



## Studies on the Compounds and its Antifungal Potentiality of Fungi Isolated from Sugarcane Field Soils of Harur, Dharmapuri District

Ramasamy Mahalingam\*, Varatharaju Ambikapathy, Annamalai Panneerselvam

P.G. and Research Dept, of Botany and Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi, Thanjavur (DT), TN, India.

\*Corresponding author's E-mail: [lingammahalingam@gmail.com](mailto:lingammahalingam@gmail.com)

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### ABSTRACT

Totally 41 species belonged to 17 genera were recorded. A preliminary screening of all the species isolated from soils were made for antifungal (antagonistic) activity against *Ceratocystis paradoxa*, a known soil borne fungal pathogen. Among the species tested. The *Hypocrea virens* inhibited the pathogenic fungus to the maximum both in dual culture and in food poisoning technique. Gas Chromatography mass spectrum analysis of methanol extract of the filtrates of *Hypocrea virens* revealed the presence of five compounds represents five major peaks. The peaks correspond with 3 – Ethyl – 3 Methylheptane, Undecane, Heptadecane, 9 – hexyl, Octadecane, 3 – Ethyl – 5 – (2- ethylbutyl), Tetratetracontane.

**Keywords:** Fungal extract, compounds, GC – MS.

### INTRODUCTION

Inventory of biologically active compounds has gained importance in recent years. This involves the process such as extraction, separation, purification and characterization. The compound resulted in the process are proved to their structure and effective activity against various pathogens. Moreover the compounds (both extra and intra – cellular) are considered as a key factor to identify the organisms.

Now a days the diseases are managed with the application of chemical pesticides. Use of chemical pesticides causes environmental problem, as they don't undergo biodegradation so minimizing the application of pesticides has become order of the day. To achieve this goal the biological control methods can be effectively used along with other methods of diseases control. *Trichoderma* sp. is filamentous soil fungus known to be effective biocontrol agents (BCAS) against plant pathogens. Weindling and Emerson, (1936), stated that they could excrete extra cellular compound called gliotoxin. Since then many antibiotics and extra cellular enzymes were isolated and characterized. Their biocontrol mechanisms were also established.<sup>1,2</sup>

### MATERIALS AND METHODS

#### Fungal Isolates

About 41 species were isolated from Harur sugarcane soil, Dharmapuri Dt, Tamilnadu, India. All these strains were screened for their antifungal activity against pathogenic fungi.

#### Antibiotic Interactions Assay

A preliminary screening was conducted against *C.paradoxa* with all the fungi isolated. Based on this, ten species were selected for the study of antagonistic activity. Colony interaction between the test organism and the soil fungi namely *Aspergillus awamori*, *A.niger*,

*Penicillium citrinum*, *Hypocrea virens*, *Trichoderma glaucum*, *T. harzianum*, *T.hirusta*, *T.koenigii*, *T.viride* and *Trichothecium* sp. was studied invitro in dual culture experiments. In the dual culture experiments *H.virens* inhibited the growth of the pathogen to the maximum extent. Hence, *H.virens* was taken for further studies.

#### Gas chromatography – Mass Spectrum analysis of the culture filtrate<sup>3</sup>

##### Extraction of antifungal compounds

The fungus which showed promising activity against the pathogen was cultured in liquid potato dextrose medium at 24°C in darkness for three weeks. After incubation, the culture was filtered twice through whatman No. 1 filter paper and seitz filter (G.S). To 100 ml of culture filtrate, 10 ml of ethyl acetate was added in a separation funnel (250 ml), shaken well for 3 min and the solvent and aqueous layer were separated. The methanol layer of the culture filtrates was used for further analysis.

##### Gas Chromatography – Mass Spectrometry (GC – MS)

Volatile components were identified by GC – MS using a column Elite – 1 (100% Dimethyl poly siloxane), 30x0.24mm x 1µm 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 2µl. The column temperature was maintained initially at 110°C for 2min (hold) followed by increases up to 200°C at the rate of 5 to– 9 min (hold). The injector temperature was 250°C and this temperature was held constant for 36min. 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 (EX) mass scan (m/z) was recorded in the 45-450 aMU range.



Using computer searches on the NIST ver.2.1MS data library and comparing the spectrum obtained through GC-MS the compounds present in the crude sample were identified.

## RESULTS AND DISCUSSION

Understanding the mechanisms involved in the antagonistic effect of *Trichoderma* sp. against plant pathogen are important in selection of suitable biocontrol agent for effective and safe utilization. Different isolates of *Trichoderma* have various effects of fungal antagonism and on the plant health. The possible mechanism of antagonism employed by *Trichoderma* sp. realized so far include competitions, antibiosis by producing non volatile antibiotics and exploitation.<sup>4</sup>

When the extract of methanol culture filtrate of *H.virens* was subjected to GC – MS analysis to find out the components produced by the fungus, it yielded five prominent peaks with retention time 2.64, 3.02, 4.97, 5.57, 5.57min. The peaks with reaction time 2.64 min, corresponds to 3 – ethyl – 3 – Methylheptane with 34.94% of peak area; 3.02 min, corresponds to the undecane with 36.22% of peak area; 4.97min, corresponds to the Heptadecane, 9 – hexyl with 8.65% of peak area; 5.57min, corresponds to the octadecane, 3 – ethyl – 5 – (2 – ethylbutyl) with 4.49% of peak area and biological activity and chemical structure of phytocompound were identified (Table-1 and Fig-1).

This proved that *Hypocrea virens* is capable of producing many compounds that are produced by many other fungal species. The antimicrobial activity of the 3 – Ethyl – 3 – Methylheptane have already been reported by Ushadevi, (2008) from the marine isolates of *P. lividum* and *T. lignorum*. Thus, these compounds were also isolated in the present investigation, individually and in combination with other compounds such as undecane, Heptadecane, 9 – hexyl, octadecane, 3 – ethyl – 5 – (2 – ethylbutyl) and Tetratetracontane. Thus the present investigation concludes that 3 – Ethyl – 3 – methylheptane along with other compounds would have suppressed the growth of *C.paradoxa*.<sup>5</sup>

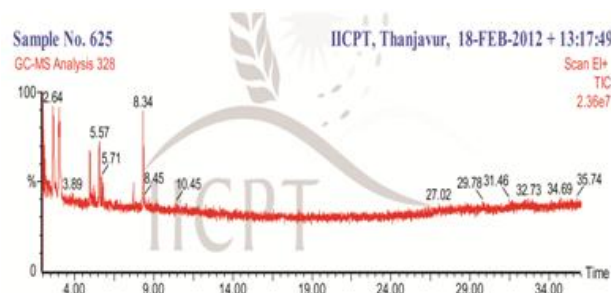
Squalene isolated from *Rhizoctonia solani*, *Aspergillus flavus*, *A. fumigatus*, *Penicillium afrovenetum*, *Phytophthora cinnamomi*, *P. cactorum*, *Pythium graminicola* and *P. ultimum* has been reported by Gottlieb, (1978) and 3 – Ethyl – 3 – Methylheptane from *Penicillium lividum*.<sup>5</sup>

Likewise there are reports on the occurrence of tetradecanoic acid, dodecanoic acid and n – hexadecanoic acid in the extract of head space of *Aspergillus versicolor*, dodecanoic acid and tetradecanoic acid from *P.chrysogenum*.<sup>6</sup> Pentadecanoic acid and oleic acid from *Mortierella alpine* and oleic acid from *Phytophthora cinnamomi*.<sup>7-10</sup>

The phytochemical analysis of *H.virens* was also studied using thin layer chromatography. The results revealed the presence of saponin, flavonoids, sterol, phenol and

alkaloids. In the present study, it was found that alkaloids showed antimicrobial activity. Hence, relatively high antimicrobial activity of *Hypocrea virens* could be attributed to the presence of these compounds.

**Figure 1:** GC-MS chromatogram of fungus *Hypocrea virens*



**Table 1:** GC-MS study

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1.	2.64	3-Ethyl-3-methylheptane	C <sub>10</sub> H <sub>22</sub>	142	34.94
2.	3.02	Undecane	C <sub>11</sub> H <sub>24</sub>	156	36.22
3.	4.97	Heptadecane, 9-hexyl-	C <sub>23</sub> H <sub>48</sub>	324	8.65
4.	5.57	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C <sub>26</sub> H <sub>54</sub>	366	9.29
5.	5.71	Tetratetracontane	C <sub>44</sub> H <sub>90</sub>	618	4.49

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