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# Secondary Metabolite Production and Terpenoid Biosynthesis in Endophytic Fungi *Cladosporium cladosporioides* Isolated from Wild *Cymbopogon martinii* (Roxb.) Wats

**Hemalatha Jayaram [1], Vinutha Marigowda [2] and Kunigal Jagadishchandra Thara Saraswathi [1,*]**

[1] Department of Microbiology and Biotechnology, Jnana Bharathi Campus, Bangalore University, Bengaluru 560056, Karnataka, India; hemalathaj24@gmail.com
[2] Department of Biotechnology and Genetics, Ramaiah College of Arts, Science and Commerce, Bengaluru 560054, Karnataka, India; vinutha_gowda@yahoo.co.in
* Correspondence: tharasaraswathi@bub.ernet.in; Tel.: +98-80-601-063

**Abstract:** Endophytic fungi *Cladosporium cladosporioides* (**F1-MH810309**) and *Cladosporium tenuissimum* (**F2-MN715834**) from the leaf of wild *Cymbopogon martinii* (**MT90507**) were isolated and selected based on the persistent occurrence during different seasons of the year. They were identified based on the morphological features and molecular characterization (ITS sequence), and later deposited at NCBI. Phytochemical studies on **F1**, **F2** and host extracts showed the presence of alkaloids, flavonoids, phenols, terpenoids and tannins. The GC-MS of **F1** extract (control) under the axenic condition revealed compounds like hexadecane, heptadecane,2,4-Ditert-butylphenol, E-14 hexadecenal, geraniol, geranyl acetate and cubenol similar to the host. The GC-MS of **F2** extract (control) revealed metabolites that were unique. Further, both **F1** and **F2** were cultured in the supplementation of different concentrations (5%, 10%, 15% and 20%) of the host plant extract (an-axenic condition). The GC-MS of **F1** extracts (test) exhibited good growth and showed the gradual increased production of terpenoid compounds whereas the **F2** (test) did not show any growth. These compounds such as hyrdoxymenthol, nor-borneol, cedralacetate, α-cyclogeraniol, campesterol, β-cyclogeraniol, linalool oxide,2,3-boranediol, citronellyltiglate and 2,3-pinanediol were produced in a minor quantity and were known as biotransformed forms of the precursor compounds present in the host extract. In comparison, only **F1** was able to produce terpenoids similar to the host species both in axenic and an-axenic conditions. Hence from the current study, the endophytic fungus **F1** isolated from wild *C. martinii* for the first time can serve as a better resource for the bioprospection of an important terpenoid and its metabolites.

**Keywords:** *Cymbopogon martinii*; endophytic fungi; *Cladosporium cladosporioides*; *Cladosporium tenuissimum*; terpenoids; biotransformation



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## 1. Introduction

*Cymbopogon martinii* (Roxb.) commonly called "Rosha" grass belongs to the *Rusae* series and serves as an important species of Cymbopogon, yielding essential oil rich in geraniol [1,2]. The species is found widely distributed in south India and exists in two distinct forms referred to as "motia and sofia" varieties producing quality essential oil [3,4]. The essential oil of *C. martinii* possesses significant traditional medicinal properties and finds extensive usage in the treatment of rheumatism, arthritis, lumbago and spasms past from ancient times [5]. Furthermore, because of its potential activity it is used in preventing chronic diseases like cancer, diabetes and coronary heart diseases as well as showing antimicrobial, antifungal, antiviral, anthelmintic, antioxidant and cytotoxicity [6,7]. These compounds are produced against biotic and abiotic stress and help in defense mechanisms, communication and attracting pollinators [8]. The essential oil compounds of *C. martinii* have been extensively used in aromatic industries in the prepa-

ration of perfumes, soaps, cosmetics, food and beverage industries. These essential oil compounds show high biodegradability, volatility and negligible toxicity and are replacing the synthetic metabolic compounds in aromatic industries [9]. Even the extracts of Cymbopogons have been known to possess biological activities such as antimicrobial, antioxidant, anti-hypercholesterolaemic potential, and anti-inflammatory and antimutagenic activities [10–12] and are commonly used in food preservation, pharmaceuticals and natural medicines [13].

Wild plants species growing in typical agro-geo climatic conditions offer unique niches and lodge a variety of fungi within them [14]. These large groups of endophytic fungi are ubiquitously found asymptomatically associated with the plant tissues during their life cycle without negatively affecting the host [15–17] and are variable depending on components, plant genotype, growth stage, physiological status, tissue type and other environmental conditions [18]. They help the host plant adopt to various biotic and abiotic stresses and produce secondary metabolites including alkaloids, flavonoids, phenols, phenylpropanoids, quinines, indole derivatives, steroids, amines and terpenoids maintaining specific bioactivities [15,19,20]. The endophytic fungi associated with the host are identified using traditional taxonomic means and molecular tools like flanking internal transcribed spacers (ITS). They are also known to be a repository of secondary metabolites [17,21] possessing various biological activities, which are used in pharmaceutical applications [22,23].

The association of endophytic fungi with aromatic and medicinal plants is considered to be the prominent source for producing volatile organic or terpenoid compounds [24,25] and they show potential bioactivity and are hence used in pharmaceutical industries [26,27]. The biosynthetic potential of endophytes in producing terpenoid compounds similar to the host plant has gained a lot of commercial importance. Some of the known terpenoid compounds such as Taxol, HupA, 2-Carene, 5,8-diol, bisabolene and cadinene are produced from endophytic fungi along with other secondary metabolites [28,29]. A more intriguing and inexplicable issue with many endophytes that has to be critically evaluated is their ability to produce host metabolites, which can be harnessed on a large scale for potential use in diverse areas [19]. In the present study, the endophytic fungi isolated from wild *Cymbopogon martinii* were studied for the biogenesis of common terpenoid compounds between them and the biotransformation of plant metabolites into commercially useful terpenoids.

## 2. Materials and Methods

### *2.1. Collection and Authentication of Plant*

The wild genotype of *Cymbopogon martinii* was collected from Devarayana Durga hills, Tumkur, Karnataka. The plant was identified and authenticated from Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD), Ministry of AYUSH, Bengaluru, under the accession no. SMPU/RARIMD/BNG/2019-20/352/RRCBI-**1052**. The herbarium (**AC-25/2021)** and seeds were deposited at ICAR- National Bureau of Plant Genetic Resources (NBPGR), Pusa, New Delhi and the DNA barcoding sequence of the plant was deposited in GenBank NCBI under accession no. **MT90507**.

### *2.2. Plant Extraction Method*

The powdered leaf sample (50 g) of the plant was mixed with 150 mL ethyl acetate solvent and kept in a shaker at 120 rpm for 48 h. The extract was filtered through Whatman filter paper grade no. 1. The filtrate obtained was dried at 40 °C using a rotary vacuum evaporator. The residue was dissolved in 1 mL of ethyl acetate and the extract was used for the GC-MS analysis. Furthermore, the powdered leaf (50 g) of the plant was used as the host plant extract for supplementing in the broth culture of endophytic fungi by subjecting it to 48 h of shaking in distilled water and filtering it through Whatman filter paper grade no. 1.

### *2.3. Isolation and Characterization of Endophytic Fungi*

The leaf samples of wild *C. martinii* were collected and rinsed in distilled water, blot dried and cut into small pieces (10–15 mm length). The leaves were surface sterilized by immersing in Tween-20 for 5 min, followed by two times wash with distilled water; 70% ethanol (3 min), two times wash with distilled water; 4% sodium hypochlorite solution (3 min) and washed thoroughly 4–5 times with distilled water to remove traces of surfactants [30]. The edges of the leaves were trimmed and placed onto the petri plates containing potato dextrose agar (PDA) media and incubated at room temperature for 8–9 days. The endophytic fungi emerging after fourteen days in the culture media were transferred to fresh PDA to obtain the pure culture. The morphological characters of the endophytic fungi were studied based on the macroscopic and microscopic observations.

### *2.4. Characterization of Endophytic Fungi*

The genomic DNA extraction from endophytic fungi was carried out by following the [31]. Extracted DNA was subjected to PCR amplification using forward (ITS1-F CTTGGTCATTTAGAGGAAGTAA) and reverse (ITS4-R TCCGTAGGTGAACCTGCGG) primers. The PCR products were run on agarose gel (0.8%) and the bands were purified using the Nucleo-pore PCR Clean-up Gel Extraction kit (Genetix Biotech Asia Pvt. Ltd., Delhi, India). The amplified DNA was subjected to sequencing using the 3130XL genetic analyzer (Applied Biosystems, Waltham, MA, USA). The DNA sequences of endophytic fungi were aligned using Clustal W and Bio Edit tool version 7.0 and the phylogenetic tree was constructed. The DNA sequences of endophytic fungi were deposited in the NCBI Gen Bank.

### *2.5. Extraction of Secondary Metabolites*

The endophytic fungi were mass cultured using 250 mL of potato dextrose broth (PDB) without supplementing the host plant extract (control—axenic culture) and incubated at room temperature for 21 days under static conditions. The broth culture was filtered through Whatman filter paper grade no. 1 and the filtrate obtained was subjected to extraction using ethyl acetate as the solvent system (1:2). The mixture was subjected for 20 min and was kept aside until two immiscible layers were formed. The ethyl acetate layer was collected using a separating funnel. The extract was concentrated under reduced pressure and evaporated to dryness at 40 °C using the rotary vacuum evaporator, further mixed with 1 mL of ethyl acetate before storing at 4 °C.

The endophytic fungi supplemented with host plant extract (test—an-axenic culture) (5%, 10%, 15% and 20%), were mass cultured using 250 mL of PDB medium under static conditions and incubated at room temperature for 21 days. The broth culture was filtered through Whatman filter paper grade no. 1 and the filtrate obtained was subjected to extraction using ethyl acetate as the solvent system (1:2). The mixture was subjected to mixing for 20 min and was kept aside until two immiscible layers were formed. The extract was concentrated under reduced pressure and evaporated to dryness at 40 °C using the rotary vacuum evaporator and mixed with 1 mL of ethyl acetate before storing at 4 °C. The experiments were conducted in three replicates.

### *2.6. Phytochemical Analysis*

The plant and fungal extracts (**F1** and **F2**) were analyzed for the presence of phytochemical compounds using standard methods [32,33] and screened for the presence of secondary metabolites.

Alkaloids (Wagner's reagent): About 1 mL plant extract was treated with 4–5 drops of Wagner's reagent and observed for the formation of a reddish-brown precipitate. Wagner's reagent is a mixture of 1.27 g of iodine and 2 g potassium iodide in 100 mL of water [34].

Tannins: The fungal crude extract was treated with alcoholic FeCl3 reagent. A bluish-black color, which disappears on the addition of a drop of dilute H2SO4 was followed by the formation of yellowish-brown precipitate [35].

Saponins (foam test): About 6 mL of water was added to 2 mL of extract in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam confirming saponins' presence [36].

Terpenoids (Salkowski's test): About 1 mL of chloroform was added to 2 mL of extract. A reddish-brown precipitate is formed immediately on the addition of a few drops of concentrated HCl [37].

Quinones: About 2 mL of the fungal extract was treated with concentrated HCl. The formation of yellow precipitate or coloration indicated the presence of quinones [38].

Phenols: About 2 mL of fungal extract was taken and mixed with aqueous 5% FeCl3 and observed for deep blue or black color formation [39].

Flavonoids: About 0.5 mL of crude extract was taken in a test to which 5–10 drops of diluted HCl and a small piece of zinc were added and the solution was boiled for a few minutes. Reddish-pink or brown color precipitate indicates the presence of flavonoids [40].

### *2.7. Gas Chromatography and Mass Spectroscopy-(GC-MS)*

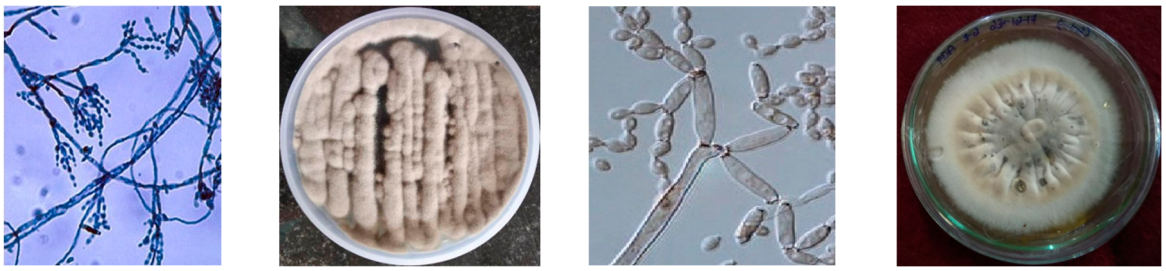
The GC-MS analysis of the extracts of endophytic fungi and the host plant was carried out at Kerala Forest Research Institute (KFRI), Kerala. The analysis was carried out on Shimadzu GC-MS gas chromatogram QP2010S using two capillary columns (0.25 mmID), one coated with dimethylsiloxane and the other with Carbowax 30M. Nitrogen was used as a carrier gas at 65.2 kpa inlet pressure and organized the temperature between 26 to 28 °C for 6 °C/min under the dimethylsiloxane column. The split ratio used was 1:50. Helium was used as a carrier gas (1 mL/min) along with the ion source temperature of 20 °C. The identification of the compounds was made by analogizing the relative retention indices of the peaks with literature data 3 ± 10 and peak development co-injection with authentic original samples. Mass spectral data were compared against NIST05 (National Institute of Standards and Technology, Washington, DC, USA), WILEY 8 and FFNSCI.3 (Flavour and Fragrance Natural and Synthetic Compounds) libraries for the identification of bioactive compounds.

## 3. Results

### *3.1. Isolation and Identification of Endophytic Fungi*

A total of eight isolates of endophytic fungi such as Alternaria species, Aspergillus species, Fusarium species, Trichoderma species, Curvularia species and Cladosporium species were isolated from the inoculants at different seasons of the year. Of the eight isolates, two endophytic fungi such as *Cladosporium cladosporioides* (**F1**) and *Cladosporium tenuissimum* (**F2**) were isolated consistently at different seasons of the year and hence selected for further studies. The morphological studies and molecular characterization confirmed that the fungi were the *Cladosporium* species belonging to the *Dothideomycetes* group of *Cladosporiaceae* family. Endophytic fungi **F1** produced olivaceous colonies with diffused aerial mycelia, woolly-mat finished texture, in reverse of its olive-black color. Conidiophores were straight solitary, unbranched terminal or lateral mycelia bearing 8–9 conidia in chains. Conidia were limoniform, ovoid, light brown and aseptate. Whereas **F2** produced velvety, olive gray with white margins, reversed with its reddish-white color; conidiophores were straight, solitary, unbranched, terminal mycelia bearing 3–4 conidia in chains. Conidia were limoniform, ovoid, aseptate and stained blue. The results of the ITS sequences from the genomic DNA were submitted to NCBI Gen Bank with accession nos. **F1-MH810309** and **F2-MN715834,** respectively (Figures 1 and 2). The phylogenetic tree for the same was constructed using the Clustal W tool (Figure 3).

**Figure 1.** Wild genotype of *Cymbopogon martinii* (**MT905074**).



(**a**) (**b**)

**Figure 2.** Morphological identification of endophytic fungi (**F1** and **F2**) isolated from wild *C. martinii* (leaf). (**a**): Endophytic fungus **F1 (*Cladosporium cladosporioides*-MH810309)** showing gray colored mat bearing cladospores stained with lactophenol cotton blue; (**b**): Endophytic fungus **F2 (*Cladosporium tenuissimum*-MN71583**) showing gray-white colored mat bearing cladospores stained with lactophenol cotton blue; the scale of the Micrograph: 40× magnification.

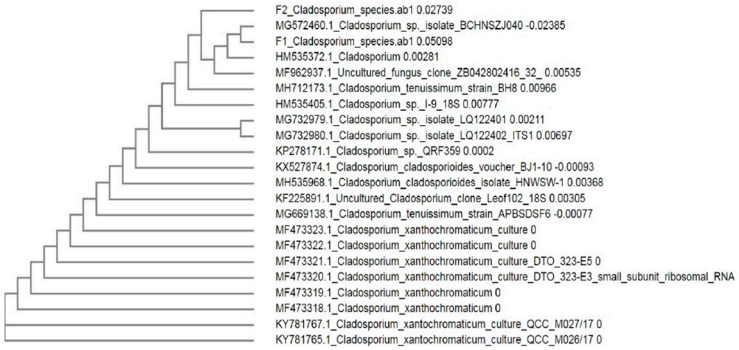



**Figure 3.** Dendrogram showing phylogenetic relationship between endophytic fungi (**F1** and **F2**) on the basis of ITS sequencing. Phylogenies were inferred by neighbor-joining analysis and trees generated using MEGA 6.0 software. Numbers at branch points indicate bootstrap values. The scale bar represents estimated differences in nucleotide sequence.

### *3.2. Phytochemical Screening of Endophytic Fungi*

The phytochemical analysis of fungal extracts (**F1** and **F2**) revealed the presence of alkaloids, flavonoids, phenols, terpenoids and tannins (Table 1).

**Table 1.** Phytochemical analysis of the extracts from endophytic fungi and host plant.

| Sl No. | Test | Host Plant Extract (*C. martinii*) | PDB (Control) (without Supplementation of Plant Extract) | | PDB (Test) (with Supplementation of Plant Extract) |
|---|---|---|---|---|---|
| | | | *Cladosporium cladosporioides* (F1) | *Cladosporium tenuissimum* (F2) | *Cladosporium cladosporioides* (F1) |
| **1** | Alkaloid | +ve | +ve | +ve | +ve |
| 2 | Flavonoid | +ve | +ve | +ve | +ve |
| 3 | Phenol | +ve | +ve | +ve | +ve |
| 4 | Tannin | +ve | +ve | −ve | +ve |
| 5 | Terpenoid | +ve | +ve | +ve | +ve |
| 6 | Quinine | +ve | −ve | −ve | −ve |
| 7 | Essential oil | +ve | +ve | −ve | +ve |

(+) Presence, (−) Absence.

### *3.3. Analysis of Secondary Metabolites (GC-MS)*

The GC-MS analysis of **F1** (control—axenic culture) showed the presence of important compounds like hexadecane (19.4%), octadecane (20.99%), eicosane (10.69%), 2,4-ditert-butyl-phenol (9.34%), hexadecanal (9.01%), heptadecanal (6.78%), tricosane (5.23%), geraniol (1.2%), geranyl acetate (0.91%) and cubenol (0.39%). **F2** (control—axenic culture) showed the presence of compounds like 4,5,7-trihydroxy-2-octanoic acid (42.26%), 3-(tert-butyl di methyl silyl) oxyiminobutan-2-one (12.48%), 1,2-benzenedicarboxylic acid (6.24%), cyclo- octanone (7.01%) and hexadecane (4.59%) (Figure 4; Table 2). The GC-MS analysis of the host plant extract also revealed the presence of compounds such as geranyl acetate (21.44%), geraniol (5.45%), heptadecane (30.39%), hexadecane (10.95%), cubenol (9.81%), bornyl acetate (3.73%), tridecane (4.39%) and beta-caryophyllene epoxide (3.09%) in higher percentages along with the production of other compounds.

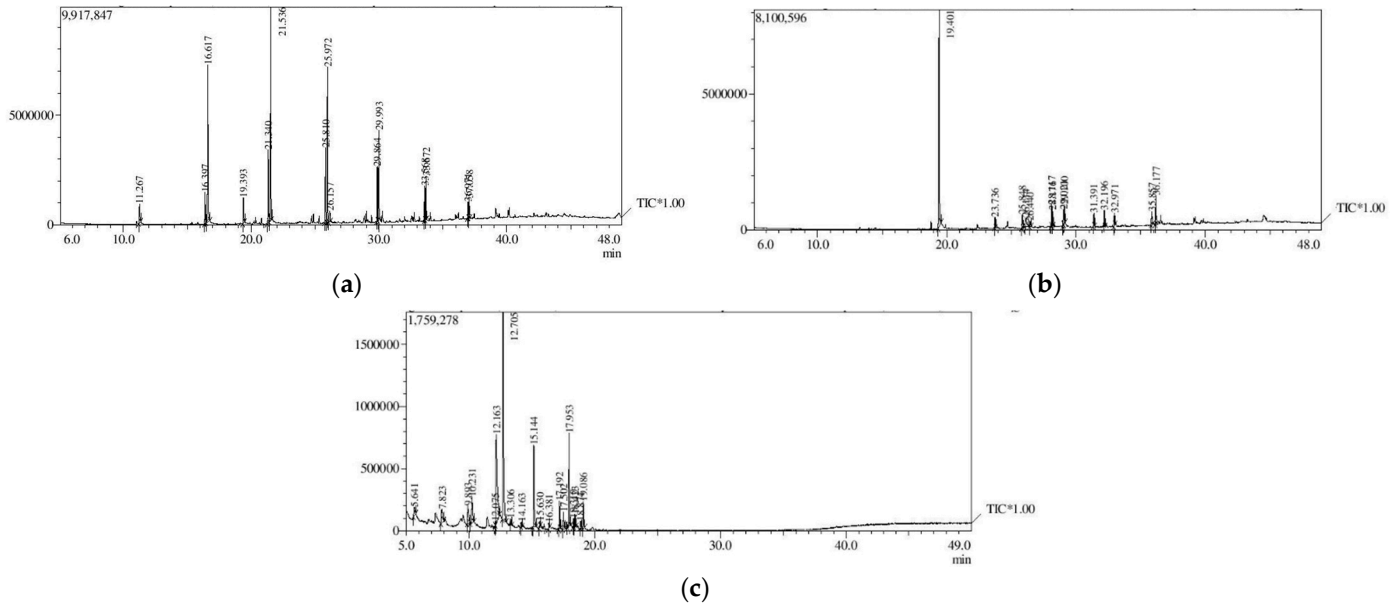



**Figure 4.** Gas chromatogram of ethyl acetate extract (**a**). Host plant (**b**). Endophytic fungus (**F1**) (**c**). Endophytic fungus (**F2**).

**Table 2.** GC-MS analysis of the extracts of endophytic fungi (**F1a** and **F2**) without supplementation of host plant extract.

| Sl No. | Compound | Area% | | |
|---|---|---|---|---|
| | | Extract of Plant *C. martinii* | Extract of Endophytes | |
| | | | F1a | F2 |
| **1** | **Tetradecane** | 2.08 | 0.97 | 2.12 |
| 2 | Dodecane | 2.88 | - | - |
| 3 | 1S-Endo-bornyl acetate | 3.73 | - | - |
| 4 | Tridecane | 4.39 | - | - |
| **5** | **Geraniol** | **5.45** | **1.2** | - |
| **6** | Farnesane | 0.34 | - | - |
| **7** | **Geranyl acetate** | **21.44** | **0.91** | **-** |
| 8 | **Heptadecane** | **30.39** | **3.17** | **-** |
| 9 | (-)-Beta-caryophyllene | 0.63 | - | - |
| **10** | Decane, 2,3,5,8-tetramethyl- | 0.44 | - | - |
| 11 | **Hexadecane** | **10.95** | **19.4** | **4.59** |
| 12 | Gamma-cadinene | 0.63 | - | |
| 13 | 2-(3-Isopropenyl-4-methyl-4-vinylcyclohexyl)-2-propanol | 0.50 | - | - |
| 14 | (-)-Beta-caryophyllene epoxide | 3.09 | - | - |
| **15** | Tridecane | 2.07 | - | - |
| 16 | **Cubenol** | **9.81** | **0.39** | **-** |
| 17 | (-)-Guaiol | 0.83 | - | - |
| 18 | Agarospirol | 1.79 | - | - |
| 19 | Beta-eudesmol | 0.94 | - | - |
| 20 | Globulol | 3.07 | - | - |
| 21 | **2,4-Ditert-butylphenol** | - | 9.34 | - |
| **22** | E-14-Hexadecanal | - | 9.01 | - |
| 23 | **Octadecane** | - | **20.99** | - |
| 24 | 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione | - | 2.10 | 1.40 |
| 25 | Heneicosane, 4-cyclohexyl- | - | 2.20 | - |
| **26** | E-15-heptadecenal | - | 6.78 | - |
| 27 | **Eicosane** | - | **10.69** | - |
| 28 | Decane 4-cycloheyl-, 4-cyclohexyl- | - | 1.25 | - |
| 29 | Tricosane | - | 5.23 | - |
| 30 | 1-Heneicosanol | - | 1.96 | - |
| 31 | Tetracosane | - | 2.28 | - |
| 32 | 1,2-Benzenedicarboxylic acid | - | 3.62 | 6.24 |
| 33 | Cyclooctanone | - | - | 7.01 |
| 34 | Cycloheptanone | - | - | 2.66 |
| 35 | 3-Methyl-2-butenoic acid, pentadecyl ester | - | - | 2.61 |
| 36 | Valeric acid, 2-pentadecyl ester | - | - | 1.21 |
| 37 | 3-(tert-Butyl dimethylsilyl) oxyiminobutan-2-one | - | - | 12.48 |
| 38 | Pentadecane | - | - | 3.12 |
| 39 | 4,5,7-Trihydroxy-2-octenoic acid | - | - | 42.26 |
| 40 | Erythro-cis (1,4), trans (1,4)-4,4-dihyrdoxybicyclooctyl | - | - | 9.04 |
| 41 | Nonadecane | - | - | 1.37 |

Test organism **F1** (an-axenic culture) supplemented with different concentrations of the host plant extract (5%, 10%, 15% and 20%) produced a higher number of secondary

metabolites when compared to control (Figure 5; Table 3). With supplementation of 5 mL (5%) of the host plant extract, the fungi produced compounds such as exo-nor borneol (0.15%), cedrol acetate (2.92%), hydroxy menthol (0.11%), nonadecene (12.6%), hexadecenoic acid (20.98%), 1,4-epoxynaphthalene-1(2H)-menthol (1.63%) and more (Table 4); with 10 mL (10%) supplementation it produced 2-hydroxymentol (0.14%), oxo-borneol (0.28%), alpha cyclo geraniol (0.4%), cetonal (0.36%), phthalic acid (14.64%), hexadecenoic acid (7.45%), campesterol (0.41%), 1,4-epoxynaphthalene-1(2H)-menthol (0.55%), gamma-sitosterol (2.22%) and more (Table 5); under 15 mL (15%) it produced 2-hydroxymenthol (0.15%), elemicin (1.26%), cedrane (1.06%), cetonal (0.35%), decanoic acid (11.9%), phthalic acid (21.69%), dibutyl phthalate (11.7%) along with other compounds (Table 6); and 20 mL (20%) supplementation produced limonene (0.4%), beta-cyclo homo geraniol (0.13%), linalool oxide (0.24%), 2,3-bornanediol (1.4%), nor borneol (0.38%), 2,3-pinanediol (0.1%), epiglobulol (0.84%), di isobutyl phthalate (7.9%), hexadecenoic acid (8.03%), phthalic acid (9.23%) and citronellyl tiglate (1.11%) along with many other compounds (Table 7).

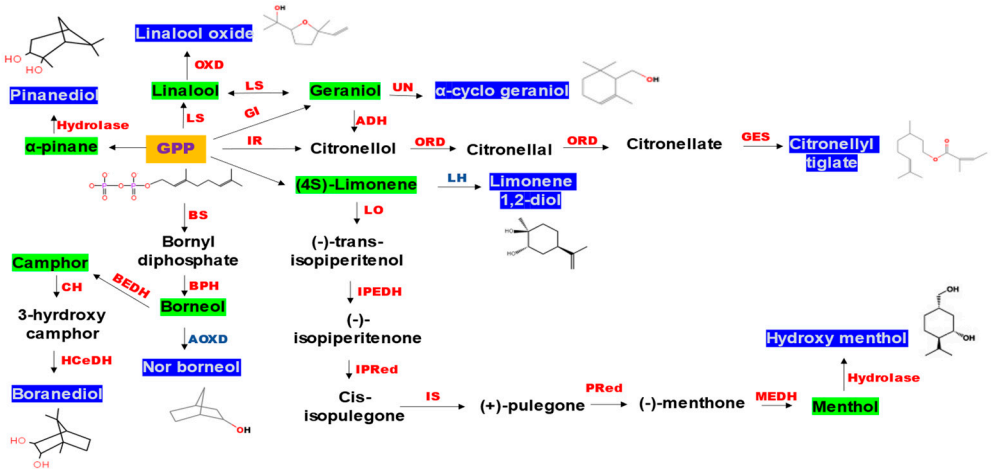



**Figure 5.** Biosynthesis of monoterpenoids in *Cladosporium cladosporioides* (F1-MH810309) with host plant by cross-talk. The green color represents precursor compounds from the plant extract. The blue color represents compounds produced by **F1** in support of the plant extract. Orange color indicates the enzymes involved: GPP geraniol diphosphate, LS linalool synthase, *OXD* oxidase, *GI* geraniol isomerase, *UN* unclear reaction, *ADH* alcohol dehydrogenase, *ORD* oxidoreductase, *GES* geraniol synthase, *BS* bornyl synthase, *BPH* bornyl pyrophosphate hydrolase, *AOXD* asymmetric oxidase, *BEDH* borneol dehydrogenase, *CH* camphor hydroxylase, *HCeDH* hydroxy camphor dehydrogenase, *LO* limonene-3-monooxygenase, *IPEDH* iso-piperitenol dehydrogenase, *IPRed* isopiperitone reductase, *IS* isomerase, *PRed* pulegone reductase and *MEDH* menthol dehydrogenase, *UN* unclear reaction.

**Table 3.** GC-MS analysis of secondary metabolites of endophytic fungi **F1** (*Cladosporium cladosporioides* isolated from wild *C. martnii* with supplementation of host plant extract at different concentrations).

| Sl No. | Compound | Area % | | | |
|---|---|---|---|---|---|
| | | 5 mL | 10 mL | 15 mL | 20 mL |
| 1. | 2-Hydroxy menthol | 0.11 | 0.14 | 0.15 | - |
| 2. | Nor-borneol | 0.15 | 0.28 | - | 0.38 |
| 3. | Cedrane | 0.14 | - | 1.06 | - |
| 4. | Cedral acetate | 2.92 | - | - | - |
| 5. | Alpha cyclo geraniol | - | 0.4 | - | - |
| 6. | Campesterol | - | 0.41 | - | - |

**Table 3.** *Cont.*

| Sl No. | Compound | Area % | | | |
|---|---|---|---|---|---|
| | | 5 mL | 10 mL | 15 mL | 20 mL |
| 7. | 1,4-Epoxynaphthalene-menthol | - | 0.55 | - | - |
| 8. | Elemicin | - | - | 1.26 | - |
| 9. | Cetonal | - | - | 0.35 | - |
| 10. | Limonene 1,2-diol | - | - | - | 0.4 |
| 11. | Beta-cyclo homo geraniol | - | - | - | 0.13 |
| 12. | Linalool oxide | - | - | - | 0.24 |
| 13. | 2,3-Bornanediol | - | - | - | 1.4 |
| 14. | 2,3-pinanediol | - | - | - | 0.11 |
| 15. | Epiglobulol | - | - | - | 0.84 |
| 16. | Calamenene | - | - | - | 0.66 |
| 17. | Citronellyl tiglate | - | - | - | 1.11 |

**Table 4.** GC-MS analysis of endophytic fungus **F1** (cultured with 5 mL host extract).

| Sl No. | Compound | Area % | RT |
|---|---|---|---|
| 1. | Nonanal | 0.04 | 6.074 |
| 2. | 1,3-Butanediol, diacetate | 0.03 | 6.361 |
| 3. | 3-tert-Butyl-2-pyrazolin-5-one | 0.06 | 8.167 |
| 4. | Nonanoic acid | 0.05 | 8.292 |
| 5. | 1-Tetradecene | 0.08 | 9.964 |
| 6. | **2-hydroxy menthol** | **0.11** | **10.054** |
| 7. | Cyclohexane butanal, | 0.6 | 10.14 |
| 8. | **exo-nor borneol** | **0.15** | **10.283** |
| 9. | Phenol, | 0.23 | 11.542 |
| 10. | Benzoic acid, | 0.05 | 11.75 |
| 11. | 1,7-Dioxaspiro [5.5] undec-2-ene | 0.06 | 11.918 |
| 12. | Dodecanoic acid | 0.18 | 12.076 |
| 13. | 1-Heptadecene | 0.85 | 12.431 |
| 14. | Octadecane | 0.63 | 12.512 |
| 15. | Oxirane | 1.44 | 12.816 |
| **16.** | **Cedrane** | **0.14** | **13.547** |
| 17. | Tridecanal | 0.08 | 13.859 |
| 18. | E-8-Methyl-9-tetradecen-1-ol acetate | 0.92 | 14.334 |
| 19. | 1-Nonadecene | 12.61 | 14.662 |
| 20. | Spiro [4.5] decan-7-one | 0.29 | 15.334 |
| **21.** | **Cedrol acetate** | **2.92** | **16.019** |
| 22. | Widdrol hydroxyether | 1.43 | 16.295 |
| 23. | n-hexa decanoic acid | 20.98 | 16.447 |
| 24. | Oleic Acid | 3.32 | 17.276 |
| 25. | 1-Octadecanol | 0.27 | 17.568 |

**Table 4.** *Cont.*

| Sl No. | Compound | Area % | RT |
|---|---|---|---|
| 26. | Octadecanoic acid | 7.9 | 18.307 |
| 27. | Decanoic acid, | 0.37 | 19.100 |
| 28. | 1-Tetracosanol | 1.38 | 20.234 |
| 29. | Octadecanal | 0.3 | 20.553 |
| 30. | 2-palmitoyl glycerol | 1.72 | 21.228 |
| 31. | 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl | 1.32 | 21.553 |
| 32. | 1-Triacontanol | 1.86 | 21.805 |
| 33. | 10,12,14-Nonacosatriynoic acid | 0.72 | 22.263 |
| 34. | Abietic acid | 1.3 | 22.385 |
| 35. | Retinoic acid | 0.43 | 22.544 |
| 36. | Glyceryl mono acetate | 3.99 | 22.629 |
| 37. | Stearin | 0.97 | 22.787 |
| 38. | Squalene | 0.93 | 23.568 |
| 39. | Corticosterone 21-acetate | 0.63 | 23.796 |
| 40. | Trans-beta-lonone | 0.58 | 23.934 |
| 41. | Dehydroergosterol 3,5-dinitrobenzoate | 0.92 | 24.848 |
| 42. | Cholest-5-en-3-ol (3. beta) | 0.24 | 26.134 |
| 43. | Cycloeucalenol | 0.36 | 26.73 |
| 44. | Stigmasta-4,7,22-trien-3. alpha-ol | 0.88 | 30.15 |
| 45. | Hexa decanoic acid | 1.42 | 30.562 |
| 46. | Rhodopin | 0.83 | 31.201 |
| 47. | 1,4-Epoxynaphthalene-1(2H)-methanol | 1.63 | 32.554 |
| 48. | Ergosta-8,24(28)-dien-3-ol, 14-methyl-, (3. beta,5. alpha) | 1.79 | 33.576 |

Red color indicates the compounds with high percentages. Bold indicates similar compounds present in the essential oil of the host plant. RT: retention time.

**Table 5.** GC-MS analysis of endophytic fungus **F1** (cultured with 10 mL host extract).

| Sl No. | Compound | Area % | R Time |
|---|---|---|---|
| 1. | 1-Tetradecene | 0.1 | 9.951 |
| 2. | 2-hydroxymenthol | 0.14 | 10.041 |
| 3. | 2-hydroxybutyl acrylate | 0.45 | 10.129 |
| **4.** | **Oxo-borneol** | **0.28** | **10.271** |
| 5. | 1-Heptadecene | 0.93 | 12.418 |
| **6.** | **Alpha-cyclo geraniol** | **0.4** | **14.319** |
| 7. | 1-Nonadecene | 2.12 | 14.649 |
| 8. | 2,11-Dodecadiene | 0.4 | 14.906 |
| 9. | Cetonal | 0.36 | 15.318 |
| 10. | diisobutyl phthalate | 19.28 | 15.547 |
| 11. | l-(+)-Ascorbic acid 2,6-dihexadecanoate | 1.12 | 16.297 |
| 12. | n-Hexadecanoic acid | 7.45 | 16.389 |
| 13. | Dibutyl phthalate | 12.3 | 16.494 |

**Table 5.** *Cont.*

| Sl No. | Compound | Area % | R Time |
|---|---|---|---|
| 14. | Phthalic acid, | 14.64 | 16.69 |
| 15. | Oleic Acid | 6.87 | 18.074 |
| 16. | Benzenedicarboxylic acid | 2.51 | 17.017 |
| 17. | 9-Tricosene | 2.52 | 18.522 |
| 18. | 1-Tetracosanol | 1.22 | 20.219 |
| 19. | 1-Triacontanol | 0.61 | 21.793 |
| 20. | Oleoyl chloride | 0.86 | 22.617 |
| 21. | Tetrapentacontane | 0.49 | 23.255 |
| 22. | Tetracosamethyl-cyclododecasiloxane | 1.92 | 24.362 |
| 23. | Dehydroergosterol 3,5-dinitrobenzoate | 0.36 | 24.838 |
| 24. | Dithianone | 0.37 | 25.048 |
| 25. | Cyclononasiloxane | 0.88 | 25.359 |
| 26. | Stigmast-5-en-3-ol | 1.64 | 25.909 |
| 27. | Isoindole-1,3(1H,3H)-dione | 0.78 | 27.085 |
| **28.** | **Campesterol** | **0.41** | **27.241** |
| 29. | Chondrillasterol | 0.57 | 27.581 |
| 30. | gamma-Sitosterol | 2.22 | 28.297 |
| **31.** | **1,4-Epoxynaphthalene-1(2H)-methanol** | **0.55** | **32.525** |

Red color indicates the compounds in high percentages. Bold indicates similar compounds present in the essential oil of host plant.

**Table 6.** GC-MS analysis of endophytic fungus **F1** (cultured with 15 mL host extract).

| Sl No. | Compound | Area % | R Time |
|---|---|---|---|
| 1. | Propanoic acid | 0.35 | 5.478 |
| 2. | Malic acid | 0.18 | 5.68 |
| 3. | Butanedioic acid | 0.13 | 5.856 |
| 4. | 2-Octenoic acid | 0.4 | 7.513 |
| 5. | 2-Decenoic acid | 11.95 | 9.538 |
| 6. | 1-Tetradecene | 0.12 | 9.958 |
| 7. | 2-Hydroxymenthol | 0.15 | 10.05 |
| **8.** | **Elemicin** | **1.26** | **12.069** |
| 9. | 1-Hexadecanol | 1.46 | 12.438 |
| 10. | Cyclodecanamine | 3.46 | 12.629 |
| 11. | D-Galactose | 0.54 | 12.945 |
| 12. | **Cedrane** | **0.1** | **13.547** |
| 13. | 9,10-Dimethyltricyclo [4.2.1.1(2,5)] decane-9,10-diol | 0.87 | 14.333 |
| 14. | 1-Nonadecene | 1.54 | 14.658 |
| 15. | 2-Cyclohexen-1-one | 0.18 | 14.83 |
| 16. | 3.alpha,7 beta-Dihydroxy-5 beta,6 beta-epoxycholestane | 0.1 | 15.127 |
| 17. | 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol | 0.04 | 15.24 |

**Table 6.** *Cont.*

| Sl No. | Compound | Area % | R Time |
|---|---|---|---|
| 18. | **Cetonal** | **0.35** | **15.327** |
| 19. | Di isobutyl phthalate | 7.35 | 15.556 |
| 20. | Phthalic acid | 21.69 | 16.236 |
| 21. | n-Hexadecanoic acid | 9.16 | 16.417 |
| 22. | Dibutyl phthalate | 11.78 | 16.505 |
| 23. | Oleic acid | 7.73 | 17.261 |
| 24. | Acetophenone | 0.49 | 18.384 |
| 25. | Pentaerythrityl tetrachloride | 0.36 | 18.815 |
| 26. | 4-Bromobutanoic acid | 0.29 | 19.057 |
| 27. | Pentaerythrityl tetrachloride | 0.91 | 19.826 |
| 28. | 1-Tetracosanol | 1.02 | 20.226 |
| 29. | Carbamazepine | 0.33 | 20.651 |
| 30. | 1-Triacontanol | 0.55 | 21.795 |
| 31. | glyceryl mono oleate | 2.36 | 22.621 |
| 32. | 1-Hexacosanol | 0.35 | 23.255 |
| 33. | 1-Nonadecene | 2.01 | 18.53 |

Red color indicates the compounds in high percentages. Bold indicates similar compounds present in the essential oil of host plant.

**Table 7.** GC-MS analysis of endophytic fungus **F1** (cultured with 20 mL host extract).

| Sl No. | Compound | Area % | R Time |
|---|---|---|---|
| 1. | Glycerine | 0.11 | 4.264 |
| 2. | 2-Nonadecanone | 0.13 | 7.756 |
| **3.** | **Limonene glycol or P-menth-8-ene** | **0.4** | **9.191** |
| **4.** | **Beta-cyclohomo geraniol** | **0.13** | **9.487** |
| **5.** | **Linalool oxide** | **0.24** | **9.56** |
| **6.** | **2,3-Bornanediol** | **1.4** | **10.046** |
| 7. | Cyclohexanebutanal | 1.15 | 10.146 |
| **8.** | **2-Norborneol** | **0.38** | **10.278** |
| 9. | Alpha-campholene aldehyde | 0.18 | 10.418 |
| 10. | Lomustine | 0.13 | 10.637 |
| 11. | Cyclohexanol | 0.14 | 10.754 |
| 12. | 10-Methyl-8-tetradecen-1-ol acetate | 0.18 | 11.021 |
| 13. | 4-Nonanone | 0.22 | 11.245 |
| 14. | Benzoic acid, 4-ethoxy-, ethyl ester | 0.11 | 11.742 |
| 15. | 1,7-Dioxaspiro [5.5] undec-2-ene | 0.58 | 11.908 |
| 16. | 1-Heptadecene | 1.12 | 12.427 |
| 17. | 6-Acetyl-4,4,7-trimethylbicyclo [4.1.0] heptan-2-one | 0.42 | 12.735 |
| **18.** | **2,3-pinanediol** | **0.1** | **13.03** |
| 19. | Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl- | 0.67 | 13.544 |
| 20. | 1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl- | 0.23 | 14.18 |

**Table 7.** *Cont.*

| Sl No. | Compound | Area % | R Time |
|---|---|---|---|
| 21. | 1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl) | 7.3 | 14.337 |
| 22. | 1-Nonadecene | 3.41 | 14.662 |
| 23. | 2,5,9-Tetradecatriene, 3,12-diethyl- | 0.23 | 14.839 |
| 24. | 2-Dodecen-1-yl (-) succinic anhydride | 1.57 | 14.998 |
| **25.** | **Epiglobulol** | **0.84** | **15.131** |
| 26. | 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol | 1.28 | 15.246 |
| 27. | Spiro [4.5] decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl- | 0.42 | 15.326 |
| 28. | Diisobutyl phthalate | 7.91 | 15.556 |
| 29. | 3-Methyl-5-(1,4,4-trimethylcyclohex-2-enyl) pentan-1-ol | 1.72 | 15.666 |
| **30.** | **Calamenene** | **0.66** | **15.780** |
| 31. | 2,6-Bis-(acetamido)-pyridine | 0.93 | 16.115 |
| 32. | Phthalic acid, isobutyl 2-pentyl ester | 1.21 | 16.231 |
| 33. | Widdrol hydroxyether | 3.35 | 16.302 |
| 34. | n-Hexadecanoic acid | 8.03 | 16.429 |
| 35. | Dibutyl phthalate | 4.65 | 16.504 |
| 36. | 1,2-Benzenedicarboxylic acid, butyl decyl ester | 1.53 | 16.579 |
| 37. | Phthalic acid, isobutyl octadecyl ester | 9.23 | 16.709 |
| 38. | 2,4,7,14-Tetramethyl-4-vinyl-tricyclo [5.4.3.0(1,8)]t | 1.38 | 16.945 |
| 39. | 1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester | 0.79 | 17.024 |
| 40. | 2-naphthalene butanoic acid | 5.94 | 17.161 |
| 41. | 2-Methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pentane-2,3-diol | 0.35 | 17.336 |
| 42. | Phthalic acid, isobutyl 2-pentyl ester | 0.56 | 17.416 |
| 43. | 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl]cy | 1.11 | 17.516 |
| **44.** | **Citronellyl tiglate** | **1.11** | **17.761** |
| 45. | Oleic acid | 5.41 | 18.110 |
| 46. | Octadecanoic acid | 1.68 | 18.288 |
| 47. | Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl- | 1.6 | 18.390 |
| 48. | 1-Tricosanol | 2.78 | 18.529 |
| 49. | Beta-sitosterol | 0.17 | 18.867 |
| 50. | 1-Tetracosanol | 1.67 | 20.226 |
| 51. | Oxirane, hexadecyl | 0.15 | 20.546 |
| 52. | Hexadecanoic acid, | 0.41 | 21.223 |
| 53. | 1,2-Benzenedicarboxylic acid, | 3.68 | 21.548 |
| 54. | 1-Triacontanol | 1.06 | 21.795 |
| 55. | 10,12,14-Nonacosatriynoic acid | 0.24 | 22.259 |
| 56. | Glyceryl monooleate | 1.73 | 22.620 |
| 57. | 1-Hexacosanol | 0.77 | 23.252 |
| 58. | 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- | 0.21 | 23.559 |

**Table 7.** *Cont.*

| Sl No. | Compound | Area % | R Time |
|---|---|---|---|
| 59. | Dehydroergosterol 3,5-dinitrobenzoate | 0.44 | 24.836 |
| 60. | 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, | 0.18 | 26.716 |
| 61. | Ergosta-5,8,22-trien-3-ol, (3 beta,22E) | 0.54 | 27.008 |
| 62. | Anthraergostatetraenol | 0.13 | 27.239 |
| 63. | Stigmasta-7,25-dien-3-ol, (3 beta, 5 alpha) | 0.11 | 27.831 |
| 64. | 17-Pentatriacontene | 0.14 | 28.588 |
| 65. | Rhodopin | 0.96 | 31.174 |
| 66. | 1,4-Epoxynaphthalene-1(2H)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3 | 0.72 | 32.520 |
| 67. | Cholesta-8,14-dien-3-ol, 4,4-dimethyl- | 0.43 | 33.532 |

Red color indicates the compounds in high percentages. Bold indicates similar compounds present in the essential oil of host plant.

## 4. Discussion

During the present study, six endophytic fungal species like *Alternaria*, *Aspergillus*, *Fusarium*, *Trichoderma*, *Curvularia* and *Cladosporium* were isolated from wild *Cymbopogon martinii* (**MT90507**). Furthermore, the Cladosporium species were studied (*C. cladosporioides* and *C. tenuissimum*) based on their consistent growth throughout the year. The *C. cladosporioides* is also reported as endophytic fungi in the genus Cymbopogon such as *C. citratus*, *C. flexuosus* and *C. caesius* [41,42] expressing tremendous ecological adaptability [43].

The phytochemical analysis of *C. cladosporioides* (**F1**) and *C. tenuissimum* (**F2**) showed the presence of secondary compounds belonging to alkaloids, flavonoids, phenols, terpenoids and tannins which are similar to those of the host plant (wild *C. martinii*). The GC-MS analysis of ethyl acetate extracts of endophytic fungi **F1** and **F2** (control-without adding host plant extract) revealed the presence of compounds like 1-octen-3-ol, 3-methyl-1-butanol, hexanol, trans-2-octanol and hexadecanol in similarity with the host plant extract. These compounds are known to possess antimicrobial, anti-inflammatory and antioxidant activities [44]. Sharing of the above mentioned similar secondary metabolite compounds between endophytic fungi and the host plant has been reported earlier [9,45]. Likewise, common compounds were shared between endophytic fungi **F1** (control) and the host plant such as octadecane, eicosane, hexadecane, heptadecane and 2,4-ditert-butyl phenol coming under alkanes producing in higher percentages along with the production of geranyl acetate, cubenol and geraniol belonging to monoterpenoids. The production of such an alkane group of compounds has been reported in endophytic fungi including also *C. cladosporioides* and *C. tenuissimum* and these are known to possess potential biological activities [46–48]. Under in vitro conditions, the endophytic fungus **F1** having a pre-existing terpenoid pathway could produce compounds similar to that of the host plant. The transcriptome studies made on endophytic fungus *C. cladosporioides* expressed the presence of a pathway for terpenoid biosynthesis [49–51].

The endophytic fungus **F1** (test) produced a large number of plant derived secondary metabolites belonging to hydrocarbons, hydrocarbons esters and monoterpenoid. There are reports on *C. cladosporioides* as endophyte producing compounds belonging to a monoterpenoid group [26,52–55]. Studies made on *C. cladosporioides* isolated from *Taxus brevifolia, T. baccata*, *H. serrata* and Tobacco *species* expressed the production of monoterpenoid compounds similar to that of host plants [28,29,56,57]. Supplementation of the host plant extract at different concentrations (5–20%) to broth culture resulted in variation in monoterpenoid compound production in the endophytic fungi (Table 3). The **F1** (test) might utilize the precursor components from the host plant extract and convert them into biotransformed monoterpenoid compounds (Figure 5), and similar studies have been made earlier (Vijay Lakshmi et al., 2019, 56]. These monoterpenoid compounds were produced in low percent-

ages due to the poor catalytic activity of plant derived enzymes in the endophytic system (Sun et al., 2019). The monoterpenoid compounds and their precursors are known to express antioxidant, anticancer and antimicrobial activities [7,58,59]. The present study revealed that the endophytic fungus **F1**[1] (*C. cladosporioides*) and the host plant share common Mevalonate (MVA) and Phenylpropanoid (PP) pathways as also documented in earlier studies [7,50].

The endophytes show a strong relationship and long-term association with the host plant and thus mimic the plant for secondary metabolite production [60]. The relationship shared between endophyte and plant could lead to coevolution and adaptation [61], which results in horizontal gene transfer, establishes cross-talk between gene clusters and helps in the biosynthesis of common terpenoid compounds [43,51,62]. There are several reports on revealing the capability of *C. cladosporioides* for adaptation within the host plant environment thus producing secondary metabolites similar to the host plant [9,21,43,63,64]. The in vitro cultured endophytic fungus **F2** (*C. tenisimum*) could not produce secondary metabolites similar to **F1** (*C. cladosporioides*) or the host plant as the metabolites were produced for their basic survival activity within the host system and thus were not involved in cross-talk with the plant [65].

In the current study, the endophytic fungus **F1** has expressed high adoptability and a stronger relationship with the wild genotype of *C. martinii* and has been successful in producing secondary metabolites similar to the host plant. Further, the mechanism of the molecular interactions happening during secondary metabolite production between endophytic fungi and the host plant needs elaborate studies using '*omic*' approaches. The interaction of the endophytic fungi with the host plant explains the vital activities taken up by the sessile natured plant.

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