Biocatalysis and Agricultural Biotechnology 18 (2019) 101043

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Evaluation of antimicrobial potential of endophytic fungi associated with healthy plants and characterization of compounds produced by endophytic *Cephalosporium* and *Fusarium solani*

Hafiza Farhat[a], Faizah Urooj[a], Amna Tariq[b], Viqar Sultana[c], Madeeha Ansari[d], Viqar Uddin Ahmad[d], Syed Ehteshamul-Haque[a,*]

[a] Agricultural Biotechnology & Phytopathology Laboratory, Department of Botany, University of Karachi, Karachi, 75270, Pakistan
[b] Postharvest & Food Biochemistry Laboratory, Department of Food Science & Technology, University of Karachi, Karachi, 75270, Pakistan
[c] Biotechnology & Drug Development Laboratory, Department of Biochemistry, University of Karachi, Karachi, 75270, Pakistan
[d] H.E.J. Research Institute of Chemistry & International Center for Chemical & Biological Sciences (ICCBS), University of Karachi, Karachi, 75270, Pakistan

ARTICLE INFO

Keywords:
Endophytic fungi
Cephalosporium
Fusarium solani
Antimicrobial activity
Secondary metabolites

ABSTRACT

The modern medicines have revolutionized drug therapy, but due to emergence of new infectious disease and resistance among infectious agents towards drugs, it is necessary to discover new antimicrobial agents. The fungal derivatives play a key role in human life and their compounds are the main source of drug production. In this study, endophytic fungi isolated from healthy plants have showed strong antibacterial and antifungal activity against common laboratory bacteria and root infecting fungi. They also showed strong nematicidal potential against *Meloidogyne javanica,* the root knot nematode. GC-MS spectroscopy of oily fractions from mycelium of *Fusarium solani* and *n*-hexane fraction of culture filtrate of *Cephalosporium* revealed the presence of several compounds and some of them are new from this source. The ability of endophytic fungi to produce bioactive compounds with pharmaceutical and agricultural potential may lead in the pursuit of new biological agents for drug discovery and agrochemicals.

1. Introduction

Endophytic fungi are the organism widely found inside the tissues of most of the plant species (Strobel and Daisy, 2003). But they are different from pathogens in a way that they lead disease development and lower the fitness of plants, while endophytic fungi inhabit above and below ground tissues of hosts without causing any disease and are mostly found in the divisions of land plants (Zimmerman and Vitousek, 2012). Endophytes often form interactions with their host in a way both get benefit from each other (Deshmukh et al., 2006). Land plants and fungal endophytes interacted approximately 400 million years (Krings et al., 2007; Jia et al., 2016) and established an interactions with their hosts, such as growth stimulation, tolerance to salt stress, and induction of systemic and local resistance to phytopathogens (Lahrmann et al., 2013). *Phomopsis* sp., produce indole-3-acetic acid, piperine, and gibberellic acid to enhance plant growth (Chithra et al., 2017) and *Penicillium* may produce gibberellins to reduce salt stress (Leitao and Enguita, 2016). Secondary metabolites from endophytic fungus *Nigrospora oryzae*, isolated from leaves of *Combretum dolichopetalum*, have potential against diabetic (Uzor et al., 2017). Many bioactive molecules obtained from most of the endophytic fungi (Strobel, 2006) are considered more active metabolically because of their role in the environment and they activate numerous metabolic pathways in order to exist in their host tissues (Strobel and Daisy, 2003).

Food production must be increased in order to meet the rapidly growing population of the world, but plant diseases may cause severe crop losses (Aliye et al., 2008). According to estimates plant diseases, may cause 26% yield losses to agricultural crops (Khan et al., 2009). Each year billion of dollars losses occur, just because of plant pathogens such as species of *Rhizoctonia, Verticillium, Fusarium, Macrophomina* and *Sclerotinia* (Shafique et al., 2016). Biological control of plant disease is an alternate to chemicals but actual use of biological control agents in disease management is very few, due to their limited efficacy and inconsistent performance under filed condition. Discovery of new potential biocontrol agent active against wide range of pathogens is utmost need of time. Similarly, emergence of virulent strains of infectious agents, occurrence of resistance among them against well-known antibiotics, besides their side effects they are advocating to continue the

* Corresponding author.
E-mail address: sehaq@uok.edu.pk (S. Ehteshamul-Haque).

https://doi.org/10.1016/j.bcab.2019.101043
Received 20 November 2018; Received in revised form 10 February 2019; Accepted 12 February 2019
Available online 25 February 2019
1878-8181/

discovery of new antimicrobial agents. The aim of the study is to isolate endophytic fungi from healthy plants and evaluate the production of secondary metabolites from potential isolates based on their activity against common laboratory bacteria, pathogenic fungi and plant parasitic nematode. Isolation and characterization of compounds are also reported here from two potential fungi.

2. Methods

2.1. Collection of plant samples

Wild and cultivated healthy plants were collected from University of Karachi and different agricultural fields of Karachi like Memon Goth, Malir and Kathor and isolation of endophytic fungi were made within 24 h.

2.2. Isolation and identification of endophytic fungi

To isolate endophytic fungi, 1 g of plant samples (stem, root and leaf) were washed carefully with tap water and 1% bleach [Ca $(OCl)_2$] was used for sterilization for 3 min, then with (70%) alcohol for 3 min and finally washed with distilled water. Each sample was crushed in sterile grinder with sterilized water (50 ml) and dilutions was made up to $1:10^4$ and 0.1 mL suspension from final dilution was poured onto a Petri-dish having Potato Dextrose Agar (containing penicillin 100000 units/liter and streptomycin 0.2 gm/liter). At 28 °C, plates were incubated for 5 days and identification of endophytic fungi was made with reference to, Barnett and Hunter (1998); Booth (1971); Domsch et al. (1980); Dugan (2006); Ellis (1971) and Nelson et al. (1983).

2.3. Determination of antifungal activity of endophytic fungi in dual culture plate assay

To test antifungal activity of endophytic fungi, common root rotting fungi such as *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina*, and *Rhizoctonia solani* were used. Disc of 5 mm of endophytic fungi was placed on one sides of the Petri dish (90 mm) containing Czapek's Dox Agar having pH (7.2). On other side of the Petri dish, disc of 5 mm of pathogenic fungus was placed and at 28 °C plates were incubated for 5 days. To check their activity, inhibition zone was measured and expressed in terms of mm (Korejo et al., 2014).

2.4. Preparation of culture filtrate and their antimicrobial activity

Endophytic fungi were grown in conical flasks of 500 ml containing Czapek's Dox broth (300 ml). 5 mm disc of fungi was inoculated in each flask. The flasks were incubated for 15 days (25–30 °C). Fungal broth was filtered over Whatman filter paper after 15 days and filtrate was exposed to chloroform vapors to kill any propagule of fungi, if left. For antifungal activity of secondary metabolites of fungi, disc of thick filter paper were loaded with different concentration of culture filtrate (20, 40 and 60 µl/disc) and dried under laminar flow hood. Discs were placed at periphery of plates containing Czapek'sDox Agar. Test fungus was inoculated in the center of the plates. Discs of sterile broth was used as control, while disc of 20 µg/disc of carbendazim considered as a positive control. At 30 °C plates was incubated for 5–7 days and distance was measured between disc and test fungus (Qureshi, 2003). For the determination of antibacterial activity of culture filtrate, bacterial lawn was prepared with the help of sterilized cotton swab and disc of control and culture filtrate were placed at different places in Petri dish having Trypticase Soya Agar. Streptomycin 10µg/disc was used as positive control. At 30 °C, plates were incubated for 24 h. Diameter of inhibition zone were measured, and expressed in mm.

2.5. In vitro juvenile mortality test

Freshly hatched 1 mL suspension of second stage of juvenile (20 juveniles) of *Meloidogyne javanica*, a root knot nematode was add along with culture filtrate (1 ml) of fungus in cavity blocks. Each test had 3 replicates and placed at 26 °C ± 5. Mortality rate of juveniles were observed after 24 and 48 h under stereo-microscope. Dead nematode did not show movement when probed with a needle (Cayrol et al., 1989).

2.6. Fractionation of culture filtrates of endophytic fungi

Culture filtrate was extracted three times with *n*-hexane and then with chloroform by shaking vigorously in a separating funnel. Solvent layer was allowed to separated out and run off from the aqueous layer. The extracted volume of each solvent was approximately half of that filtrate. The *n*-hexane and chloroform fractions were pooled separately and dried on a rotary vacuum evaporator (Eyela-NE) and weighed. The fraction was oily in nature (Hameed et al., 2009).

2.7. In vitro antimicrobial activity of fractions of culture filtrate

A dilution of 1.5 mg/ml of *n*-hexane soluble fractions were made in their respective solvent and loaded on a disc of sterilized thick filter paper (30µg/disc). These discs were used for the determination of antimicrobial activity as described in 2.4 (Korejo et al., 2017).

2.8. Extraction of metabolites from mycelium of Fusarium solani and identification of compounds

10 gm mycelium of *Fusarium solani* was thoroughly washed with *n*-hexane to remove excess water. Then mycelium was extracted in *n*-hexane (200 ml) by using soxhlet apparatus for 8 h. Fraction extracted from mycelium of *F. solani* was evaporated at 40 °C on rotary vacuum evaporator till semi gummy mass was obtained. The *n*-hexane fraction was oily in nature.

2.9. Analysis of n-hexane fraction by gas chromatography and mass spectrometry (GC-MS)

Both oily fractions were subjected to GC-MS analysis. GC-MS was carried out on Agilent 6890 Gas Chromatograph equipped with ZB-SMS 30 m x 0.32ID and 0.25 µm thick film which was hyphenated with Mass Spectrometer, Jeol, JMS- 600H operational mode was EI having the ion source at 50 °C and their electron energy was maintained at 70 eV. Volume of carrier gas was placed in between 1.0 and 5.0 µL. Individual peaks of compounds were assigned by comparing their retention indices and mass spectra by computer matching against NIST Mass Spectrometry Data Center (mainlib) NIST#: 352898 ID#: 113419 DB), National Institute of Standards and Technology, USA and finally compared with Science finder.

3. Results

3.1. In vitro antifungal activity

Cephalosporium sp., *Fusarium moniliforme, Fusarium solani* were found to produce more than 15 mm inhibition zones against test fungi *F. oxysporum*, *F.solani, Rhizoctonia solani* and *M.phaseolina* in dual culture plate assay. While *Curvularia* sp., produced inhibition zone more than 10 mm against all four root rotting fungi (Table 1).

3.2. Antimicrobial activity of culture filtrate and its fractions

The culture filtrate of the endophytic fungi produced more than 10 mm zone of inhibition at 60 µl/disc against *M.phaseolina, F.*

Table 1
Growth inhibition of *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum* in dual culture plate assay by the endophytic fungi isolated from different wild and cultivated plants.

Fungi isolated	Code #	Name of host	Plant part	*M. phaseolina*	*R. solani*	*F. solani*	*F. oxysporum*
			Zone of inhibition (mm)[a]				
Cephalosporium sp.	SR1	*Helianthus annuus* L.	root	32	19	38	37
Fusarium moniliforme	SR111	*Helianthus annuus L.*	root	30	32	38	39
Fusarium solani	SXR	*Solanum xanthocarpum* Schrad. & H. Wendl	root	17	16	26	19
Curvularia sp.	OniR	*Allium cepa L.*	root	18	16	30	25
Curvularia sp.	BrijS	*Solanum melongena L.*	stem	10	11	17	15

[a] Average of three replicates.

Table 2
In vitro growth inhibition of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F.oxysporum* by culture filtrates of endophytic fungi isolated from wild and cultivated plant species.

Endophytic fungi	Treatments	*M.phaseolina*	*R.solani*	*F.solani*	F.oxysporum
		Zone of inhibition (mm)			
	Control	0	0	0	0
	+ve Control (Carbendazim 20 µg*/disc)*	5	7	10	9
Cephalosporium sp.	20 µl/disc	7	11	10	14
	40 µl/disc	14	12	13	18
	60 µl/disc	18	15	19	21
Fusarium moniliforme	20 µl/disc	7	5	7	9
	40 µl/disc	12	11	13	12
	60 µl/disc	18	13	18	17
Fusarium solani	20 µl/disc	11	8	6	9
	40 µl/disc	17	14	9	15
	60 µl/disc	20	17	13	19
Curvularia sp.	20 µl/disc	5	0	4	5
	40 µl/disc	11	8	7	9
	60 µl/disc	16	9	10	12
Curvularia sp.	20 µl/disc	4	2	5	4
	40 µl/disc	7	5	7	6
	60 µl/disc	11	10	13	9

Table 3
In vitro growth inhibition of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Bacillus subtilis* and *Escherichia coli* by culture filtrates of endophytic fungi isolated from wild and cultivated plant species.

Isolates	*S.aureus*	*P.aeruginosa*	*S.typhimurium*	*B.subtilis*	*E.coli*
		Zone of inhibition (mm)			
Control	0	0	0	0	0
Cephalosporium (+ve)[a]	8	11	13	7	9
20 µl	7	9	7	0	6
40 µl	13	16	11	9	14
60 µl	19	20	17	16	19
Fusarium moniliforme (+ve)[a]	8	11	13	7	9
20 µl	0	6	7	0	9
40 µl	11	13	9	7	12
60 µl	16	18	15	12	17
Fusarium solani (+ve)[a]	8	11	13	7	9
20 µl	7	9	7	0	9
40 µl	12	14	13	8	10
60 µl	16	19	15	14	11
Curvularia sp. (+ve)[a]	8	11	13	7	9
20 µl	0	8	7	0	7
40 µl	12	11	11	0	10
60 µl	15	17	12	0	14
Curvularia sp. (+ve)[a]	8	11	13	0	8
20 µl	0	0	0	0	6
40 µl	7	7	7	0	11
60 µl	9	10	11	0	13

[a] (+ve) = +ve control (streptomycin 10 µg/disc).

oxysporum, *Rhizoctonia solani* and *F.solani* (Table 2). Whereas, against Gram negative bacteria such as *Salmonella typhimurium, Pseudomonas aeruginosa,* and *E.coli* at 60 µl/disc produced more than 10 mm zone of inhibition, while, less than 10 mm zone was measured against *Bacillus subtilis* and *Staphylococcus aureus* at 60 µl/disc (Table 3).

n-Hexane soluble fraction of culture filtrate of endophytic fungi was found effective against *E. coli*, *S.aureus*, *P.aeruginosa* and produced upto 15 mm zone of inhibition at 60 µl/disc. *n*-Hexane fraction was also found effective against root rotting fungi by producing inhibition zone more than 10 mm at 40 µl/disc and 20 mm zone of inhibition at 60 µl/disc against *M. phaseolina*. More than 15 mm zone of inhibition was measured at 60 µl/disc against *F.solani, R. solani* and *F. oxysporum* (Tables 4 and 5). Chloroform soluble fraction of *Cephalosporium* and *F.solani* showed strong activity against *E.coli* and *P.aeruginosa* and produced zone of inhibition upto 15 mm at 60 µl/disc (Table 4). Chloroform soluble fraction of all endophytic isolates showed a zone ranging from 10 to 15 mm against *M.phaseolina, F. oxysporum*, *F.solani*, and *R. solani* at 60 µl/disc (Table 5).

3.3. In vitro juvenile's mortality of root knot nematode

Cultural filtrates of endophytic fungi caused killing of nematodes within 24 and 48 h at different degree level. Culture filtrates of *Cephalosporium, F. moniliforme* and *F.solani* showed more than 50% killing of nematodes after 24 h, while after 48 h 100% activity was shown by *Cephalosporium* sp., (Table 6).

3.4. Spectral data of endophytic Cephalosporium sp. and Fusarium solani

On the basis of mass spectral data hexane soluble fraction

Table 4
In vitro antibacterial activity of *n*-hexane and chloroform soluble fractionations of culture filtrate.

Isolates	*S. aureus*		*P. aeruginosa*		*S .typhimurium*		*B. subtilis*		*E. coli*	
	hexane	chloroform	hexane	chloroform	hexane	chloroform	hexane	chloroform	hexane	chloroform
Control	0	0	0	0	0	0	0	0	0	0
Cephalosporium sp. (+ve)[a]	18	12	10	7	11	9	7	6	7	7
20 µl	7	8	8	7	6	0	8	0	7	9
40 µl	10	8	12	11	13	9	10	8	15	14
60 µl	18	14	21	15	19	11	16	9	21	17
Fusarium moniliforme (+ve)[a]	7	12	10	7	0	9	11	0	12	7
20 µl	7	8	7	7	0	0	7	7	14	9
40 µl	8	9	11	11	8	8	9	8	18	12
60 µl	9	13	16	16	17	11	11	10	21	19
Fusarium solani (+ve)[a]	15	12	9	7	12	9	6	7	8	7
20 µl	8	7	10	10	8	8	9	7	9	9
40 µl	9	9	11	11	14	14	13	12	11	11
60 µl	12	11	16	14	17	16	14	15	15	15

[a] (+ve) = +ve control (streptomycin 10 µg/disc).

(emulsion) of *Cephalosporium* was found to be mixture of different volatile compounds such as normal hydrocarbon, cyclopenten, fatty acid, alcohol, benzene derivatives and terpenoids (Table 7; Fig. 1).

GC/MS spectrum of hexane soluble fraction of *F.solani* mycelium indicated the presence of benzaldehyde, benzylAlcohol, 1-propene, and methyl 3,3-dichloropropenoate, 1,1,2-trichloro-3,3,3-trifluoro (Table 8; Fig. 2).

4. Discussion

Endophytic fungi colonized healthy plant tissues but do not always produce noticeable symptoms. This type of interaction is termed as mutualistic (Carroll, 1988). According to Schulz and Boyle (2005), this mutualistic interaction can only be temporary and can change with the passage of time. Therefore, endophytic fungi could assign for those fungi with an epiphytic phase as well as latent pathogens that live asymptomatically in the host plant for some period in their life time (Petrini, 1991). In this study, endophytic fungi isolated from healthy plants are identified as *Cephalosporium* sp., *Fusarium solani, F. moniliforme* and *Curvularia* sp. *Fusarium solani* and *F.moniliforme* are well known plant pathogens attacking a number of plants (Domsch et al., 1980; Hameed et al., 2009). It is suggested that well known plant pathogens should be deal with great care even though, they have isolated from healthy plants.

Endophytic fungi are a sources of wide range of bioactive compounds including terpenoids, alkaloids, quinones steroids, isocoumarins, phenols, phenylpropanoids, lignans, and lactones (Radic and Strukelj, 2012); (Deshmukh et al., 2014). The drug development program from the endophytic fungi may play a crucial role in the search of new leads. Endophytic fungi have a potential in order to produce bioactive compounds which protect host plant from harmful organisms (Tan and Zou, 2001). Secondary metabolites produced from endophytes are reported as a source of inhibitors of different variety of animal and plant pathogens (Gunatilaka, 2006; Zhao et al., 2011).

In the current study, all five endophytic isolates of fungi showed promising result against root rotting fungi and gram negative and gram

Table 6
Effect of culture filtrates of different isolates of endophytic fungi on juvenile mortality of *Meloidogyne javanica*, the root knot nematode.

Isolates	Nematode mortality (%)	
	24 h	48 h
Control (Czapek'sDox broth)	0	24.5
Cephalosporium sp.	51	100
Fusarium moniliforme	61	85.6
Fusarium solani	54	94.5
Curvularia sp.	39	79
Curvularia sp.	42	82

LSD($p < 0.05$) 1.4 1.6.
[1]Mean values in the column showing difference greater than LSD value are significantly different at $p < 0.05$.

Table 5
In vitro antifungal activity of hexane and chloroform soluble fractionations of culture filtrate.

Isolates	Concentration of culture filtrate	Zone of Inhibition (mm)							
		M. phaseolina		*R. solani*		*F. solani*		*F. oxysporum*	
	Control	Hexane	Chloroform	Hexane	Chloroform	Hexane	Chloroform	Hexane	Chloroform
		0	0	0	0	0	0	0	0
Cephalosporium sp.	+ve control[a]	5	10	12	9	10	10	9	15
	20µl/disc	9	9	13	12	10	10	15	7
	40µl/disc	12	12	15	14	15	13	19	11
	60µl/disc	20	17	17	15	23	21	24	14
Fusarium moniliforme	+ve control	5	10	7	9	9	10	9	15
	20µl/disc	8	8	13	0	10	8	10	5
	40µl/disc	13	13	15	9	11	14	14	9
	60µl/disc	20	15	19	12	18	17	18	13
Fusarium solani	+ve control	5	10	12	9	6	10	9	15
	20µl/disc	15	14	8	8	6	6	12	5
	40µl/disc	18	17	12	12	11	11	14	9
	60µl/disc	21	19	19	15	20	16	15	13

[a] +ve control (Carbendazim 20 µg/disc).

Table 7
Spectral data of endophytic *Cephalosporium* sp. isolated from hexane soluble fraction of culture filtrate.

Systemic name	Molecular formula	Mol.wt.	t_R (min)
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	64.175
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	49.311
Eicosanoic acid	$C_{21}H_{42}O_2$	326	45.06
Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326	45.065
Octadecanoic acid	$C_{19}H_{38}O_2$	298	93.0
Methyl 16-methyl-heptadecanoate	$C_{19}H_{38}O_2$	298	39.7
Heptadecanoic acid, 16- methyl	$C_{19}H_{38}O_2$	298	39.749
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	42.46
Phytol	$C_{20}H_{40}O$	296	42.465
Methyl 9-cis, 11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294	38.56
Methyl 10-trans, 12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	38.563
9,12,15-Octadecatrienoic acid	$C_{19}H_{32}O_2$	292	38.812
4,14-Retro-retinol	$C_{20}H_{30}O$	286	44.43
Podocarp-7-en-3-one, 13β-methyl-13-vinyl	$C_{20}H_{30}O$	286	44.435
3-Eicosyne	$C_{20}H_{38}$	278	42.465
Hexadecanoic acid	$C_{17}H_{34}O_2$	270	30.919
2-Pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}O$	268	28.078
Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$	268	30.74
9-Hexadecenoic acid	$C_{17}H_{32}O_2$	268	30.743
Methyl 7,10,13-hexadecatrienoate	$C_{17}H_{28}O_2$	264	29.864
2-Cyclopenten-1-one, 2-(2-butenyl)-4-hydroxy-3-methyl	$C_{10}H_{14}O_2$	166	17.344

positive bacteria. Endophytic fungi are known to produce substances, inhibitory to growth of microbes (Atmosukarto et al., 2005; Chomchoen et al., 2005; Ezra et al., 2004; Liu et al., 2004; Stinson et al., 2003). In this study, *n*-hexane soluble and chloroform soluble fractions of endophytic fungi also showed strong antibacterial as well as antifungal activity. Culture filtrate of these endophytic fungi also showed effective result against nematode and killed more than 75% of second stage juvenile after 48 h. Health problems caused by pathogenic bacteria and fungi are increasing day by day, whereas endophytic fungi are being recognized as important sources of secondary metabolites (Schulz et al., 2002; Strobel and Daisy, 2003). A recent study showed that 51% of biologically active metabolites isolated from endophytic fungi, most of them were previously unknown (Schulz et al., 2002). Two potential fungi that showed a strong anti-microbial property were then brought for further study and their chemical compounds were analyzed by GCMS. Chemical compounds obtained from *Cephalosporium* are Ethyl iso-allocholate, 3-Eicosyne, Octadecanoic acid, Methyl 16-methyl-heptadecanoate, Heptadecanoic acid, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, Methyl 9-cis, 11-trans-octadecadienoate, Methyl 10-trans, 12-cis-octadecadienoate, 4, 14-Retro-retinol, 2-Pentadecanone, 6, 10, 14-trimethy, Eicosanoic acid. The fungus *Cephalosporium acremonium* have been reported to possess potent antibiotic activity (Crawford et al., 1952). In our study, twenty one different volatile compounds were identified from hexane soluble fraction (emulsion) of *Cephalosporium* in which eleven are new compounds from this source. Endophytic fungi *Fusarium solani*, from Apodytes (lcacinaceae) produced 9-methoxycamptothecin, 10-hydroxycamptothecin (Shweta et al., 2010). In our study four different compounds were isolated from *Fusarium solani* and are characterized as new one as it has not been reported earlier. *Fusarium solani* is known to produce naphthoquinone compounds (Baker et al., 1990; Hameed et al., 2009); including fusarubin, anhydrofusarubin, javanicin, norjavanicin, methyl etherfusarubin, marticin, isomarticin, bostrycoidia, ethyl ether-fusarubin, solaniol, nectriafusarubin and dihydrofusarubin lactone (James and Robert, 1983; Tatum and Baker, 1983; Tatum et al., 1985). In our study 1-Propene,1,1,2 -trichloro-3,3,3-trifluoro compound isolated from endophytic *F. solani* has not been reported previously.

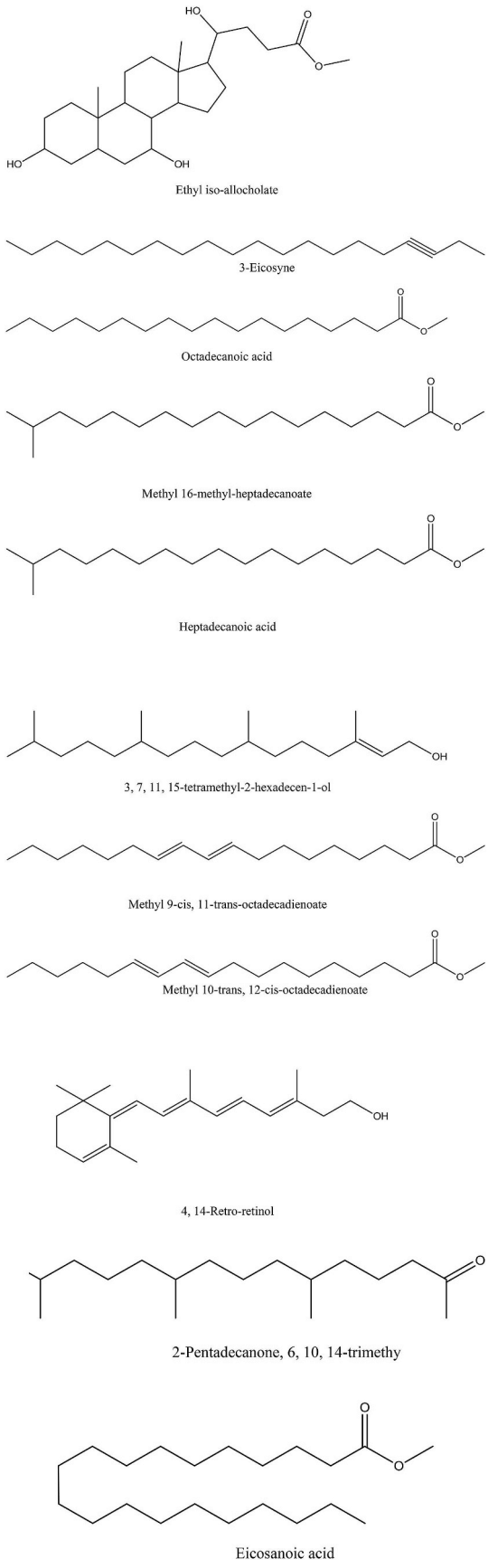
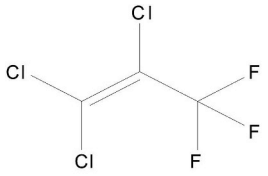


Fig. 1. New compounds from endophytic fungi *Cephalosporium* sp.

Table 8
Spectral data of hexane extract of mycelium (oily fraction) of *Fusarium solani*.

Systemic name	Molecular formula	Mol.wt.	t_R (min)
1-Propene,1,1,2-trichloro-3,3,3-trifluoro	$C_3Cl_3F_3$	198	13.009
Methyl 3,3-dichloropropenoate	$C_4H_4Cl_2O_2$	154	6.119
BenzylAlcohol	C_7H_8O	108	11.450
Benzaldehyde	C_7H_6O	106	8.140



1,1,2-trichloro-3,3,3-trifluoroprop-1-ene

Fig. 2. New compound from endophytic fungi *Fusarium solani*.

5. Conclusions

In this study endophytic *Cephalosporium* and *F. solani* showed strong antifungal, antibacterial and nematicidal activity. This study also resulted in the isolation and identification of new compounds from these fungi. Findings suggested endophytic fungi have a wide range of novel antimicrobial compounds, which could be used in drug development and agrochemical production for protecting agricultural crops from plant root diseases.

Author Contribution statement

Hafiza Farhat and Faizah urooj carried out the collection of plants, isolation and identification of endophytic fungi and conducted experiments. Amna Tariq and Viqar Sultana extracted the metabolites from endophytic fungi and improved manuscript. GC-MS analysis was done by Madeeha Ansari and Viqar Uddin ahmad. Syed Ehteshamul-Haque helped in plant collection, supervised research work and improve the quality of final version of manuscript. All authors read and approved the final manuscript.

Acknowledgement

Financial Assistance provided by the Higher Education Commission, Pakistan (Grant #. NRPU/4491) is sincerely acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101043.

References

Aliye, N., Fininsa, C., Hiskias, Y., 2008. Evaluation of rhizosphere bacterial antagonists for their potential to bioprotect potato *(Solanumtuberosum)* against bacterial wilt (*Ralstoniasolanacearum)*. Biol. Control 47, 282–288.
Atmosukarto, I., Castillo, U., Hess, W.M., Sears, J., Strobel, G., 2005. Isolation and characterization of *Muscodoralbus* I-41.3s, a volatile antibiotic producing fungus. Plant Sci. 169, 854–861.
Baker, R.A., Tatum, J.H., Nemec, S., 1990. Antimicrobial activity of naphthoquinones from Fusaria. Mycopathologia 111, 9–15.
Barnett, H.L., Hunter, B.B., 1998. Illustrated Genera of Imperfect Fungi, fourth ed. APS press, Minnesota, USA.
Booth, C., 1971. The Genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England., pp. 237.
Carroll, G., 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69, 2–9.
Cayrol, J.C., Djian, C., Pijarowski, L., 1989. Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyceslilacinus*. Rev. Nematol. 12, 331–336.
Chithra, S., Jasim, B., Mathew, J., Radhakrishnan, E.K., 2017. Endophytic Phomopsissp. colonization in Oryza sativa was found to result in plant growth promotion and piperine production. Physiol. Plantarum 160, 437–446.
Chomchoen, P., Wiyakrutta, S., Sriubolmas, N., Ngamrojanavanich, N., Isarangkul, D., Kittakoop, P., 2005. 3-Nitropropionic acid (3-NPA), a potent antimycobacterial agent from endophytic fungi: is 3-NPA in some plants produced by endophytes. J. Nat. Prod. 68, 1103–1105.
Crawford, K., Heatley, N.G., Boyd, P.F., Hale, C.W., Kelly, B.K., Miller, G.A., et al., 1952. Antibiotic production by a species of Cephalosporium. J. Gen. Microbiol. 6, 47–59.
Deshmukh, S., Hückelhoven, R., Schäfer, P., Imani, J., Sharma, M., Weiss, M., et al., 2006. The root endophytic fungus *Piriformosporaindica* requires host cell death for proliferation during mutualistic symbiosis with barley. Proc. Natl. Acad. Sci. Unit. States Am. 103, 18450–18457.
Deshmukh, H.S., Liu, Y., Menkiti, O.R., Mei, J., Dai, N., Oleary, C.E., et al., 2014. The microbiota regulates neutrophil homeostasis and host resistance to Escherichia coli K1 sepsis in neonatal mice. Nat. Med. 20, 524.
Domsch, K.H., Gams, W., Anderson, T.H., 1980. Compendium of Soil Fungi'. Academic Press (London) Ltd.
Dugan, F.M., 2006. The Identification of Fungi: an Illustrated Introduction with Key, Glossary and Guide to Literature. The American Phytopathological Society, St. Paul. Minnesota., pp. 184.
Ellis, M.B., 1971. DematiaceousHyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, UK.
Ezra, D., Hess, W.M., Strobel, G.A., 2004. New endophytic isolates of Muscodoralbus, a volatile-antibiotic-producing fungus. Microbiol. 1500, 4023–4031.
Gunatilaka, A.L., 2006. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J. Nat. Prod. 69, 509–526.
Hameed, S., Sultana, V., Ara, J., Ehteshamul-Haque, S., Athar, M., 2009. Toxicity of *FusariumsolaniStrains* on brine shrimp (*Artemiasalina*). Zool. Res. 30, 468–472.
James, H.T., Robert, A.B., 1983. Naphthaquinones produced by *Fusariumsolani*isolated from citrus. Phytochemistry 22, 543–547.
Jia, M., Chen, L., Xin, H.L., Zheng, C.J., Rahman, K., Han, T., et al., 2016. A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Front. Microbiol. 7, 906.
Khan, M.S., Khan, A., Zaidi, P.A., Wani, M., 2009. Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ. Chem. Lett. 1–19.
Korejo, F., Ali, S.A., Shafique, H.A., Sultana, V., Ara, J., Ehteshamul-Haque, S., 2014. Antifungal and antibacterial activity of endophytic *Penicillium* species isolated from Salvadora species. Pakistan J. Bot. 46, 2313–2318.
Korejo, F., Noreen, R., Ali, S.A., Humayun, F., Rahman, A., Sultana, V., Ehteshamul-Haque, S., 2017. Evaluation of antibacterial and antifungal potential of endophytic fluorescent Pseudomonas associated with Salvadora persica L. and Salvadora oleoides Decne. Pakistan J. Bot. 49, 1995–2004.
Krings, M., Taylor, T.N., Hass, H., Kerp, H., Dotzler, N., Hermsen, E.J., 2007. Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. New Phytol. 174, 648–657.
Lahrmann, U., Ding, Y., Banhara, A., Rath, M., Hajirezaei, M.R., Dohlemann, S., et al., 2013. Host-related metabolic cues affect colonization strategies of a root endophyte. Proc. Natl. Acad. Sci. Unit. States Am. 34, 13965–13970.
Leitao, A.L., Enguita, F.J., 2016. Gibberellins in*Penicillium* strains: challenges for endophyte-plant host interactions under salinity stress. Microbiol. Res. 183, 8–18.
Liu, J.Y., Song, Y.C., Zhang, Z., Wang, L., Guo, Z.J., Zou, W.X., et al., 2004. *Aspergillusfumigatus* CY018, an endophytic fungus in Cynodondactylon as a versatile producer of new and bioactive metabolites. J. Biotechnol. 114, 279–287.
Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. Fusarium Species: an Illustrated Manual for Identification. Pennsylvania State University Press, Pennsylvania, USA.
Petrini, O., 1991. Fungal endophytes of tree leaves. In: Microbial Ecology of Leaves. Springer, New York, NY, pp. 179–197.
Qureshi, S.A., 2003. Studies on Antibiotics from Soil Fungi. Ph.D. thesis. University of Karachi at Pakistan.
Radic, N., Strukelj, B., 2012. Endophytic fungi. The treasure chest of antibacterial substances. Phytomedicine 19, 1270–1284.
Schulz, B., Boyle, C., Draeger, S., Rommert, A.K., Krohn, K., 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol. Res. 106, 996–1004.
Schulz, B., Boyle, C., 2005. The endophytic continuum. Mycol. Res. 109, 661–686.
Shafique, H.A., Sultana, V., Ehteshamul-Haque, S., Athar, M., 2016. Management of soil-borne diseases of organic vegetables. J. Plant Protect. Res. 56, 221–230.
Shweta, S., Zuehlke, S., Ramesha, B.T., Priti, V., Kumar, P.M., Ravikanth, G., et al., 2010. Endophytic fungal strains of Fusariumsolani, from Apodytesdimidiata E. Mey. exArn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. Phytochemistry 71, 117–122.
Stinson, M., Ezra, D., Hess, W.M., Sears, J., Strobel, G.A., 2003. An endophytic *Gliocladium* sp. of *Eucryphiacordifolia* producing selective volatile antimicrobial compounds. Plant Sci. 165, 913–922.
Strobel, G., 2006. Harnessing endophytes for industrial microbiology. Curr. Opin. Microbiol. 9, 240–244.

Strobel, G., Daisy, B., 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol. Mol. Biol. Rev. 67, 491–502.
Tan, R.X., Zou, W.X., 2001. Endophytes: a rich source of functional metabolites. Nat. Prod. Rep. 18, 448–459.
Tatum, J.H., Baker, R.A., 1983. Naphthoquinones produced by Fusariumsolani isolated from citrus. Phytochemistry 22, 543–547.
Tatum, J.H., Baler, R.A., Bessy, R.E., 1985. Three further naphthaquinones produced by *Fusariumsolani*. Phytochemistry 24, 3019–3021.
Uzor, P.F., Osadebe, P.O., Nwodo, N.J., 2017. Antidiabetic activity of extract and compounds from an endophytic fungus Nigrosporaoryzae. Drug Res. 67, 308–311.
Zhao, K., Penttinen, P., Guan, T., Xiao, J., Chen, Q., Xu, J., et al., 2011. The diversity and anti-microbial activity of endophytic*actinomycetes* isolated from medicinal plants in Panxi plateau, China. Curr. Microbiol. 62, 182–190.
Zimmerman, N.B., Vitousek, P.M., 2012. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proc. Natl. Acad. Sci. U.S.A. 109, 13022–13027.