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Detection of Phytotoxin Produced from Leaf, Neck and Finger Blast Disease Causing *Magnaporthe grisea* through GC-MS Analysis

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Authors' contributions

This work was carried out in collaboration between all authors. Author TR designed the study, supervised the research and wrote the first draft of the manuscript. Authors SS and SP performed the experiments and analyzed the results obtained in the study. All authors read and approved the final manuscript.

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ABSTRACT

Finger millet blast is the most devastating disease affecting different aerial parts of the plant at all growth stages starting from seedling to grain formation. *Magnaporthe grisea* being a ubiquitous pathogen with many hosts, understand the basis for host pathogen interaction and role of toxins will be useful in development of resistant varieties and their screening procedures. In this study, the presence of toxic volatile compounds were detected by Thin Layer Chromatography (TLC) in the culture filtrate of leaf blast, neck blast and finger blast pathogen at different bands indicating various retention factor (R*f*). Likewise the several toxic compounds were detected from *M. grisea* through GC-MS analysis from the semi-purified crude toxin *in vitro viz.,* 1-Hexadecene (CAS), 1-Octadecene (CAS), Quinic acid, 1,2-Benzenedicarboxylic acid- bis (2-ethylhexyl) ester (CAS) from leaf blast isolate, Hippocasine, Holothurinogenin-2, Neophytadiene, 1,2-Benzene-dicarboxylic acid, diisooctyl ester (CAS), à-Patchoulene (CAS), Synaptogenin B, 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene), Zingiberene (CAS) from neck blast isolate and 2H-Pyran-2-one, 6-hexyltetrahydro-delta-Hexylvalerolactone from finger blast isolate were detected.

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Keywords: Blast disease; GC-MS; Magnaporthe grisea; finger millet; toxic compounds.

1. INTRODUCTION

The transformation of agriculture from a stable to more productive systems has been through crop distribution and diversification. Currently, the area and production of traditional crops are showing a declining trend in most developing countries. One such traditional group of cereal crops is the minor coarse cereals (small millets). Among the small millets, finger millet a widely grown traditional grain cereal cultivated in semi-arid areas of East and Southern Africa and South Asia, is a staple food and generates income for millions of poor people. In India, the important finger millet growing states are Karnataka, Odisha, Maharashtra, Tamil Nadu, Andhra Pradesh, Uttarakhand, Uttar Pradesh and Bihar. Finger millet constitutes about 81% of the minor millets produced in India [1]. In recent years, the overall production and productivity of finger millet has been declining due to several biotic and abiotic stresses. Of the biotic stresses, diseases caused by fungi, bacteria, viruses and MLOs are common. Among the fungal diseases, blast disease caused by *Magnaporthe grisea* (anamorph-*Pyricularia grisea* (Cooke) Sacc.) is a major problem in India and Africa causing substantial yield losses. In India, the disease was first reported from the Tanjore delta of Tamil Nadu by [2] with an estimated loss of 50% [3]. The average loss due to blast has been reported to be around 28-36% and in endemic areas, yield losses could be as high as 80-90% [4,5]. The blast pathogen *M. grisea* (Cooke) Sacc. [6] is a heterothallic, filamentous fungus, pathogenic to almost 50 plant species in 30 genera of *Poaceae* including economically important crops like rice, wheat, barley and millets [7].

Toxic metabolomics is a recently developed tool of systems biology which has enriched our knowledge on the regulation of metabolic networks [8]. A number of metabolomic studies on plant-pathogen interactions have been published [9,10]. The toxic volatiles organic compounds released from *M. grisea* was found to be a pathogenecity factor to enable epidemic disease. The different elicitors have resulted in some qualitative and quantitative differences in the production of volatiles. Monoterpenes and sesquiterpenes were identified as the rice blast fungus- induced toxic volatiles [11]. Several host selective and host non-selective toxins produced by plant pathogens have been isolated and their structures were determined during the last decade [12]. [13] reported that the toxic compound 1,5-Pentanedioic acid was detected at the retention time of 20.95 from *C. falcatum* through GC/MS. Similarly, [14] proved that the Oxalic acid is a major pathogenicity factor for *Sclerotinia sclerotiorum.* [15] reported that the oxalic acid compound was detected in the GC/MS analysis of ripe tomato fruits inoculated with *Aspergillus niger*. They further proved that oxalic acid compoundis the key factor for pathogenecity. [16] identified the toxic compound as lycomarasmic acid (aspergillomarasmin B) through thin layer chromatography which was produced by *C. gloeosporioides*. [17] reported that the toxic volatile compounds *viz*., Boronic acid, ethyl, 1,4-Cyclohexadiene, 1-methyl and Thujol were detected, from mango infected with *C. gloeosporioides* through portable GC/MS. [18] reported the production of secondary metabolite compounds *viz.*, 2-benezenedicarboxylic acid, bis (2-methylpropyl) ester, hexadecanoic acid, methyl ester, 1,2 benzenedicarboxylic acid, butyl 2-methylpropyl ester, 1,4-napththalenedione, 2-hydroxy-3-(3-methyl-2-butenyl)-, 9-octadecenoic acid (Z), methyl ester, 10,13-octadecadienoic acid, methyl ester and 1,2-benzenecarboxylic acid from endophytic fungi *Alternaria alternata.* 1,2-benezenedicarboxylic acid, bis (2-methylpropyl) ester have also been proved as a toxic compounds produced by plant pathogens. Metabolomic approach enables identification of bioactive fungal-derived metabolites and it provides valuable information that could be exploited in pathogenesis, chemical elicitor induced susceptibility or resistance, and detection for the development of early infection detector with advanced trapping and profiling tools.

2. MATERIALS AND METHODS

2.1 Collection and Isolation of Pathogen

In major finger millets growing regions of Tamil Nadu and from the All India Coordinated Small Millet Improvement Project (AICSMIP) centers, the blast infected finger millet plant parts *viz.*, leaf or neck or finger blast infected samples were taken at the time of survey. The collected samples were air dried, separately bagged and stored under refrigerated condition at 4°C for the isolation of the pathogen. The pathogen (*M. grisea*) of different samples collected was isolated by using the standard tissue isolation method [19]. Blast infected plant tissues were cut

into small pieces and washed in sterile water twice and surface sterilized with 0.1 per cent mercuric chloride solution for 30 sec. followed by rinsing in sterilized water twice and transferred to plates containing Oat Meal Agar Medium (OMA). After 4 days for obtaining monoconidial isolate, a dilute spore suspension was prepared in sterilized distilled water and plated onto 0.8% water agar in Petri plates. After 15 days of incubation at 26±1°C, single germinating conidium was marked under a microscope and transferred to fresh Petri dish containing OMA medium and then the plates were incubated at 26±1°C for 10 days to get monoconidial isolates [7]. Among the 24 isolates [20], the most virulent pathogenic isolates from leaf (TNLB1), neck (TNNB8) and finger blast (BIFB13) were selected for further studies.

2.2 Extraction of *M. grisea* Toxin *in vitro*

The toxin was isolated from the leaf, neck and finger blast isolates of *M. grisea viz.,* TNLB1, TNNB8 and BIFB13. The *in vitro* toxin was partially purified as per the procedure described by [21]. Erlenmeyer conical flasks containing 100 mL of Richard's broth were inoculated with 9 mm mycelial disc of two week old *M. grisea* isolates separately. After 20 days of growth under stationary conditions at laboratory temperature (25±1°C), the culture filtrates were pooled, filtered through three layers of cheese cloth under sterile conditions and concentrated *in vacuo* at 45°C using rotary evaporator to 10 per cent of its original volume. The condensed material was treated with equal volume of methanol and allowed to precipitate overnight at 4°C. Precipitates were removed by filtering through Whatman No. 1 filter paper. Methanol was evaporated *in vacuo* and the aqueous fraction was extracted three times with equal volumes of chloroform, ethyl acetate, carbon tetra chloride, hexane and ether using a separating funnel. The water fraction containing toxin activity was evaporated to dryness *in vacuo* at 40°C and dissolved in 10 mL of distilled water and used for further purification.

2.3 Purification of Toxin

The aqueous fraction after solvent separation containing toxic activity was applied to a Sephadex G-75 (Sigma, USA) superfine column (2.5x2.5 cm, Pharmacia, USA) and eluted with double distilled water at room temperature. Fractions (5 mL) were collected at a flow rate of 5 mL min^{-1} using Bio-Rad automated econosystem (Biorad, USA) and the column elute was monitored by UV monitor and recorder of the chromatography unit based on the absorbance at 280 nm. The obtained fractions were combined and evaporated to dryness *in vacuo* at 40°C, dissolved in 5 mL of distilled water to get a clear homogeneous syrup, freeze dried and stored at -20°C. The partially purified toxin was used to analyze the biological functions of the toxin in all further studies.

2.4 Detection of Toxins by Thin Layer Chromatography (TLC)

2.4.1 Separation of toxic compounds

Toxin produced by virulent isolates of *M. grisea* as determined by running the concentrated oily residues on TLC plates. Pure HPLC grade methanol (Sisco) and di-methyl sulfoxide (DMSO) were used as reference. The crude extract of each isolates of *M. grisea* as dissolved separately in methanol and DMSO (1:10) and spotted on the silica gel coated (Merk, Silica gel 60 F_{254}) TLC plates and placed in tanks containing solvents of Chloroform: glacial acetic acid: ethanol (3:1:1) for *M. grisea.*

The solvent system was poured into TLC tanks with approximately 0.5 cm immersed into the solvent at the bottom. The tanks were closed with a glass lids so as to have the chamber completely filled with the solvent vapour. Within 30 minutes, solvent reached the end of the TLC plates. Then the plates were removed from the tank and kept in open air at room temperature so as to enable the solvent to get evaporated and to leave the separated toxic compounds of *M. grisea.*

2.5 Identification of the Compound by TLC

2.5.1 Visual observation

Spots were visualized by spraying with various spraying reagents to find different compounds present in the extract. Compounds were detected by spraying with 1% Ferric chloride reagent for flavonoids, Dragendeoff's reagent for alkaloids, Liebermann-Burchard reagent for steroids and Anisaldehyde-sulphuric acid for sugars. Presence of compound was indicated by specific colour spots. All the spots were observed under UV light (254 nm). The relation to front (R*f*) of the spots developed on the TLC plate.

2.5.2 Detached leaf bioassay

Leaf sheaths of 20-day-old finger millet plants (variety KM 252) were detached and cut into 4 cm pieces. Each leaf sheath was placed on a glass slide and its ends were fixed with gum tape so the leaf sheath wouldn't curl. An injury was made with the tip of a ballpoint pen. The slide was kept inside a Petri dish lined with wet blotting paper. The test toxin sample was placed on a four mm diameter filter paper disk that was placed on the injured leaf sheath section. The Petri dishes were incubated under laboratory conditions (25±2°C; 12 h of light and 12 h of darkness). After 5 days of incubation, symptom development was assessed.

2.6 Gas Chromatography Mass Spectrometry (GC/MS) Studies

2.6.1 Preparation of sample

The isolates of *M. grisea* collected from leaf, neck and finger were grown on OMA plates for 15 days at 28°C in dark. Three pieces of 6 mm plug of the isolate was cut from the margin of the plate and inoculated on Erlenmeyer flasks (250 ml) containing 150 ml of oat meal broth. The flasks were then incubated in shaking incubator at 120 rpm at 28°C. The culture was harvested after 15 days. The mycelium was separated with Whatman No 1 filter paper and cell free supernatant was prepared through endotoxin free 0.2 µm PES syringe filter for GS-MS assay. For GC-MS analysis, the mycelium was separated by vacuum filtration using bottle top filter to collect the culture filtrate. The filtrate was extracted with Ethyl acetate in 1:1 ratio and allowed to shake for 2 hrs in rotary shaking incubator. The filtrate was air dried in a closed chamber in a dark room for GC-MS analysis.

2.6.2 Detection of toxic compound from *M. grisea* toxin through GC-MS analysis

The toxic compounds produced by the virulent isolates of *M. grisea* were analyzed through GC/MS (Thermo scientific Trace GC Ultra DSQ II) equipped with column (30 mm × 0.25 mm × 0.25 µm) under the following conditions. Helium was used as carrier gas with a flow rate at 1ml per minute, 1 µl sample injection with pre injection of solvent by AI/AS 3000 method with split-less mode injection with 30 seconds of sampling time. The column temperature was maintained initially at 50°C at the increasing rate of 10°C/min, no hold was followed by increasing up to 200°C and kept at the same temperature for 2 minutes hold with surge pressure 3kPa and 220 base temperature at right SSL method and 250 base temperature at right ECD method with the Aux 1 MS transfer line at 250°C. The electron impact energy was 70eV, Julet line temperature was set at 2000°C and the source temperature was set at 200°C. Electron impact (EI) mass scan (m/z) was recorded in the 45-450 aMU range. An ion mass spectrometer and OMA detector were used to monitor the eluted compounds. Compounds were identified by absorbance at nm over 10 to 25 min (total analysis time 35 min). Particular compounds structures were putatively identified and evaluated by comparing the molecular masses (m/z values) of the eluted compounds with literature data and standards.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Detection of *M. grisea* toxin through TLC

The toxic metabolite productions by *M. grisea* were detected using Thin Layer Chromatography (TLC). The presence of toxin substance was detected under ultraviolet light and iodine tank test. The leaf blast (TNLB1), neck blast (TNNB8) and finger blast (BIFB13) pathogen produced various bands at various retention factor (R*f*) value *viz.,* 0.92, 0.90 and 0.78 respectively from the crude toxin fraction produced *in vitro*. The TLC plates after development with iodine tank test showed distinct spots of golden yellow and dark green colour (Plate 1; Table 1).

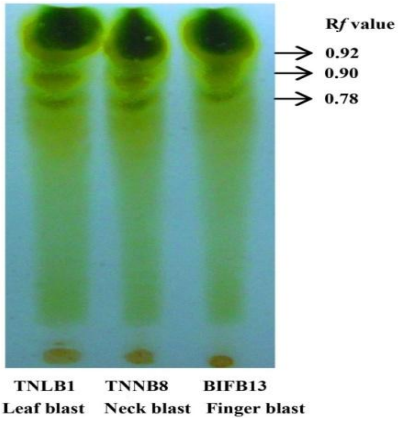


Plate 1. *In vitro* detection of *M. grisea* crude toxin by TLC

Table 1. Detection of *M. grisea* toxin by TLC produced under *in vitro*

Sl. No.	Isolates	Number of spots	Colour of band	Distance traveled (cm)	R*f* value
1.	TNLB1	3	Golden yellow	8.8, 8.6, 7.5	0.92, 0.90, 0.78
2.	TNNB8	3	Golden yellow	8.8, 8.6, 7.5	0.92, 0.90, 0.78
3.	BIFB13	3	Golden yellow	8.8, 8.6, 7.5	0.92, 0.90, 0.78

TNLB1- Leaf blast; TNNB8 – Neck blast; BIFB13 – Finger blast

3.1.2 Toxicity assay

Toxins characters revealed that the typical symptoms of blast disease with oval shaped spot contain gray centre and dark brown margin developed on the leaves 7 days after inoculation (Plate 2). When these infected leaves were removed and re-isolated for the toxic compounds from the spots, which were similar to the original toxin isolated from the *M. grisea*. This indicated that toxin produced by *M. grisea* was the primary causative factor for blast disease of finger millet.

3.1.3 Identification of toxic compounds from crude toxin of leaf blast causing *M. grisea* through GC-MS analysis

The toxic compounds from *M. grisea* were analyzed through GC/MS to detect the novel compounds and secondary metabolites responsible for pathogenicity. The compound identity was confirmed through NIST library 2005 AMDIS software programme. The total amounts of compounds 132 and104 were detected *in vitro*, among these few compounds were selected based on the unique nature and relative abundance of the peaks. The compounds detected *in vitro* were 1-Hexadecene (CAS) (0.83), 1-Octadecene (CAS) (1.49), Quinic acid (13.23) and 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) (2.56). Among the four toxic compounds maximum peak area was observed in the compound Quinic acid (13.23) (Plate 3; Table 2).

3.1.4 Identification of toxic compounds from crude toxin of neck blast causing *M. grisea* through GC-MS analysis

The toxic compounds from *M. grisea* produced *in vitro* were analyzed through GC/MS. The toxin produced *in vitro* yielded eight prominent peaks with retention time of 5.59, 10.02, 12.12, 12.72, 14.76, 25.15, 28.52 and 31.54 min. The peaks with retention time 5.59 corresponds to Hippocasine with 6.45 per cent peak area; 10.02 min represent to the Holothurinogenin-2 with 0.76 per cent of peak area; 12.12 min corresponds to Neophytadiene with 0.30 per cent of peak area; 12.72 min corresponds to 1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS) with 1.82 per cent of peak area; 14.76 min corresponds to à-Patchoulene (CAS) with 2.06 per cent of peak area; 25.15 min corresponds to Synaptogenin B with 0.76 per cent of peak area; 28.52 min corresponds to 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene) with 4.84 per cent of peak area and 31.54 min corresponds to Zingiberene (CAS) with 14.36 per cent of peak area were detected (Plate 4; Table 3).

Plate 2. Symptoms produced by crude toxin on finger millet leaves

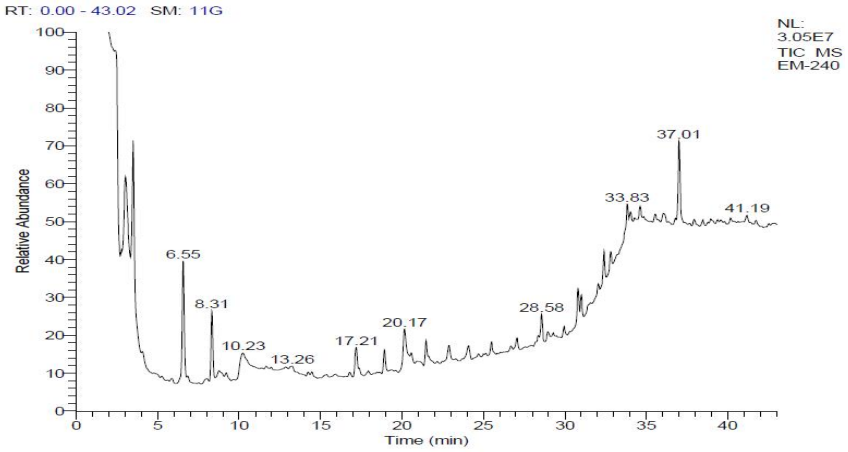


Plate 3. GC-MS chromatogram of toxic compounds from leaf blast causing *M. grisea*

Table 2. Toxic compounds identified from leaf blast causing *M. grisea* through GC/MS under *in vitro*

Sl. No.	RT	Name of the compound	Mol. formula	MW	Peak area (%)	CAS	Structure
1.	8.31	1-Hexadecene (CAS)	$C_{16}H_{32}$	224	0.83	629-73-2	
2.	13.26	1-Octadecene (CAS)	$C_{18}H_{36}$	252	1.49	112-88-9	
3.	17.21	Quinic acid	C7H12O6	180	13.23	87-89-8	
4.	28.58	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS)	$C_{24}H_{38}O_4$	390	2.56	117-81-7	

RT: Retention Time, MW: Molecular Weight, CAS: Chemical Abstracts Service

3.1.5 Identification of toxic compounds from crude toxin of finger blast causing *M. grisea* through GC-MS analysis

Results revealed the presence of toxin compound belonging to volatile compound group. *In vitro* analysis showed the presence of two volatile compounds with the retention time of 21.08 and 22.83. The identified compounds were 2H-Pyran-2-one, 6-hexyltetrahydro- delta-hexylvalerolactone and 1-Octadecene, octadec-1-ene, alpha-octadecene acid (Plate 5; Table 4).

3.2 Discussion

Identification of major biotic constituent present in the *M. grisea* isolates would help to acquire the mode and source of infection and further it will help to develop effective disease management strategies. In the present study, the toxic volatile compounds which are responsible for toxic substances in *M. grisea* isolates were identified through GC-MS method.In the present study, the major toxic volatile compounds *viz.,* 1-Hexadecene (CAS), 1-Octadecene (CAS), Quinic acid and 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) were identified on leaf blast. Subsequently, the compounds *viz.,*Hippocasine, Holothurinogenin-2, Neophytadiene, 1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS), à-Patchoulene (CAS), Synaptogenin B, 1-Naphthalenol, decahydro-1, 4a-dimethyl-7- (1-methylethylidene), Zingiberene (CAS) were identified on neck blast. Whereas in

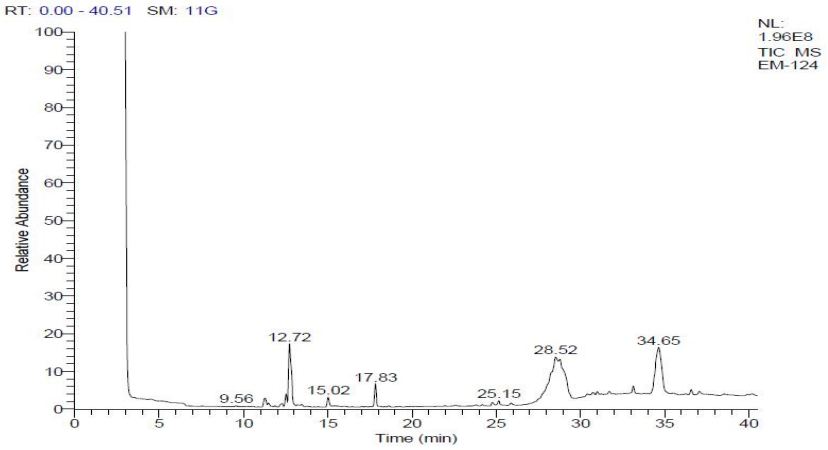


Plate 4. GC-MS chromatogram of toxin from neck blast causing *M. grisea*

fingers the compounds viz., 2H-Pyran-2-one, 6-hexyltetrahydro-delta-hexylvalerolactone and 1-Octadecene, octadec-1-ene, alpha-octadecene acid were identified in culture filtrate crude toxin. The results are in accordance with [18] reported that metabolites compounds viz., 2-benezenedicarboxylic acid, bis (2-methylpropyl) ester, hexadecanoic acid, methyl ester, 1,2 benzenedicarboxylic acid, butyl 2-methylpropyl ester, 1,4-napththalenedione, 2-hydroxy-3-(3-methyl-2-butenyl), 9-octadecenoic acid (Z), methyl ester, 10,13-octadecadienoic acid, methyl ester and 1,2-benzenecarboxylic acid were present in culture filtrate of endophytic fungi Alternaria alternata. This is also in concordance with [14] who reported that the benzene dicarboxalic and oxalic acid were major pathogenicity factors for Sclerotinia sclerotiorum. Earliar work reported by [22] indicated that the rice blast pathogen P. grisea has divergent physiological races distributed around the world and has been reported to produce several phytotoxic compounds such as pyricularin, α-picolinic acid, pyriculol and tenuazonic acid are potent inhibitors of seed germination. [23] reported that brown spot of rice pathogen B. oryzae to produce a host specific toxin ophiobolin which inhibits seed germination, growth of root and coleoptile in rice plant. Later, [24] reported that effects of plant age on toxin inoculation of detached leaves and whole plant conditions. For detached leaves inoculation, it was found that 20 day old plants were the most ideal to test the sensitive of crude extract toxin derived from culture filtrate of P. oryzae. Forty day old plants were found to be the most appropriate age for crude extract toxin inoculation as they produced typical blast

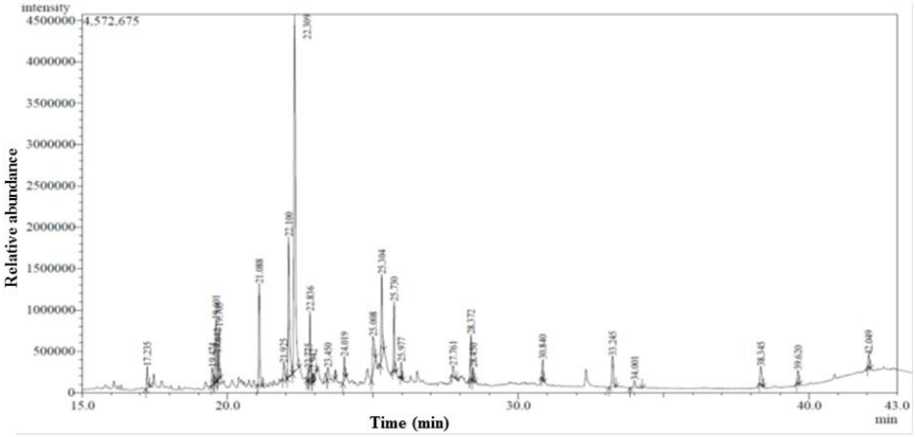
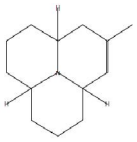
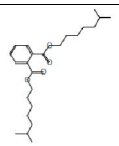
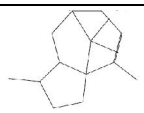
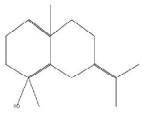
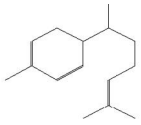


Plate 5. GC-MS chromatogram of toxins from finger blast causing *M. grisea*

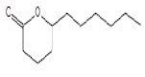
symptom while tenuazonic acid could produce similar typical blast symptoms on leaves at all plant ages.The results of the present study was in agreement with the earlier work of [15] who also studied the presence of toxic compounds in endophytic fungal pathogens and reported that the oxalic acid compound detected in the GC/MS analysis of ripe tomato fruits inoculated with *Aspergillus niger* may be the key factor for pathogenecity of *A. niger*. Similarly, several studies assessed the presence of toxic compounds in different endophytic fungal pathogens and found various toxic volatile compounds *viz*., 2-methylpropan-1-ol, 3-methylbutan-1-ol and oct-1-en-3-ol, 1,2-benezenedicarboxylic acid, bis (2-methylpropyl) ester, Hexadecanoic acid, methyl ester, 1,4-napththalenedione, 2-hydroxy-3-(3-methyl-2-butenyl), 10,13-octadecadienoic acid and 9-octadecenoic acid (Z), methyl ester [25,26,27, 28,29,30,31,32].

Table 3. Toxic compounds identified from neck blast causing *M. grisea* through GC/MS under *in vitro*

S. No.	RT	Name of the compound	Mol. formula	MW	Peak area (%)	CAS	Structure
1.	5.59	Hippocasine	$C_{13}H_{21}N$	191	6.45	75556-13-7	
2.	10.02	Holothurinogenin-2	$C_{30}H_{48}O_5$	488	0.76	27554-26-3	
3.	12.12	Neophytadiene	$C_{20}H_{38}$	278	0.30	504-96-1	
4.	12.72	1,2-Benzene-dicarboxylic acid, diisooctyl ester (CAS)	$C_{24}H_{38}O_4$	390	1.82	117-81-7	
5.	14.76	à-Patchoulene (CAS)	$C_{15}H_{24}$	204	2.06	560-32-7	
6.	25.15	Synaptogenin B	$C_{30}H_{46}O_4$	470	0.76	75556-13-7	
7.	28.52	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)	$C_{15}H_{26}O$	222	4.84	473-04-1	
8.	31.54	Zingiberene (CAS)	$C_{15}H_{24}$	204	14.36	495-60-3	

RT: Retention Time, MW: Molecular Weight, CAS: Chemical Abstracts Service

Table 4. Toxic compounds identified from finger blast causing *M. grisea* through GC/MS under *in vitro*

Sl. No.	RT	Name of the compound	Mol. formula	MW	Peak area (%)	CAS	Structure
1.	21.08	2H-Pyran-2-one, 6-hexyltetrahydro- delta-Hexylvalerolactone	$C_{11}H_{20}O_2$	184	71.05	710-04-3	
2.	22.83	1-Octadecene, octadec-1-ene, alpha-octadecene	C18H36	252	83.10	112-88-9	

RT: Retention Time, MW: Molecular Weight, CAS: Chemical Abstracts Service

4. CONCLUSION

Results of the present studies revealed the ability of *M. grisea* to produce phytotoxic compound in the culture filtrate and its toxicity on finger millet plant tissues. Thus, the involvement of this toxin in the development of blast symptoms is a possibility. Proper understanding of toxin chemistry and its role in pathogenesis requires further investigations and the current investigations provide a proper base for this.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Latha AM, Rao KV, Reddy VD. Production of transgenic plants resistant to leaf blast disease in finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Sci. 2005;169(4):657-667.
2. Mc Rae W. Detailed administration report of the government mycologist for the year. 1920;19:19-20.
3. Venkatarayan SV. Diseases of ragi. Mysore J. Agric. Sci. 1946;24:50-57.
4. Vishwanath S, Sanne Gowda S, Seetharam A, Shankare Gowda BT. Reaction to blast disease of released and pre-released varieties of finger millet from different states. Millet Newsletter. 1986;5: 31.
5. Rao ANS. Estimates of losses in finger millet (*Eleusine coracana*) due to blast disease (*Pyricularia grisea*). J. Agric. Sci., 1990;24:57–60.
6. Rossman AY, Howard RJ, Valent B. *Pyricularia grisea*, the correct name of the rice blast disease fungus. Mycologia. 1990;82:509–512.
7. Ou SH. Rice diseases. Commonwealth Mycological Institute, Kew, Surrey, England; 1985.
8. Aliferis KA, Jabaji S. Metabolomics-a robust bioanalytical approach for the discovery of the modes-of-action of pesticides: A review. Pestic Biochem. Phys. and Physiology. 2011;100:105-117.
9. Hong L, Hemert JV, Dash S, Roger P, Dickerson A. PLEXdb: Gene expression resources for plants and plant pathogens. Life Sci. 2012;40:1194-1201.
10. Lopez-Gresa Kim M, Choi HK, Verpoorte YHR, Conejero V. Metabolic fingerprinting of tomato mosaic virus infected *Solanum lycopersicum*. J. Plant Physiol. 2012;169: 1586-1596.
11. Obara N, Hasegawa M, Kodama O. Induced volatiles in elicitor-treated and rice blast fungus-inoculated rice leaves. Biosci. Biotechnol. Biochem. 2002;66(12):2549-2559.
12. Scheffer RP, Livingston RS. Sensitivity of sugarcane clones to toxin from *Helminthosporium sacchari* as determined by electrolyte leakage. Phytopathology. 1980;70:400-404.
13. Jayakumar V. Studies on biological control of sugarcane (*Saccharum officinarum* L.) red rot caused by *Colletotrichum falcatum* Went. *Ph. D.* Thesis, Tamil Nadu Agricultural University, Coimbatore, India. 2004;193.
14. Cessna SG, Sears VE, Dickman MB, Low PS. Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum,* suppresses the oxidative burst of the host plant. Plant Cell. 2000;12:2191-2199.
15. Ibrahim AD, Sani A, Manga SB, Aliero AA, Joseph RU, Yakubu SE, Ibafidon H. Volatile metabolites profiling to discriminate diseases of tomato fruits inoculated with three toxigenic fungal pathogens. Res. Biotech. 2011;2(3):14-22.
16. Ballio A, Botallicao A, Buonocore V, Carilli A, Divittoria V, Graniti A.. Production and isolation of aspergillomarasmin-B (Lycomarasmic acid) from *Colletotrichum gloeosporioides* (*Gloeosporium olivarum*). Phytopathol. Mediterr. 1969;8:187-196.
17. Moalemiyan M, Vikram A, Kushalappa AC. Detection and discrimination of two fungal diseases of mango (cv. Keitt) fruits based on volatile metabolite profiles using GC/MS. Post harvest Biol. Technol. 2007; 45:117-125.
18. Govindappa M, Channabasava R, Sadananda TS, Chandrappa CP, Umashankar T. Identification of bioactive

metabolites by GC-MS from an endophytic fungus, *Alternaria alternata* from *Tabebuia argentea* and their *in vitro* cytotoxic activity. Int. J. Pharm. Biol. Sci. 2014;5(6): 527-534.

19. Tuite J. Plant pathological methods, fungi and bacteria. Burges Publishing Company, USA 1969;239.
20. Shanmugapackiam S, Parthasarathy S, Raguchander T. Detection of finger millet blast pathogen *Magnaporthe grisea* using FTIR spectroscopy. Chem. Sci. Rev. Lett. 2017;6(22):956-960.
21. Samiyappan R, Amutha G, Kandan A, Nandakumar R, Babu S, Vijayasamundeeswari A, Radjacommare R, Ramanathan A, Balasubramanian P. Purification and partial characterization of a phytotoxin produced by *Sarocladium oryzae*, the rice sheath rot pathogen. Arch. Phytopathol. Plant Protect. 2003;36:247-256.
22. Umetsu N, Kaji J, Tamari K. Investigation on the toxin production by several blast fungus strains and isolation of tenuazonic acid as novel toxin. Agr. Biol. Chem. 1972; 36:859-866.
23. Kulkarni S, Ramakrishnan K, Hegde RK. Incidence of brown leaf spot of rice caused by *Drechslera oryzae* (Breda de Haan) subram and jain under different agroclimatic conditions of Karnataka. Mysore J. Agri. Sci. 1980;14:321-322.
24. Singburaudom N, Chaudhary RN, Sommartya T, Sarobo E. Effects of plant age on symptom development produced by *Pyricularia oryzae* toxin. Kasetsart J. Nat. Sci. 1998;32:90–101.
25. Bechtel DB, Kaleikau LA, Gaines RL, Seitz LM. The effects of *Fusarium graminearum* infection on wheat kernels. Cereal Chem. 1985;62:191–197.
26. Hayashi Y, Nishikawa Y, Mori H, Tamura H, Matsushita YI, Matsu T. Antitumor activity of (1OE, 122)-9-Hydroxy- 10, 12-octadecadienoic acid from rice bran. J. Ferment. Bioeng. 1998;86(2):149-153.
27. Oliveira MF, Lemos TG, de Mattos MC, Segundo TA, Santiago GMP, Braz-Filho R. New enamine derivatives of lapachol and biological activity. An. Acad. Bras. Cienc. 2002;74(2):211-221.
28. Jelen HH. Use of solid phase microextraction (SPME) for profiling fungal volatile metabolites. Lett. Appl. Microbiol. 2003;36:263–267.
29. Holopainen JK, Gershenzon J. Multiple stress factors and the emission of plant VOCs. Trends Plant Sci. 2010;15:176–184.
30. Wei LS, Wee W, Fu Siong JY, Syamsumir DF. Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of *Peperomia pellucida* leaf extract. Acta Med. Iran. 2011;49(10):671-674.
31. Sudha S, Masilamani SM. Characterization of cytotoxic compound from marine sediment derived actinomycete *Streptomyces avidinii* strain SU4. Asian Pac. J. Trop. Biomed. 2012; 2(10):770–773.
32. Amanian RS, Brindha P. *In vitro* cytotoxic, antioxidant and GC-MS studies on *Centratherum punctatum* Cass. Int. J. Pharm. Pharm. Sci. 2013;5(3):364-367.

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