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Original article

Identification and *in silico* screening of biologically active secondary metabolites isolated from *Trichoderma harzianum*

Abhishek Mishra, Supriya Dixit, Ved Ratan, Mukesh Srivastava, Shubha Trivedi and Yatindra Kumar Srivastava
Department of Plant Pathology, C. S. Azad University of Agriculture and Technology, Kanpur-208002, Uttar Pradesh, India

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Abstract

The genus *Trichoderma* contains more 100 species which are used as bioprotectants for the management of a number of fungal and plant pathogens by virtue of their broad-spectrum action. The present study focuses to evaluate four *Trichoderma* isolates (CA-03, CA-06, CA-07 and CA-08) ability to produce volatile and non-volatile metabolites against *Fusarium oxysporum* f. sp. *capsici* (FOC) and *Colletotrichum capsici* (CC) by using inverse plate technique and poisoned food technique which enables the identification of secondary metabolites from culture filtrate and mycelial mass of potential *Trichoderma* isolates through GC/MS analysis. Furthermore, molecular property and bioactivity score of those secondary metabolites were predicted using molinspiration software. Studies indicated that volatile metabolites from *Trichoderma* isolate code CA-07 (26.2% and 40.9%) was found to be most effective in reducing the mycelial growth followed by CA-06 isolate (22.0% and 31.9%) of FOC and CC, respectively. However, non-volatile compounds from *Trichoderma* isolate code CA-07, followed by CA-06 were effectively inhibiting mycelial growth of tested pathogens at 5%, 10% and 15% concentration. GC-MS analysis was carried out in order to identify active secondary metabolites of potential *Trichoderma* isolates CA-07 and CA-06. A total of 19 and 15 peaks were identified for CA-07 and CA-06, respectively. These compounds are mainly carboxylic acid and fatty acid esters. Among 34 compounds, 12 compounds followed Lipinski's rule of five and can also act as a good binder towards few receptors. 1H-Indene-1,5(6H)-dione, 2, 3, 7, 7a - tetrahydro-7a-methyl-(0.53) was found to have lowest log P value which indicates its good drug likeness score also, it showed highest score towards enzyme inhibitor (0.32) which exhibited antibiotic nature and also used as a drug for treatment of dermatological disorder. Thus, it can be further considered for *in vivo* study for management of FOC and CC.

Keywords: Volatile metabolites, non-volatile metabolites GC-MS analysis, molinspiration, molecular property, bioactivity score, lipinski's rule of five

1. Introduction

Soil is the natural habitat of the majority of beneficial and harmful fungal micro-organisms. Beneficial ones produces antifungal, antioxidant, antibiotic, anti-inflammatory natural metabolites and various lytic enzymes when grown on laboratory culture media. Some of these compounds inhibit the germination of fungal spores and the growth of certain fungal phytopathogens. Therefore, the soil flora could be induced those natural metabolites or enzymes which could be possible to control soil-borne pathogens which and causing wilt, root-rot, damping off, agricultural soil slow and insufficient germination of seeds in different vegetable crops (Cota *et al*., 2007). Chilli (*Capsicum annuum* L.) is one of the most important spice and vegetable crop, cultivated throughout in India but several biotic agents like fungi, bacteria and virus drastically reduced the yield potential and the quality (Egea *et al*., 2002). Wilt and anthracnose of chilli is caused by *Fusarium oxysporum* f.sp. *capsici* (FOC) and *Colletotrichum capsici* (CC), respectively which are reported as the most important fungal pathogen. Due to these diseases, more than 50% crop losses have been reported from different parts of India (Ramchandran *et al*., 2007). The fungal phytopathogens colonized and produce mycotoxin which leads to crop diseases and deterioration of food. Now-a-day, fungal pathogens acquiring resistance to many commonly used chemical preservatives and fungicides. In addition to the yield losses due to fungal growth, their mycotoxin causes risk in animal and human health. Therefore, an effective alternative strategy should be the used which can control fungal growth and, thus overcome the production of mycotoxin. For the management of phytopathogens and their mycotoxin, enhance the usage of potential antifungal biocontrol agents. Fungal based bio control agents are widely used because of their borderer spectrum in terms of disease control and yield (Ouda, 2014). It is believed to be that natural and biologically active drugs are safe for consumption with undesirable toxic side effects (Rahman, 2017). *Trichoderma* spp are considered as potential antagonistic fungi and their secondary metabolites production had been found to be involved in biocontrol activity (Reino *et al*., 2008). *Trichoderma* spp produces over 40 different secondary metabolite which plays significant role in mycoparasitic action. The goal of this work is to obtain the potent *Trichoderma* isolate which are able to produce natural metabolites which plays vital for chilli disease resistance against FOC and CC. Thus, the present study focuses to evaluate

Author for correspondence: Dr. Abhishek Mishra
Department of Plant Pathology, C. S. Azad University of Agriculture and Technology, Kanpur-208002, Uttar Pradesh, India
E-mail: abhishekmishracsa@gmail.com
Tel.: +91-8317013768

the antifungal activity of a *Trichoderma* isolates against FOC and CC by employing various antifungal assays. The study also reports the identification of various volatile and non-volatile secondary metabolites obtained from the isolates by GC/MS analysis. *In silico* study has been done for the prediction of molecular properties and bioactive score of secondary metabolites through molinspiration software.

2. Materials and Methods

Four potential *Trichoderma* isolates, *Trichoderma asperellum* (CA-03), *Trichoderma harzianum* (CA-06 and CA-07) and *Trichoderma longibrachiatum* (CA-08) were selected for present study.

2.1 Effect of volatile compounds of *Trichoderma* isolates on radial growth of fungal pathogens

The effect of volatile metabolites produced by *Trichoderma* spp/ isolates against FOC and CC spp were evaluated by inverse plate technique. A 5 mm mycelial disc of *Trichoderma* isolates and test pathogens were placed in the centre of two separate bottom portions of petriplates containing PDA, and one of the plates was placed in an inverted position over the other. The plates were sealed with parafilm tape and incubated at 28^0C for 8 days. Observation was recorded after one week and proportion of inhibition was calculated. The percent growth inhibition was calculated by using equation

$$I = (C-T)/C \times 100$$

where, C is mycelial growth in control plate, T is mycelial growth in test organism inoculated plate and I is inhibition of mycelial growth.

2.2 Effect of non-volatile compounds of *Trichoderma* isolates against fungal pathogens

The production of non-volatile compounds by *Trichoderma* spp/ isolates were evaluated by poisoned food technique. *Trichoderma* spp/isolates were inoculated in 100 ml sterilized potato dextrose broth and incubated at 25 ± 1°C for 12-14 days. *Trichoderma* mycelium was collected after 12-14 days and filtered through whatman filter paper. Different volumes of filtrates were added to the PDA medium at 40 ± 3°C to obtain final concentrations of 5, 10 and 15. The medium placed into petriplate was inoculated with 5 mm mycelial disc of pathogen at the centre. The plates were incubated at 25 ± 1°C for 7 days. Plates without culture filtrate were maintained which served as a control. Percentage of mycelial growth inhibition was calculated as below:

Percent mycelial growth inhibition = $((A-B)/A) \times 100$

where, A is diameter of fungal colony (mean) in control, B is diameter of fungal colony (mean) with plant extract.

2.3 Extraction and evaluation of secondary metabolites

Trichoderma isolates were grown on potato dextrose broth in and incubated 28°C for 25 days. Afterwards, the content of flasks was filtered through muslin clothes. After filtration, the obtained liquid phase is used for the extraction of volatile/non-volatile metabolites. The method is used for extraction is solvent extraction method in which metabolites were mixed into ethyl acetate at ration of 1:1 (v/v). According to Dubey *et al.* (2011), upper phase of the solvent which contains metabolites were collected through the separating funnel into conical flasks. Ethyl acetate was evaporated from the collected upper phase and further remain residues were dissolved in acetone which was used for GC-MS analysis.

GC-MS analysis was done by Indian Institute of Science Education and Research, Bhopal. The samples were analysed using Agilent 7890A GC with 5975C MS system, used for identification and quantification with oven temperature of 75 °C for 1 min and 30°C/ min to 300°C for 2 min; inlet and transfer line temperature is programmed at 250°C and 290^0C, respectively. The flow rate of helium gas is of 1.3 ml/min. 1 µ; samples were injected under split of 3:1. The ionization mass spectroscopic analysis was done with 70eV. Interpretation of mass spectrum GC-MS analysis is done by matching list of known compound's spectrum with Agilent's GC/ MSD ChemStation, NIST MS Library and NIST's Automated Mass Spectral Deconvolution and Identification Software.

2.4 Prediction of bioactivity

Structures of selected *Trichoderma* secondary metabolites for research work were taken from pubchem database in smiley format and prediction of bioactivity properties and drug-likeness were calculated using Molinspiration tool and comparison was made.

i. Molecular property

Molecular properties of the *Trichoderma* secondary metabolites were calculated using molinspiration and the values were given in Table 1 and Table 2.

ii. Bioactivity scores

The bioactivity scores of the *Trichoderma* secondary metabolites towards GPCR ligand, and as ion channel modulator, nuclear receptor ligand, kinase inhibitor, protease inhibitor and enzyme inhibitors were given in Table 3.

3. Results and Discussion

3.1 Antagonistic effects of *Trichoderma* isolates and evaluation of secondary metabolites

Four *Trichoderma* isolates CA-03, CA-06, CA-07 and CA-08 (Table 4) were tested to check their ability to produce toxic volatile metabolites against *Fusarium* and *Colletotrichum* isolates and it was found that they significantly reduced the radial growth of pathogen isolates. The average radial growth rate of *Fusarium* and *Colletotrichum* isolates ranges from 60-70 mm and 35-46 mm, respectively. The maximum growth inhibition effect of volatile compounds of isolates CA-06 and CA-07 against both FOC and CC isolates and isolate CA-08 showed least inhibitory effect against both pathogens data is shown in Table 5.

In order to check the efficiency of culture filtrate of four *Trichoderma* isolates were collected after 14 days of incubation and all of them showed maximum inhibitory effect against both phytopathogen isolates at different concentration. From the data (Table 6), it was clearly shown that all isolates having increased inhibitory effect of non-volatile compounds with their increasing concentration that signifies reduction of the mycelial growth of *Fusarium* and *Colletotrichum* isolates. At 15% concentration isolates, CA-06 and CA-07 causes 73% and 77% inhibition to FOC, respectively while in case of CC the same isolates causes 62% and 66% inhibition, respectively. The maximum growth inhibition was observed by isolate CA-07 over control, followed by isolate CA-06. The lowest

growth inhibition is caused by isolate CA-08. From the results of antifungal assay it was confirmed that isolate CA-07 and CA-06 have some potential volatile or non-volatile antifungal secondary metabolite which considered to be promising antimicrobial/ antioxidant / anti-inflammatory potential because of the presence of phytochemical constituents, *i.e.*, alkaloids, steroids, triterpenoids and flavonoids (Aslam *et al*., 2017).Thus, the culture filtrate of those isolates were collected and taken for identification and extraction of active antifungal metabolites. Earlier studies showed that *Trichoderma* spp having more than 40 metabolites which play significant role in biological control mechanism. Muthumeenakshi *et al*. (1998) observed production of toxic volatile metabolites of *T. harzianum* biotypes Th1, Th2, and Th3 against *Agaricus bisporus*. Similarly, Tarus *et al*. (2003) demonstrated that *T. harzianum* and *T. longibrachiatum* produces some metabolites having antifungal and antibacterial activity. From the bioautography assay, it was revealed that *T. harzianum* isolates T16 and T23 excreted several secondary metabolites which showed antifungal activity against *Fusarium moniliforme* (Hasan *et al*., 2009). Vinale *et al*. (2009) evaluated numerous secondary metabolites by *Trichoderma* strain T22 and T39 against *Leptosphaeria maculans*, *Phytophthora cinnamomi* and *Botrytis cinerea*. Li *et al*. (2015) demonstrated inhibitory effect of *Trichoderma* strain T-33 against *Cytospora chrysosperma* and obtained an active antifungal compound by using various separation techniques. The present study focuses to identification of naturally occurring secondary metabolites from potent *Trichoderma* isolates CA-07 and CA-06 which will reduces the risk of yield loss of chilli.

Table 1: Prediction of molecular properties of compound identified from isolate CA-07

Secondary metabolites	LogP	TPSA	Natoms	NON	Nohnh	Nviolations	Nrotb	Volume	MW
3-Eicosene, (E)-	9.08	0.00	20	0	0	1	16	342	280.54
3-Trifluoroacetoxytetradecane	7.45	26.30	21	2	0	1	14	306.67	310.40
10-Heneicosene	9.32	0.00	21	0	0	1	17	358.80	294.57
Dichloroacetic acid, tetradecyl ester	7.76	26.30	20	2	0	1	14	319.27	325.32
Trichloroacetic acid, hexadecyl ester	8.93	26.30	23	2	0	1	17	366.30	387.82
2-Hexadecanol	7.04	20.23	17	1	1	1	13	289.03	242.45
1-Decanol, 2-hexyl-	6.97	20.23	17	1	1	1	13	289.03	242.45
Acetic acid, chloro-, hexadecyl ester	8.25	26.30	21	2	0	1	17	339.53	318.93
1-Hexadecanol, 2-methyl-	7.42	20.23	18	1	1	1	14	305.83	256.47
Acetic acid, 2-chloro- ,octadecyl ester	8.83	26.30	23	1	0	1	19	373.13	346.98
E-15-Heptadecenal	7.36	17.07	18	1	0	1	18	294.02	252.44
Dichloroacetic acid, heptadecyl ester	8.86	26.30	23	2	0	1	18	369.89	367.40
Cyclotetracosane	9.67	0.00	24	0	0	1	0	405.04	336.65
n-Tetracosanol-1	9.44	20.23	25	1	1	1	22	423.65	354.66
1-Octacosanol	9.83	20.23	29	1	1	1	26	490.86	410.77
Docosyl pentafluoropropionate	9.66	26.30	32	1	0	1	24	467.67	472.62
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	4.43	52.61	20	4	0	0	10	273.91	278.35
1,2-Benzenedicarboxylic acid, diisooctyl ester	8.79	52.61	28	4	0	1	18	408.33	390.56
1,2-Benzenedicarboxylic acid, isodecyl octyl ester	8.67	52.61	30	4	0	1	19	441.62	418.62

The ethyl acetate and methanol extract of *Trichoderma* culture filtrate of potential isolates (CA-07 and CA-06) were subjected to gas chromatography/mass spectroscopy (GC/MS) analysis. The GC/MS analysis of *Trichoderma* isolates CA-07 and CA-06 revealed 19 and 13 compounds data shown in Figure 1 and Figure 2, respectively.

Extraction of *Trichoderm*a secondary metabolites of isolates CA-07 and CA-06 with ethyl acetate and methanol generates, organic and aqueous phases, in which large amount of metabolites were identified having antifungal property against *Fusarium oxysporum*

f.sp. *capsici*. Identified compounds are free long chain fatty acids and fatty acid esters in nature and having chain length ranging from 10 to 33 carbons. The compounds shared the common feature of being small molecules with molecular mass ranging from 162.14 g/mol to 354.61 g/mol. The sequential order of major compounds identified from CA-07 based on their probability of occurrence are 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (51.7%) and 1,2-benzenedicarboxylic acid, diisooctyl ester (17.4%) whereas compounds identified from isolate CA-06 are 2(3H)-naphthalenone,4,4a,5,6,7,8-hexahydro-4a-methyl (16.4%),1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (12.2%) and 2H-Pyran-2-one, 6-pentyl (9.81%).

Table 2: Prediction of molecular properties of compound identified from isolate CA-06

Secondary metabolite	LogP	TPSA	Natoms	NON	Nohnh	Nviolations	Nrotb	Volume	MW
2H-Pyran-2-one, 6-pentyl-	3.04	30.21	12	2	0	0	4	168.36	166.22
Spiro[4.5]decane-1,6-dione	0.81	34.14	12	2	0	0	0	163.03	166.22
11- Oxadispiro[4.0.4.1] undecan-1-one	1.95	32.76	12	2	1	0	0	164.56	168.24
Spiro[5.6]dodecane	4.72	0.00	12	0	0	0	0	192.28	166.31
2,7(1H,3H)-Naphthalenedione, hexahydro-	0.74	34.14	12	2	0	0	0	163.38	166.22
1,5-Dodecadiene	5.68	0.00	12	0	0	1	8	201.96	166.31
2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro -4a-methyl-	2.37	17.07	12	1	0	0	0	171.23	164.25
1H-Indene-1,5(6H)-dione, 2,3,7,7a-tetrahydro-7a-methyl-	0.53	34.14	12	2	0	0	0	156.61	164.20
1-Methyl-2-methylene-trans-decalin	4.20	0	12	0	0	0	0	186.54	164.29
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	4.11	52.61	20	4	0	0	9	273.70	278.35
1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	4.90	52.61	22	4	0	0	8	296.94	304.39
Phthalic acid, isobutyl 2-pentyl ester	4.48	52.61	21	4	0	0	9	290.29	292.38
Phthalic acid, butyl nonyl ester	6.96	52.61	25	4	0	1	15	357.92	348.48

3.2 Calculation of drug likeness properties and bioactivity score

Prediction of drug likeness properties of identified secondary metabolites is referred to the similarity of identified compounds to oral drugs. Lipinski's rule is most promising parameters to predict the molecular properties for analysis of pharmacokinetics of drug candidate. Lipinski defines it as compounds that have sufficiently acceptable ADME properties and toxicity properties to survive through the completion of phase I clinical trial (Vieth *et al*., 2004). According to Lipinski rule of five (Lipinski *et al*., 1997), drug candidate should obey five rules as: (i) log P is less than 5, (ii) there are not more than 5 hydrogen bond donors, (iii) there are not more than 10 hydrogen bond acceptors and (iv) the molecular weight is under 500. Previously, Khan *et al*. (2013) predicts molecular and bioactivity score through molinspiration software of boswellic acid derivatives in search of a those lead compound having anti-inflammatory activity. Among 12 boswellic acid derivatives, only compound number 2 is predicted to be orally active and is considered as a potential drug candidate. Similarly, another study is done by Valli and Geetha (2015), sixteen flavonoids of *Erythrina varigata* were compared and it was found that Erysenegalensin-F compound have lowest log P values among all flavonoids which indicate good drug likeness score also other compounds showed good binding capabilities towards receptors.

Molinspiration is a java based on-line free cheminformatics software which calculates molecular properties like logP, total polar surface area (TPSA), number of hydrogen bond donors and acceptors and molecular weight, it also predicts of bioactivity score, in order to find out potential secondary metabolite of *Trichoderma* isolates CA-07 and CA-06. Log P parameter is useful for understanding the behavior of drug candidate in terms of molecular hydrophobicity, factors such as absorption, excretion and penetration of the CNS are related to the log P value of a drug candidates. From the data (Table 2), it was observed that compound 1,2-Benzenedicarboxylic acid (4.43) from isolate CA-07 and from isolate CA-06 compound 1H-Indene-1,5(6H)-dione, 2,3,7,7a-tetrahydro-7a-methyl- (0.53) have lowest predicted logP score which is good drug likeness score indicating their permeability across the cell membrane. Compounds having high logP score are considered to be more toxic in nature. Another important parameter was calculated which is related to the drug transport properties like human intestinal absorption, Caco-2 monolayers permeability and blood-brain barrier penetration

known as total polar surface area (TPSA). The predicted TPSA of all identified compounds are below 160Å limit. Compounds having low molecular weight can be easily transported, diffusible and absorbed as compared to heavy molecular weight compounds. Molecular weight of all identified compounds is found to be less than 500. In identified compounds, number of hydrogen bond acceptors (O and N atoms) and number of hydrogen bond donors (NH and OH) were found to be less than 10 and 5, respectively. Molecular volume is a function of molecular weight and structure which enables to access all conformations of compounds under physiological conditions which is further relatable with presence of number of rotatable bonds and the number of rings in compounds. To measure the flexibility of identified compounds, number of rotatable bonds were calculated by software which ranges from 0 to 26, it means compounds having more rotatable bonds are considered as more flexible and compounds having 0 rotatable bonds are considered as rigid in nature. This parameter is considered as a good descriptor of oral bioavailability of drugs, only if when compounds obey all five rules. 1-octacosanol compound is identified from CA-07 isolate having 26 rotatable bonds but the calculated log P value is 9.67 which is more than 5 indicate increased toxicity in nature, hence this compound will not be considered as drug candidate.

Table 3: Prediction of bioactivity score of identified compound from *Trichoderma harzianum*

Secondary metabolite	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Proteaseinhibitor	Enzyme inhibitor
2H-Pyran-2-one, 6-pentyl-	-0.96	-0.76	-1.52	-1.27	-0.74	-0.28
Spiro[4.5]decane-1,6-dione	-0.60	-0.05	-1.50	-0.39	-0.85	-0.06
11-Oxadispiro[4.0.4.] undecan-1-one	-0.66	-0.06	-0.94	-0.56	-0.59	-0.01
Spiro[5.6]dodecane	-0.66	-0.14	-0.94	-0.75	-0.82	-0.27
2,7(1H,3H)-Naphthalenedione, hexahydro-	-0.51	-0.12	-1.19	-0.33	-0.40	-0.06
2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-4a-methyl-	-0.98	-0.63	-2.12	0.04	-0.97	0.03
1H-Indene-1,5(6H)-dione, 2,3,7,7a-tetrahydro-7a-methyl-	-1.01	-0.21	-1.93	0.16	-0.76	0.32
1-Methyl-2-methylene-trans-decalin	-0.70	-0.04	-1.20	-0.36	-0.88	-0.10
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	-0.15	-0.08	-0.34	-0.07	-0.18	-0.08
1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	-0.05	-0.03	-0.23	-0.05	-0.10	0.01
1,2-Benzenedicarboxylic acid (CA-07)	-0.16	-0.09	-0.27	-0.12	-0.25	-0.07
Phthalic acid, isobutyl 2-pentyl ester	-0.19	-0.10	-0.31	-0.02	-0.17	-0.10

After the observation of molecular property of identified compounds, it was found that from isolate CA-07 and CA-06 among 22 and 13 identified compounds, only 1 and 11 compounds were found to obey rule of five and will take account for prediction of bioactivity scores (Table 6). The identified compounds are naturally produced by *Trichoderma* species with high medicinal use and so have pharmaceutical values are reported based on their biological activity. According to Khan *et al*. (2013), the larger bioactivity score has higher probability that compound will be active. Therefore, if a molecule having bioactivity score more than 0.00 is most likely to possess considerable biological activities, while values –0.50 to 0.00 are expected to be moderately active and if score is less than –0.50, it is assumed to be inactive. In this present study, prediction of bioactivity scores were aimed to identify those biological active compounds which are able to produce the significant physiological actions by interacting with GPCR ligands, nuclear receptor ligands, and inhibit protease and other enzymes. The present observations reveled that 1H-indene-1,5(6H)-dione, 2,3,7,7a-tetrahydro-7a-methyl- identified from CA-06 is active nuclear receptor ligand (0.16) and it also exhibited the higher enzyme inhibitory (0.32) action among 12 compounds, followed by 2(3H)-naphthalenone,4,4a,5,6,7,8-hexahydro-4a-methyl which exhibited similar activity towards nuclear receptor ligand (0.04) and enzyme inhibitory (0.03). The highest peak of gas chromatogram of both compounds is shown in Figure 3 and 4, respectively. Compound 1,2-benzenedicarboxylic acid identified from isolate CA-07 is moderately active towards all receptors whereas butyl 2-methylpropyl ester (-0.15 and -0.34), 1,2-benzenedicarboxylic acid, butyl cyclohexyl ester (-0.05 and -0.23) and phthalic acid, isobutyl 2-pentyl ester (-0.19 and -0.31) are found to be moderately active towards GPCR receptor and kinase inhibitor, respectively. Thus, it can be concluded that some structural modifications should be carried out with improved molecular property like logP to obtain the molecules with better pharmacological activity.

Table 4: Identification of *Trichoderma* spp.

Isolate code	Source	ITCC No.	*Trichoderma* spp.	National center for biotechnology accession no	GPS coordinates
CA-03	Kadipur, Sulatanpur	9861.15	*Trichoderma asperellum*	KU821782	26°9'23.46.84"N82 °23'4.8228"E
CA-06	Nani, Allahabad	9864.15	*Trichoderma harzianum*	KU947032	25°24'33.0516"N8 1°51'3.2472"E
CA-07	Pipri Faizabad	9865.15	*Trichoderma harzianum*	KX579941	26°38'41.5"N82°08 '03.2"E
CA-08	Sikrara, Jaunpur	9866.15	*Trichoderma longibrachiatum*	-	25°42'29.736"N82° 32'4.7436"E

Table 5 : Per cent mycelial growth inhibition by four *Trichoderma* isolates producing volatile metabolites against two phytopathogens

Isolates	*Fusarium oxysporum* f. sp. *capsici*		*Colletotrichum capsici*	
	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control
CA-03	62.33	22.0	38.33	38.8
CA-06	62.33	22.0	42.67	31.9
CA-07	59.00	26.2	37.00	40.9
CA-08	70.00	12.5	46.33	26.0
Control	80.00	0	62.67	0

Table 6: Growth inhibition of *Fusarium oxysporum* f.sp. *capsici* (FOC) and *Colletotrichum capsici* (CC) by non-volatile compounds produces by four *Trichoderma* isolates at concentration of 5, 10 and 15

Treatment	*Fusarium oxysporum* f.sp. *capsici*					
	5%		10%		15%	
	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control
CA-03	44.50	29.9	31.00	51.1	22.00	65.3
CA-06	**40.50**	36.2	**29.50**	53.5	**17.00**	73.2
CA-07	**39.00**	38.5	**27.50**	56.6	**14.00**	77.9
CA-08	46.00	27.5	31.50	50.3	28.00	55.9
CONTROL	63.50	0	63.50	0	63.50	0
Treatment	*Colletotrichum capsici*					
	5%		10%		15%	
	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control
CA-03	45.5	20.8	34	40.8	22.5	60.6
CA-06	**44.5**	22.6	**31.83**	44.6	**21.5**	62.6
CA-07	**42.5**	26.08	**30.17**	47.5	**19.0**	66.9
CA-08	48.5	15.65	34.6	39.7	29.0	49.5
Control	57.5	0	57.5	0	57.5	0

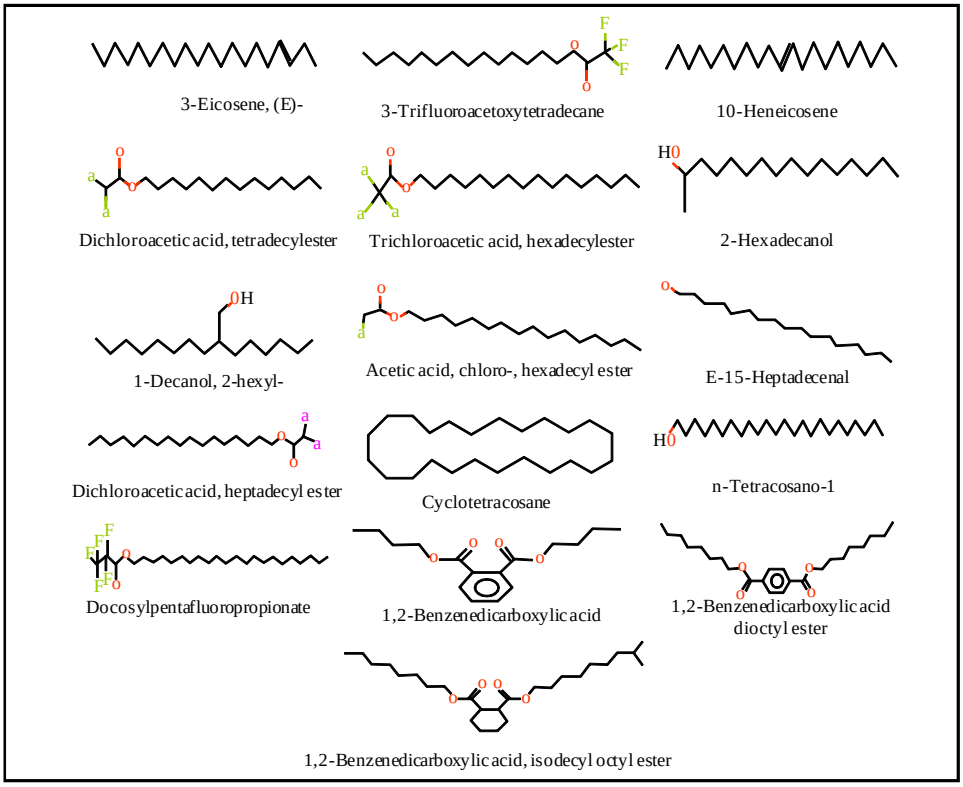

Figure 1: Secondary metabolites identified from cultural filtrate of *Trichoderma* isolate CA-07

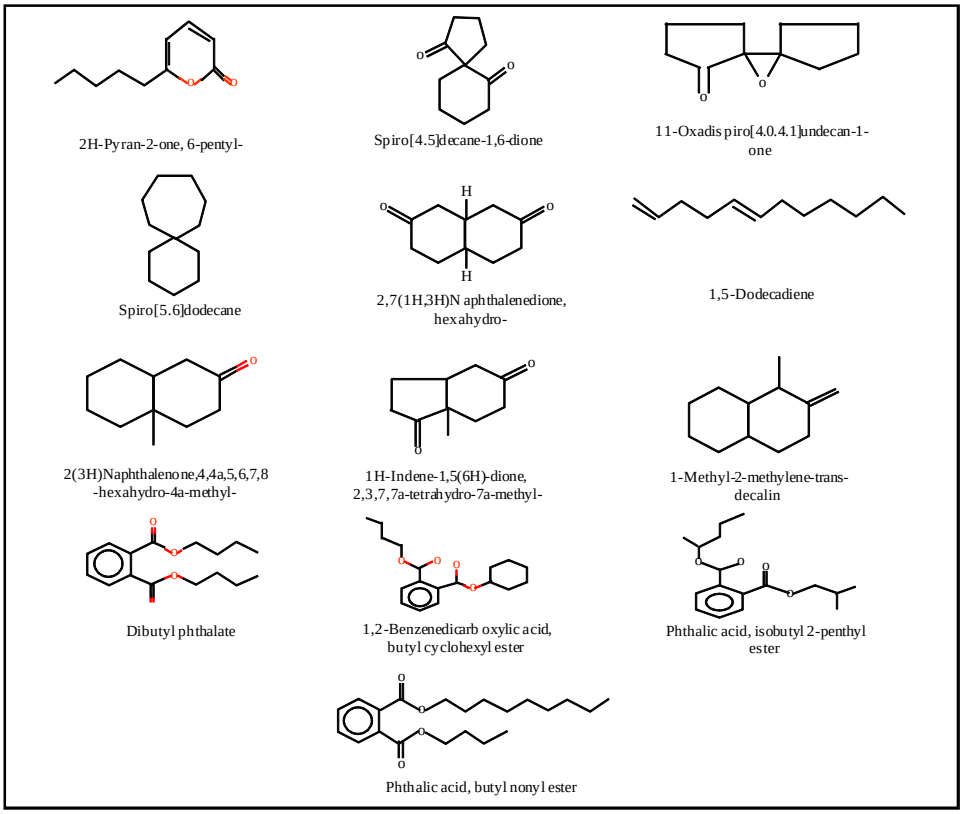

Figure 2: Secondary metabolites identified from cultural filtrate of *Trichoderma* isolate CA-06.

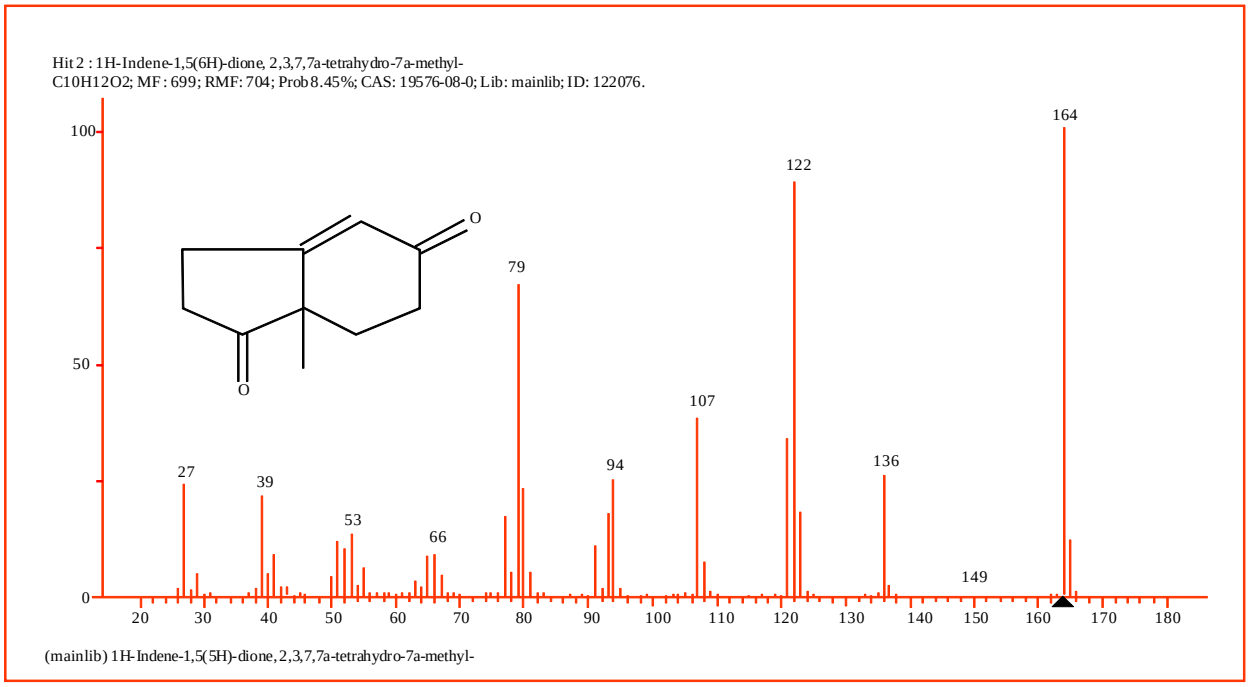

Figure 3: The highest peak of gas chromatogram of compound 1H-Indene-1,5(6H)-dione, 2,3,7,7a-tetrahydro-7a-methyl.

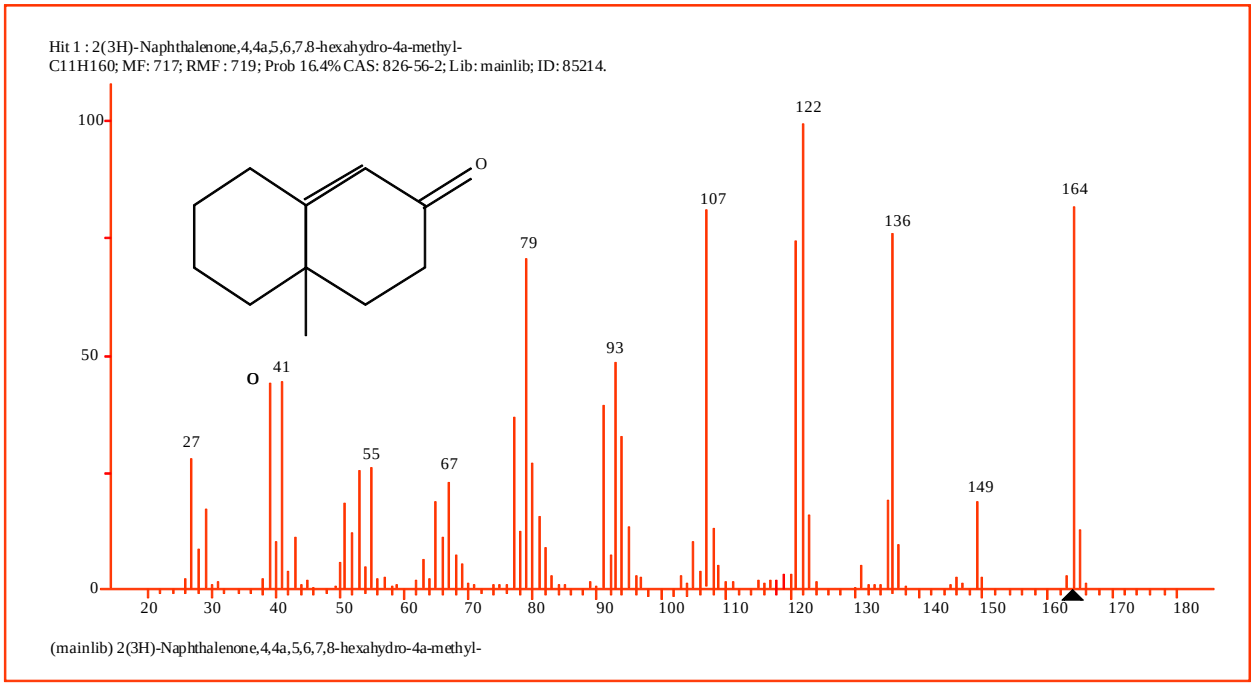

Figure 4: The highest peak of gas chromatogram of compound 2(3H)-naphthalenone,4,4a,5,6,7,8-hexahydro-4a-methyl.

4. Conclusion

Identification of secondary metabolites from *Trichoderma* isolates are carried out further for the *in silico* evaluation of molecular properties and bioactivity score in order to find out the potential compound with high pharmaceutical value which can be proposed for the development of drug against FOC and CC. Compound 1H-indene-1,5(6H)-dione, 2,3,7,7a-tetrahydro-7a-methyl is found as promising lead molecules with anti-infective property and it showed high enzyme inhibitory effect among identified compound from *Trichoderma* isolates which can be further consider for *in vivo* studies for the development of new potential drug compound.

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Conflict of interest

We declare that we have no conflict of interest.

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