Volatile organic compound profile of *Trichoderma koeningii*, inhabiting in marine soil: An explorative studing using GC-MS

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Abstract

Trichoderma koeningii, a fungus known for their potential as biocontrol agent against many fungal diseases, was isolated from marine soil, cultured *in vitro* in PDA broth prepared using seawater and freshwater (1:1v/v) and the filtrate was subjected to Gas chromatography - Mass spectrum analysis. The analysis revealed the presence of eight volatile organic compounds (VOCs) viz., dodecanoic acid, tetradecanoic acid, pentadecanoic acid, 9-hexadecanoic acid, n-hexadecanoic acid, oleic acid, 1,2-benzene dicarboxylic acid, diisooctyl ester and squalene with1.16, 2.19, 1.51, 3.51, 21.82, 5.32, 28.84 and 35.65 % peak areas respectively. The compounds identified are discussed in the light of their biological activity.

Key words: Biocontrol, GCMS profile, Marine soil fungi, *Trichoderma koeningii,* Volatile organic compounds(VOCs).

INTRODUCTION

Volatile Organic Compounds (VOCs) are the chemically diverse small molecules emitted by plants and microbes, including fungi. The VOCs are having the bio-physical property, *viz.*,diffusiveness, and this property enables their easy movement through liquid and air spaces and pores in the soil matrix and there by influences the functions of communication, interaction, and defense in their surroundings (Schulz-Bohm *et al*., 2017).

Considering that over 5 million fungal species are predicted to live on earth (Blackwell, 2011), in the recent years, studies have been conducted by several research groups intensively on the ecological and biological roles of fungal VOCs (Penuelas *et al*., 2014; Schenkel *et al*., 2015; Werner *et al*., 2016). On the other side, as the VOC profiles are reported to species specific, the studies have also been conducted to use the VOC as a tool for the identification of species (Guo Yuan, 2019). However, in all, emission profiles of around 600 microbial and fungal species have been obtained (Schulz-Bohm *et al*., 2017) so far.

The genus *Trichoderma* comprises 254 species and 2 varieties (Bissett *et al*., 2015) and they are living ubiquitous in soils of terrestrial environs. Most of the members of this genus are well established to compete against pathogenic microbes and to promote the plant fitness in terrestrial ecosystems (Bitas *et al*., 2013). Approximately 480 different VOCs have been detected from *Trichoderma* species altogether (Siddiquee, 2014).

Among the different species of *Trichoderma,* a few species are reported from the marine realm and *T. koeningii* is one among them. Though this fungus is well known for their use in agriculture and forestry as an effective and alternative biopesticide to control fungi-induced plant diseases and to promote the plant growth, its role and potential in the marine soils are poorly understood and hence the present study.

MATERIALS AND METHODS

To isolate the fungi from saline habitat, samples were collected from Nagapattinam coast, Tamilnadu, India, between 10°15′ to 11°30′ N and 79°39′ to 79°55′ E, following the standard mycological procedures. Dilution plating technique(Warcup, 1950) was adopted to isolate the fungi from soils using PDA medium prepared with 50% seawater (1:1 v/v seawater of 30 ppt salinity :tap water) and supplemented with the antibiotics, streptomycin (1% solution @16ml/L). Based on the colony morphology, *Trichoderma koeningii* was picked up among the colonies of fungi on PDA plates and confirmed its identity by referring the standard works (Ellis, 1976). The fungus was sub-cultured in the same PDA

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medium repeatedly, ensured its axenity and maintained as pure culture at 5°C.

The pure isolate of *T. koeningii* was cultured in Potato Dextrose Broth at 25°C, in darkness for three weeks in Erlenmeyer flasks. It was filtered twice with Whatman No.1 filterpaper and then through a Seitz filter. This filtrate was taken in to a separation funnel and added with ethylacetate(@ 100ml/L), shaken well for 3 min and the solvent and aqueous layers were separated. The ethyl acetate layer of the culture filtrate was used for GC-MS analysis (Watts *et al*., 1988).

Volatile components were identified by GC-MS using a column Elite-1 (100% Dimethyl poly siloxane), 30 ´ 0.25 mm ´ 1 mm 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 per min, split 10:1, and injected volumes were 2 µl. The column temperature was maintained initially at 110°C for 2 min(hold) followed by increases upto200°C at the rate of 10°C /min (nohold), upto 280°C at the rate of 5°/ min-9 min (hold). The injector temperature was 250°C and this temperature was held constant for 36 min.

Table1. Compounds separated from culture filterate of *T. koeningii*, their RT, molecular formula, molecular weight and peakarea% using GC-MS analysis.

S. No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	11.2	Dodecanoicacid	C12H24O2	200	1.16
2	13.7	Tetradecanoicacid	C14H28O2	228	2.19
3	15.1	Pentadecanoicacid	C15H30O2	242	1.51
4	16.3	9-Hexadecanoicacid	C16H30O2	252	3.51
5	16.6	n-Hexadecanoicacid	C16H32O2	256	21.82
6	19.3	Oleicacid	C18H34O2	282	5.32
7	25.2	1,2-Benzenedicarboxylicacid, diisooctylester	C24H38O4	390	28.84
8	29.6	Squalene	C30H50	410	35.65

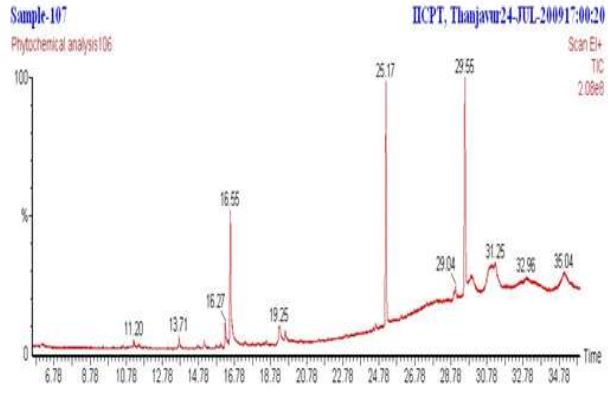

Fig.1. GC-MS spectrum of the culture filterate of *Trichoderma koeningii*

The electron impact energy was 70eV Julet, line temperature was set at 200°C and the source temperature was set at 200°C. Electron impact (EI) mass scan(m/z) were recorded in the 45-450aM U range. Using computer searches on the NISTVer.2.1M S data library and comparing the spectrum obtained through GC-MS, the compounds present in the crude sample were identified.

RESULTS AND DISCUSSION

The GC-MS analysis of the culture filtrate of *T. koeningii* yielded eight prominent peaks (Fig. 1) with retention time 11.20, 13.71,15.10, 16.27, 16.55, 19.25, 25.17 and 29.55 min indicating the presence of eight volatile organic compounds *viz*., dodecanoic acid, tetradecanoic acid, pentadecanoic acid, 9-hexadecanoic acid, n-hexadecanonic acid, oleic acid, 1,2-benzenedicarboxylicacid, diisooctyl ester and squalene, respectively (Fig. 1) Their molecular weight, molecular formula and percentage of the peak are given in table -1.

The components identified from the profile are belonging to the category, fatty acids and known widely from different plant (Speert *et al*., 1979; Farina Mujeeb *et al,* 2014; Rigoberto, 2017) and microbial (Gottilied *et al*.,1978; Zaki *et al*., 1983; Griffith *et al*., 2007; Ushadevi, 2008) sources.

The biological activity of all the components identified in the study are already reported by different workers. The activity ranges from one to many. Tetradecanoicacid is known to have antifungal, antioxidant, cancer preventive, nematicide, hypercholesterolemic and Lubricant properties, while Pentadecanoic acid(Farina Mujeeb *et al,* 2014) and Oleic acid (Speert *et al*., 1979) are reported with antibacterial properties and 9-hexadecenoic acid(Rahman *et. al*., 2014) and Dodecanoicacid (Parsaeimehr and Lutzu, 2016) are reported to have antimicrobial property. The components that are sharing major peak are as are n-Hexadecanoic acid, and it is known to have Antioxidant, Pesticide, Flavor, 5-Alpha reductase inhibitor, anti fibrinolytic,hemolytic,lubricant, nematicide and antialopecic properties (Rigoberto, 2017) and 1,2-Benzenedicarboxylicacid diisooctyl ester, to possess fungi toxic and cyto toxic activities (Rahman and Anwar, 2006).

Squalene was identified with 35.65% peak area, and among all, in the present study, it is a notable one. It is an intermediate of sterol biosynthesis in wide variety of organisms (Spanova and Daum, 2011) and it is found in varying contents among the organisms (Bhattacharjee *et al*., 2001). Squalene play a significant role in fungus and plant interactions. By regulating the genes, it increases the plant responses against fungi and there by protects the plants from fungal

infections (Lindo *et al*., 2020). Squalene has been used as a natural antioxidant, adjuvant for vaccines, dietary supplement and skin moisturizer for therapeutic, pharmacological and cosmetic purposes (Reddy and Couvreur, 2009;Spanova and Daum, 2011) and shark liver serves as be the principal source squalene.

Thus the volatile organic compounds identified in the study from marine isolate of *T. koningii* are known to act against micro-organisms and regulate the host against the fugal pathogens. Hence this fungus can be explored for the possible use as biocontrol agents in the saline influenced soils. Further, squalene production reported in the present study leads to think about *T. koningii* as an alternative source for squalene for commercial production which in turn will ease the sharks from hunting pressures for squalene.

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