Chemical Science Review and Letters ISSN 2278-6783

Research Article

Detection of finger millet blast pathogen *Magnaporthe grisea* using FTIR spectroscopy

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Abstract

The functional group of crude toxin produced by pathogenic *Magnaporthe grisea* isolates were detected and identified through Furrier Transformed Infrared (FTIR) spectroscopy. The culture filtrates from leaf, neck and finger blast pathogen showed spectral peaks region from 3718.76 to 756.10 cm^{-1}, 3726.47 to 756.10 cm^{-1} and 3726.47 to 717.52 cm^{-1} in the different frequency ranges respectively. Among the three regions, the toxin produced at leaf region by *M. grisea* was higher with proof in spectral peak intensity become strong compared with finger and neck region toxin functional groups which were analyzed through FTIR spectroscopy.

Keywords: Blast disease, detection, finger millet, FTIR, *Magnaporthe grisea*

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Introduction

Plant infecting fungal pathogens produce several toxic substances and few of them plays a major role for causing disease symptoms in plants. The identification and quantification of such primary toxic substances produced by the fungal pathogens is essential for the development of diagnostic tools and effective disease management strategy through reducing the activity of toxins. Enzymes and protein based detection techniques are shows reduced specificity. Nucleic acid based detection techniques are highly expensive and time consuming. Using these advanced detection tools it is very difficult to diagnose new pathogens or unknown organisms, as a result these methods are not suitable for rapidly screening of large numbers of samples [7].The Furrier Transformed Infrared Spectroscopy (FTIR) techniques widely used and easy method for the identification and quantification of toxic substances and other secondary metabolites produced by pathogens. FTIR spectroscopy is one of the methods that have been successfully used for detecting and identifying microorganisms, especially in food products [8, 9]. Some of these studies showed that discrimination was possible not only at the genus level, but also at the species [10, 11] and strain levels. The first application of infrared spectroscopy for detecting the microorganisms dates from 1950s [2] published their first work on the detection toxic metabolites from *Botrytis cinerea* and *Alternaria tenuissima* by infrared spectroscopy. Later, [1] have studied the presence of toxic substances in fungal pathogens and demonstrated the structure of suberin. [3, 6] studied the presence of toxic substances in *Rhizoctonia*, *Colletotrichum* and *Verticillium* and reported the presence of lipids, amide I and amide II through spectroscopy having wave length between 2800 and 3020 cm^{-1}. Later, [4] reported the presence of toxic substances in *Fusarium* genus. Recently, [5] reported the stretching frequency of alkaloid and terpenoids through FTIR analysis and explained that the compounds in the crude extract more possibly may belong to alkaloid or terpenoid group of compounds. Stretching frequency at 1664.62 cm^{-1} confirmed the presence of conjugated aromatic compound and six atom ring which gives information about basic ring structure system of alkaloid and terpenoid structure.

Experimental Methods

Collection and Isolation of Pathogen

Field level collection conducted during Kharif in major finger millets growing regions of Tamil Nadu and the blast infected finger millet plant parts *viz.*, either leaf or neck or finger blast infected samples were taken based on the crop stage available at the time of survey. In addition, the blast infected finger millet samples were also received from the All India Coordinated Small Millet Improvement Project (AICSMIP) centers in India. 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 [12]. 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 [13]. Among these, the most virulent pathogenic isolates from leaf, neck and finger blast were chosen for further studies.

Sample preparation

Samples of these *M. grisea* fungi were purified from the culture broth by spinning at 2000 rpm for 5 min, washing 4 times with H_2O and the pellet was suspended in appropriate volume of H_2O (about 1 ml). For attenuated total reflection (ATR) examination about 500 µl of the above obtained fungal suspension as flattened and spread on the ATR crystal producing a flat layer covering the entire crystal. Then the samples were air dried for about 30 minutes and examined by ATR sampling technique used in conjugation with infrared spectroscopy.

FTIR spectral measurement

The ATR measurements were performed using FTIR spectrometer (Bruker Tensor 127). Measurements were performed by using the FTIR–ATR with a LN_2 cooled MCT detector (mercury-cadmium-telluride). Around 128 coded scans were collected in each measurement in the wave numbers region 600– 4000 cm^{-1}, after the samples were dried. Spectral resolution was set at 4 cm^{-1}. All spectra were baseline corrected and vector normalized using OPUS software.

Results

Detection of volatile compound from M. grisea toxin through Fourier Transform Infrared (FTIR) spectroscopy

The aqueous extract from blast infected leaf, neck and finger regions produced various functional groups of toxins and they were analyzed through FTIR spectroscopy analysis. The leaf blast extract showed spectral peaks at 3718 cm^{-1} (Phenols), 2924 cm^{-1} (Lipids), 1728 cm^{-1} (Alkyl groups), 1450 cm^{-1}, 1373 cm^{-1}, 1219 cm^{-1} (Amide I, II) and 756 cm^{-1} (Alkenes). The neck blast extract showed peaks at 3726 cm^{-1} (Phenols), 2924 (Lipids), 1604 (Amide I), 1458 cm^{-1} (Amide II, III), 840 cm^{-1} (Carbohydrate) and 756 cm^{-1} (alkenes) and the finger blast extract showed peaks at 3726 cm^{-1} (phenols), 2924 cm^{-1} (lipids), 1720 cm^{-1} (alkyl group), 1373 cm^{-1} (Amide II, III) and 756 cm^{-1} (alkenes). Among the three regions, the toxin produced at leaf region by *M. grisea* was higher with proof in spectral peak intensity become strong compared with finger and neck region toxin functional groups which were analyzed through FTIR spectroscopy (**Tables 1-3**) (**Figures 1-3**).

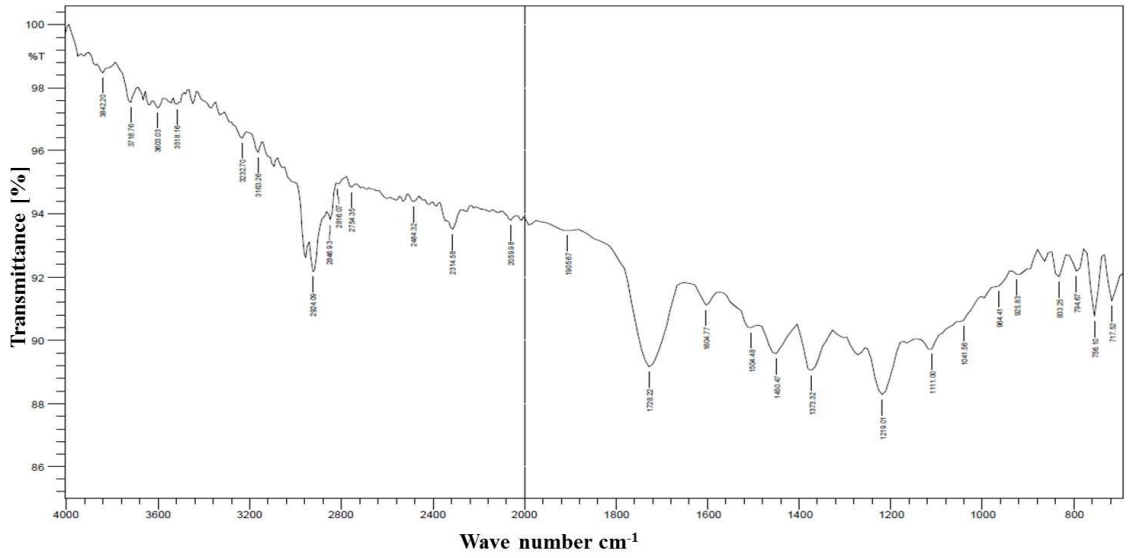

Figure 1 FT-IR analysis showing characteristic absorption peaks of crude toxin from leaf blast causing *M. grisea*

Table 1 FT-IR spectroscopic analysis of crude toxin from leaf blast pathogen showing IR range and functional groups

S.No.	Wave number (cm^{-1})	Intensity	Bond	Functional groups
1	3718.76	Weak	O-H stretch	Phenols
2	2924.04	Strong	C-H stretch	Lipids
3	2845.93	Strong		
4	2314.58	Medium		
5	1728.22	Strong	C=H stretch	Alkyl groups/lipids
6	1450.22	Strong		
7	1373.32	Strong	C-H stretch	Amide II, III – Protein
8	1219.01	Strong		
9	833.25	Medium	C-O stretch	Carbohydrate
10	794.67	Strong	CH_2 bending	Alkenes
11	756.10	Medium		

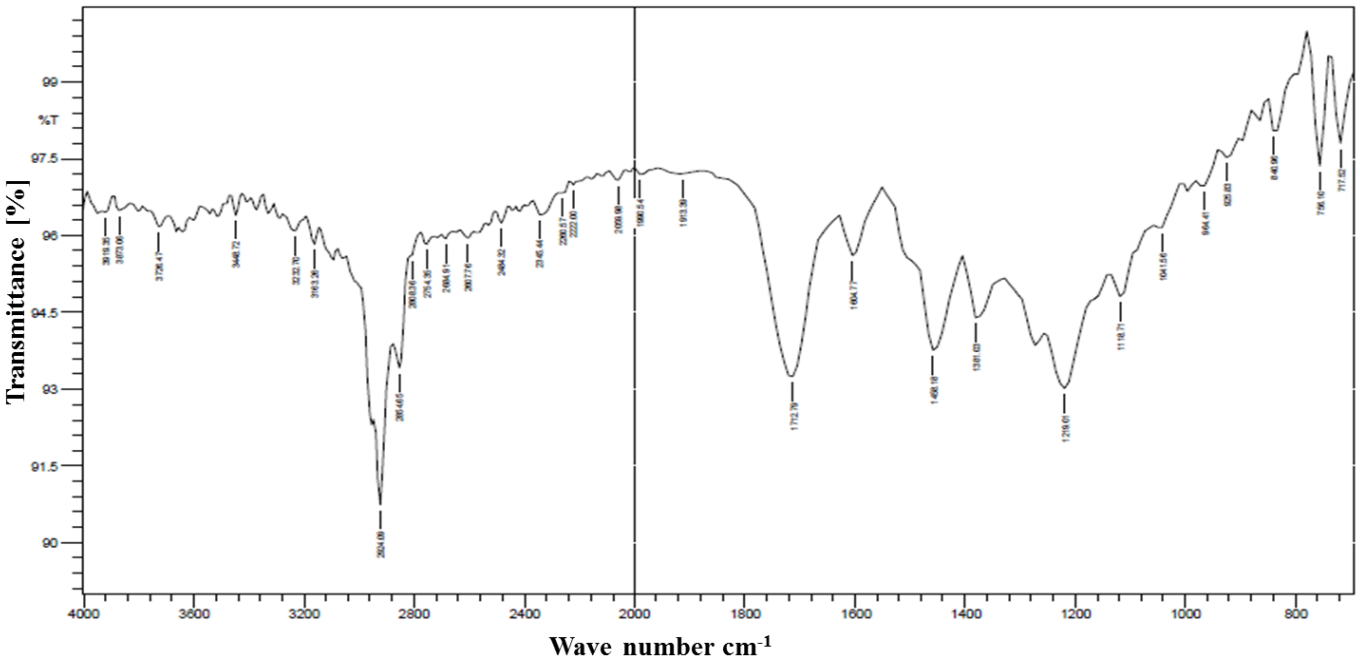

Figure 2 FT-IR analysis showing characteristic absorption peaks of crude toxin from neck blast causing *M. grisea*

Table 2 FT-IR spectroscopic analysis of crude toxin from neck blast pathogen showing IR range and functional groups

S.No.	Wave number (cm^{-1})	Intensity	Bond	Functional groups
1	3726.47	Weak	O-H stretch	Phenols
2	2924.09	Strong	C-H stretch	Lipids
3	2854.65	Strong		
4	2345.44	Weak		
5	1722.79	Strong	C=H stretch	Alkyl groups/lipids
6	1604.71	Medium	C-O stretch	Amide I – Protein
7	1458.18	Strong	C-H stretch	Amide II, III – Protein
8	1318.03	Medium		
9	1219.01	Strong		
10	840.96	Medium	C-O stretch	Carbohydrate
11	756.10	Medium	CH_2 bending	Alkenes

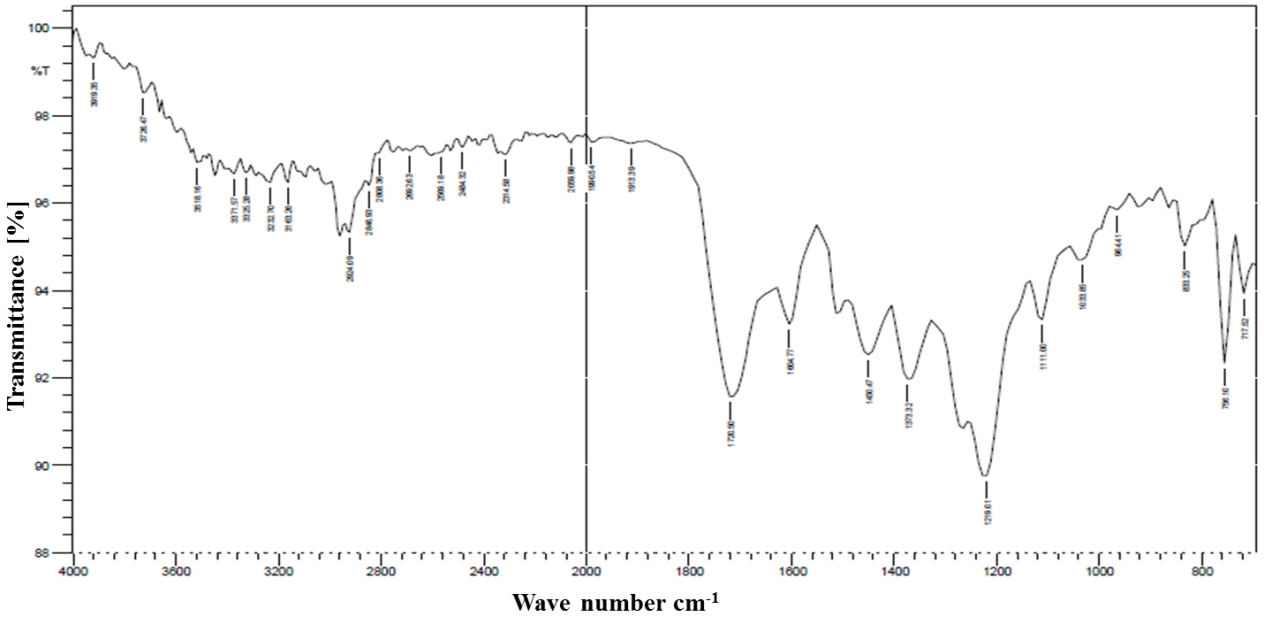

Figure 3 FT-IR analysis showing characteristic absorption peaks of crude toxin from finger blast causing *M. grisea*

Table 3 FT-IR spectroscopic analysis of crude toxin from finger blast pathogen showing IR range and functional groups

S.No.	Wave number (cm^{-1})	Intensity	Bond	Functional groups
1	3726.47	Weak	O-H stretch	Phenols
2	2924.09	Strong	C-H stretch	Lipids
3	2314.58	Weak		
4	1720.50	Strong	C=H stretch	Alkyl groups/lipids
5	1450.47	Medium	C-H stretch	Amide II, III – Protein
6	1373.32	Strong		
7	1219.01	Strong		
8	1111.10	Medium	C-O stretch	Carbohydrate
9	833.25	Medium		
10	756.10	Strong	CH_2 bending	Alkenes
11	717.52	Medium		

Discussion

Crude toxin obtained from culture filtrate and infected sample was purified and analyzed through FTIR, which indicated number of spectral peaks. The present data revealed that FTIR spectroscopy has been widely used to provide information on a range of vibrationally active functional groups including O-H, C-H, C=H and CH2. FTIR spectra of aqueous extracts of leaf, neck and finger blast infected samples have been detected in the region from 3718.76 to 756.10 cm^{-1}, 3726.47 to 756.10 cm^{-1} and 3726.47 to 717.52 cm^{-1} in the different frequency ranges respectively. Similar results were reported that in higher wave numbers region, the spectrum is dominated by the water absorption bands at 3350 cm^{-1} [3,6]. Bands at 2849, 2917 and 3008 cm^{-1} are mainly due to lipids absorbance in *Fusarium* genus. Also observed a higher wave numbers region (2800–3020 cm^{-1}) and the low wave number region (1740 cm^{-1}) in the *Fusarium* genus based spectra [4]. While in an another study, three genera *viz.*, *Rhizoctonia*, *Colletotrichum* and *Verticillium* have some differences in the lipids, Amide I and Amide II bands. Stretching frequency at 1664.62 cm^{-1} has confirmed the presence of conjugated, aromatic and six atom ring which gives information about basic ring structure system of alkaloid and terpenoid [5].

Conclusion

The identification and discrimination of pathogenic microorganisms using FTIR spectroscopic techniques is most attractive and valuable technique because of its rapid-sensitivity and low cost. It gives a unique finger print detection

of given sample even in a large scale screening and routine application. In this present FTIR analysis given the profile of functional group exist in the toxic metabolites produced by the *Magnaporthe grisea*, these results is most useful for the development of rapid diagnostic kit, reduce the virulence of pathogen by altering the nature of toxic substances and also favors to develop the integrated disease management module under field condition.

Acknowledgements

This work was partially supported by University Grants Commission - Rajiv Gandhi National Fellowship (UGC-RGNF) and technically facilitated by to All India Coordinated Small Millet Improvement Project Centre (AICSMIP).

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Publication History
Received 08th Apr 2017
Revised 17th Apr 2017
Accepted 19th Apr 2017
Online 30th Apr 2017