INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–1863/2019/21–6–1256–1262
DOI: 10.17957/IJAB/15.1019
http://www.fspublishers.org

Full Length Article

Molecular Characterization of *Pyricularia oryzae* and its Management by Stem Extract of *Tribulus terrestris*

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Abstract

Rice blast disease caused by *Pyricluaria oryzae* Cavar is one of the most destructive diseases of rice responsible for higher yield losses all around the world. In the present study, morphological and molecular characterization of *P. oryzae* and its management by stem extracts of *Tribulus terrestris* L. was carried out. In a laboratory bioassay, methanolic stem extract of 1, 2, 3, 4 and 5% concentrations significantly reduced biomass of *P. oryzae* by 35–43%. Methanolic extract was successively fractionated using four organic solvents. Bioassays with various concentrations (1.562, 3.125, 6.25……., 200 mg mL^{-1}) of these fractions showed that chloroform and ethyl acetate fractions were highly antifungal resulting in 54–82% and 16–85% suppression in biomass of *P. oryzae*, respectively. Chloroform fraction was analyzed by GC-MS that revealed 17 compounds. 1, 3-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (20.69%), octacosane (11.20%), heptacosane (9.67%), octadecane, 6-methyl- (5.40%), *n*-hexadecanoic acid (4.82%), pentadecanoic acid, 14 methyl-, methyl ester (3.80%) and apiol (3.71%) were the predominant compounds. In conclusion, stem of *T. terrestris* contains potent antifungal compounds which can be exploited for management of *P. oryzae.* © 2019 Friends Science Publishers

Keywords: Antifungal activity; Stem extract; *Pyricluaria oryzae*; *Tribulus terrestris*

Introduction

Rice (*Oryza sativa* L.) is the third most-produced agricultural product in the globe that fulfils about twenty percent of daily calories of more than 3.5 billion individuals around the world (IRRI, 2012; USDA, 2015). It is grown in at least 114 countries with China and India as leading producers contributing 28.4% and 21.2% of the world's production, respectively. The total production of rice is about 610 million metric tons worldwide (FAOSTAT, 2014). Its average consumption is about 65 kg $person^{-1}$ $year^{-1}$; however, the pattern of consumption varies around the world (Crawferd and Lee, 2003). In Pakistan, rice is an important cash crop and second essential staple food after wheat (*Triticum aestivum* L.). It was cultivated on an area of 2899 thousand ha with a record production of 7442 thousand tons in 2017–2018. It is one of the major export items contributing 0.6% of GDP and 3.1% of value added in agriculture (Pakistan Economic Survey, 2017–2018).

Rice is attacked by a number of fungal, bacterial and viral diseases which significantly contribute to reduced annual yield. Rice blast is among the most serious fungal diseases causing recurring problems in about 85 countries of the world (Gilbert *et al*., 2004). First reported in Asia (Rao, 1994), the disease now has become a major threat to global food security as it has the potential to reduce rice production up to 50% under favorable environment. The disease can attack on almost all parts of rice plants at all growth stages (Zheng *et al*., 2000). Formation of diamond shaped lesions is the diagnostic symptom of the disease; however, symptoms can vary depending on age of the host plant, level of resistance and environmental conditions (Meena, 2006). In Pakistan, this disease is a limiting factor for rice production as most of the cultivated basmati varieties of rice are susceptible to this disease (Khan *et al*., 2002). The disease is caused by *Pyricularia oryzae* Cav. that belongs to phylum Ascomycota. The pathogen has a wide host range and can attack more than 50 host plants of family Poaceae (Tosa *et al*., 2004). On the bases of phenotypic and genotypic variations, a number of populations of this pathogen occur in the world (Kumar *et al*., 1999).

Certain cultural practices such as management of nutrients and water, removal of crop residues and planting time can reduce the disease development, however, these practices cannot eradicate the disease completely (Pooja and Katoch, 2014). Application of fungicides is very effective method to control rice blast disease, however, too much dependence on these agrochemicals also adversely affect the beneficial microorganisms and environment, and also causes health risks to consumers (Awla *et al.,* 2017). In recent years, there is an increasing trend of using plant products for the management of phytopathogens in an environmental friendly way. These strategies include use of crude plant extracts (Ali *et al.,* 2017; Banaras *et al.,* 2017;

To cite this paper: Javaid, A., F. Anjum and N. Akhtar, 2019. Molecular characterization of *Pyricularia oryzae* and its management by stem extract of *Tribulus terrestris*. *Intl. J. Agric. Biol*., 21: 1256–1262

Javaid *et al.,* 2018a), use of purified plant products (Jabeen *et al.,* 2011) and soil amendment with plant materials for management of plant diseases (Javaid *et al.,* 2017a; Akhtar and Javaid, 2018). Recently, Shafaullah and Khan (2016) reported that crude extracts of various plant species especially *Eucalyptus* is very effective against *P. oryzae.*

Tribulus terrestris, family Zygophylaceae, is noxious weed that also has medicinal and pharmaceutical importance especially because of presence of steroidal saponins (Hashim *et al.,* 2014). The plant has its uses in asthma, urinary dysfunction and opthalmia (Qureshi *et al.,* 2010) and also possesses vasodilatory, antihelmentic, antihypertensive, cytotoxic and antitumor properties (Hashim *et al.,* 2014; Yanala *et al.,* 2016). Studies regarding antimicrobial activities of *T. terrestris* are limited. In addition, previous studies regarding antibacterial and antifungal activities of this plant were generally carried out using its fruits, leaves and roots (Al-Bayati and Al-Mola, 2008; Javaid and Anjum, 2019). The present study was therefore, undertaken to characterize *P. oryzae* on molecular basis and assess the potential of stem extract of *T. terrestris* for its antifungal activity against this pathogen.

Materials and Methods

Molecular Characterization

Pure culture of fungus causing rice blast disease in fields of district Sialkot, Pakistan was recovered and identified on the basis of cultural and morphological characteristics. Morphology based identification of *P. oryzae* was confirmed by nucleotide sequence analysis of Internal Transcribed Spacer (ITS) region of rDNA (Javaid *et al.,* 2016; Nayab and Akhtar, 2017). Conidia from one week old pure culture were ground to fine powder in liquid nitrogen for DNA isolation following the procedure as described by Akhtar *et al.* (2014). Quality of the isolated DNA was checked by agarose gel electrophoresis. Partial ITS sequence from ITS1-5.8S-ITS2 region of rDNA was amplified by universal primer pair ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) from the total genomic DNA (White *et al*., 1990; Nayab and Akhtar, 2016). Resulting nucleotide sequence of amplified DNA fragment was deposited to GenBank under the accession number KT021877 and used as query for homology studies by Basic Local Alignment Search Tool (BLAST).

Antifungal Activity of Stem Extract

Mature plants of *T. terrestris* were collected from Raiwind Road Lahore, Pakistan. Plants were washed under tap water. Stems were separated, cut into small pieces, dried in shade for one week and crushed thoroughly. For the extraction of methanol soluble compounds from the plant material, 1700 g of crushed stem were soaked in 4 L methanol in closed bottle for 15 days. Thereafter, filtration of the material was done first by cheesecloth and then by filter paper. Residues were re-soaked in 2 L of methanol for 7 days and again filtered. The filtrates were combined and evaporated in a rotary evaporator at 45^{o}C to reduce the volume to about 40 mL. The extracts were transferred to clean beakers and placed in dry heat oven at 45^{o}C to completely evaporate the solvent. Crude 77 g methanolic extract (gummy biomass) of stem was obtained and stored in sterilized glass bottle.

In vitro bioassay was carried out with methanolic extract against *P. oryzae*. Stock solution was prepared by dissolving 4.725 g crude methanolic stem extract in 4 mL of DMSO (Dimethyl sulfoxide) and final volume was raised up to 10.5 mL with autoclaved distilled water. Six concentrations *viz*., 0.5, 1, …, 3% were prepared by adding appropriate quantity of stock solution to autoclaved malt extract (ME) broth to raise the volume up to 45 mL that was later on divided in to three equal portions in 100 mL conical flasks. In all the treatments, amount of DMSO was kept constant. Control treatment was without extract but had the same amount of DMSO as was in experimental flasks. Flasks were inoculated with fresh *P. oryzae* culture using 5 mm diameter borer. Incubation was done at 27^{o}C for 10 days. Thereafter, the fungal biomass in each flask was filtered and weighed after drying at 60^{o}C (Javaid *et al.,* 2017b).

To the remaining methanolic extract (72.275 g), 300 mL autoclaved distilled water was added fractionated with 500 mL of *n*-hexane and the process was repeated 5 times to completely separate *n*-hexane soluble components. The aqueous phase was further fractionated using 500 mL of each of chloroform, ethyl acetate and *n*-butanol followed by evaporation of solvents on a rotary evaporator to yield various organic solvent fractions. Different fractions of methanolic stem extract were assessed for their bioactivity against *P. oryzae* in 10 mL test tubes using malt extract broth. Weighed amount (1.2 g) of each fraction was dissolved in 1 mL DMSO and raised the volume stock solution to 6 mL of 200 mg mL^{-1} concentration by addition of autoclaved ME broth. Stock solution was serially double diluted by addition of ME broth to prepare lower concentrations up to1.51 mg mL^{-1}. Control solution was prepared by adding 1 mL of DMSO in 5 mL of sterilized ME broth and serially double diluted as in case of extract treatments. Malt extract (1 mL) was transferred in each 10 mL test tube and 20 µL of spore suspension (10^{7} mL^{-1}) of *P. oryzae* was added to it under aseptic conditions. Three replicates were made for each treatment. After incubation at 27^{o}C for ten days, fungal biomass was collected on filter papers, dried at 60^{o}C and weighed (Javaid *et al.,* 2017c).

GC-MS Analysis

Chloroform fraction of *T. terrestris* stem extract was analyzed by GC-MS on an Agilant technologies model GC-7890A machine coupled to MS 5975C mass spectrometer following Ali *et al.* (2017).

Statistical Analysis

Statistical analysis was done by applying ANOVA followed by application of LSD test at $P = 0.05$ using Statistix 8.1 software.

Results

Molecular Characterization

The colony color of isolated fungal pathogen on PDA and MEA was grayish black. Diameter of colony reached to 4.5–5 cm in 7 days. Mycelia were branched, septate and olivaceous. Conidia were pale olive and borne sympodially, usually 2–3 septate and 17–25×8^{-11} μm in size. Based on Morphology fungus was identified as *Pyricularia oryzae*. Identification of pathogen was verified by nucleotide sequence analysis of rDNA-ITS region. Primer pair ITS1/ITS4 amplified approximately 600 bp DNA pragment from ITS1–5.8S rDNA-ITS4 region of rDNA (Fig. 1).

Nucleotide BLAST results of amplified DNA fragment indicated that this sequence had 100% homology to many different isolates of *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*) present in the database of GenBank. For example *M. oryzae* strain w11-1 (gb|KM816793), strain C2P21B.2 (gb|JQ936264), strain CBS 365.52 (gb|KM484890), strain BF0028 (gb|KM484886) etc. (Fig. 2).

Antifungal Activity of Stem Extract

Various concentrations of stem extract used in experiment significantly reduced biomass of *P. oryzae* by 35–43% as compared to control. However, the variation among different extract concentrations (0.5 to 3.0%) was not very much pronounced (Fig. 3).

Among the various fractions of methanolic extract of *T. terrestris* stem, chloroform fraction showed the best antifungal activity against *P. oryzae.* All the concentrations of chloroform fraction significantly suppressed biomass of *P. oryzae* by 54–81% over corresponding control treatments (Fig. 4B and Fig. 5). The second most effective fraction of methanolic stem extract against *P. oryzae* was ethyl acetate one. Lower concentration of this fraction ranging from 1.562 to 6.25 mg mL^{-1} showed an insignificant effect. However, 12.5 mg mL^{-1} and higher concentrations significantly reduced biomass of *P. oryzae* by 50–86% over control. Likewise, higher concentrations of *n*-butanol fractions (50–200 mg mL^{-1}) suppressed fungal biomass production by 76–80% (Fig. 4C, D and Fig. 5). In contrast, all the concentrations of *n*-hexane and aqueous fraction except 200 mg mL^{-1} enhanced pathogen growth (Fig. 4A and E).

GC-MS Analysis

GC-MS chromatogram presented in Fig. 6 showed the presence of 17 compounds in chloroform fraction of

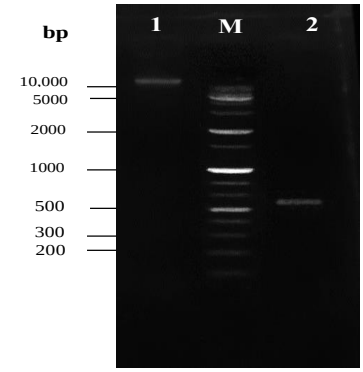

Fig. 1: *Pyricularia oryzae.* (**1**): Genomic DNA, (**M**): 1 Kb marker (**2**): ITS1/ITS4 amplified PCR product

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FCBP1526 TGTCGTTGCTTCGGCGGGCACGCCCGCCGGAGGTTCAAAACTCTTAttttttttCAGTATC   60
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
w11-1    TGTCGTTGCTTCGGCGGGCACGCCCGCCGGAGGTTCAAAACTCTTATTTTTTTTCAGTATC   136

FCBP1526 TCTGAGCCTAAAAGACAAATAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC   120
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
w11-1    TCTGAGCCTAAAAGACAAATAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC   196

FCBP1526 GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG   180
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
w11-1    GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG   256

FCBP1526 AATCTTTGAACGCACATTGCGCCCGCCGGTATTCCGGCGGGCATGCCTGTTCGAGCGTCA   240
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
w11-1    AATCTTTGAACGCACATTGCGCCCGCCGGTATTCCGGCGGGCATGCCTGTTCGAGCGTCA   316

FCBP1526 TTTCAACCCTCAAGCCTCGGCTTGGTGTTGGGGCGCCCGGGCCCTCCGCGGCCCGGGGCC   300
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
w11-1    TTTCAACCCTCAAGCCTCGGCTTGGTGTTGGGGCGCCCGGGCCCTCCGCGGCCCGGGGCC   376

FCBP1526 CCCAAGTTCATCGGCGGGCTCGTCGGTACACTGAGCGCAGTAAAACGCGGTAAAACGCGA   360
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
w11-1    CCCAAGTTCATCGGCGGGCTCGTCGGTACACTGAGCGCAGTAAAACGCGGTAAAACGCGA   436

FCBP1526 ACCTCGTTCGGATCGTCCCGGCGTGCTCCAGCCGCTAAACCCCCAATTTTTTAAAGGTTG   420
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
w11-1    ACCTCGTTCGGATCGTCCCGGCGTGCTCCAGCCGCTAAACCCCCAATTTTTTAAAGGTTG   496

FCBP1526 ACCTCGGATC  430
         ||||||||||
w11-1    ACCTCGGATC  506
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Fig. 2: ITS sequence alignment of *M. oryzae* (*P. oryzae*). The BLAST results revealed 100% identity of FCBP1526 with the strain w11-1 in the GenBank database

methanolic stem extract of *T. terrestris.* Retention time of these 17 compounds ranged from 10.399 to 26.922 min, molecular weight from 143 to 394 a.m.u. and peak areas from 1.15% to 20.69% (Table 1). 1, 3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester was the most abundant compound with peak area 20.69% followed by octacosane and heptacosane with peak areas 11.20% and 9.67%, respectively. Other frequently occurring compounds were octadecane, 6-methyl- (5.40%), *n*-hexadecanoic acid (4.82%), pentadecanoic acid, 14 methyl-, methyl ester (3.80%) and apiol (3.71%). Less frequently occurring compounds included 6,9,12,15-Docosatetraenoic acid, methyl ester (2.77%), 1, 2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (2.50%), 9,12-octadecadienoic acid, methyl ester.(E,E)- (2.30%), phenol,2-methoxy-4-(1-propenyl)-,(Z)- (2.11%), cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans- (1.99%), stigmasterol (1.87%), oleic acid (1.43%), ethyl iso-allocholate (1.28%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (1.27%), and DL-proline,5oxo-, methyl ester (1.15%) (Table 1). Structures of the identified compounds are presented in Fig. 7.

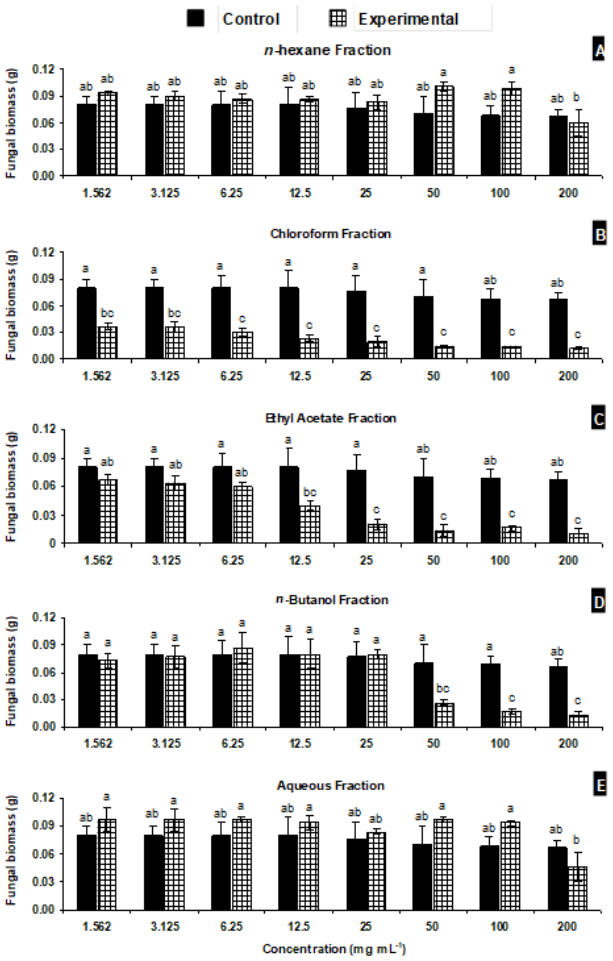

Fig. 4 (A-E): Effect of different concentrations of sub-fractions of methanolic stem extract of *T. terrestris* on growth of *P. oryzae*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test

Table 1: Compounds identified from chloroform fraction of methanolic stem extract of *T. terrestris* through GC-MS analysis

Comp. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	DL.Proline,5oxo methyl ester	$C_6H_9NO_3$	143	10.40	1.15
2	Phenol,2-methoxy-4-(1-propenyl)-,(Z)-	$C_{10}H_{12}O_2$	164	10.92	2.11
3	Apiol	$C_{12}H_{14}O_4$	222	12.84	3.71
4	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	14.74	1.27
5	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	346	15.26	2.77
6	Pentadecanoic acid, 14 methyl-, methyl ester	$C_{17}H_{34}O_2$	270	15.87	3.80
7	9,12-Octadecadienoic acid, methyl ester.(E,E)-	$C_{19}H_{34}O_2$	294	17.54	2.30
8	Cyclopropanepentanoic acid, 2-undecyl. methyl ester, trans-	$C_{20}H_{38}O_2$	310	17.79	1.99
9	Oleic acid	$C_{18}H_{34}O_2$	282	18.32	1.43
10	Palmitic acid	$C_{16}H_{32}O_2$	256	18.41	4.82
11	Octadecane, 6-methyl-	$C_{19}H_{40}$	268	19.30	5.40
12	Heptacosane	$C_{27}H_{56}$	380	20.14	9.67
13	Octacosane	$C_{28}H_{58}$	394	20.95	11.20
14	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	21.41	2.50
15	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	22.98	20.69
16	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	25.80	1.28
17	Stigmasterol	$C_{29}H_{48}O$	412	26.92	1.87

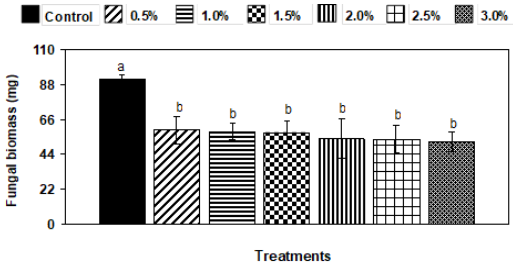

Fig. 3: Effect of different concentrations of methanolic stem extract of *T. terrestris* on growth of *P. oryzae*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test

Discussion

In the present study, ascomycete *P. oryzae* was recovered from the infected plants of rice having the characteristic three celled pear-shaped conidia (Barnett and Hunter, 1960; Kariaga *et al.*, 2016). Due to the complexity and non-stability in morphology of fungi grown under laboratory conditions, molecular and phylogenetic characterization of fungi is always recommended (White *et al.*, 1990; Akhtar *et al.*, 2014; Javaid *et al.*, 2016). Nucleotide sequence data of Inter Transcribed Spacer region of rDNA provides valuable information regarding the molecular identification of *P. oryzae* (Putri *et al.*, 2014; Xue *et al.*, 2017). Similarly, current study provided 100% homology of our pathogen to various strains of *P. oryzae* in GenBank hence confirming the pathogen identification.

Methanolic stem extract reduced fungal biomass up to 43%. Methanolic extract is proffered over aqueous extract in such types of study to avoid contamination and this method has been used in many recent studies (Akhtar and Javaid, 2018; Khurshid *et al.*, 2018). Earlier little work has been carried out regarding antifungal activity of *T. terrestris.* Few studies carried out in this filed revealed that extracts of different parts of *T. terrestris* showed antifungal activity against *Candida albicans* (Al-Bayati and Al-Mola, 2008; Kim *et al.*, 2018). Bioactive constituents such as phenols, alkaloids, terpenoids, saponins, tannins flavonoids and glycosides have been identified in methanolic extract of *T. terrestis* (Vasait, 2017), which could be responsible for its antifungal activity against *P. oryzae* (Chapagain *et al.*, 2007; Kanwal *et al.*, 2011; Zabka and Pavela, 2013). Methanolic extract was partitioned using various organic solvents in order of increasing polarity starting from non-polar *n*-hexane and ending at highly polar *n*-butanol. This technique is very useful to separate compounds present in methanolic extracts on the basis of their polarity nature

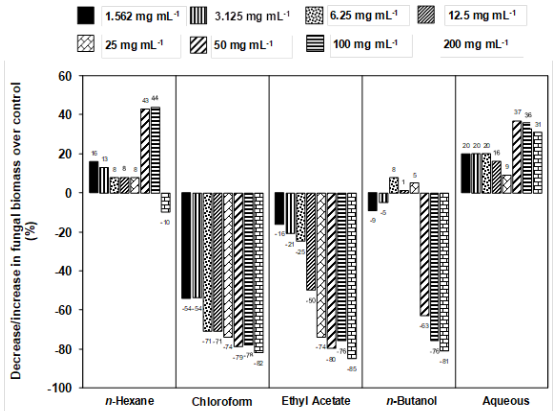

Fig. 5: Percentage increase/decrease in biomass of *P. oryzae* due to different concentrations of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions of methanol stem extract of *T. terrestris* over control

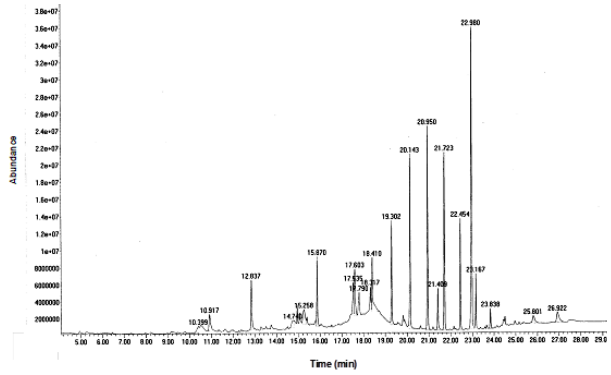

Fig. 6: GC-MS chromatogram of chloroform fraction of methanolic stem extract of *T. terrestris*

(Javaid *et al.*, 2018b). Bioassays revealed that among the various fractions of methanolic stem extract, chloroform fraction displayed the highest antifungal activity followed by ethyl acetate fraction causing 54–82% and 16–85% reduction in biomass of *P. oryzae*. On the other hand, *n*-hexane fraction stimulated the fungal growth. In various earlier studies, mostly chloroform fraction of methanolic extracts of *Datura metel, Senna occidentatlis* and other plant species showed the highest antifungal activity (Rauf and Javaid, 2013; Jabeen *et al.*, 2014; Javaid *et al.*, 2017b). Steroidal saponins and oligosaccharides have been identified in aerial parts of *T. terrestris* with antifungal activity against *Cryptococcus neoformans* and *Candida* spp. (Zhang *et al.*, 2005; Hammoda *et al.*, 2013).

A total of 17 compounds were identified in chloroform fraction of methanolic stem extract through GC-MS analysis. The most abundant compound 1, 3-benzenedicarboxylic acid, bis (2-ethylhexyl) ester also known as bis (2-ethylhexyl) isophthalate is a phthalate. Another less abundant compound 1, 2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester or mono (2-ethylhexyl) phthalate is also a phthalate.

Both of these compounds are plasticizers. Natural occurrence of phthalates in a variety of living organisms including plants (Ramalakshmi and Muthuchelian, 2011), algae (Dong *et al.*, 2016), bacteria (Al-Bari *et al.*, 2006) and fungi (Zhang *et al.*, 2018) has been reported in literature. Phthalates are known for their antibacterial and antifungal activities (Srivinasan *et al.*, 2009). Zhang *et al.* (2018) isolated compound 1, 2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester from *Trichoderma longibrachiatum* and reported it antifungal activity against pathogenic fungi.

Out of 17 compounds identified in GC-MS analysis, four namely 6,9,12,15-docosatetraenoic acid, methyl ester; pentadecanoic acid, 14 methyl-, methyl ester; 9,12-octadecadienoic acid, methyl ester (E,E)- and cyclopropanepentanoic acid, 2-undecyl-, methyl ester,trans-belonged to fatty acid methyl esters (FAME) and constituted 10.86% of the total. Fatty acid methyl esters are well known for their antifungal activity against a variety of fungal species (Ali *et al.*, 2017). FAME extract of *Excoecaria agallocha* suppressed growth of various *Candida* spp. namely *C. albicans*, *C. krusei*, *C. tropicalis* and *C. prapsilosis* with MIC value of 0.5–1.0 mg mL^{-1} (Agoramoorthy *et al.*, 2007). FAME extract of *Salicornia brachiata* (family Chenopodiaceae) showed strong antifungal activity against various *Candida* spp. while other species of the same family namely *Suaeda monoica, S. maritime* and *Arthrocnemum indicum* showed moderate anticandidal activity (Chandrasekaran *et al.*, 2007). Three FAMEs isolated from vegetable oils of sunflower and soybean showed strong antifungal activity against various isolates of *Paracoccidioides brasiliensis* and *P. lutzii*, the cause of paracoccidioidomycosis (Pinto *et al.*, 2017).

Among the identified compounds, oleic acid and palmitic acid belonged to free fatty acids. Fatty acids are known to exhibit antifungal activity against a number of fungal species (Pohl *et al.*, 2014). Palmitic acid is a saturated fatty acid with 16 carbon atoms. It is known for its antifungal properties against a number of fungal species including *Alernaria solani, Fusarium oxysporum, Cucumerinum lagenarium* (Liu *et al.*, 2008), *Emericella nidulans, Aspergillus terreus* and *Aspergillus niger* (Altieri, *et al.*, 2007). Oleic acid is an unsaturated fatty acid with 18 carbon atoms and is known to possess antifungal behaviour against *Pythium ultimum* and *Crinipellis pernicosa* (Walters *et al.*, 2004). Fungal cell membrane is the target site of fatty acids. Fatty acids increase the fluidity of cell membrane resulting in leakage of cellular components and ultimately cell death (Pohl *et al.*, 2014).

Conclusion

Methanolic stem extract has antifungal potential against rice blast fungus *P. oryzae*. Antifungal activity of methanolic extract is possibly because of free fatty acids namely oleic and palmitic acids; fatty acid methyl esters and phthalates

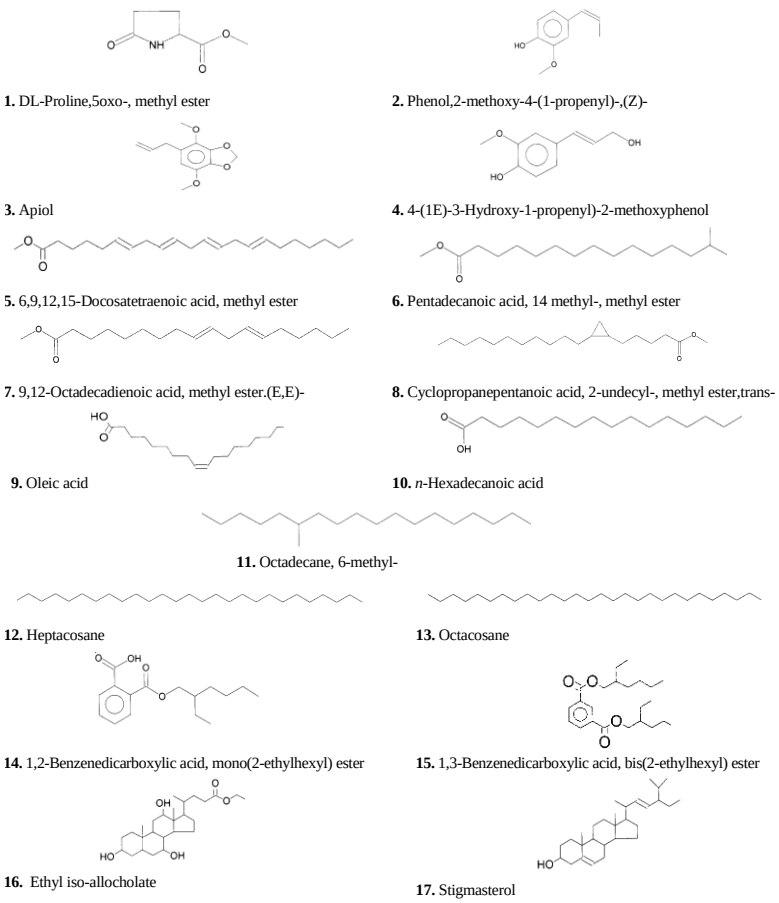

Fig. 7: Structures of compounds identified from chloroform sub-fraction of methanolic stem extract of *T. terrestris* through GC-MS analysis

which are present in chloroform fraction of the extract. Further studies are suggested to isolate and identify the individual compounds in chloroform sub-fraction through various chromatographic and spectroscopic techniques. Moreover, green house and field studies for management of rice blast disease using *T. terrestris* stem extract are also suggested.

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(Received 15 December 2018; Accepted 19 January 2019)