(trimethylsiloxy) phenyl] - 2- propen – 1–one) (Figure 3 and Table 3).

Table 1: Per cent recovery of bioactive compounds from fruit bodies of *P. tinctorius*.

Solvent used @100 ml	Fruit bodies	
	(mg)	Per cent
Ethyl acetate	40	0.04
Methanol	32	0.032
Chloroform	28	0.028
Water	10	0.01

*Each sample of biomolecules mixture was weighed after condensing the solvents in a vacuum flask evaporator at 45^{0}C for 30 min.

Table 2: Effect of various fractions of bioactive molecules of *P. tinctorius* (fruiting bodies) against different soil-borne plant pathogens

Bioactive molecules	Concentrations (µl)	Inhibition (mm^2)			
		F.oxysporum* f. sp. *lycopersici	***M. phaseolina***	***R. solani***	***S. rolfsii***
Ethyl acetate fraction	50	310.00^{c} (17.64)	420.00^{c} (20.52)	330^{c} (18.20)	0.00 (1.12)
	100	470.00^{b} (21.70)	540.00^{b} (23.26)	620^{b} (24.95)	0.00 (1.12)
	150	680.00^{a} (26.70)	790.00^{a} (28.13)	840^{a} (29.00)	0.00 (1.12)
Methanol fraction	50	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
	100	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
	150	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
Chloroform fraction	50	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
	100	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
	150	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
Water residue	50	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
	100	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
	150	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
Control (+) Sterile water	150	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
Control (-) DMSO	150	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)	0.00 (1.12)
CD		136	158	168	0.0

Values in parentheses are square-root transformed, means followed by a common letter is not significantly different by DMRT (p = 0.05 per cent).

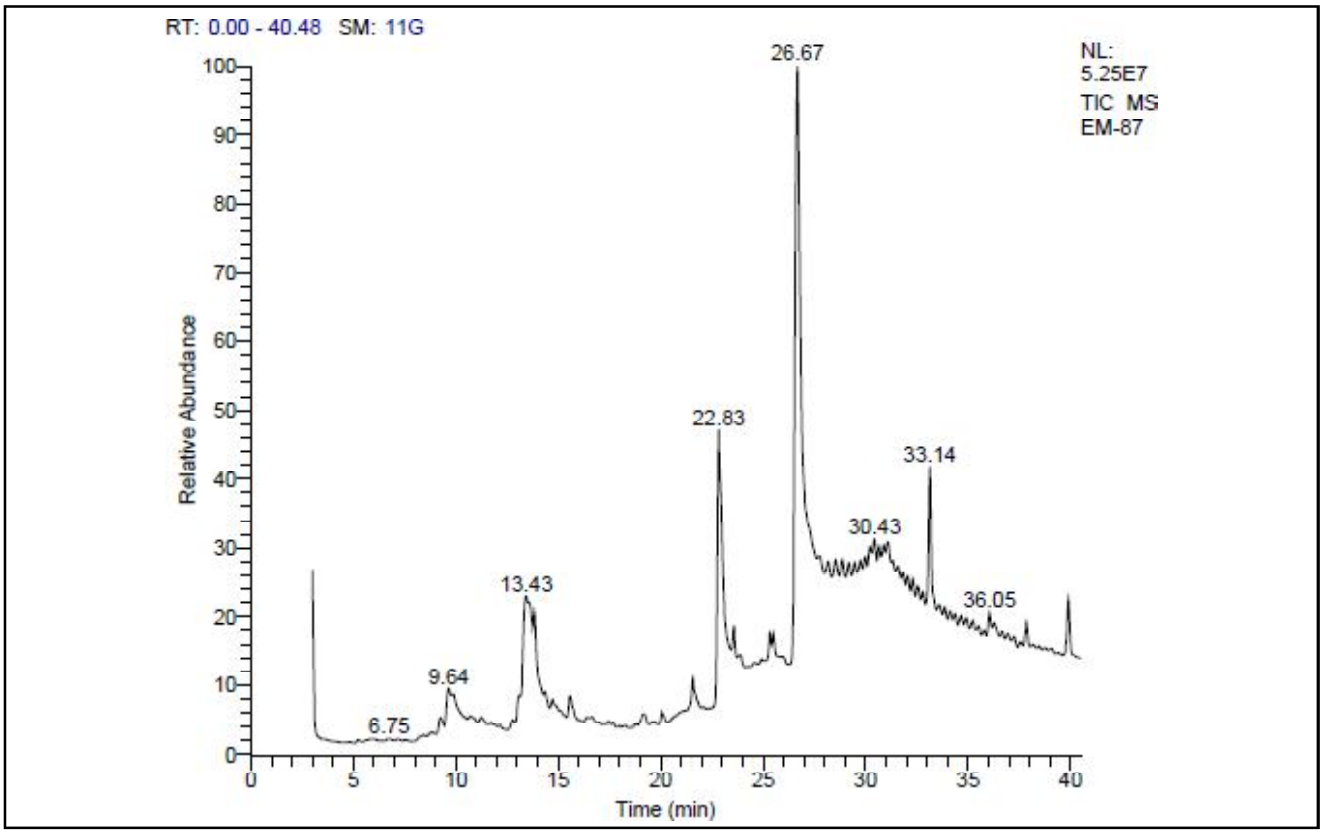
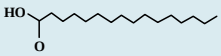
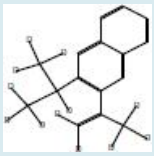
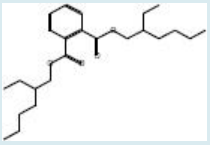
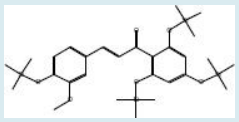


Figure 3: Total ion chromatogram (TIC) of biomolecules identified from fruiting bodies of *P. tinctorius* through GC-MS.

Table 3: GC-MS analysis of fruiting body extract (ethyl acetate fraction) of *P. tinctorius*

RT*	Compound	Structure	Molecular formula	MW*	Peak area (%)	Nature of compounds
22.83	Hexadecanoic acid		C16H32O2	256	10.98	Fatty acid
26.67	2Pentadeuterioisopropenyl-3heptadeuterioisopropylnaphthalene		C16H6D12	210	35.18	Not known
30.43	Tetrakis (Dimethylsilyl-carbodiimide)	Unidentified	C12H24N8Si	392	2.09	Not known
33.14	Di-(2-ethylhexyl)phthalate		C24H38O4	390	5.16	Not known
36.05	1-[2,4,6-tris(trimethylsiloxy) phenyl]- 3-[3-methoxy-4-(trimethylsiloxy)phenyl]-2-propen-1-one (Hesperetin)		C28H46O6Si4	590	1.02	Flavanoids

*RT-Retention time; *MW-Molecular weight.

Likewise, diethyl ether fraction of mycelial mat contained cyclohexane, 1,4-dimethyl-2-octadecyl-, methyl -2- (3', 3' – dimethyl - 1' – butyn - 1' – yl) -1 cyclohexene carboxylate, 7, 9 - di-tert-butyl-1 oxaspiro (4,5) deca -6, 9 – diene -2, 8-dione (Figure 4 and Table 4).

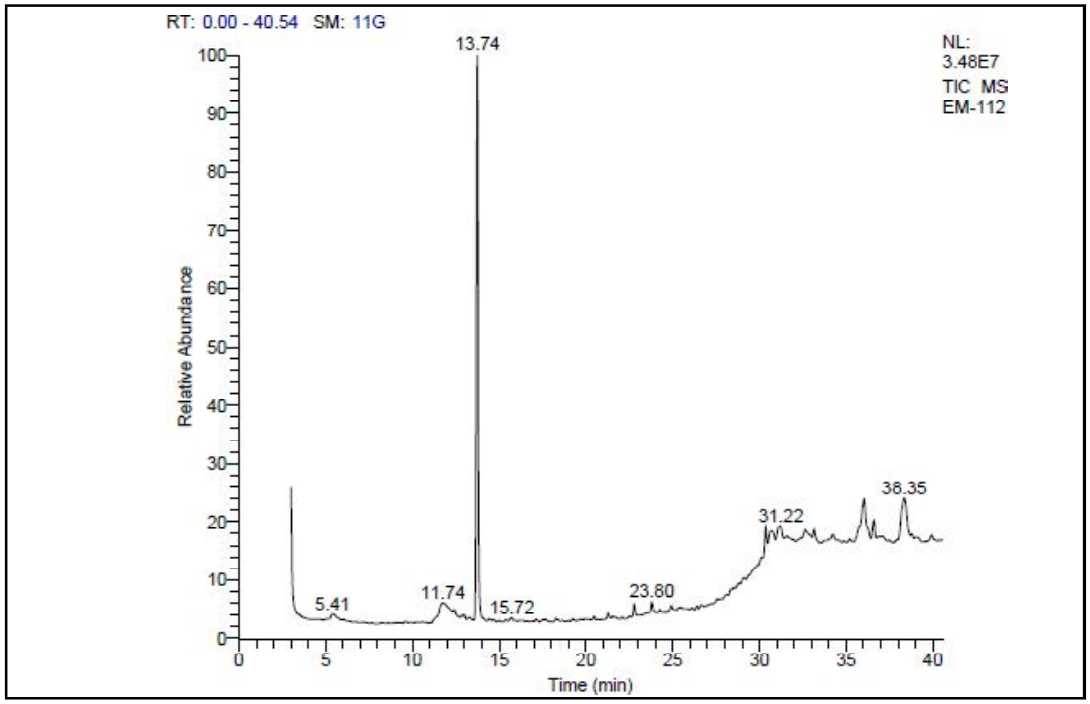
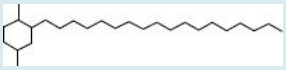
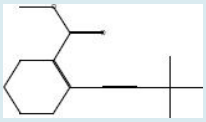
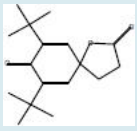


Figure 4: Total ion chromatogram (TIC) of biomolecules identified from mycelial mat of *P. tinctorius* through GC-MS.

Table 4: GC-MS analysis of mycelial mat (diethyl ether extract) of *P. tinctorius*

RT*	Compound	Structure	Molecular formula	MW*	Peak area Per cent	Nature of compounds
5.41	Cyclohexane, 1,4-dimethy l- 2-octadecyl		$C_{26}H_{52}$	364	1.48	Terpenoid
13.74	Methyl 2-(3',3'-dimethyl-1'-butyn-1'-yl)-1cyclohexene-carboxylate		$C_{14}H_{20}O_2$	220	42.42	Terpenoid
23.80	7,9-Di-tert-butyl-1- oxaspiro (4, 5)deca-6, 9-diene- 2,8-dione		$C_{17}H_{24}O_3$	276	0.80	Steroid

RT*- Retention time; MW*-Molecular weight.

4. Discussion

Soil-borne plant pathogens are very difficult to destroy because their inoculums survive in the soil throughout the year even absence of host. Several fungicides are available in market but no one could control the pathogen completely with recommended dose. The farmers are using a lot of pesticides with high concentration that has led to environmental pollution and health hazards as well the high cost of production. Keeping all these constrain in mind, the current investigation is mainly focused on search of noval antimicrobial compounds from the beneficial fungi to develop biopesticides. Some of the novel compounds were initiated and tested against soil-borne plant pathogens, *viz.*, *F. o.* f. sp. *lycopersici*, *M. phaseolina*, *R. solani* and *S. rolfsii*. The results obtained are discussed hereunder.

4.1 Morphological characterization of *P. tinctorius* isolate MTP1

Macro and micro morphological differences, in carpophores; and of isolated cultures, have been observed in *P. tinctorius* in several countries. The basidiomes of *P. tinctorius* exhibit polymorphism and vary greatly in size, shape, diameter, and colour of glebal chambers and nature of the exterior of the peridium, but are commonly characterized by globose basidiospores, 7-12 µm in diameter covered with densely packed spines up to 1.5 µm in length. However, an examination of basidiospores collected from North America, Europe, Africa, and Australia had revealed three distinct basidiospore types (Grand, 1976). Moreover, Burgess *et al.* (1995) had reported that basidiomes from Western Australia, New South Wales and Queensland varied considerably in size (2-20 cm), shape (globose, pyriform, ellipsoid), length and type of sterile stipe (absent, short, well developed with rhizome, long and woody); and peridium features (smooth, cracked, rugulose, striated, patchy). Two new species of *Pisolithus*, namely; *Pisolithus aurantioscabrosus* collected under *Shorea macroprera* from Malaysia (Martin *et al.*, 2002) and *P. adbitus* collected under *Dipterocarpus alatus* from Thailand (Kanchanaprayudh *et al.*, 2003) have been described from dipterocarp forests. These reports on morphological variability suggest that *P. tinctorius* is taxonomically more diverse than currently recognized, and needs further taxonomic revision. Reddy *et al.* (2005) identified a new species of *Pisolithus indicus*, which to forms ectomycorrhizal association with dipterocarps of native forests in India. The preliminary morphological observations of *P. tinctorius* isolate, MTP1 is similar to *P. indicus* reported by Reddy *et al.* (2005) and Cullings *et al.* (2020). But, the Annual report of the Institute of Forest Genetics and Tree Breading (IFGTB), Coimbatore for the year 2003-2004 indicates that the ectomycorrhizal fungus found in association with *Eucalyptus* plantation from the same geographical coordinates at Mettupalayam as *P. tinctorius*. Taking this report as strong evidence, *Pisolithus* isolate MTP1 is rightly placed under the species "*tinctorius*".

4.2 Extraction and recovery of biomolecules from *P. tinctorius* isolate MTP1

In the present study, biomolecules were extracted from the whole fruiting body inclusive of basidiospores and mycelial mat using different solvents and water. After, a series of extraction processes, the per cent recovery of biomolecules in solvent and water fractions was calculated on a dry weight basis. Ethyl acetate fraction of the samples was always found to give the maximum recovery of biomolecules (0.2% from whole fruiting bodies). In a similar study, Ameri *et al.* (2011) reported that the ethyl acetate fraction of the sporocarps of *Pisolithus albus* exhibited increased levels of recovery of biomolecules (up to 40 mg per 100 ml of solvent which is equivalent to 0.4 per cent). The poor recovery of biomolecules in water fraction indicates that the composite biomolecules do not dissolve in an aqueous solution. As the recovery percentage of biomolecules from the mycelial mat was very minimum (0.0010 percent), no further experiment was conducted using this composite; but, it was subjected to GC-MS analysis, just for profiling the compounds that were present in it.

4.3 Effects of ethyl acetate fraction of bioactive molecules of *P. tinctorius* isolate MTP1

Bioactive compounds were found to be diversely distributed in the fruiting bodies and the mycelial mat of *P. tinctorius*. Among the different solvents used for extraction and recovery of bioactive compounds, ethyl acetate fractions of fruiting body extract had exhibited considerable antifungal activity against *F. o.* f.

sp. *lycopersici*, *M. phaseolina*, and *R. solani* whereas, methanol, chloroform, and aqueous extracts were not effective in controlling any of the test pathogens. The results also indicated the ineffectiveness of even the ethyl acetate fraction against *S. rolfsii*. More interestingly, the ethyl acetate fraction of whole fruiting body extract showed only 680 mm^2 inhibition against *F. o.* f. sp. *lycopersici*. And in the case of *R. solani* and *M. phaseolina*, whole fruiting body extracts showed only 840 mm^2 and 790 mm^2 inhibition, respectively. The reason for this differential expression of antimicrobial activities might be due to the differences in the bioactive compositions, concentrations, methods of extraction, and mechanism of action of the available active ingredients. Imtiaj and Lee (2007) also reported that the ethanol extract of non-edible macrofungi, *Stereum ostrea* had contained antifungal properties against certain plant pathogenic fungi including *Colletotrichum gloeosporioides*, *C. miyabeanus*, and *Botrytis cinerea*; and the extract was found to significantly reduce the mycelial growth of the pathogenic fungi.

In a similar study, Shrestha *et al.* (2005) reported the antibacterial activity of metabolites of *Pisolithus* spp against a spectrum of Gram-negative and Gram-positive bacteria. Ameri *et al.* (2011) also concluded that the ethyl acetate fractions of the sporocarps of *P. albus* showed the maximum inhibition for *Staphylococcus aureus.* Earlier, Waser (2002) reported that many of the macrofungi had contained bioactive compounds, with antifungal, antibacterial and antiviral activities. In a separate study, the methanol extract of *Phellinus* was reported to possess better antifungal potentials than aqueous extract (Balakumar *et al.*, 2011). The methanol extract was found to significantly inhibit the mycelial growth of five fungal pathogens, *viz.*, *Penicillium* sp., *Aspergillus fumigatus*, *A. niger*, *A. flavus,* and *Mucor indicus*.

4.4 Characterization of bioactive compounds through GC-MS

The ethyl acetate fraction of the whole fruiting body (including basidiospores) and diethyl ether fraction of mycelial mat was subjected to GC-MS analysis and the result revealed that fruiting body and mycelia mat contained compounds, *viz*., hexadecanoic acid; 2Pentadeuterioisopropenyl-3heptadeuterioisopropylnaphthalene; Tetrakis (Dimethylsilylcarbodiimide); Di-(2-ethylhexyl) phthalate and 1-[2,4,6-tris(trimethylsiloxy) phenyl]- 3-[3-methoxy-4- (trimethylsiloxy) phenyl] -2- propen-1-one (Hesperetin). Among these, benzene-ethanol (0.25 per cent) is aromatic alcohol expressed at 7.85 RT (Retention time). Fraud *et al.* (2003) had observed antioxidant and antibacterial activity of this compound against certain Gram-negative bacteria and mycobacteria.

Nevertheless, the biomolecules composite of sporocarps of *P. tinctorius* contained several other organic compounds including hexadecanoic acid, a fatty acid fraction (10.98 per cent) was identified at 22.83 RT. Praveen Kumar *et al.* (2010) reported that hexadecanoic acid had acted as an antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor compound, hemolytic, and 5-alpha reductase inhibitor. The biological activity of 2-Penta-deuterio-isopropenyl-3-hepta-deuterio-isopropyl naphthalene (35.18 percent) found to be expressed at 26.67 RT is not known. Likewise, tetrakis (dimethylsilylcarbodimide) found expressed to a limited extent of up to 2.09 per cent at 30.43 RT has already been reported to induce severe salt tolerance in tomato plants when applied through the foliage. Hence, it is assumed that the symbiotic association of *P. tinctorius* would help its host to withstand any of the physiological stress. This concept is further supported by the fact that *Eucalyptus* is known for its stress tolerance; and *P. tinctorius* was always found to be in association with *Eucalyptus* plantations. At 33.14 RT a compound namely, di-(2-ethylhexyl) phthalate was found to be meagrely expressed (up to 5.16 percent). However, Habib and Karim (2009) reported about the antifungal activity of di-(2-ethylhexyl) phthalate against *A. flavus*, *A. niger,* and a *Fusarium* sp. Another phenolic compound namely, hesperetin (1-[2,4,6-tris (trimethylsiloxy) phenyl] 3- [3-methoxy-4-(trimethylsiloxy) phenyl] -2- propen-1-one) was found to be expressed up to 1.02 per cent at 36.05 RT. Zaat *et al.* (1989) reported that this compound had induced nodulation in leguminous plants.

In addition, certain terpenoids and steroidal compounds such as cyclohexane 1,4-dimethyl-2-octadecyl; methyl -2- (3', 3' – dimethyl - 1' – butyn - 1' – yl) -1 cyclohexene carboxylate; 7, 9 - di-tert-butyl-1 oxaspiro (4,5) deca -6, 9 – diene -2, 8-dione were identified from the diethyl ether fraction of mycelial mat. Cyclohexane, carboxylic acid, and diene compounds are more valuable and major components of many of the pharmaceutical and pesticidal preparations (Praveen Kumar *et al.*, 2010). Further studies to recover these useful compounds in large quantities from *P. tinctorius* will have multifaceted benefits. Interestingly, several unknown compounds were also found expressed during GC-MS analysis; and their potential role in inhibiting the test pathogens, *viz.*, *F. o.* f. sp. *lycopersici*, *M. phaseolina*, *R. solani*, and even *S. rolfsii* need to be elucidated. Conclusively, the results of the present investigations reveal evidence that the bioactive compounds of *P. tinctorius* isolate MTP1 could be potentially explored for the management of soil-borne plant pathogens that induce wilt, root rot, and sheath blight diseases in crop plants. Also, such compounds may be useful in plant growth promotion, quorum sensing, nodulation, and stress tolerance.

5. Conclusion

The current study revealed the antifungal potential of bioactive compounds of *P. tinctorius* isolate MTP1 against soil borne plant pathogens, *viz.*, *F. oxysporum* f. sp. *lycopersici*, *M. phaseolina*, *R. solani* and *S. rolfsii*. The ethyl acetate fraction of whole fruiting body (inclusive of basidiospores) extract yielded 0.2% biomolecule composite that could have maximum inhibition of *R. solani* (840 mm^2), *M. phaseolina* (790 mm^2), and *F. oxysporum* f. sp. *lycopersici* colony (680 mm^2). Characterization of biomolecules through GC-MS indicated the presence of antimicrobial compounds that belonged to the nature of fatty acids, aromatic alcohol, terpenoids, steroids, and flavonoids. Further study is warranted to explore the mode of action of these biomolecules against plant pathogens.

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Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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