Research Article

Identification of Secondary Metabolites from Finger Millet Parts Infected with *Magnaporthe grisea* by GC-MS Analysis

S. Shanmugapackiam[1]*, P. Murali Sankar[2], S. Irulandi[3] and T. Raguchander[2]

[1]ICAR – Krishi Vigyan Kendra, Vamban, Pudukkottai – 622 303
[2]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641 003
[3]Department of Entomology, Tamil Nadu Agricultural University, Periyakulam – 625 601

Abstract

In this paper, volatile compounds from leaf, neck and finger blast plant samples of ragi infected with *Magnaporthe grisea* were investigated. The presence of volatile compounds were detected by using Thinlayer chromatography(TLC) in the infected plant extract of leaf, neck and finger blast at different bands indicating various retention factor *(Rf)* value *viz*., 0.88, 0.77, 0.68, 0.83, 0.66, 0.86 and 0.80 respectively. Likewise the volatile compounds were also detected through GC-MS analysis from the crude metabolite of plant extract from infected blast plant parts *viz*., leaf, neck and finger blast. The crude volatile compound obtained from leaf blast samples yielded certain compounds *viz*., Octadecane (CAS), Pentadecanoic acid (CAS), Quinic acid, 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) and Desulphosinigrin. From neck infected plant samples, compounds *viz*., Zingiberene (CAS), à-Patchoulene (CAS), 1-Naphthalenol, 1-Naphthalenol, decahydro-1, 4a-dimethyl-7-(1methylethylidene), Synaptogenin B, Holothurinogenin-2 were detected. Similarly, in finger blast samples compounds like 1-Naphthalenol, Isopulegol 1, 2H-Pyran-2-one, 6 hexyl tetrahydro - delta-hexyl valerolactone, delta-hexyl-delta-valerolactone, delta Undecalactone, Z-9-Pentadecenol, 1-Octadecanol, octadecan-1-ol, 1- octadecanol, 1 Hydroxyoctadecane and Phenylacetic acid, 2-(1-adamantyl) ethyl esterwere detected.

Keywords: Finger millet, Blast disease, *Magnaporthe grisea*, Toxic Compounds, GC-MS

***Correspondence**
Author: S. Shanmugapackiam
Email: spmpatho@gmail.com

Introduction

Finger millet (*Eleusine coracana* (l.) Gaertner. is one of the stable food in the rural community of Tamil Nadu, Andhra Pradesh, Karnataka and Maharashtra. Blast disease incited by *Magnaporthe grisea* (hebert) barr; anamorph of *Pyricularia grisea* (cooke) sacc. is a heterothallic, filamentous fungus, one of the major destructive disease causing excessive damage to this crop from seedling to ear head forming stages. The disease occurs during all growing seasons and on almost all finger millet varieties cultivated. Yield loss due to blast can be as high as 50% when the disease occurs in epidemic proportions. Yield loss due to blast may be around 28 per cent but under favorable conditions it may be higher than 80 – 90 per cent. Toxic metabolomics is a recently developed tool of systems biology which has enriched our knowledge on the regulation of metabolic networks [15]. A number of metabolomic studies on plant-pathogen interactions have been published [16]. The toxic volatiles organic compounds released from *M. grisea* were found to be a pathogenecity factor that initiate epidemic disease. The different elicitors have resulted in some qualitative and quantitative differences in the production of volatiles. Several host selective and host non-selective toxins produced by plant pathogens have been isolated and their structures were determined during the last decade [1]. [8] Reported the 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 sensitivity 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 symptom while tenuazonic acid could produce similar typical blast symptoms on leaves at all plant ages. [2] Proved that the Oxalic acid is a major pathogenicity factor for *Sclerotinia sclerotiorum*. Similarly, [3] 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. High-pressure liquid chromatography studies of toxin produced by *C. dematium* showed the presence of four toxic fractions in the extract obtained from anthracnose lesions [4]. [5] The volatile compounds *viz*., Boronic acid, ethyl, 1,4-Cyclohexadiene, 1-methyl and Thujol were detected at the 0.1×10^{-5},0.1×10^{-5} and 0.1×10^{-5} relative abundance respectively, from mango infected with *C. gloeosporioides* through portable GC/MS. In the present study, efforts have been made to identify the volatile compound from *in planta* sample infected by *Magnaporthe grisea* through GC-MS analysis.

Materials and Methods

Collection and Extraction of crude metabolites

Blast infected parts of ragi from susceptible variety KM 252 *viz.*, Leaf, neck and finger were collected from glasshouse and inoculated separately with virulent isolates *viz.*, TNLB1 (Leaf), TNNB8 (Neck) and BIFB13 (Finger) under controlled condition at experimental pots of Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The collected samples were air dried, separately bagged and stored under refrigerated condition at 4°C. The blast infected parts (leaf, neck and finger) were powdered and stored. This was further extracted with 10g in 100 ml of 100 mM carbonate buffer (pH 9.2) in a prechilled mortar. The homogenate was centrifuged at 10000 rpm for 20 min. at 4 ^{0}C. The step was repeated till the supernatant became clear from chlorophyll content. The *in planta* toxin was further partially purified as per the procedure described by [6].

Purification of toxin

The aqueous fraction after solvent separation containing toxic activity was applied to a Sephadex G-75 (Sigma, USA) superfine column (2.5x25 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 peak fractions were combined and evaporated to dryness *in vacuo*at 40°C, redissolved in 5 mL of distilled water to get a clear homogeneous syrup, freeze dried and stored at -20°C. The plant extracts with Ethyl acetate in 1:1 ratio and allowed to shake for 2 hrs in rotary shaking incubator. The extract was air dried in a closed chamber in a dark room. The partially purified toxin was used to analyze the biological functions of the toxin in all further studies.

Detection of toxins by Thin Layer Chromatography (TLC)

Separation of toxic compounds

Toxin produced by *M. grisea* in different plant parts (leaf, neck and finger) were 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 blast infected samples of *M. grisea* was 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.*

Identification of the compound by TLC

Visual observation

Spots were visualized by spraying with various spraying reagents (identify the various spraying agents) 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 (Rf) of the spots developed on the TLC plate.

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.

Preparation of sample

The 20g of powdered leaf, neck and finger blast were soaked in 95% ethanol for 12 h. The extracts were then filtered through 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 infected plant extract was separated by vacuum filtration using bottle top filter to collect the extract. The plant extracted with Ethyl acetate in 1:1 ratio and allowed to shake for 2 hrs in rotary shaking incubator. The extract was air dried in a closed chamber in a dark room for GC-MS analysis.

Detection of volatile compound from in planta *toxin through GC-MS analysis*

The volatile compounds produced by the virulent *M. grisea* isolates (TNLB1, TNNB8 and BIFB13) on different parts *viz.*, leaf, neck and finger were analyzed through GC/MS (Thermo scientific Trace GC Ultra DSQ II) equipped with column (30mm × 0.25mm × 0.25µm) under the following conditions. Helium was used as carrier gas with a flow rate at 1ml per minute. 1µl 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.

Results

Detection of in plantaM. grisea toxin through TLC

The toxic metabolite productions by *M. grisea* were detected using Thin Layer Chromatography (TLC). The presence of volatile substance was detected under ultraviolet light and iodine tank test. The infected samples of leaf, neck and finger by the pathogen isolates *viz.,* TNLB1, TNNB8 and BIFB13 produced bands at various retention factors (R*f*) value *viz.,* 0.88, 0.77, 0.68, 0.83, 0.66, 0.86 and 0.80 indicating various components in the toxin produced in living host of finger millet. The TLC plates after development with iodine tank test showed distinct spots of golden yellow and dark green colour (**Table 1**; **Plate 1**).

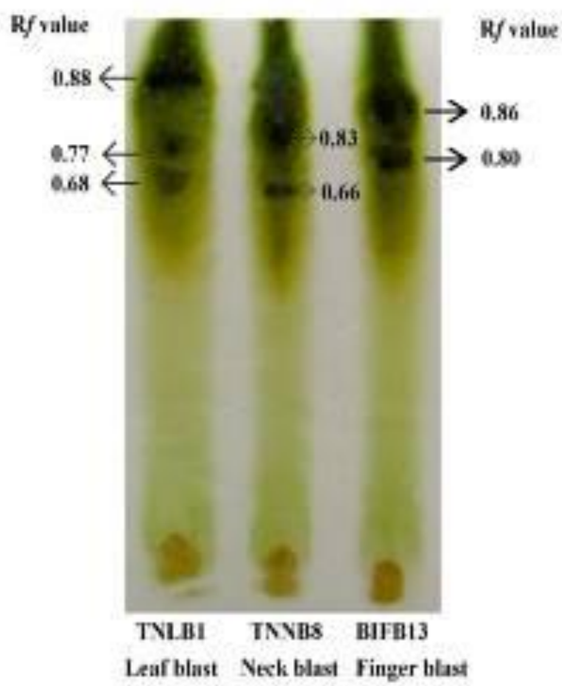


Plate 1 Detection of *in plantaM. grisea* crude toxin by TLC

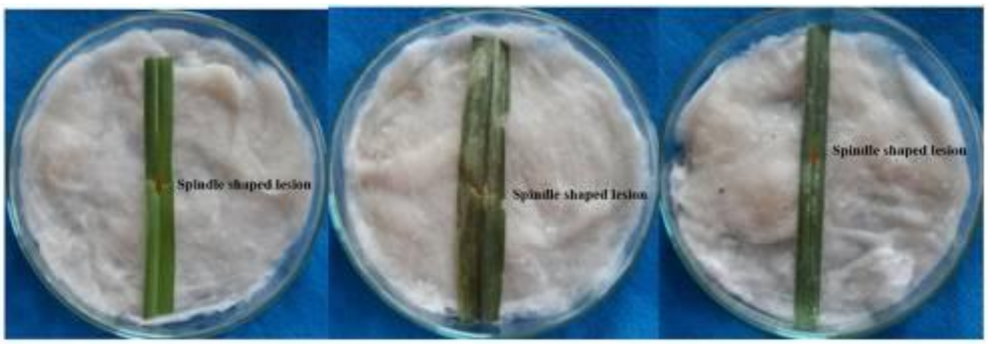
Table 1 Detection of *in plantaM. grisea* toxin by TLC method

S. No.	Isolates	Number of spots	Colour of band	Distance traveled (cm)	R*f* value
1	TNLB1	3	Dark green	8.4, 7.4, 6.5	0.88, 0.77, 0.68
2	TNNB8	2	Dark green	7.5, 6.0	0.83, 0.66
3	BIFB13	2	Dark green	7.8, 7.2	0.86, 0.80

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

Toxicity Assay

Toxic 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, they showed similarity with 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.



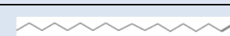
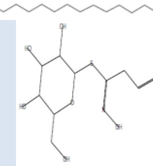
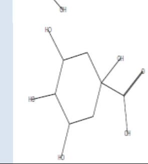
TNLB1 (Leaf blast isolate) **TNNB8** (Neck blast isolate) **BIFB13** (Finger blast isolate)

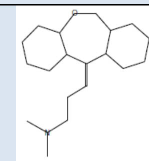
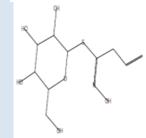
Plate 2 Symptoms produced by crude toxin on finger millet leaves

Identification of toxin compounds from in planta leaf blast crude toxin

The toxin compounds from *M. grisea* infected *in planta* extract were analyzed using GC/MS to detect the compounds and secondary metabolites responsible for pathogenicity. The compound identity was confirmed through NIST library 2005 AMDIS software programme. The total amounts of compounds (104) were detected *in planta*, among these; few compounds were selected based on the unique nature and relative abundance of the peaks. The compounds detected *in planta* were Octadecane (CAS), Pentadecanoic acid (CAS), Quinic acid, 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) and Desulphosinigrin detected with per cent peak height range of relative abundance from 1.08 to 8.50 (**Table 2**; **Figure 1**). Among the toxic compounds highest relative abundance were found to be Desulphosinigrin (8.50) followed by Quinic acid (8.50), Octadecane (CAS) (3.89), 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) (2.18) and Pentadecanoic acid (CAS) (1.08).

Table 2 Volatile compounds identified from *in planta* crude toxin of leaf blast through GC/MS

S. No.	RT	Name of the compound	Mol. Formula	MW	Peak Area (%)	CAS	Structure
1.	11.50	Octadecane (CAS)	$C_{18}H_{38}$	254	3.89	593-45-3	
2.	15.08	Pentadecanoic acid (CAS)	$C_{15}H_{30}O_2$	242	1.08	5115-81-1	
3.	19.90	Quinic acid	$C_7H_{12}O_6$	192	8.50	77-95-2	

S. No.	RT	Name of the compound	Mol. Formula	MW	Peak Area (%)	CAS	Structure
4.	26.08	1,2-Benzene-dicarboxylic acid, bis(2-ethylhexyl) ester (CAS)	$C_{24}H_{38}O_4$	390	2.18	117-81-7	
5.	31.34	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	8.50	5115-81-1	

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

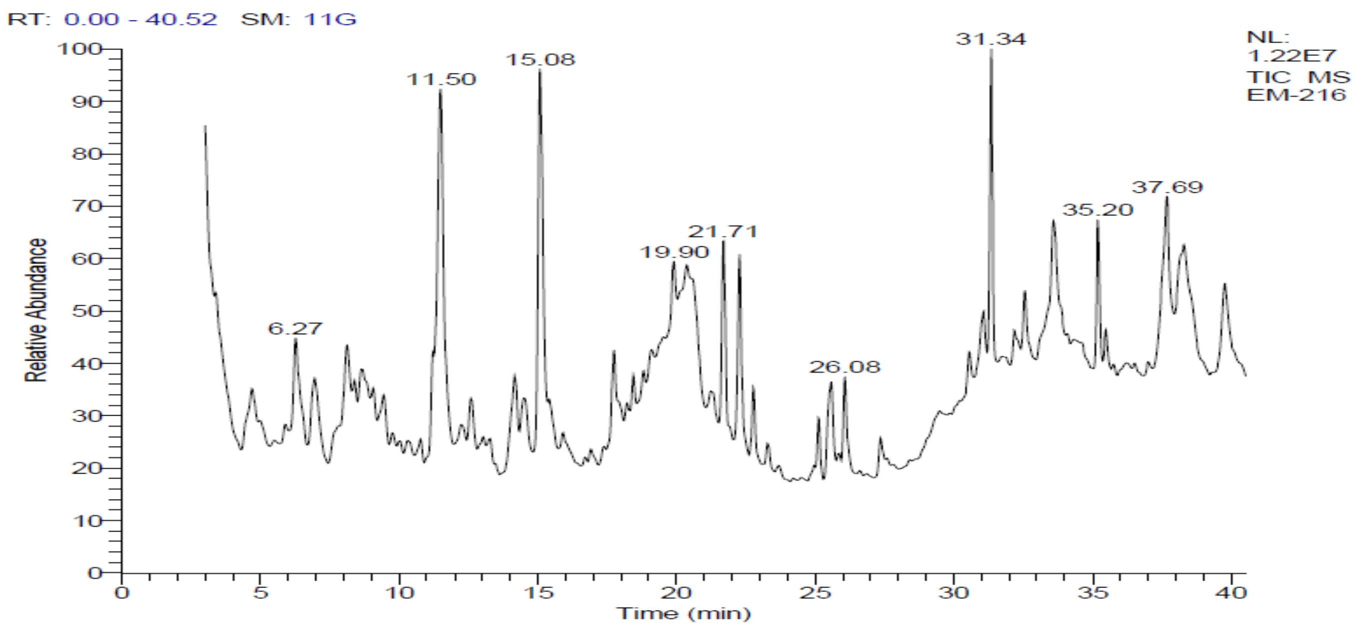
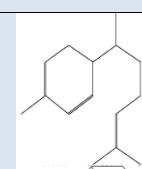
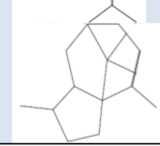


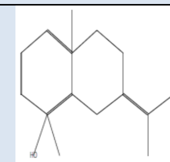
Figure 1 GC-MS chromatogram of volatile compounds from *in planta* crude toxin of leaf blast

Identification of toxin compounds from in planta neck blast crude toxin

The toxin compounds from *M. grisea* produced *in planta* were analyzed through GC/MS. The crude toxin from *in planta* condition, produced five prominent peaks with retention time of 12.74, 15.04, 17.83, 30.98 and 31.93 min. The peaks with retention time 12.74 min corresponds to the Zingiberene (CAS) with 10.45 per cent of peak area; 15.04 min corresponds to à-Patchoulene (CAS) with 1.66 per cent of peak area; 17.83 min corresponds to 1-Naphthalenol, 1-Naphthalenol, decahydro-1, 4a-dimethyl-7-(1methylethylidene) with 4.04 per cent of peak area; 30.98 min corresponds to Synaptogenin B with 1.63 per cent of peak area and 31.93 min corresponds to Holothurinogenin-2 with 0.90 per cent of peak area. Among the five toxin compounds, maximum peak area was observed in the compound Zingiberene (CAS) with 10.45 per cent peak area (**Table 3**; **Figure 2**).

Table 3 Volatile compounds identified from *in planta* crude toxin of neck blast through GC/MS

S. No.	RT	Name of the compound	Mol. Formula	MW	Peak Area (%)	CAS	Structure
1.	12.74	Zingiberene (CAS)	$C_{15}H_{24}$	204	10.45	495-60-3	
2.	15.04	à-Patchoulene (CAS)	$C_{15}H_{24}$	204	1.66	560-32-7	

S. No.	RT	Name of the compound	Mol. Formula	MW	Peak Area (%)	CAS	Structure
3.	17.83	1-Naphthalenol, 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1methylethylidene)	$C_{15}H_{26}O$	222	4.04	473-04-1	
4.	30.98	Synaptogenin B	$C_{30}H_{46}O_4$	470	1.63	64144-79-2	
5.	31.93	Holothurinogenin-2	$C_{30}H_{48}O_5$	488	0.90	64144-79-2	

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

Identification of toxin compounds from in planta finger blast crude toxin

Results revealed the presence of toxin compound belonging to volatile compound group. The six volatile compounds were detected from the finger blast infected plant sample with varied retention time of 19.60, 20.37, 21.08, 22.10, 22.83 and 39.20 min. The molecular weight of the compound pertaining to retention time are 286, 154, 184, 226, 270 and 298 respectively with corresponding peak area of 21.05, 55.05, 55.05, 55.05, 83.10 and 12.77 (**Table 4**; **Figure 3**). The identified volatile compounds are as follows:,1-Naphthalenol, Isopulegol 1, 2H-Pyran-2-one, 6 hexyl tetrahydro- delta-hexyl valerolactone, delta-hexyl-delta-valerolactone, delta Undecalactone, Z-9-Pentadecenol, 1-Octadecanol, octadecan-1-ol, 1- octadecanol, 1 Hydroxyoctadecane and Phenylacetic acid, 2-(1-adamantyl) ethyl ester.

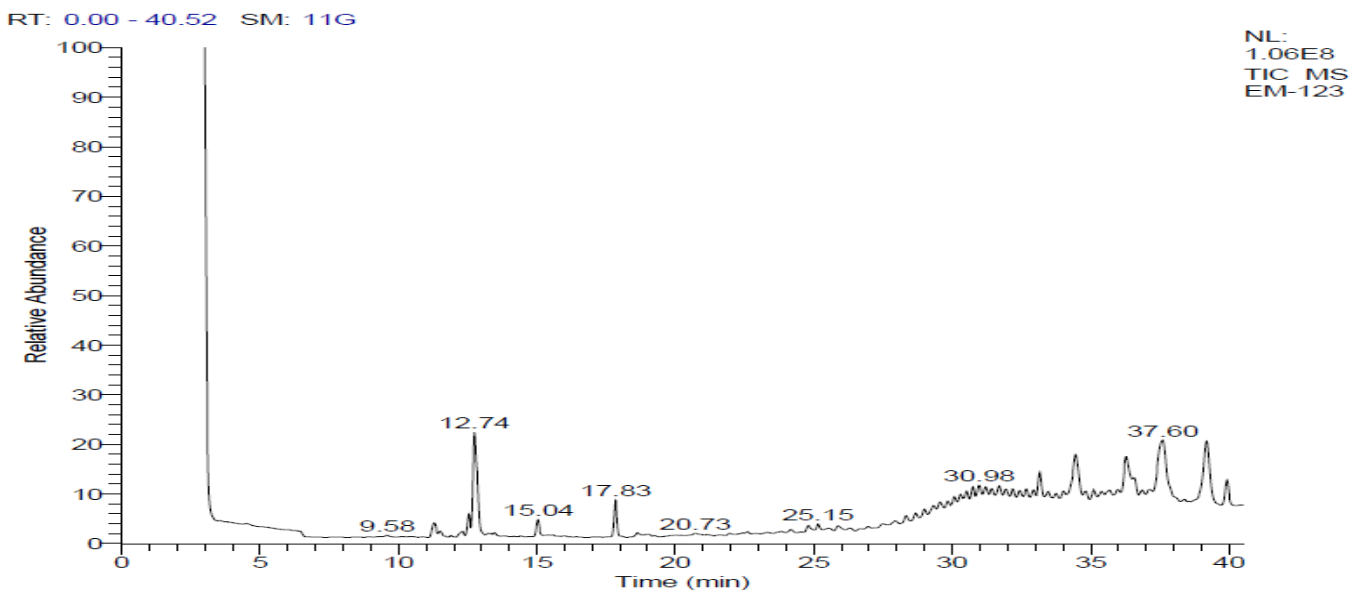
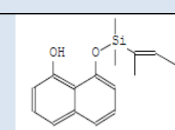
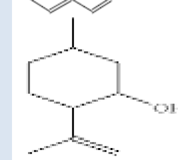


Figure 2 GC-MS chromatogram of volatile compounds from *in planta* crude toxin of neck blast

Table 4 Volatile compounds identified from *in planta* crude toxin of finger blast through GC/MS

S. No.	RT	Name of the compound	Mol. Formula	MW	Peak Area (%)	CAS	Structure
1.	19.60	1-Naphthalenol	$C_{17}H_{22}O_2Si$	286	21.05	125452-20-2	
2.	20.37	Isopulegol 1	$C_{10}H_{18}O$	154	55.05	56797-40-1	
3.	21.08	2H-Pyran-2-one,6-hexyltetrahydro-delta-Hexylvalerolactone, delta-Hexyl-delta-valerolactone, delta-Undecalactone	$C_{11}H_{20}O_2$	184	55.05	710-04-3	
4.	22.10	Z-9-Pentadecenol	$C_{15}H_{30}O$	226	55.05	470-40-	

S. No.	RT	Name of the compound	Mol. Formula	MW	Peak Area (%)	CAS	Structure
5.	22.83	1-Octadecanol, octadecan-1-ol, 1-octadecanol, 1-Hydroxyoctadecane	$C_{18}H_{38}O$	270	83.10	6 112-92-5	
6.	39.20	Phenylacetic acid, 2-(1-adamantyl) ethyl ester	$C_{20}H_{26}O_2$	298	12.77	64144-79-2	

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

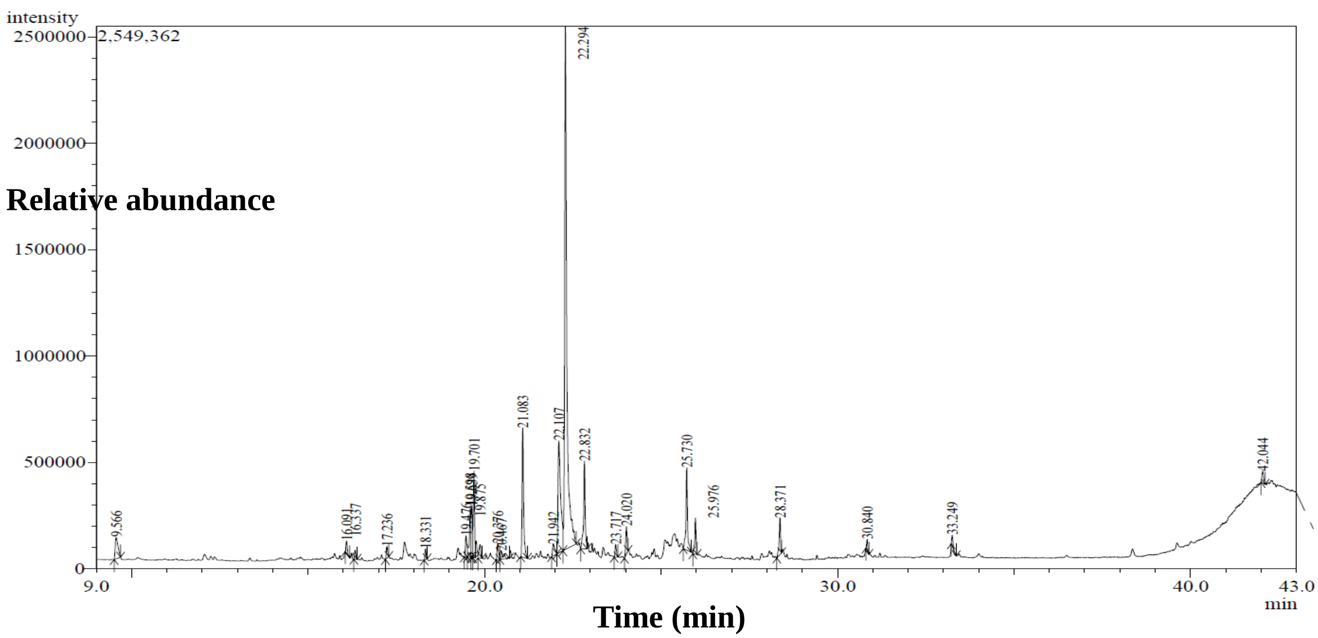


Figure 3 GC-MS chromatogram of volatile compounds from *in planta* crude toxin of finger blast

Discussion

Identification of major biotic constituent present in the *M. grisea* infected plant extract 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* extract were identified through GC-MS method. The major toxic volatile compounds *viz.*, Octadecane (CAS), Pentadecanoic acid (CAS), Quinic acid, 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) and Desulphosinigrin were identified on leaf blast. Subsequently, the compounds *viz.*, Zingiberene (CAS), à-Patchoulene (CAS), 1-Naphthalenol, 1-Naphthalenol, decahydro-1, 4a-dimethyl-7-(1methylethylidene), Synaptogenin B, Holothurinogenin-2 were identified on neck blast. Whereas in fingers the compounds *viz.*, 1-Naphthalenol, Isopulegol 1, 2H-Pyran-2-one, 6 hexyl tetrahydro-delta-hexyl valerolactone, delta-hexyl-delta-valerolactone, delta Undecalactone, Z-9-Pentadecenol, 1-Octadecanol, octadecan-1-ol, 1- 0ctadecanol, 1 Hydroxyoctadecane and Phenylacetic acid, 2-(1-adamantyl) ethyl esterwere identified in blast infected *in planta*crude toxin.

The results are in accordance with [7] who 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 which are potent inhibitors of seed germination. [9] Finger millet blast fungus *M. grisea* produced maximum quantity of crude toxins. Crude toxins were partially purified and characterized by UV and mass spectra. Pyrichalasin H, aphytotoxic metabolite has been identified and found to be toxic when tested for inhibition of seed germination and seedling growth of blast resistant and susceptible finger millet. (3) 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*., Several studies assessed the presence of toxic compounds in different endophytic fungal pathogens and found various 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 [11-14]. Similarly, crude volatile compound obtained from leaf blast samples compounds *viz*., Octadecane (CAS), Pentadecanoic acid (CAS), Quinic acid, 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) and Desulphosinigrin. neck infected plant samples, compounds *viz*., Zingiberene (CAS), à-Patchoulene (CAS), 1-Naphthalenol, 1-Naphthalenol, decahydro-1, 4a-dimethyl-7-(1methylethylidene), Synaptogenin B, Holothurinogenin-2., and finger blast samples compounds like 1-Naphthalenol, Isopulegol 1, 2H-Pyran-2-one, 6 hexyl tetrahydro - delta-hexyl valerolactone, delta-hexyl-delta-valerolactone, delta Undecalactone, Z-9-Pentadecenol, 1-Octadecanol, octadecan-1-ol, 1- octadecanol, 1 Hydroxyoctadecane and Phenylacetic acid, 2-(1-adamantyl) ethyl ester [17].

Conclusion

Results of the present studies revealed the ability of *M. grisea* to produce phytotoxic compound in the infected plant 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.

Acknowledgement

Authors have acknowledged UGC for providing Rajiv Gandhi National Fellowship, Government of India to carry out this research and technically facilitated by All India Coordinated Small Millet Improvement Project Centre (AICSMIP), Coimbatore centre.

References

[1] Scheffer RP, Livingston RS. Sensitivity of sugarcane clones to toxin from Helminthosporium sacchari as determined by electrolyte leakage. Phytopathology 1980, 70, 400-404.

[2] 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.

[3] 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.

[4] Yoshida S, Hiradate S, Fujii Y, Shirata A. Colletotrichum dematium produces phytotoxins in anthracnose lesions of mulberry leaves. Phytopathology 2000, 90, 285-291.

[5] 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.

[6] Samiyappan R, Amutha G, Kandan A, Nandakumar R, Babu , 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.

[7] 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.

[8] Singburaudom N, Chaudhary RN, Sommartya T, Sarobo E. Effects of plant age on symptom development producedby Pyricularia oryzae toxin. Kasetsart J Nat Sci 1998, 32, 90 – 101.

[9] Sanmathi Kumar RP, Sudharshana L, Anilkumar TB. Bioassay of the phytotoxins produced by Pyricularia grisea, the blast fungus of finger millet. In: Abstr. Proc.IPS (SZ) Symp. on Plant Disease Scenerio in Southern India 2006, 19 (21), 16.

[10] 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.

[11] Holopainen JK, Gershenzon J. Multiple stress factors and the emission of plant VOCs. Trends Plant Sci 2010, 15, 176–184.

[12] 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.

[13] 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.
[14] Amanian RS, Brindha P. In vitro cytotoxic, antioxidant and GC-MS studies on Centratherum punctatum Cass. Int J Phar Pharm Sci 2013, 5(3), 364-367.
[15] 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.
[16] Hong L, Hemert JV, Dash S, Roger P, Dickerson A. PLEXdb: gene expression resources for plant and pathogens. Life Sci 2012, 40, 1194-1201.
[17] Shanmugapackiam S, Parthasarathy S, Raguchander T. Detection of Phytotoxin produced from leaf, neck and finger blast disease causing Magnaporthe grisea through GC-MS analysis. Int. J. Biochem. Res. Rev., 2017, 19(3), 1-10.

Publication History

Received	31.10.2019
Revised	15.11.2019
Accepted	16.11.2019
Online	30.11.2019