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Research Article

Bioactive Compound Produced By *Trichoderma harzianum* For Controlling *Sarocladium oryzae*

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Abstract

Rice (*Oryzae sativa* L.) is an important cereal in agriculturally based economy of Thanjavur district, Tamilnadu. Several microbial diseases have been found to occur on the rice resulting in extensive damage to grain. The most prevalent one is sheath rot disease in paddy caused by *Sarocladium oryzae*. In recent years, the increasing uses of pesticides in agriculture have been the subject of growing concern for both environment and public health authorities. The possible use of fungal antagonists on rice pathogen has been viewed as an alternative disease management. Among different biocontrol agents *Trichoderma* sps. has proved effective and selective enough against most of the fungal disease. The separation of phytocompounds such as alkaloids, flavonoids, phenols, sterols, saponins, tannins and pigments from *T.harzianum* by the differential migration of solute between two phases, a stationary and mobile phase of TLC, the major bands were scrapped out and extracted individually with methanol. This paper reports the characterization of seven compounds extracted from culture filtrates of *Trichoderma harzianum* isolate to act against *Sarocladium oryzae*. Maximum inhibition zone occur in the presence of sterol compound 16.8 mm. The present studies indicated that the isolate *T.harzianum* have the potential to inhibit mycelia growth of *Sarocladium oryzae* due to the presence of bioactive compound.

Keywords: Rice, Sarocladium oryzae, Trichoderma harzianum, sheath rot, Phytocompounds

INTRODUCTION

Rice (*Oryzae sativa* L.) is an important cereal in agriculturally based economy of Thanjavur district, Tamilnadu. Several microbial diseases have been found to occur on the rice resulting in extensive damage to grain. The most prevalent one is sheath rot disease in paddy caused by *Sarocladium oryzae*. *Trichoderma* strains are among the most studied fungal biocontrol agents and are successfully used as biopesticides and biofertilizers in greenhouse and field plant production[1]. These applications are related to their ability to control plant diseases and to promote plant growth and development. Different formulations using a variety of *Trichoderma* strains are available commercially for crop production worldwide[2]. The strains of Trichoderma used as biocontrol agents show different mechanisms of action in their antagonistic interactions with fungal pathogens. These include antibiosis, through the

production of a variety of compounds with antibiotic activity[3], mycoparasitism or hyperparasitism[4], competition for nutrients[5]. Of these, antibiotic production is considered to play an important role during biocontrol events, although the interactions in soil and plants are not yet fully elucidated. Different volatile and nonvolatile secondary metabolites have been characterized from *Trichoderma* spp. by antibiotic assay-guided isolation and the production of antibiotic molecules by certain strains is often well correlated with their biocontrol ability[6]. This paper reports the characterization of seven compounds extracted from culture filtrates of *Trichoderma harzianum* isolate to act against *Sarocladium oryzae* .

MATERIAL AND METHODS

Trichoderma spp. isolates were isolated from soil samples obtained from paddy field of Thanjavur district, Tamilnadu. These isolates were identified and characterized by using the identification key techniques which based on the macro- and micro morphological characteristics. The characterization through macro morphology includes examination on the colony appearance, growth rate on Potato Dextrose Agar (PDA), while the shapes and size of conidia, the branching patterns of conidiophores and phialides were observed through micromorphology under the microscope. These isolates of *Trichoderma* species were identified and ascribed to two species namely as *T. harzianum* (TH) and *T. viride* (TV). As the *Trichoderma* spp. is commonly used as biological control agent against pathogenic fungi. *Trichoderma* isolates were also tested as antagonistic activity against *Sarocladium oryzae*, a pathogen for sheath rot disease in paddy by dual culture experiment.

Preliminary qualitative screening of fungal metabolites produced by *Trichoderma harzianum*

Thin layer chromatography (TLC) The separation of phytocompounds such as alkaloids, flavonoids, phenols, sterols, saponins, tannins and pigments from *T.harzianum* by the differential migration of solute between two phases, a stationary and mobile phase of TLC[7] .

Preparation of sample for Thin layer Chromatoraphy

One gram of fungal culture filterate (*T.harzianum*) was taken and these samples were added to 10ml of corresponding organic solvent and allowed to stand for 18h. After 18h, the sample was filtered. The filtrates were concentrated on a water bath and used as a sample to carry out TLC. The sample was dissolved in ethanol and spotted using a capillary tube on silica gel G plate (Silica gel 60F 254 precoated Aluminium) using respective solvents as mobile phase. After running of chromatogram, the plates were dried and observed in UV 253nm and UV 336nm. The plates were sprayed with corresponding spraying reagent. The colour developed in the chromatogram was photographed and Rf values were calculated

Rf value: It is a ratio of distance travelled by the sample and distance travelled by the solvent. The Rf value was determined by the following standard formula:

$$\text{Rf value} = \frac{\text{Distance travelled by the sample (solute)}}{\text{Distance travelled by the solvent}}$$

T.harzianum revealed the presence of alkaloids, flavonoids, phenols, sterols, saponins, tannins and pigments

Alkaloids : The fungal crude extract was dissolved in 2 N HCl solutions. The mixture was treated with a few drops of Meyer's reagent (3.0 ml of potassium iodide solution mixed 2.0 ml of mercuric chloride solution). The creamish precipitate indicates the presence of alkaloids.

Flavonoids: The test tube containing 1.0 ml of the fungal crude extract was added with a few drops of 20% NaOH solution. Change to yellow which on addition of acid changed to colorless solution depicted the presence of flavonoids.

Phenols: The fungal extract was dissolved in 5 ml of distilled water. To this, few drops of 5% ferric chloride solution were added. A dark green indicated the presence of phenolic compounds.

Saponins: The presence of saponins was determined by the frothing test. The fungal extract was vigorously shaken with distilled water and was allowed to stand for 10 minutes. Formations of a fairly stable emulsion indicated the presence of saponins.

Tannins: The fungal crude extract was treated with alcoholic $FeCl_3$ reagent, a bluish black, which disappears on the addition of a little dilute H_2SO_4 was followed by the formation of yellowish brown precipitate indicated the presence of tannins.

Antifungal activity of phytochemical compounds isolated from *T.harzinum*

Bioactive compounds were screened for their antifungal activity against *S.oryzae* by agar well diffusion method[8]. 48h old grown culture of *S.oryzae* on PDA were used for this study. An aliquot (0.02ml) of inoculums was introduced to molten PDA and poured into a petridish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using sterile cork borer. About 0.05 ml of each bioactive compound was introduced into separate well. Then the plates were incubated for 24-48 h at 28°C. The antifungal activity was evaluated by measuring zones of inhibition (mm) of fungal growth surrounding the bioactive compounds. The experiment was carried out in triplicates.

RESULTS AND DISCUSSION

In the present investigation, a survey was conducted to find out the fungi harbouring the paddy filed soils of Thanjavur district, Tamilnadu. *Trichoderma* species grow over the pathogen *Sarocladium oryzae* and it may be possible due to the growth rate of *Trichoderma* species being greater than pathogen *S.oryzae*[9]. The maximum percentage inhibition of *S.oryzae* was observed with *Trichoderma harzianum* (79%) followed by *T.viride* (78%). The inhibitory activites of *T. harzianum* against the pathogen of the present study was similar to the findings of Abdollahzadeh *et al*.[10] and Amin *et al*.[11].

Separation of the bioactive compounds by using TLC

From the preparative TLC plates, the bioactive compounds were separated from *T.harzianum* revealed that the presence of alkaloids, flavonoids, phenols, sterols, saponins, tannins and pigments were scrapped out and extracted individually with methanol. The Rf values of bioactive compounds were recorded (Table.1). The present study was similar to Ramesha and Srinivas[12].

Table 1: Separation of the bioactive compounds by using TLC

S.No.	Bioactive compound	Result	Rf value
1.	Alkaloids	+	0.6
2.	Flavonoids	+	0.53
3.	Phenols	+	0.6
4.	Saponins	+	0.83
5.	Sterols	+	0.76
6.	Tannins	-	-
7.	Pigments Chlorophyll	+	0.17

Rf-Retention factor., +-Presence., - - Absence

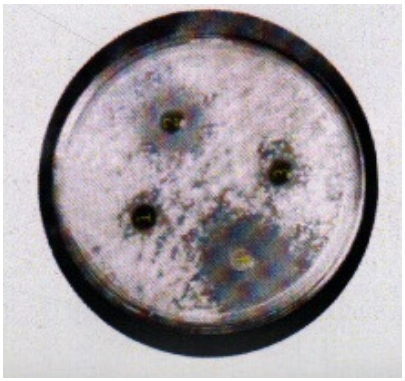
Antifungal activity of bioactive compounds from *T.harzianum*

The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the bioactive compounds. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates. Maximum inhibition zone occur in the presence of sterols compound 16.8 mm (Table 2, Plate I).

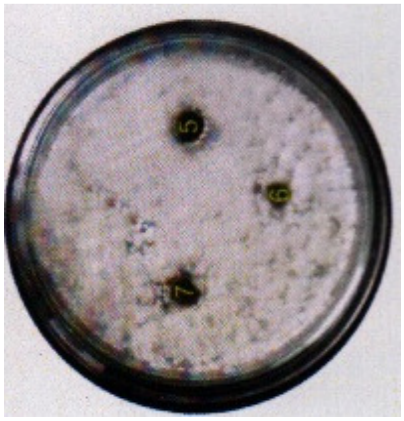
Table 2: In invitro antibiosis of the seven TLC purified fractions against Sarocladium oryzae with respective Rf values.

S.No.	Bioactive compound	Zone of inhibition(mm)
1.	Alkaloids	2.5±1.80
2.	Flavonoids	11.8±1.25
3.	Phenols	10.3±2.02
4.	Saponins	14.8 ±2.25
5.	Sterols	16.8±2.25
6.	Tannins	Nil
7.	Pigments Chlorophyll	Nil

Plate I: Antifungal activity of bioactive compounds against *Sarocladium oryzae*



1. Flavonoids
2. Saponins
3. Phenols
4. Sterols



5. Alkaloids

6.Tannins

7. Pigments

The present studies indicated that the isolate *T.harizianum* have the potential to inhibit mycelia growth of *Sarocladium oryzae* due to the presence of bioactive compound.

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