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Role of Secondary Metabolites Produced by Commercial *Trichoderma* Species and their Effect Against Soil Borne Pathogens

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Abstract

The study was focused on the identification of the major secondary metabolites produced by the *Trichoderma spp* and soil borne pathogens. *Trichoderma harzianum (Th. Azad)* and *Trichoderma viride (01PP)* are two microorganisms used as active agents in a variety of commercial bio pesticides and bio fertilizers and widely applied on field and greenhouse crops. The production, isolation, biological and chemical characterization of the major secondary metabolites produced by these strains is done during this study. Metabolites are organic compounds that are used in, or created by, the chemical reactions happening in every cell of living organisms. In this paper we are studying about the production of secondary metabolites from *Trichoderma* species against soil borne pathogens at 27°C at different time intervals. Higher the concentration of secondary metabolites, greater will be the efficiency against soil borne pathogens.

Keywords: Secondary metabolites; *Trichoderma*; Soil borne pathogens

Introduction

The biocontrol mechanism of *Trichoderma spp*. is a complex process mediated by the secretion of extracelluar enzymes, such as chitinase, β-glucanase and proteinases, as well as secondary metabolites. Although some antibiotics may be the major factor for the biocontrol activity of certain strain, this may not be the case of others. In addition, there is still no clear evidence on the production of antibiotic compounds by *Trichoderma spp*. in the rhizosphere. Metabolites are organic compounds that are used in, or created by, the chemical reactions happening in every cell of living organisms [1]. This process, known as metabolism, is responsible for breaking down food and other chemicals into energy and materials needed for health, growth, and reproduction. Metabolism is also responsible for the removal of toxic substances from the body. Metabolites can be the starting materials, intermediate materials, or end products of these chemical reactions. A variety of metabolites and reactions combine to produce all the effects that allow an organism to sustain life. A primary metabolite is a kind of metabolite that is directly involved in normal growth, development, and reproduction. The main primary metabolites are carbohydrates, proteins, nucleic acids, and lipids. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. Secondary metabolites often play an important role in plant defense against herbivores and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings, and recreational drugs. The main secondary metabolites are alkeloids, terpenoids, phenolics etc.

Trichoderma spp. [2] are fungi that are present in nearly all soils and other diverse habitats. In addition to colonizing roots, *Trichoderma* spp. attack, parasitize and otherwise gain nutrition from other fungi [3]. Several new general methods for both bio control and for causing enhancement of plant growth have recently been demonstrated [4] and it is now clear that there must be hundreds of separate genes and gene products involved in this processes. *Trichoderma* spp. possess innate resistance to most agricultural chemicals, including fungicides [5] although individual strains differ in their resistance. Soil borne pathogens is a fungal pathogen, producing a disease in tomato and potato plants called early blight. It produces small, darkened lesions on the plants that spread into growing black spots of dead tissue, often killing most of the plant in the long run. Seeds infected with the disease may even damp off during germination.

Material and Methods

Media preparation

TSM (*Trichoderma* Selective Media) contains 0.2 g $MgSO_4.7H_20$, 0.9 g K_2HPO_4, 0.15 g KCl, 3.0 g NH_4NO_3, 3.0 g Dextrose, 0.25 g Chloramphenicol, 0.305 g, Fenaminosulf, 0.20 g PCNB, 0.15 g Rose Bengal, 1000 ml distilled water, 18.0 g AGAR. After that pH were adjusted 7.0. PCNB has been added after autoclaving of media. After the media preparation it has been kept in autoclave machine for 30 min at 121°C. Then serial dilution has been done, for serial dilution three samples of soils has been taken. The samples from which soil has been taken are *Rofflia serpentine, Parthenium and Carissa carandus*. From 10^{-4}, 10^{-5}, 10^{-6} dilution vials 100 µl sample has been taken and spread on the TSM plate. After 2-3 days *Trichoderma* growth has been observed. The colour of colony is green. Potato Dextrose Broth contains 100 g Potato, 10 g dextrose, 500 ml distilled water and pH was adjusted 6.0. After the broth is prepared, six conical flasks of 500 ml is taken and 50 ml broth is poured in each flask. After that each broth has been kept in autoclave machine for 30 min at 121°C and 15 psi. An autoclaved broth is taken and kept into Laminar Air Flow for the inoculation of *Trichoderma* and soil borne pathogens into each flask. Then the flask is kept for 5 days at room temperature. After 5 days all the six conical flask is kept at shaker for 1 week for better mixing. After this long term process the broth is filtered with sterile 0.22 micron filter paper. For getting higher concentration of secondary metabolites we will make three different

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volume of broth by mixing two flask media into one and three flask media into one. Now we have three broth which is indicated as 1st, 2nd, 3rd. The fresh broth is prepared which will work as control. 2 g of agar is added is added into each flask and all the four broth is kept to autoclave machine for 30 min at 121°C. Pour the 1st, 2nd, 3rd and Control media into 1st, 2nd, 3rd and CONTROL written sterile petri plates respectively and inoculate the pathogen in all plates. After the inoculation the plates are incubated at 27°C and the growth is recorded after 24 hrs every day.

Results

Trichoderma species is filamentous fungi with high economic importance since it participate as biological control or biological pesticide agents, inhibiting the growth of phytopathogenic fungi that could destroy a large variety of crops. Result showed that secondary metabolites play an efficient role in inhibiting the growth of the pathogen. From the above study it is evident that with the increase in the concentration of metabolites the growth of the pathogen decreases Figures 1-9 and Tables 1-7, hence higher the concentration of secondary metabolites, greater will be the efficiency against pathogen.

The biological control ability of *Trichoderma* species seems to be due to multiple factors, as they have the ability to produce a variety of extracellular lytic enzymes and the production of many secondary metabolites and these metabolites are also believed to participate in induction of plant defense responses we have analyzed the plant responses at the level of CWDE gene expression in the interaction with mutant in order to establish the role of these compounds during the symbiosis. Three kinds of compounds are mainly produced by strains of *Trichoderma* i.e. peptaibols, polyketides and terpenes. Some of them have antifungal activity.

Figure 1: Concentration of Secondary metabolites

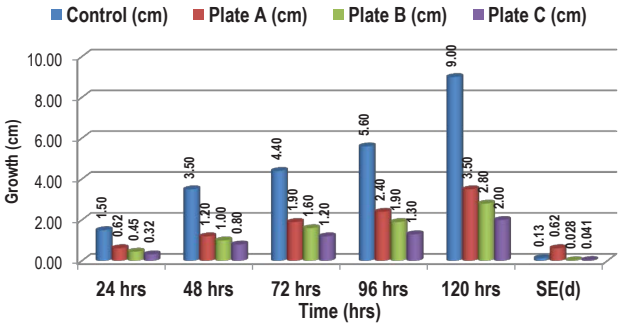

Figure 2: Growth of *Fusarium oxysporum f.sp. lentis* against *Trichoderma viride* (01PP) and *Trichoderma harzianum (Th azad)* at 26 ± 1°C.

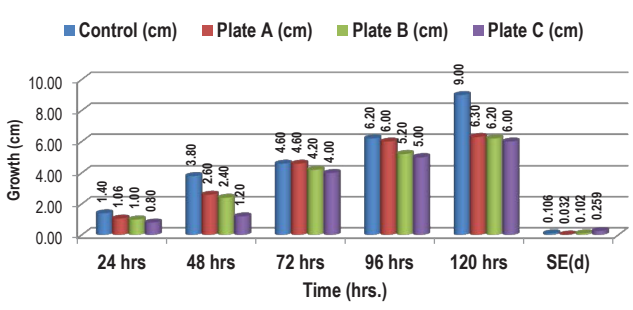

Figure 3: Growth of *Fusarium oxysporum f. sp. udum* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1°C.

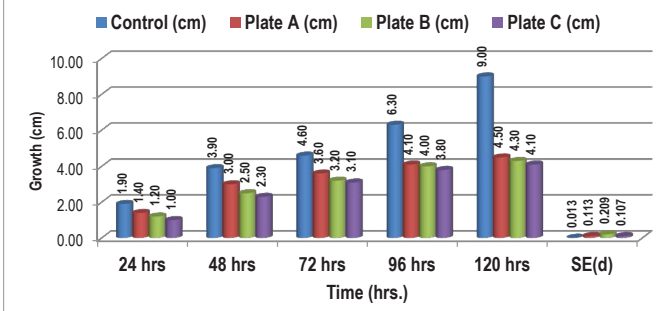

Figure 4: Growth of *Fusarium oxysporum f.sp. ciceri* against *Trichoderma harzianum (Th. Azad)* at 26±1°C.

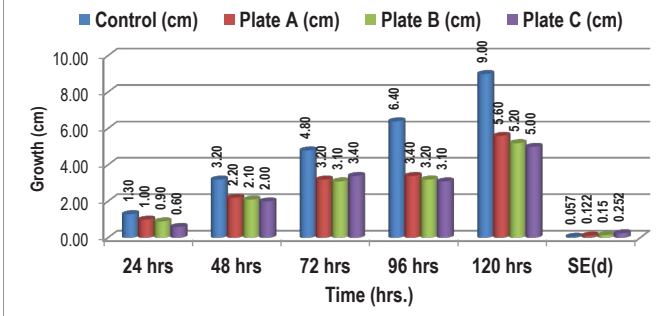

Figure 5: Growth of *Fusarium oxysporum f.sp. lentis* against *Trichoderma harzianum (Th. Azad)* at 26 ±1°C.

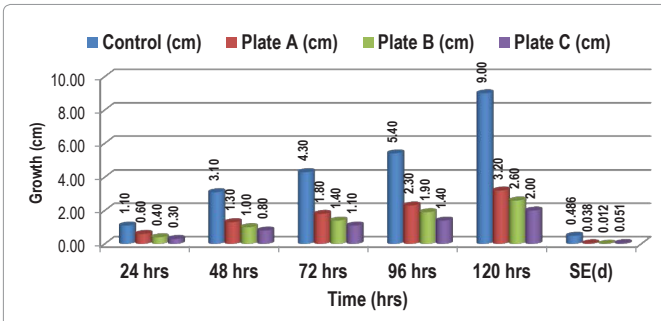

Figure 6: Growth of *Alternaria solani* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1°C.

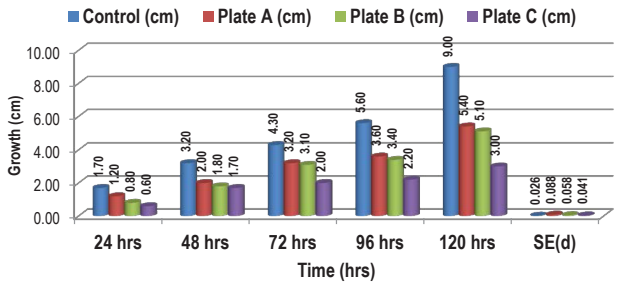

Figure 7: Growth of *Sclerotinia rolfsii* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1ºC.

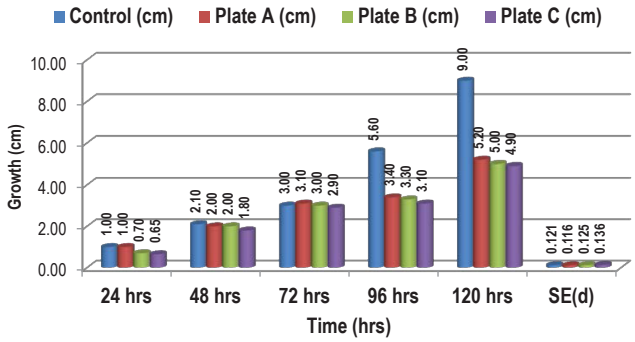

Figure 8: Growth of *Rhizoctonia solani* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1ºC.

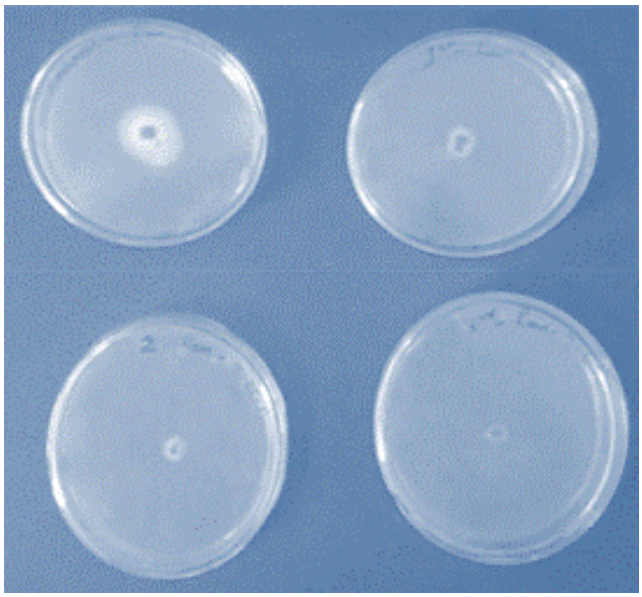

Figure 9: Growth of *Trichoderma* at 27ºC and time interval of 24 hrs.

The biocontrol mechanism of *Trichoderma* species is a complex process mediated by the secretion of extracellular enzymes, such as chitinases, β-glucanases and proteinases, as well as secondary metabolites. Although some antibiotics may be the major factor for the biocontrol activity of a certain strain, this may not be the case for others. In addition, there is still no clear evidence on the production of antibiotic compounds by *Trichoderma* spp. in the rhizosphere.

Time	Control (cm)	Plate A (cm)	Plate B (cm)	Plate C (cm)
24 hrs	1.50	0.62	0.45	0.32
48 hrs	3.50	1.20	1.00	0.80
72 hrs	4.40	1.90	1.60	1.20
96 hrs	5.60	2.40	1.90	1.30
120 hrs	9.00	3.50	2.80	2.00
SE(d)	0.130	0.620	0.028	0.041

Table 1: Growth of *Fusarium oxysporum f.sp. lentis* against *Trichoderma viride* (*01PP*) and *Trichoderma harzianum* (*Th azad*) at 26 ± 1ºC.

Time	Control (cm)	Plate A (cm)	Plate B (cm)	Plate C (cm)
24 hrs	1.40	1.06	1.00	0.80
48 hrs	3.80	2.60	2.40	1.20
72 hrs	4.60	4.60	4.20	4.00
96 hrs	6.20	6.00	5.20	5.00
120 hrs	9.00	6.30	6.20	6.00
SE(d)	0.106	0.032	0.102	0.259

Table 2: Growth of *Fusarium oxysporum f. sp.udum* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1ºC.

Time	Control (cm)	Plate A (cm)	Plate B (cm)	Plate C (cm)
24 hrs	1.90	1.40	1.20	1.00
48 hrs	3.90	3.00	2.50	2.30
72 hrs	4.60	3.60	3.20	3.10
96 hrs	6.30	4.10	4.00	3.80
120 hrs	9.00	4.50	4.30	4.10
SE(d)	0.013	0.113	0.209	0.107

Table 3: Growth of *Fusarium oxysporum f.sp. ciceri* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1ºC.

Time	Control (cm)	Plate A (cm)	Plate B (cm)	Plate C (cm)
24 hrs	1.30	1.00	0.90	0.60
48 hrs	3.20	2.20	2.10	2.00
72 hrs	4.80	3.20	3.10	3.40
96 hrs	6.40	3.40	3.20	3.10
120 hrs	9.00	5.60	5.20	5.00
SE(d)	0.057	0.122	0.150	0.252

Table 4: Growth of *Fusarium oxysporum f.sp. lentis* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1ºC.

Time	Control (cm)	Plate A (cm)	Plate B (cm)	Plate C (cm)
24 hrs	1.10	0.60	0.40	0.30
48 hrs	3.10	1.30	1.00	0.80
72 hrs	4.30	1.80	1.40	1.10
96 hrs	5.40	2.30	1.90	1.40
120 hrs	9.00	3.20	2.60	2.00
SE(d)	0.486	0.038	0.012	0.051

Table 5: Growth of *Alternaria solani* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1ºC.

Time	Control (cm)	Plate A (cm)	Plate B (cm)	Plate C (cm)
24 hrs	1.70	1.20	0.80	0.60
48 hrs	3.20	2.00	1.80	1.70
72 hrs	4.30	3.20	3.10	2.00
96 hrs	5.60	3.60	3.40	2.20
120 hrs	9.00	5.40	5.10	3.00
SE(d)	0.026	0.088	0.058	0.041

Table 6: Growth of *Sclerotinia rolfsii* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1ºC.

Time	Control (cm)	Plate A (cm)	Plate B (cm)	Plate C (cm)
24 hrs	1.00	1.00	0.70	0.65
48 hrs	2.10	2.00	2.00	1.80
72 hrs	3.00	3.10	3.00	2.90
96 hrs	5.60	3.40	3.30	3.100
120 hrs	9.00	5.20	5.00	4.90
SE(d)	0.121	0.116	0.125	0.136

Table 7: Growth of *Rhizoctonia solani* against *Trichoderma harzianum (Th. Azad)* at 26 ± 1ºC

The study was focused on the identification of the major secondary metabolites produced by *Trichoderma* species against Soil *borne pathogens*. In the in vitro interactions between species and soil borne pathogens, it is observed that secondary metabolites play an efficient role in inhibiting the growth of the pathogen. Higher the concentration of secondary metabolites, greater will be the efficiency against pathogen.

The result shows the positive effect of secondary metabolites of *Trichoderma* species against soil borne pathogens. So instead of using *Trichoderma* spp. we can use their secondary metabolites. This positive effect of secondary metabolites is a boon for agriculture. By the mass production of these secondary metabolites it can be used at large scale by which the production of crop will increase.

Discussion

Studies on the secondary metabolites isolated from T22 and T39 strains supported by a 'metabolomic approach' [6] are required to better our understanding of both the mechanisms of action of these bioactive compounds during the antagonism and their role in the interaction between biocontrol fungi, plant and microbial pathogens. The bio control mechanism of *Trichoderma* harzianum is a complex process mediated by the secretion of extracellular enzymes, such as chitinases [7] b-glucanases [8] and proteinases [9] as well as secondary metabolites [10]. The role of *Trichoderma* antibiotics in biocontrol is still a matter of discussion. Although some antibiotics may be the major factor for the biocontrol activity of a certain strain, this may not be the case for others [11]. In addition, there is still no clear evidence on the production of antibiotic compounds by *Trichoderma* spp. in the rhizosphere and spermosphere [12].

Our results support the consideration that the antibiosis occurring during the saprophytic and antagonistic growth of strains Th. azad and 01 PP could be involved, in concert with other mechanisms, during the interaction with plant pathogens [13]. Furthermore, T. harzianum is a well-known producer of cell wall-degrading enzymes and the antibiotics could act synergistically with them [14,15]. Similar work on major secondary metabolites produced by *Trichoderma* species also concluded by [16,17] reported that Tricoderma isolates are more effective and show excellent control of *S. rolfsii*, responsible for groundnut rot. These isolates could be exploited on a large scale for field application in local conditions. The two isolates will be promising bio control agents against rots caused by *S. rolfsii* in field crops with best outcome in future plant biotech applications. Barbara [18] also reported that Secondary metabolite toxins produced by fungi often play a role in triggering these responses [19] suggested that plants protect themselves by producing some compounds called as secondary metabolites. Secondary metabolites, including terpenes, phenolics and nitrogen (N) and sulphur (S) containing compounds, defend plants against a variety of herbivores and pathogenic microorganisms as well as various kinds of abiotic stresses [20] observed that the antagonists produce an array of secondary metabolites such as antibiotics and toxin, which contribute to the antagonistic activity of fungal biocontrol agents against plant pathogens. Antagonistic strains belonging to the *Trichoderma* and Fusarium genera were able to produce various secondary metabolites which can play a role in the mechanism of their biological activity [21-23].

Conclusion

This paper reports the isolation and characterization of the main secondary metabolites obtained from culture filtrates of two *Trichoderma strains (Th. Azad & 01 PP)* and their production during antagonistic interaction with soil borne pathogens. This work is done on the secondary metabolites produced by the commercially applied strains *Th. azad and 01 PP.* Our results provide a better understanding of the metabolism of these fungi, which are both widely used as biopesticides and/or biofertilizers in biocontrol.

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