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Exploration of Antifungal Metabolites of *Aspergillus terreus* (ENF12), an Endophytic Fungus Isolated from Mulberry (*Morus indica* L.) Leaf

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AMJ and KA designed the study. Author AMJ performed the laboratory experiments and produced the manuscript. Authors KA and SN revised the manuscript. Authors MT and GU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to explore the metabolic profile of endophytic fungi, *Aspergillus terreus* (ENF12) isolated from mulberry leaf and evaluate their antifungal efficacy against *Macrophomina phaseolina* causing charcoal rot disease of mulberry.
Study Design: Completely Randomized Block Design.
Place and Duration of Study: Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during August 2018 to June 2019.
Methodology: The isolation was done by serial dilution and plating method. The culture was

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identified by utilizing the morphological, microscopical and taxonomical keys as *A. terreus*. The macroscopic observations such as colony growth, color, texture, pigmentation, growth rate and microscopic observations *viz*. hyphal and conidial characters were studied. The molecular confirmation was done by DNA sequencing using ITS 1 and ITS 4 primers. The extracellular secondary metabolites were extracted from *A. terreus* and screened against *M. phaseolina* by agar well method. The composition of the metabolites was analyzed through GC-MS.

Results: The septate and hyaline mycelium along with aleurioconidia was observed under microscope and based on the DNA sequencing, the ENF12 isolate was identified as *A. terreus*. The major compounds identified from the extracellular metabolites of *A. terreus* were tromethamine, benzenediol, tetracosamethyl cyclododecasiloxane, hexadecanoic acid, benzoic acid and pregnatriene. The ethyl acetate extract of the metabolite at 4000 ppm suppressed the growth of *M. phaseolina* by 77.78%. The pathway analysis revealed that the metabolites are involved in the metabolism of drug development, cellular processes, organismal systems and other pathways. The major identified metabolites and pathways were grouped under biosynthesis of other secondary metabolites, lipid metabolism and energy metabolism.

Conclusion: The endophytic fungi, *A. terreus* from mulberry could be an effective antifungal agent against *M. phaseolina*. The metabolic pathway analysis found that the major metabolites identified from *A. terreus* were also reported in mulberry plants thereby showed the mutual interaction between the host and endophyte.

Keywords: Mulberry; endophytic fungi; antifungal; metabolites; pathway analysis.

1. INTRODUCTION

Endophytic fungi which thrive inside plant tissues as asymptomatic mutualists had been renowned as an important and novel source of bioactive compounds [1]. An array of secondary metabolites that were used as bio-control agents, immunosuppressive compounds, etc. was produced by these endophytes [2]. The metabolic interactions of endophytes with its host might favor the synthesis of biologically active secondary metabolites. The endophytes had metabolic impacts on host plants and possibly regulated the biochemical status of host plants [3]. Based on structure and biosynthetic pathway, fungal secondary metabolites were mainly classified as polyketides, nonribosomal peptides, alkaloids, or terpenes [4,5].

Plants, known for ethnomedicinal properties and applications have been found to harbor novel endophytic microflora (Ravindra et al. 2016) which showed the importance of selection of plants for endophyte isolation. Several studies had revealed that different species of *Morus* had antioxidant, antiviral, anti-inflammatory, hypolipidemic, anti-hyperglycemic, neuroprotective [6], anti-HIV, anti-hypotensive and cytotoxic activities [7]. Antibiosis is considered as the most important type of antagonism in which the antagonists produce an array of secondary metabolites such as antibiotics and toxin, which contribute to the antagonistic activity of fungal biocontrol agents against plant pathogens [8]. Root rot, due to its epidemic nature and potential to kill the plants, is a dangerous disease of mulberry. Hence, this study highlighted the exploration and documentation of metabolite profile connected with mulberry endophytic fungi, *Aspergillus terreus* and evaluation of its bio-efficacy in controlling of charcoal rot pathogen, *Macrophomina phaseolina*.

2. MATERIALS AND METHODS

2.1 Isolation of Endophytic Fungi

The surface sterilization of collected plant samples was done according to the method described by Petrini [9] with modifications. The surface sterilized samples were macerated with 1 ml of Sterile Distilled Water (SDW) in a sterile mortar and pestle, and each sample was serially diluted in test tubes containing 9 ml of SDW. The dilutions of 10^{-4} and 10^{-5} were plated on Petri plates containing PDA medium supplemented with antibiotics. The plates were then incubated at 28±2°C for 10 days and observed for fungal growth. Each colony obtained was subcultured on PDA slants and maintained for further use [10]. The isolated fungi were identified on the basis of morphological characteristics according to Domsch et al. [11]. The charcoal rot pathogen, *M. phaseolina* was isolated from infected samples by root bit method [12] and identified based on the morphological characters.

2.2 *In vitro* Antifungal Assay

The isolated endophytes were screened for *in vitro* antagonistic activity against *M. phaseolina* by using dual culture technique [13]. The per cent growth inhibition was calculated according to Fokkema [14].

2.3 Molecular Characterization of Endophytic Fungi

Based on the performance of isolates in the dual culture assay, potential isolate was further identified using molecular techniques. Genomic DNA was extracted from ground mycelium [15]. The DNA concentration and integrity were checked by electrophoresis on 0.8% agarose gel. PCR amplification of the DNA was performed using primer ITS1 (5' TCCGTAGGTGAACCTGCGG-3'), as described by Rhoden et al. [16], and primer ITS4 (5'-TCCCCGCTTATTGATATGC-3'), as described by White et al. [17]. Afterwards, samples were quantified again by electrophoresis on 1.2% agarose gel and documented. The PCR amplified products were sequenced. For the identification of endophytic fungi, per cent sequence identity and coverage were compared with available sequences in GenBank (http://www.ncbi.nlm.nih.gov) using BLASTn to search for the closest matched sequences and were submitted to GenBank.

2.4 Phylogenetic Analysis of Endophytic Fungi

Sequences were aligned using ClustalW [18] and the dendrogram was made with MEGA program, version 7.0 [19]. The evolutionary distances were computed using the p-distance method [20]. The topology of the phylogenetic tree was reconstructed using the neighbor-joining method [21].

2.5 Extraction of Extracellular Secondary Metabolites

The metabolites were extracted according to the method followed by Choudhary et al. [22] with modifications. The fungi were inoculated in Potato Dextrose Broth (PDB) and maintained at 28±2°C for 15 days. Liquid state fermentation method was followed for the production of metabolites. The culture broths were filtered and mycelia were separated. The broth containing metabolite was extracted by solvent extraction procedure using ethyl acetate as organic solvent. Equal volume of the filtrate and ethyl acetate was kept for overnight incubation in rotary shaker at 150 rpm. Then, the solvent phase was extracted using separating funnel and was dried in vacuum flask evaporator to yield the crude metabolite. The extract was scraped using HPLC grade methanol and stored for further analysis.

2.6 Gas Chromatography Mass Spectrometry (GC-MS) Analysis of Bioactive Compounds

The purified extract was subjected to GC- MS analysis to identify the bioactive compounds. The sample was analysed in PERKIN ELMER CLARUS SQ8C gas chromatograph with DB-5 MS capillary standard non-polar column (Dimension: 30 Mts, ID: 0.25 mm, Film: 0.25 IM) was used. Helium was used as a carrier gas. The identification of components was accomplished using computer searches in NIST libraries MS Search version 2.2.

2.7 *In vitro* Antifungal Activity of Crude Metabolites against *M. phaseolina*

The antifungal activity of crude metabolites was tested against mulberry charcoal rot pathogen, *M. phaseolina* by agar well diffusion assay [23].

2.8 Statistics and Informatics Analysis

The Completely Randomized Block Design method was adopted. The identified metabolites were first submitted to the KEGG pathway database (available online: http://www.kegg.jp/kegg/pathway.html) to obtain the KEGG pathway ID. Those small molecules that could not be identified from the KEGG pathway database were classified as unidentified metabolites and were not used for further analysis. The monoisotopic mass of the identified metabolites was obtained from the ChemSpider website (available online: http://www.chemspider.com/).

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Endophytic Fungi

A total of 30 endophytic fungal isolates were obtained from healthy tissues of *M. indica*. Amongst them, 13 isolates namely ENF1, ENF5, ENF8, ENF12, ENF15, ENF16, ENF18, ENF22, ENF24, ENF26, ENF28, ENF29 and ENF30

were recovered from mulberry leaf. Based on the *in vitro* antifungal efficacy against *M. phaseolina*, the best performed isolate ENF12 was selected for further studies. The morphological and microscopic characteristic of ENF12 isolate was observed. The results revealed that the young mycelium was white in color later it turned into yellowish brown color. The yellow pigmentation was observed over the media. The hyphae were septate and hyaline. The conidia were small in size, smooth and oval in shape. The aleurioconidia, asexual spores produced directly on the hyphae were noticed (Figure 1). The sequential steps in the formation and wall structure of *A. terreus* conidia were studied by Transmission Electron Microscope. Solitary or botryose globose lateral cells 'aleurioconidia' occurred on the vegetative mycelium [24]. The result of this study was collaborated with earlier findings. Pang et al. [25] isolated the endophytic fungus *Aspergillus* sp. CPCC 400735 from the plant *Kadsura longipedunculata*. Similarly, Aletaha et al. [26] isolated endophytic *A. terreus* from *Suaeda aegyptica*. The pathogen was isolated from the root rot infected plant and identified as *M. phaseolina* based on morphological characters and sclerotial structures.

3.2 *In Vitro* Screening of ENF12 Isolate against *M. phaseolina*

The ENF12 isolate was screened against mulberry charcoal rot pathogen, *M. phaseolina* by dual culture technique. The ENF12 isolate inhibited the growth of pathogen by 77.80% over control with an inhibition zone of 7.50 mm.

The interaction between ENF12 isolate and pathogen was suggested that the endophyte could antagonize the growth of pathogen by antibiosis mechanism with presence of reactive zone between endophyte and pathogen (Figure 2). The outcome of *in vitro* study was accordance with the findings of Hamdi et al. [27] who evaluated the antifungal potential of *A. terreus* against soil borne fungi (*Fusarium oxysporum f. sp. melonis* and *M. phaseolina*) of watermelon and inhibited the mycelial growth by 36.14% and 46.63% respectively.

Similarly, the biocontrol efficacy of *A. terreus* isolates 65P and 9F significantly reduced the mycelial growth of *Pythium* ausing damping off in cucumber and effectively inhibited the phytopathogenic fungi *viz*., *Botrytis cinerea*, *Rhizoctonia solani* and *P. ultimum* [28,29].

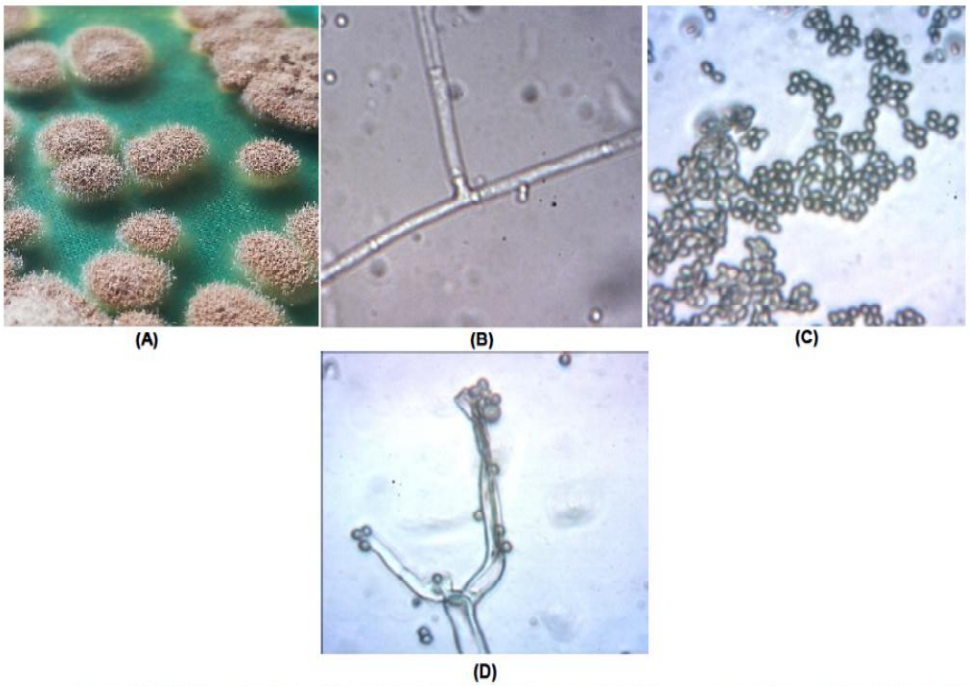


Figure 1. Morphological identification of *A. terreus* under macro and microscopic view
(A): Macroscopic view of *A. terreus*; (B): Septate and hyaline hyphae; (C): Conidiospores of *A. terreus* under 45X; (D): Aleurioconidia

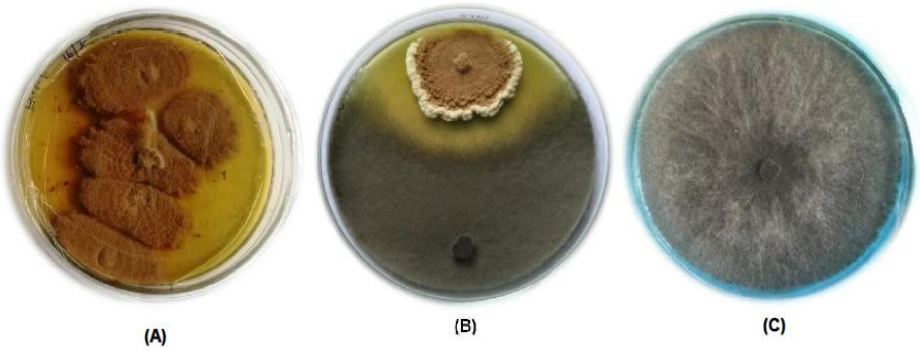


Figure 2. *In vitro* antifungal assay of *A. terreus* against *M. phaseolina*
(A): *A. terreus* on PDA; (B): *In vitro* screening of *A. terreus* against *M. phaseolina* by dual culture; (C): Control

3.3 Molecular Characterization and Phylogenetic Analysis of *A. terreus*

PCR amplification of the genomic DNA of ENF12 isolate resulted in the amplicon size of 560 bp. Based on the sequencing of the PCR product, ENF12 isolate was identified as *A. terreus*. The sequences of the *A. terreus* were deposited in GenBank (Accession number: MK963009).

The phylogenetic tree was constructed using Neighbor-joining method. The obtained sequences of ENF12 (Accession no. MK963009) was clustered with reference or closely related species. The sequences of the representative *Aspergillus* sp. were acquired from NCBI database. The phylogenetic tree resulted that nine major clades were formed with respective bootstrap values. All the clades were comprised fungal isolates only from phylum Ascomycota and genus *Aspergillus* (Figure 3). Majority of the *A. terreus* isolates were grouped under the clade 1 with bootstrap value of 40 whereas *A. terreus* isolate ENF12 and *Aspergillus terreus* isolate 720/2K was sub grouped in clade 7 separated from other species of *Aspergillus*. Clade 8 consisted *A. fumigatus* strain TMS48 and *A. citrinoterreus* isolate was clustered in clade 9. The results indicated that isolated endophytic fungi, *A. terreus* ENF12 from mulberry was closely related with *A. terreus* isolate 720/2K with the bootstrap value of 99. *A. fumigatus* strain TMS48 and *A. citrinoterreus* isolate APHM06 were clearly separated from each other by forming separate clades.

Phylogenetic analysis was useful for studying genetic relationships and to distinguish among closely related *Aspergillus* species. Molecular characterizations of *Aspergillus* sp. using ITS 1 and ITS 4 primers were performed by Yee and Zakaria [30]. Peterson [31] described the genetic relationship between *Aspergillus* species by phylogram analysis.

3.4 Metabolite Profile of *A. terreus*

The metabolic profiles of living organisms reflect features of their life activities. The metabolites were identified using GC-MS analysis showed the relative abundance of various compounds. A Total of 91 metabolites were identified from *A. terreus*. The metabolites were classified according to their nature of the compounds such as amino acids, sugars, alcohol, fatty acids, organic acids, ketone, vitamins, sterols, terpenoids, alkaloids, carboxylic esters, hydrocarbons and others (Figure 4). Among the secondary metabolites, fatty acids were significantly high followed by amino acids, terpenoids, alcohols and hydrocarbons. The metabolite array of *Aspergillus* species were supported by Pang et al. [25] who extracted thirty-three metabolites including five phenalenone derivatives (1−5), seven cytochalasins (6−12), thirteen butenolides (13−25) and eight phenyl derivatives (26−33) from endophytic *Aspergillus* sp. CPCC 400735 isolated from rice.

Monoisotopic mass (M_{mi}) is one of several types of molecular masses used in mass spectrometry. The monoisotopic mass of identified metabolites ranged from 62 to 764 Da. Based on their monoisotopic mass, the metabolites were divided into seven groups, majority of the metabolites (37) were ranged from 100 to 200 Da (Figure 5). Thirty nine fungal metabolites were identified from *A. fumigatus*, an endophytic fungus isolated from stem bark of neem plant [32]. Likewise, Kjarbolling et al. [33] studied the secondary metabolite profile of *A. novofumigatus*.

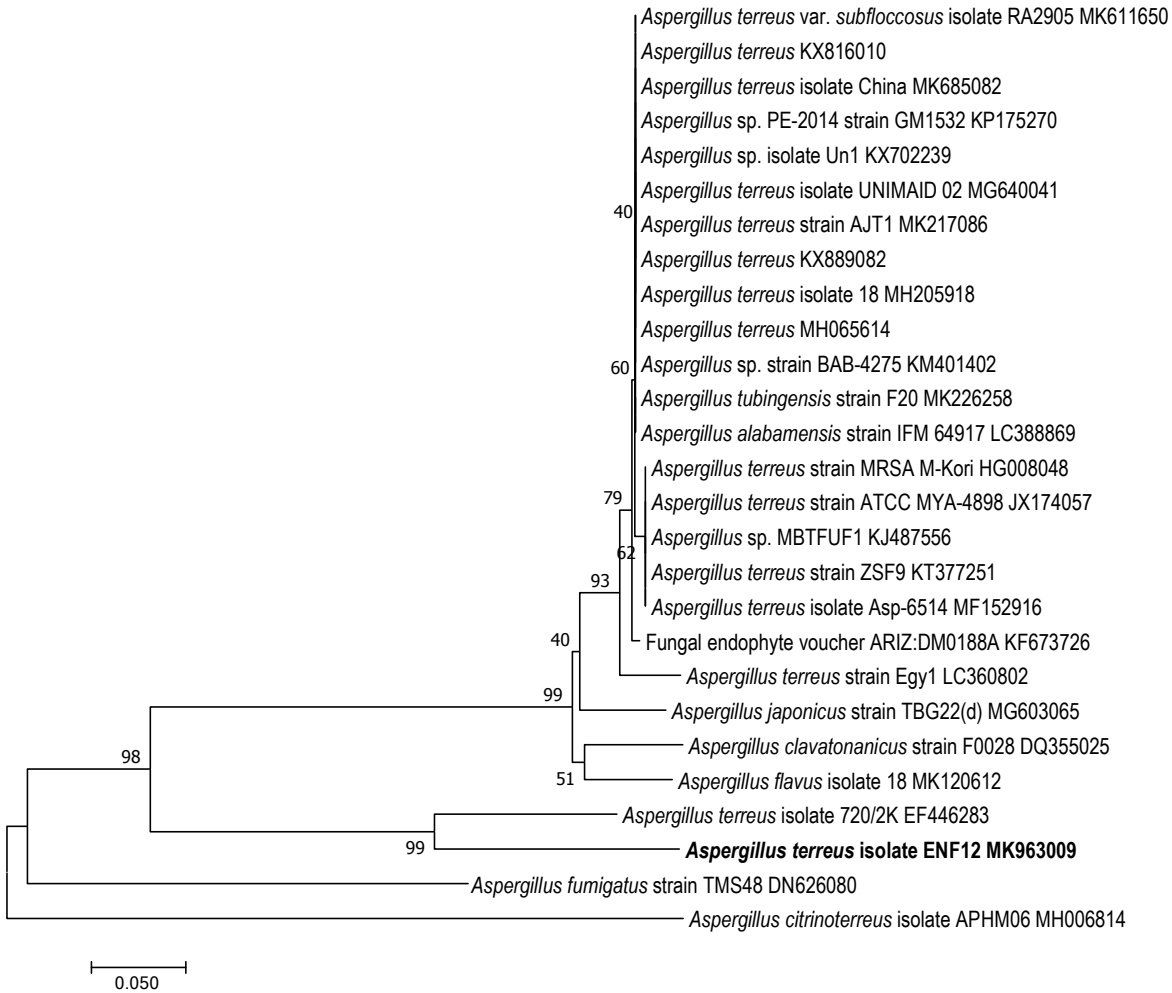


Figure 3. Neighbor-joining tree showing phylogenetic relationships among *Aspergillus* species

The compounds which were confirmed with more relative area percentage and their retention indices were presented in Table 1. Based on the peak area percentage, 1,3-Benzenediol had the highest relative abundance (7.916%). The tromethamine compound had the relative abundance of 7.283% next to Tetracosamethyl cyclododecasiloxane (7.774%). Moreover, minor compounds such as L-alanine (0.517%), octadecanoic acid (0.643%) and propanoic acid (0.830%) also displayed antifungal and antibacterial characteristics. Majority of the metabolites ensured with antimicrobial properties that confirmed the biocontrol potential of *A. terreus* (Figure 6).

Similar results were reported by Sahani and Thakur [34] who identified bioactive metabolites *viz*., Palmitic acid (24.54%), Hexadecanoic acid, Z-11-(10.57%), E,E-6,8-Tridecadien-2-ol, acetate (8.71%), Tetradecanoic acid, 12-methyl-, methyl ester (8.50%), 1-Eicosene (7.93%), Spirostan-9-ol from endophytic *Curvularia aeria* MTCC-12847 isolated from *Tribulus terrestris*. Furthermore, GC-MS analysis of crude ethyl acetate extract of endophytic *Colletotrichum gloeosporioides* isolated from *Lannea corammendalica* revealed the major compounds as 9-octadecenamide, hexadecanamide, Diethyl pythalate,2-methyl-3-methyl-3-hexene, 3-ethyl-2,4-dimethyl-pentane and exhibited antimicrobial activity [35].

3.5 Screening of Crude Metabolites against *M. phaseolina*

The extracellular antifungal metabolites were screened against mulberry charcoal rot pathogen, *M. phaseolina*. The different concentrations of crude extract was tested against the fungi resulted that the highest concentration of crude extract (4000 ppm) inhibited the growth of pathogen by 77.78% followed by 2000 ppm (73.33%). The lowest inhibition of *M. phaseolina* was recorded in minimum concentration of crude extract (250 ppm) by 15.56% (Table 2) (Figure 7). The antimicrobial activity of crude ethyl acetate extract of endophytic fungi, *Penicillium* sp. isolated from wheat was tested against *Fusarium oxysporum* f.sp. *albedinis* recorded the inhibition zone of 19 mm and *Phytopthora infestans* (14 mm) by agar well diffusion assay [36]. Moreno et al. [37] evaluated the inhibitory effect of antifungal protein from *A. giganteus* against *Botrytis cinerea* by well diffusion assay. Goutam et al. [38] extracted the crude metabolites from *A. terreus* JAS-2 and screened for antifungal activity by disc diffusion method. Nystatin and Actidione are the two bioactive metabolites derived from *Phoma* sp. isolated from *Fucus serratus* and the fungus exhibited clear inhibition zone of 20 and 50 mm respectively against the *Microbotryum violaceum* [39]. Abdallah et al. [40] studied the effect of *Aspergillus* species bioactive metabolites on the control of *Pythium ultimum*.

3.6 Metabolic Pathway Analysis

Metabolic pathway analysis, a methodology used in metabolic pathway modeling, assesses inherent network properties and identifies meaningful structural and functional units in the metabolic networks [41].

The metabolites identified from this study were submitted to KEGG pathway database to obtain the pathway details. Totally, 68 pathways were identified. The pathways were classified into five categories namely metabolism, cellular processes, organismal systems, drug development and others. Amid all, the metabolism was the premier one which contributed 13 pathways. In this analysis, the top three classes in metabolism were biosynthesis of secondary metabolites, lipid metabolism. Pathways are required for the aintenance of homeostasis within organisms. The amount of metabolites in a pathway is regulated by the requirements of cells and the availability of the substrates. The end product of a pathway might be used immediately, participate in another pathway or be stored for later use [42].

Yi et al. [43] reported that the metabolic pathway analysis became significant for evaluating intrinsic network characteristics in biochemical reaction network reconstruction. Current applications of metabolic pathway analysis involved in identifying the enzyme for the desired production, identifying pathways of optimal production, determining non-redundant pathways for drug design, and genome comparisons by alignment of pathways for missing genes identification.

The analysis revealed that many of the pathways were grouped under the category of biosynthesis of secondary metabolites included indole alkaloid biosynthesis, glucosinolate biosynthesis, biosynthesis of antibiotics and flavonoid biosynthesis. Several pathways namely fatty acid biosynthesis, arachidonic acid metabolism,

biosynthesis of unsaturated fatty acids, primary bile acid biosynthesis and steroid hormone biosynthesis were classified into lipid metabolism which shown that lipid molecules are vital for energy storage in cells and maintains the viability and flexibility of biological membrane (Figure 8). The majority of fungal species had oleic acid, palmitic acid and linoleic acid as the major acid with stearic acid, linolenic acid, arachidonic acid and palmitoleic acid as the minor ones [44,45].

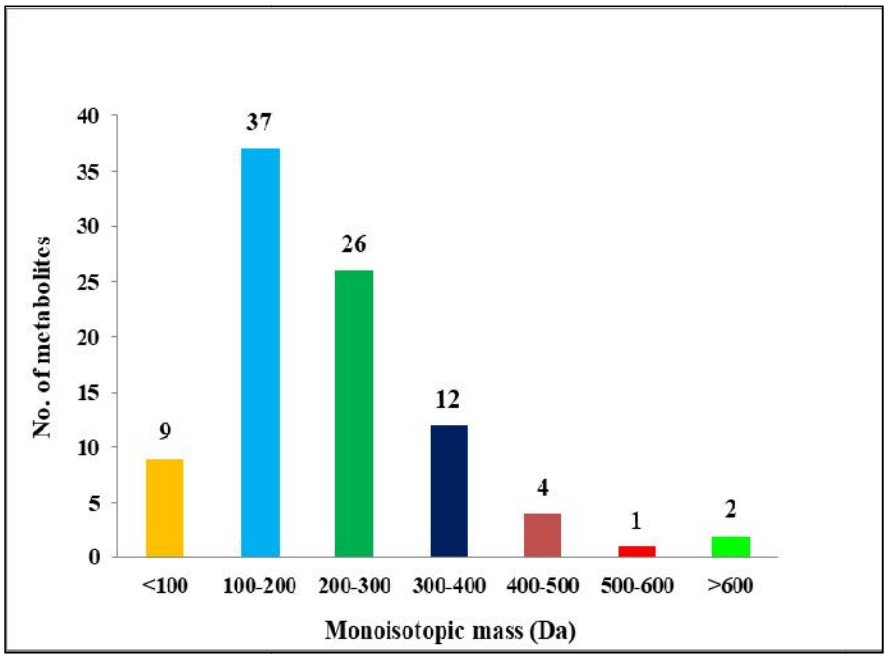


Figure 4. Monoisotopic mass of the identified metabolites

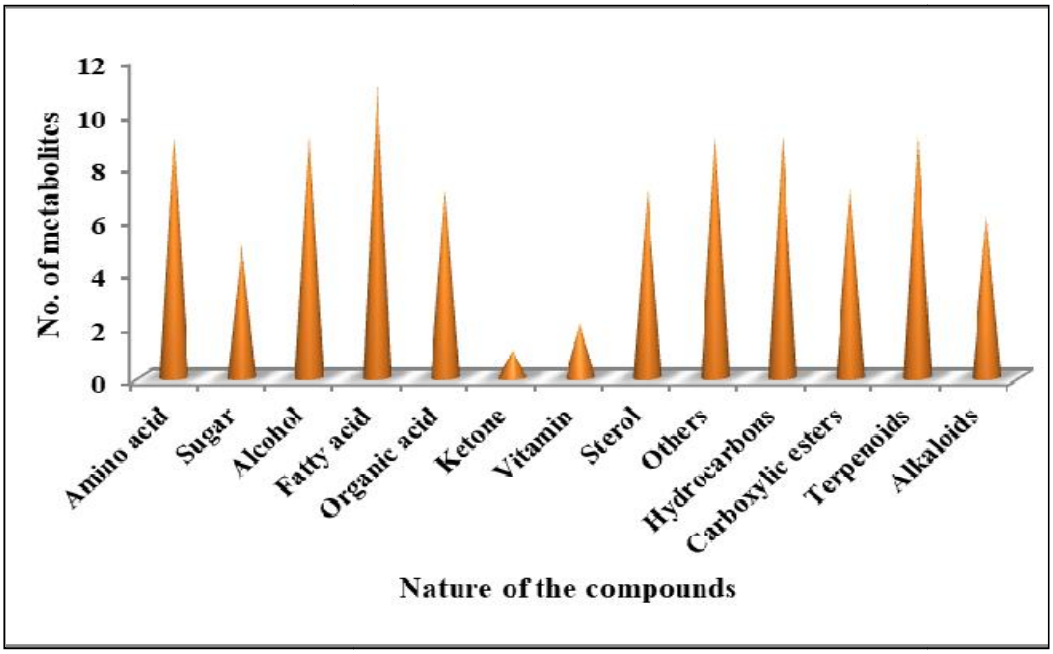
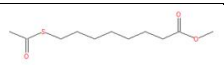
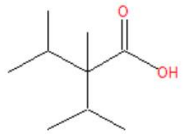
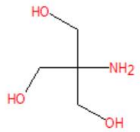
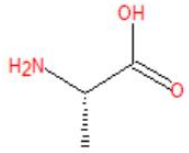
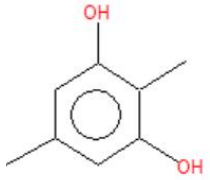
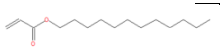


Figure 5. Classifications of the metabolites identified from *A. terreus*

Table 1. Compounds identified from *A. terreus* (ENF12) by GC-MS analysis

S. no.	Retention time (minutes)	Peak area percentage	Compound name	Molecular weight	Molecular formula	Molecular structure	Biological function	KEGG pathway ID	Reference
1.	7.345	0.926	Acetylthiooctanoic acid	232	$C_{11}H_{20}O_3S$		Antifungal Antimicrobial Antibacterial	Ko01210	Gehan et al. (2009)
2.	7.440	1.487	Butanoic acid	158	$C_9H_{18}O_2$		Antimicrobial	Ko01230	Granados-Chinchilla et al. (2016)
3.	8.345	7.283	Tromethamine	121	$C_4H_{11}NO_3$		Antibacterial Anticancer agent	Ko00590	Patel et al. (1997)
4.	8.561	0.517	L-Alanine	89	$C_3H_7NO_2$		Antibacterial	Ko04974	Shneine et al. (2017)
5.	9.796	7.916	1,3-Benzenediol	138	$C_8H_{10}O_2$		Anti-oxidant Antibacterial	Ko00361	Yang et al. [25]
6.	15.008	2.226	Dodecyl acrylate	240	$C_{15}H_{28}O_2$		Antimicrobial	-	-

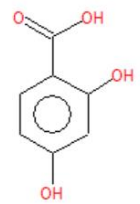
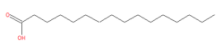
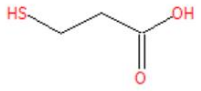
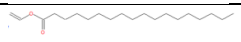
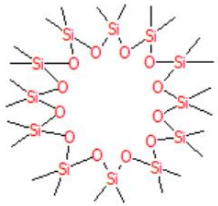
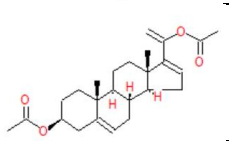
7.	18.129	3.963	Benzoic acid	154	$C_7H_6O_4$		Antibacterial	Ko01063	Wilson et al. (2004)
8.	20.430	1.106	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$		Anti-inflammatory Antioxidant Nematicidal Pesticidal Antimicrobial	Ko01040	Aparna et al. (2012) Kumar et al. (2010)
9.	21.456	0.830	Propanoic acid	106	$C_3H_6O_2S$		Antifungal Antibacterial	Ko01120	Canli et al. (2016)
10.	24.752	0.643	Octadecanoic acid	310	$C_{20}H_{38}O_2$		Antifungal, Antitumor activity, Antibacterial	Ko00061	Hsouna et al. (2011) & Gehan et al., (2009)
11.	28.463	7.774	Tetracosamethyl-cyclododecasiloxane	888	$C_{24}H_{72}O_{12}Si_{12}$		Anti-oxidant Antimicrobial	-	Esmaeili et al., (2012)
12.	29.299	6.020	5,16,20-Pregnatriene	398	$C_{25}H_{34}O_4$		Antifungal	-	Mickymaray and Alturaiki (2018)

Table 2. Bioassay of crude metabolites of *A. terreus* against *M. phaseolina* by agar well diffusion assay

Isolate	Radial growth (mm)						Growth inhibition (%)					
	4000 ppm	2000 ppm	1000 ppm	500 ppm	250 ppm	Control	4000 ppm	2000 ppm	1000 ppm	500 ppm	250 ppm	Control
A. terreus (ENF12)	20.00[a]	24.00[ab]	39.00[b]	59.00[c]	76.00[d]	90.00[e]	77.78	73.33	56.67	34.44	15.56	0.00
SE(d)	**1.22**											
CD (0.05)	**2.58**											

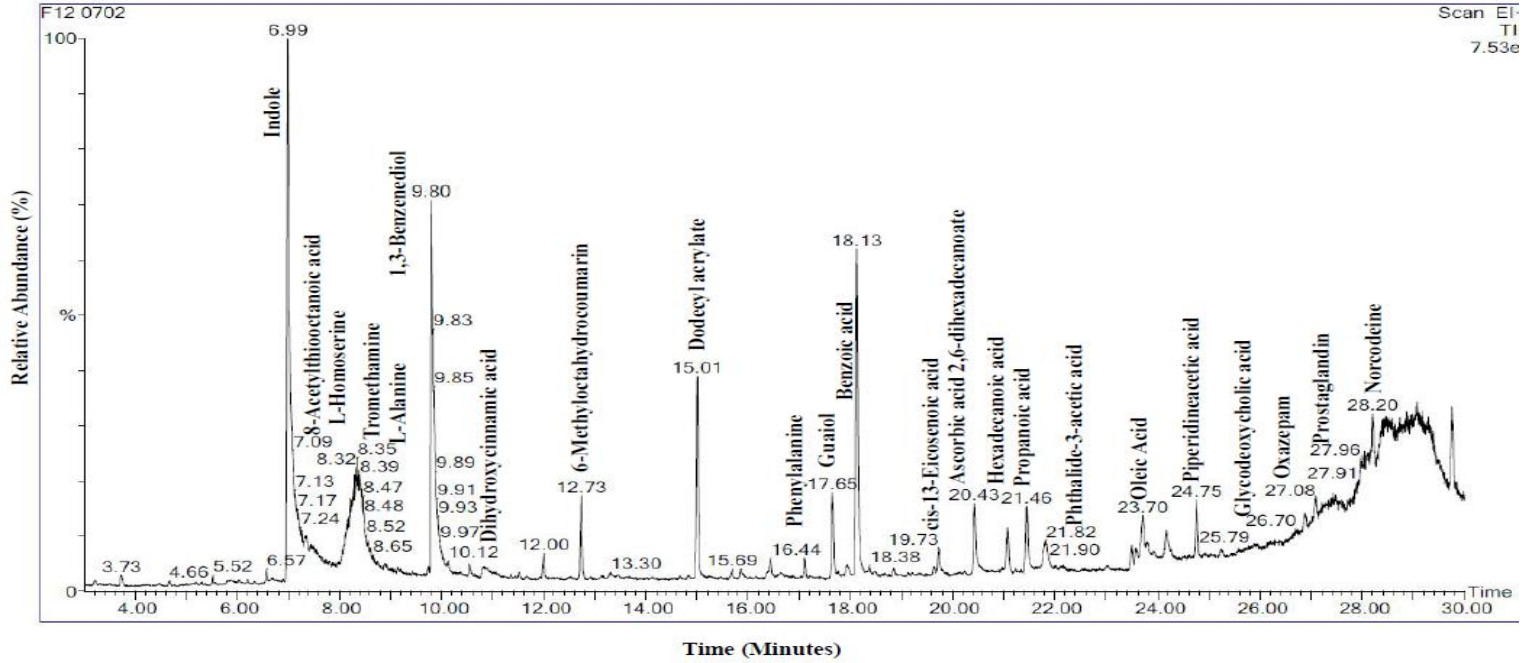


Figure 6. GC-MS chromatogram of secondary metabolites from *A. terreus* extract

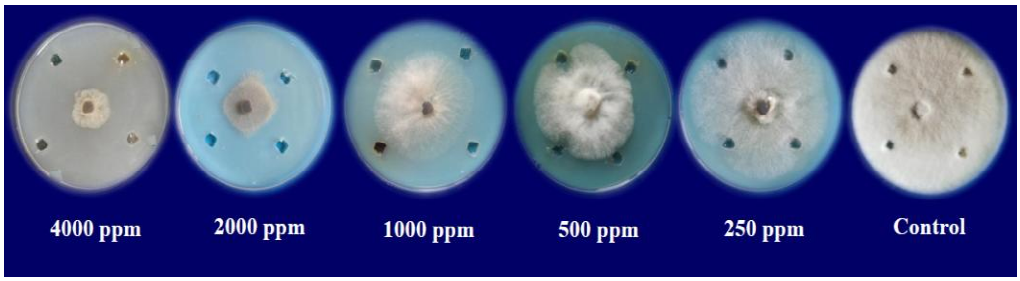


Figure 7. *In vitro* screening of crude metabolites against *M. phaseolina* by agar well method

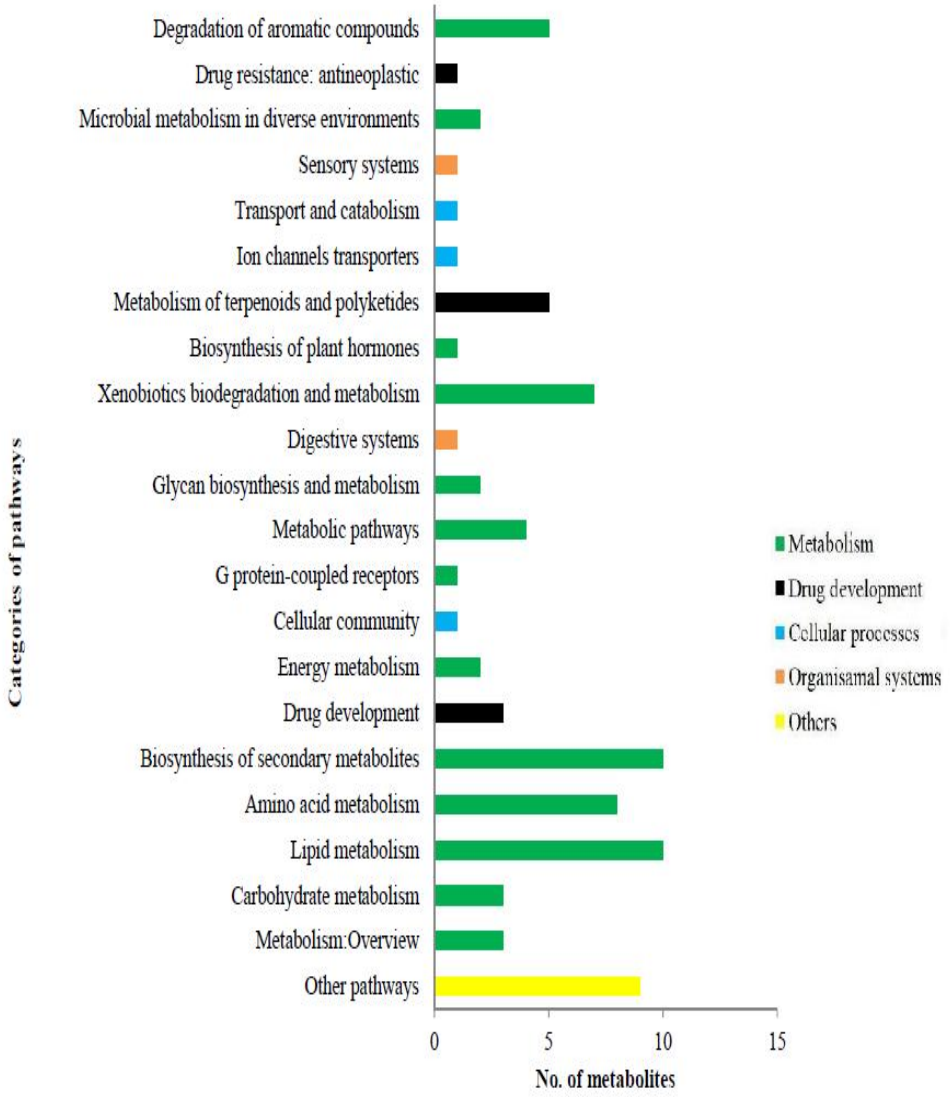


Figure 8. Classes of pathways in which the identified metabolites are involved

The fatty acids, phospholipids, sphingolipids, glycerolipids and sterols were common lipid molecules in cells, while saccharolipids, prenol lipids, and polyketides were mainly found in bacteria, fungi, and plants [46]. Phospholipids, which were the most abundant lipids in cells, comprised 40–60% of lipids in eukaryotic cells [47].

Besides, nearly eight pathways were convened under the class of amino acid metabolism that comprises tyrosine, alanine, aspartate, glutamate, phenylalanine metabolism and vitamin digestion and absorption. The pathway analysis results suggested that the amino acids were significant for protein synthesis, growth and sporulation. The antifungal protein (AFP) and α-sarcin were isolated from *Aspergillus giganteus.* AFP is a highly basic polypeptide of 51 amino acids with a high content of cysteine, tyrosine, and lysine residues [37]. In this study, the methane and sulfur metabolism were emanated under energy metabolism.

The possible functions of known metabolites in relation with host plants were summarized with relevant earlier reports. Lack of plant defense reactions against endophytic fungi [48], as well as the ability of endophytes to produce bioactive metabolites mimicking those produced by their respective host plants [49,50] also indicate the possibility of a more sustained or evolved relationship between the endophytic fungi and their host plants. Hence, the amino acids reported in this study tyrosine, alanine, aspartic acid, glutamic acid were as such as reported in mulberry by several workers [51-53] suggesting a possible genetic cross talk between the host and the endophytes and moreover the proteins are the important constituents in mulberry leaf vital for silkworm growth and development.

4. CONCLUSION

The endophytic fungi *A. terreus* isolated from mulberry leaf had potential antifungal efficacy against *M. phaseolina*. The crude ethyl acetate extracts of *A. terreus* showed strong inhibitory action against charcoal rot pathogen. The metabolite profile of *A. terreus* was studied and the identified metabolites involved pathways were analyzed. The metabolic pathway analysis revealed that the major metabolites identified from *A. terreus* were also reported in mulberry plants thus showed the mutualistic interaction between the host and endophyte.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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