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STUDIES ON THE BIOACTIVE AND ITS ANTIFUNGAL POTENTIALITY OF FUNGAL ISOLATED FROM SUGARCANE FIELD SOILS OF GUNDALAPATTI, DHARMAPUTI DISTRICT

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ABSTRACT
Totally 36 species belonged be 15 genera were recorded. A preliminary screening of all the species isolated from soils were made for antifungal (antagonistic) activity against *cephalosporium sacchari*, a known soil borne fungal pathogen, Among the species tested. The *Trichoderma harzianum* inhibited the pathogenic fungus to the maximum both in dual culture and in food poisoning technique. Gas chromatography mass spectram analysis of methanol extract of the filtrates of *Trichoderma harzianum* revealed the presence of three compounds represents three major peaks. The peaks correspond with Diacetyl dihydroennein, 3-beta-bromo-5-cholestene, 1-chlorodocosane.

KEYWORDS: Fungal extract, Bioactive 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 enivronmental problem, as the 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 use along with other control 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.[1,2]

MATERIALS AND METHODS
Fungal isolates
About 36 species were isolated from Gundalapatti 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. sacchari* 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 *Aspering flavipes, A. flavas, A. nidulans, Pcnicillum lanosum, P. notatum, Tichoderma harzianum, T. lignorum* and *ustilago scitaminea* was studied invitro in dual culture experiments. In the dual culture experiments *T. harzinaum* inhibited the growth of the pathogen to the maximum extent. Hence, *T.harzianum* was taken for further studies.

Gas chromatography – Mass Spectrum analysis of the culture filtrate

Extraction of antifungal compounds
The fungus which showed promising activity against the pathogen was cultured in liquid potato dextrose medium at 24ºC in darkness for three weeks. After incubation, the

culture was 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 indentified by GC – MS using a column Elite – 1 (100% Dimethyl poly siloxane), 30x0.24mm x 1µm df equipped with GC clarus 500perkin 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 ws maintained initially at 110ºC for 2 min (hold) followed by increases up to 200ºC at the rate of 5 to 9 min (hold). The injector temperature was 250ºC and this temperature ws held constant for 36min. The electron impact energy was 70ev. Julet line temperature was set at 200ºC and the source temperature was set 200ºC. Electron impact (EX) mass scan (m/z) ws 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.

When the extract of methanol culture filtrate of *T. harzianum* was subjected to GC – MS analysis to find out the components produced by the fungus, it yielded threa prominents peaks with retention time 2.136, 6.713, 7.238 min. The peaks with reaction time 2.136 min corresponds to *Diacetyl dihydroennein* with 61.37% of peak area, 6.713 min corresponds to the 3, beta-bromo-5-cholestene with 1.45% of peak area 7.283 min corresponds to the 1-chloradocosane with 0.87% of peak area and biological activity and chemical structure of phytocompound were identified (Table-1 & Fig. I).

This proved that *Trichoderma harzianum* is capable of producing many compounds that are produced by many other fungel species. The antimicrobial activity of the *Diacetyl dihydroennein* 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 Diacetyl dihydroennein, 3,beta-bromo-5-cholestene and 1-chlorodocosanein. Thus the present investigation concludes Diacetyl dihydroennein along with other compounds would have suppressed the growth of *C.sacchari*.

Squalene isolated from *Rhizoctonia solani, Aspergillus flavus, A. fimigatus, Pencillium afroveneturn, Phytophthora cinnamomi, P. cactorum, Phthium graminicola and P. ultimum* has been reported by Gottlieb, (1978) and Diacetyl dihydroennein Penicillium lividum.

Likewise there are reports on the occurrence of tetradeanoic acid, dodecanoic acid and n – hexadecanoic acid in the extract of head speace of *Aspergillus versicolar*, dodecanoic acid and tetradeconoic acid from P.chrysogenum. Pentadecanoic acid and oleic acid from Mortierella alpine and oleic from *Phytophthora cinnamomi*.

The phytochemical analysis of *T.harzianum* was also studied using thin layer chromatography. The reuslts 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 *T.harzianum* could be attributed to the presence of these compounds.

Table 1: GC – MS study.

No.	RT	Name of the compound	Molecular formula	MW	Peak Area No.
1.	2.136	Diacetyl dihydroennein	C24H32O8	448.5	61.37
2.	6.713	3-beta-bromo-5-cholestene	C27H45BR	449.6	1.45
3.	7.283	1-chlorodocasane	C22H45CL	345.1	0.87

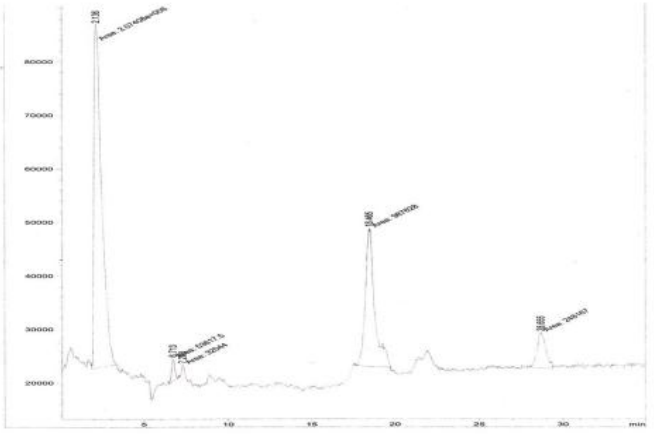

Fig I: Gas Chromatography- Mass spectrometry analysis *T. harzianum*.

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