SHORT COMMUNICATION

Identification and Characterization of Antagonism Band of Secondary Metabolite from *T. asperellum* MK045610 against *F. oxysporum* f. sp. *ciceri* and *F. oxysporum* f. sp. *lycopersici* based on HPTLC and GC-MS

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DOI: 10.18811/ijpen.v5i03.11

Abstract

The present investigation was carried out on identification and characterization of an antagonism band of extract secondary metabolites from *Trichoderma asperellum* MK045610 against *Fusarium oxysporum* f. sp. *ciceri* and *Fusarium oxysporum* f. sp. *lycopersici*. In this experiment, analysis was performed by High Performance Thin Layer Chromatography (HPTLC), paper disc assay, gas chromatography-Mass spectrometry (GC-MS). Firstly, extract crude secondary metabolites were used for partial purification based on HPTLC. Secondly, the paper disc assay method was used for the determination of antifungal property on PDA plate from the partial purified compound. Thirdly, GC-MS was used for identification of partial purified compound based on peaks. Identified compounds are named as Phenol, 3, 5-bis (1,1-dimethylethyl), Pentadecanoic Acid, 14-methyl, methyl ester, Benzenepropanoic acid, 3,5,-bis (1,1-dimethyl ethyl)-4-hydroxy-methyl ester and represented to antifungal property . Conclusively, Secondary metabolite of *Trichoderma asperellum MK045610* has a significant role in radial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* and *Fusarium oxysporum* f. sp. *lycopersici*.

Keywords: GC-MS, HPTLC, *Trichoderma asperellum*.

International Journal of Plant and Environment (2019)

Introduction

Genus *Trichoderma* (Family-Hypocreaceae and Division-Ascomycota) is filamentous, soil dwelling with most culturable fungi. *Trichoderma* produced important bioactive compound which played crucial role in plant protection from diseases, known as BCA (Biocontrol agent) (Woo *et al*., 2006; Waghunde *et al*., 2016). It's use possible to increase yield, improve health of crop or increase the natural ability to degrade toxic compounds (Waghunde *et al*., 2016). A lot of metabolite from *Trichoderma* was exhibited and showed negative response for plant pathogen i.e. *Fusarium* sp., Rhizoctonia, Pythium etc. and positive response in plants (Vinale *et al*., 2008). The biocontrol ability of *Trichoderma* present due to many factors, as production of many of extracellular lytic enzymes and secondary metabolites (Cardoza *et al*., 2005). *Trichoderma* strain exhibited various type of secondary metabolites i.e. volatile, nonvolatile, diffusible where it is responsible for protection of plant from harmful pests, nutrient support, mineral Solubilization and pharmacological activities and others mechanism viz. include as mycoparasitism, antibiosis and competition (Patil *et al*., 2016).

In this present investigation was carried out to identification and characterization of antagonism band of extract secondary metabolites from *Trichoderma asperellum* MK045610 against *Fusarium oxysporum* f. sp. *ciceri* and *Fusarium oxysporum* f. sp. *lycopersici*, were analyzed to antifungal band for prevention of *Fusarium oxysporum* f. sp. *ciceri* and *Fusarium oxysporum* f. sp. *lycopersici* (wilt causing agents) based on HPTLC, paper assay method and GC-MS technique. This finding is most applicable in agriculture and commercialization due to best substitution of agrochemical and eco-friendly method.

Materials and Methods

Pathogens were obtained from Biocontrol Lab, Chandrasekhar Azad University of Agriculture And Technology, Kanpur (U.P.).

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How to cite this article: Sonkar, P. (2019). Identification and Characterization of Antagonism Band of Secondary Metabolite from *T. asperellum* MK045610 against *F. oxysporum* f. sp. *ciceri* and *F. oxysporum* f. sp. *lycopersici* based on HPTLC and GC-MS. International Journal of Plant and Environment 5(3): 215-218

Source of support: Nil

Conflict of interest: None

Submitted: 04.01.2019 **Accepted:** 01.07.2019 **Published:** 31.07.2019

Trichoderma spp. was isolated from rhizosphere zone of chickpea healthy plant and tomato healthy plant. *Trichoderma* spp. was isolated on RBA Media (Johnson *et al*., 1995; Akhtar, 1966) and transferred to PDA plate keep it incubator at 25^{0}C. Molecular identification was conducted from NFCC, Agharkar Research Institute, Pune, India.

Screening of antagonism by *in vitro* and *in vivo*

Total number of isolate was nine and screen to best antagonism in *vitro* and *vivo* according to Ferreira *et al*. (1991). Here, we were used dual technique *in vitro* and designed to microplot with split plot technique *in vivo*. As we were determined efficiency among isolate nine species El-Mohamedy (2009), Barari (2016).

Extract secondary metabolites and its profiling

Secondary metabolites were extracted according to Dubey *et al*. (2011) and profiling of band were based on HPTLC. Moreover, antagonism of extract secondary metabolites were screened on PDA plate according to Rabinal and Bhat (2017) and Hateet (2017),

based on paper disc assay method. Isolate Antifungal compounds were characterized by GC- MS (Dubey *et al*., 2011; Srinivasa *et al*., 2017).

Result and Discussion

Further experiment 2.1, we were observed to efficiency of among nine isolate *Trichoderma* spp., T2 was showed efficiency best in compare to T0, T1, T3, T4, T5, T6, T7, T8, T9 *in vitro* and *in vivo*. T0 was control and others were number of isolate species. T2 was *Trichoderma asperellum* MK045610 based on molecular identification.

Profiling of antagonistic of partial purified HPTLC band for *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *ciceri in vitro*

Further experiment, profiling was based on HPTLC technique. Here, extract crude secondary metabolite (8 ml) was proceed for partial purified on TLC plate by syringe and separated in organic solvent based on polar and nonpolar. Rf values were used in measuring of distance on TLC plate viz. 0.10, 0.47, 0.52, 0.76, 0.83, 0.94 (Fried and Sharma, 1982) and it was visualized at 366 nm, represented in Fig. 1. Partial purified Secondary metabolites were performed against plant pathogenic fungi (Dubey *et al.,* 2011). Band was scrapped from TLC plate and resuspension in methanol and used it for further next experiment *i.e*. paper disc assay method. It was represented in Fig. 2A-B.

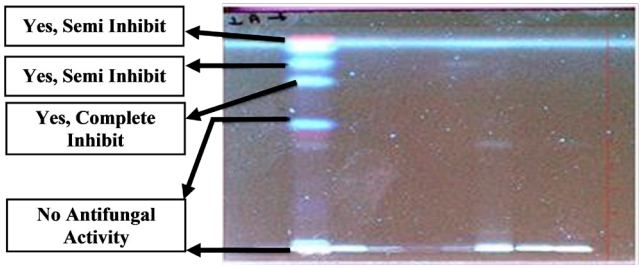
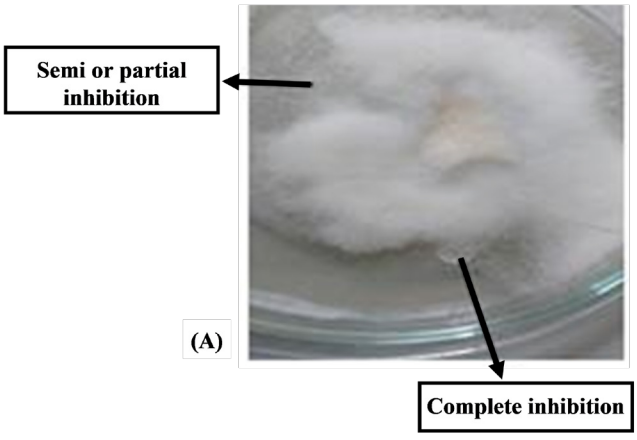


Fig. 1: Antifungal property of band was presented at 366 nm in HPTLC plate.



Antagonism of each band was characterized on PDA plate based on paper disc assay method in which was developed zone of inhibition as Fig. 2A-B. Antagonism of band against *F. oxysporum* f. sp. *ciceri* was represented Fig. 2A. Antagonism of band against *F. oxysporum* f. sp. *lycopersici* was represented in Fig. 2B. This experiment was proved that *Trichoderma* was secreted a kind of secondary metabolite which was represented antagonistic character against FOL and FOC.

Result of antagonist band was profiled in Fig. 1. The maximum band was available in T2 in comparison to other where it was extracted in hexane solvent. Thus it was characterized by paper disc assay. After screen its antifungal property, we observed three types of antifungal bands. List of antifungal band was presented in Fig. 1.

Characterization of volatile compound from partial purified HPTLC band by GC-MS Technique

Antifungal compound was characterized by GC-MASS and its identify was based on generate peak with match in NIST library. Result was presented below:

The band (4^{th}, 5^{th}, 6^{th}) was partial purified secondary metabolite which were represented to antifungal property on PDA plate (Fig. 2A-B) and observed peak of compound was named as *viz.* Phenol, 3, 5-bis (1,1-dimethylethyl), Pentadecanoic Acid, 14-methyl, methyl ester, Benzenepropanoic acid, 3,5,-bis (1,1-dimethyl ethyl)-4-hydroxy-methyl ester and its mw was presented as 206, 207, 292 as in Table 1. Each molecule peak was available in Fig. 3A-C. Moreover, peaks were match from NIST and detail of data is available in Table 1.

Hexane was proved as a best solvent for extraction of antifungal compound where it ran on TLC plate in solvent system of chloroform: acetone, as band was visualized at 366 nm, scrapped and redissolve in methanol as well as characterized to antifungal properties of each partial purified compounds by paper disc assay method. The secondary metabolites of T2 were showed antifungal properties of each partial purified compound and 6 bands were characterized by paper assay method and GC-MS. Zhang *et al. (*2014) and Qualhato *et al*. (2013) reported volatile metabolite which play role in inhibition of radial growth mycelium of FOC, *S. sclerotiorum* and *F. solani*.

Bhardwaj and Kumar (2017) reported that *Trichoderma asperellum* secreted several types of secondary metabolites as play role in different biological activities for plants and microbes. *Trichoderma asperellum* strain released different volatile secondary

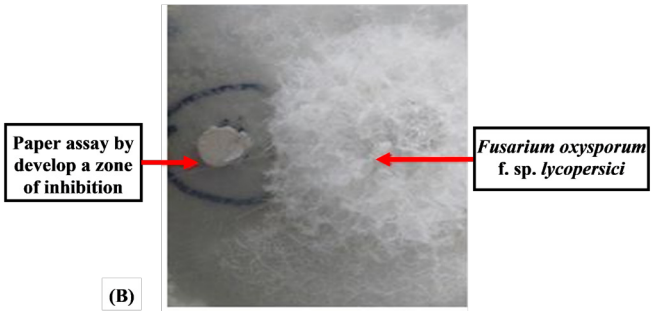


Fig. 2A-B: Zone of inhibition developed against FOC and FOL by paper disc assay method.

Table 1: List of Identification of compound From GC-MASS.

S.N.	*Band number*	*Compound Name*	*RT*	*MW*
1.	4^{th}	Phenol, 3,5-bis(1,1-dimethylethyl)	13.389 min	206
2.	5^{th}	Pentadecanoic Acid, 14-methyl, methyl ester	18.025 min	207
3.	6^{th}	Benzenepropanoic acid, 3,5,-bis (1,1-dimethyl ethyl)-4-hydroxy- methyl ester	18.115 min	292

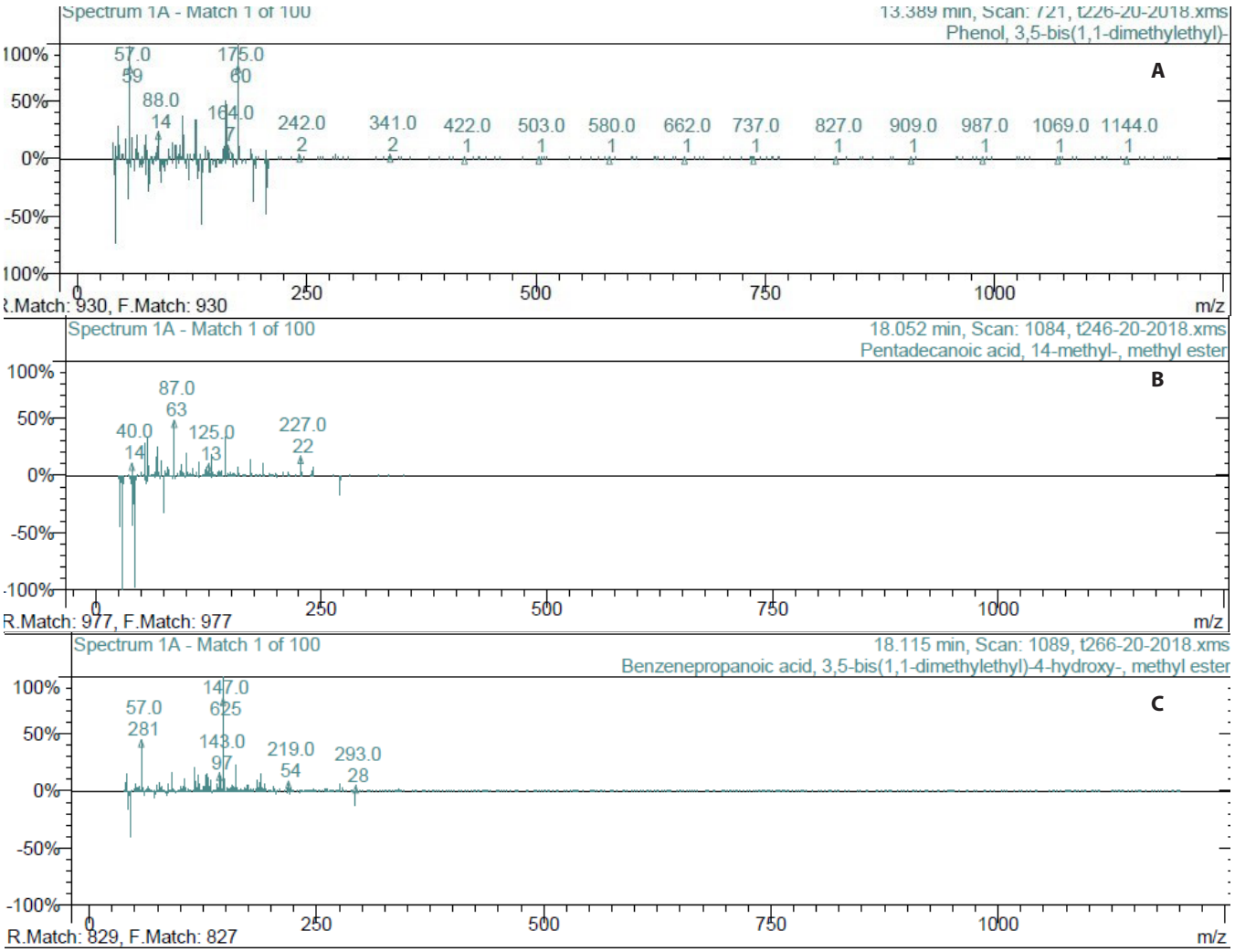


Fig. 3: Peak profiling of different compound from GC-MASS and NIST library. A. Generated peak of phenol, 3,5- bis(1,1- dimethyl) from NIST library, B. Generated peak of pentadecanoic acid, 14-methyl, methyl ester from NIST library, C. Generated peak of Benzenepropanoic acid, 3,5-bis (1,1-dimethyl)-4- hydroxy-methyl ester from NIST library.

metabolite. Secondary metabolite of *Trichoderma* was reduced growth of mycelium (Petrisor *et al*., 2017).

Conclusion

Phenol, 3, 5-bis (1,1-dimethylethyl), Pentadecanoic Acid, 14-methyl, methyl ester, Benzenepropanoic acid, 3,5,-bis (1,1-dimethyl ethyl)-4-hydroxy-methyl ester, all these compound were isolated from *Trichoderma asperellum* MK045610 strain and represented to number of band for antifungal property viz., fourth, fifth, sixth band. Conclusively, we find that secondary metabolites of *Trichoderma* have significant potential in prevention of FOL and FOC.

Acknowledgement

This manuscript includes original data that resulted from collaborative research by national staff. We thanks to Dr. Arjun Singh, and Scientist Mohit Pol,for providing lab facilities and supporting.

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