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ORIGINAL ARTICLE

Non-volatile and Volatile Metabolites of Antagonistic Trichoderma Against Collar Rot Pathogen of Mentha Arvensis

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Rahel Ratnakumari Y., A. Nagamani, S. Bhramaramba, R. Sunil kumar, Uday Chandra Kumar, Mahmood Shaik; Non-volatile and Volatile Metabolites of Antagonistic Trichoderma Against Collar Rot Pathogen of Mentha Arvensis

ABSTRACT

Mentha arvensis L. is an aromatic perennial herb, is commercially cultivated for its oil. The crop is severely affected by collar-rot disease. The disease caused by *Sclerotium rolfsii* Sacc (Saccardo) is a soil borne plant pathogen and it has wide host range. *Trichoderma* species were identified as significantly and potentially effective antagonists against the pathogen. Invitro conformation test like dual plate can be coupled to extracellular enzyme and protein analysis because they play a importent role in inactivation of fungal plant pathogens. Twelve isolates were tested for the production of volatile and Non volatile metabolites. The results indicated that *Trichoderma* isolates were not produced any volatile metabolites. All antagonists produced non-volatile metabolite/s and inhibiting the mycelial growth of *Sclerotium rolfsii*. *T. konigiopsis* (M32, M33), *T. neokoningii* (M6), *T. harzianum* (21), *T. gamsii* (M11, 15) were more aggressive in inhibiting the mycelial growth of the *S. rolfsii* and formation of sclerotial bodies. The other isolates were partially inhibiting the growth rate of *S. rolfsii.*

Key words: Volatile metabolite, Non-volatile metabolite, *Trichoderma*, collar-rot disease, Mentha arvensis

Introduction

Mentha arvensis L. is an aromatic perennial herb commercially cultivated for its oil. It has different chemical constituents of menthol, menthone, methyl acetate, terpenes are used in medicinal preparation, cosmetics, perfumery, mouth wash and flavouring agents. India is emerging as the largest producer (70%) of menthol mint oil in the world.

The crop is severely affected by collar-rot disease. The disease caused by *Sclerotium rolfsii* Sacc (Saccardo) is a soil borne plant pathogen and on a wide host range of agricultural and horticultural crops.

Several mycoparasitic strains belonging to the filamentous fungal genus *Trichoderma* are promising candidates for the biological control of plant pathogenic fungi. The genus Trichoderma is a well known group of facultative fungal saprophytes, used for the industrial production of enzymes and as biocontrol agents. *Trichoderma* species possess good antagonistic abilities against plant pathogenic fungi (Kredics *et al* 2003). In vitro conformation test like dual plate can be coupled to extracellular enzyme and protein analysis because they play a important role in inactivation of fungal plant pathogens (Chet, I 2006). *Trichoderma* spp. are reported to release active lytic enzymes that can digest these cell wall components of pathogen (Elad 1980) . The extra cellular enzymes may play a role in biological control. Trichoderma species excrete a large number of chitinolytie

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and glucanoltic enzymes, degrading cell walls and hence playing a key role in mycoparasitism (Lorito *et al* 1996).

Materials and Methods

Seven soil samples were collected (Dhingra & Sinclair 1993) from *Mentha arvensis* growing area in Central institute of medicinal and aromatic plants (CIMAP), Hyderabad, and research field are used to isolate *Trichoderma* species.

The *Sclerotium rolfsii* Sacc (Saccardo) (SR1) causative organism of collar rot disease of *Mentha arvensis* was isolated from infected plants collected from the research field.

Volatile Metabolites:

The effect of volatile metabolites produced by the effective *Trichoderma* isolates on mycelial growth was determined by the method of Dennis & Webster (1971b). The two-day old culture of *Trichoderma* isolates and *Sclerotium rolfsii* culture plates were assembled. After incubation period of 72h open the assembled plates and measure the *Sclerotium rolfsii* growth and formation of sclerotial bodied in test plates compared with control.

Non-volatile Metabolites:

The effect of non-volatile metabolites produced by the antagonistic effective *Trichoderma* isolates on mycelial growth was determined by the method of Dennis & Webster (1971a). Each antagonistic isolate was grown on a sterile cellophane disc laying on PDA (2%) in 9 cm Petri dishes for 48 h, then the cellophane with the mycelium was removed and in the same position where the mycelium was grown, a mycelia 5 mm diam of *Sclerotium rolfsii* was inoculated. Radial growth of the pathogen colonies was determined after 24 and 72 h and compared with those of the pathogen grown on PDA without metabolites (control) (Gloria *et al* 2003).

Results:

A total number of forty-five isolates are obtained from soil samples. These *Trichoderma* isolates were tested for biocontrol potency against *Sclerotium rolfsii* by using dual culture technique (Dennis & Webster 1971). All, twelve isolates significantly inhibited the growth of the *Sclerotium rolfsii* and formation of the sclerotia. These *Trichoderma* isolates were shown more aggressive in inhibiting the mycelial growth of the *Sclerotium rolfsii,* inhibition ranged from 42 to 63%.

Volatile Metabolites:

Twelve isolates were tested for the production of volatile metabolites. The results indicated that none of the isolates are able to produce any volatile metabolites.

Non-volatile Metabolites:

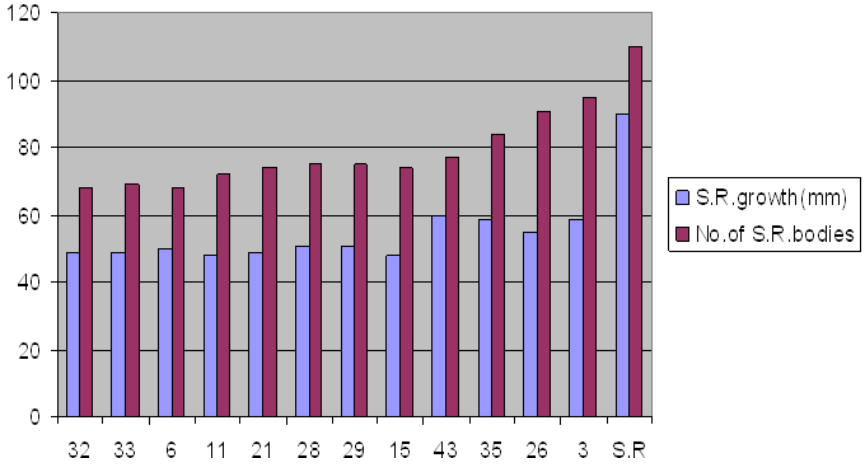
Twelve isolates were tested for the production of Non volatile metabolites. All antagonists produced non-volatile metabolite/s and inhibiting the mycelial growth of *Sclerotium rolfsii* as shown by diameter values reported in Table1. *T. konigiopsis* (M32, M33), *T. neokoningii* (M6), *T. harzianum* (21), *T. gamsii* (M11, 15) were more aggressive in inhibiting the mycelial growth of the *S. rolfsii* and formation of sclerotial bodies. The other isolates were partially inhibited the growth rate of *S. rolfsii* and reduced the formation of the sclerotial bodies.

Discussion:

All isolates tested, twelve isolates are effective in controlling the growth as well as sclerotial formation. It is attributed that the release of higher extracellular enzymes in binding and lysis of *S.rolfsii* cell wall than non effective isolates. *Trichoderma* species are known to be highly efficient against *S.rolfsii* Sacc (Elad *et al*., 1980). Results of Bosah *et al* 2010 revealed that *Trichoderma* species were identified as significantly and potentially effective antagonists against the pathogens. The cell walls of *S. rolfsii* composed of β-1,3 glucan and chitin (Elad *et al* 1983). *Trichoderma* spp are reported to release active lytic enzymes, that can digest these cell wall components of pathogen (Elad *et al*., 1983) . The extra cellular enzymes may play a role in biological control (Henis *et al* 1975).

Table 1: Non-volatile metabolites.

S.No.	Isolate.No	Isolate Name	S.R.growth (mm)	No.of S.R.bodies
1	32	*konigiopsis*	49	68
2	33	*konigiopsis*	49	69
3	6	*neokoningii*	50	68
4	11	*T.gamsii*	48	72
5	21	*harzianum*	49	74
6	28	*strictipilis*	51	75
7	29	*fasciculatum*	51	75
8	15	*T.gamsii*	48	74
9	43	*tiwances*	60	77
10	35	*Trichoderma spp*	59	84
11	26	*Trichoderma spp*	55	91
12	3	*fertile*	59	95
13	S.R	*S.rolfsii*	90	110



The study of Corona *et al* (2008) revealed that the isolate of *Trichoderma* are highly antagonistic which are producing higher enzyme production. Invitro conformation test like dual plate can be coupled to extracellular enzyme and protein analysis because they play a importent role in inactivation of fungal plant pathogens. Trichoderma species excrete a large number of chitinolytie and glucanoltic enzymes, degrading cell walls and hence playing a key role in mycoparasitism (Lorito *et al*. 1994).

The approach used was to analyze two extracellular enzymatic activities (β-1,3 glucanase and N-acetylhexosamidase). Which are considered important in the biocontrol mechanism.

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