



**ISOLATION OF SOIL FUNGI AND SCREENING OF *IN VITRO* ANTAGONISTIC POTENTIALITY OF *TRICHODERMA VIRIDE* AGAINST *PENICILLIUM CHRYSOGENUM***

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**ABSTRACT**

The investigation documented total of 8 fungal inhabitants from the paddy field at Suryamaninagar, Tripura during October 2016 to April 2017. Among them six fungi were identified as *Aspergillus flavus*, *Penicillium chrysogenum*, *Fusarium oxysporum*, *Cladosporium cladosporioides*, *Trichoderma viride* and *Curvularia lunata*, two unidentified and several sterile mycelia. Seasonal variation and percentage of contribution were analysed and calculated. Among the identified mycoflora except *Trichoderma viride* all were reported to cause infection in living plant tissue or storage tissue. *Trichoderma viride* was selected as potent bio control agent to test its influence on the growth of pathogenic fungi *Penicillium chrysogenum* which is second dominant isolated fungus in this study. The result of dual culture of *Trichoderma viride* and *Penicillium chrysogenum* showed the occurrence of 55.67% inhibition of mycelial growth of *Penicillium chrysogenum*. After 7 days onwards it was found that the hyphal tips of *Penicillium chrysogenum* become disassociated.

**KEYWORDS:** Mycoflora, Mycelial growth, Paddy field, Antagonistic activity, Dual culture.

**INTRODUCTION**

In the soil environment, fungi are one of the many members of cosmopolitan community characterized by complex interactions. In the rhizosphere, fungi play an important role for their effects on plants including beneficial associations. In fact, soil fungi can prevent infections caused by other microorganisms using defense mechanisms that involve either enzymes or secondary metabolite production (Schumann, 1991).

Paddy field soil rich in organic matters like old stubble, paddy straw, senescent roots and wastes. Soil contains numerous types of bacteria and fungi. Soil and water in paddy fields can affect the bacterial and fungal community strongly (Kogawa *et al.*, 1984). Soil microorganisms play an important role in various metabolic reactions in soil such as mineralization of soil organic nitrogen and decomposition of rice straw and compost applied in soil, which support rice production as well as maintain the fertility of paddy soil (Kikuchi *et al.*, 2007).

Fungi also use antagonism to reduce competition by producing antibodies, which suppress other microorganisms from growing. Beneficial fungi also form protective webs and nets around roots and leaves to

protect the host plants (Lowenfels and Lewis, 2006; Sylvia *et al.*, 2005). Fungus also protects plants by supplying a protective health to supply both water and phosphorus to the plant roots during droughts (Magdoff and VanEs, 2009).

An antagonist organism which has inhibitory relationship with other organism. In nature microbes grow in various association ranging from antibiosis, symbiosis, synergism to commensalisms. These associations may exist within or between different groups of microbes.

Fertilizer effect the soil fertility and crop products. These days many non chemical and eco-friendly fertilizers are in use for a better and healthy crop. Many bacteria, algae, fungi used as bio control agent play a significant role in the field of plant disease control, thereby increasing plant productivity. The potential efficacy of *Trichoderma harzianum* against the pathogenic fungi like *Cladosporium sphaerospermum*, *Aspergillus niger* and *Fusarium oxysporum* was evaluated on the fungal growth by culture pattern (Mansoor Ahmad Lone, 2012) For sustainable crop production, eco-friendly pest management is essentially required. To fulfil these requirement mass production of *Trichoderma* spp as a biocontrol agent is becoming a very popular and useful

research tool so that farmers can replace chemical fungicides with *Trichoderma* sp.

The aim of the present study was to identify fungal community from soil samples collected from paddy field and screening the antagonistic activity of *Trichoderma viride* against *Penicillium chrysogenum*.

## MATERIALS AND METHODOLOGY

### Study area

The soil samples were collected from paddy fields at Suryamaninagar (23°45'N91°15'E), Agartala, Tripura.

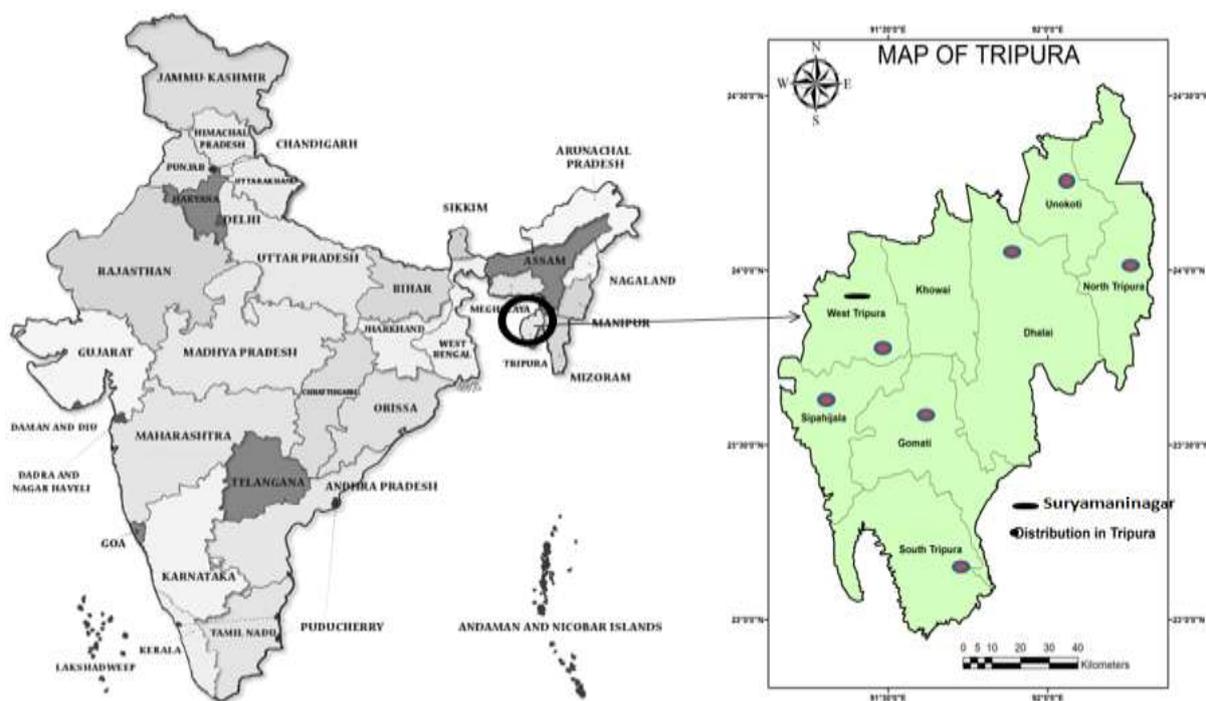


Fig 1: Map showing the study site.

### Method for collection of soil samples

Soil samples were collected during October 2016 to April 2017. The soil samples were collected at a depth of 15cm from paddy fields into a small sterilized polythene bags and brought to laboratory for further studies.

### Isolation of fungi from the soil samples

The soil micro fungi were enumerated by serial dilution methods (Waksman, 1927). One gm of soil sample was suspended in 10ml of double distilled water to make microbial suspensions ( $10^{-5}$ ). 100 $\mu$ l of microbial suspension of each concentration was added to sterile Petri dishes (triplicate of dilution) containing 15 ml of sterile Malt extract Agar (Himedia) and Rose Bengal Agar (Himedia). The Petri dishes were then incubated at  $28 \pm 2$  C in dark. The plates were observed everyday up to five days.

### Identification of the soil fungi

Fungal morphology were studied macroscopically by observing colony features (Colour and Texture) by staining with lacto phenol cotton blue and observed under compound microscope (Olympus, CX-21i) for the conidia, conidiophores and arrangement of spores. The fungi were identified with the help of literature (Ellis. M. B. 1971, Domsch *et al.*, 1980 and Watanabe T. 2002).

### Soil characteristics

The pH of the soil was determined in 1:1 (w/v) soil water suspension with pH meter (Eutech, Model: pH 700). The electrical conductivity of the soil was determined by using electric conductivity meter (Systronics, Model: 304). Moisture content of the soil was determined by oven dry method and soil temperature was determined by digital thermometer (Omron MC-246).

### Determination of *in vitro* antagonistic efficacy of *Trichoderma viride*

Antagonistic efficacy of *Trichoderma viride* was tested based on dual culture technique (Zlata *et al.* 2008) method by plating both the organisms on the same petriplate containing malt extract medium. The fungus *Penicillium chrysogenum* was separately inoculated and labelled as control. In another plates in one side *Trichoderma viride* was inoculated and the other side was inoculated with *Penicillium chrysogenum*. Plates were incubated at  $28 \pm 2$ °C for 7 days, then the plates were observed for development of zone of inhibition as antagonistic activity.

### DATA ANALYSIS

Mycelial growth inhibition of *Penicillium chrysogenum* was calculated using the formula (Vincent 1947).

$$L = \left[ \frac{C - T}{C} \right] \times 100$$

Where L is the per cent of growth inhibition; C is the mycelial growth of *Penicillium*. In control plates; and T is the mycelial growth of *Penicillium* with *Trichoderma*.

#### Colony forming Unit (CFU) (Subha *et al.* 2013)

$$\text{CFU per gm of soil} = \frac{\text{No. of colonies}}{\text{Dry weight of the soil}} \times \text{Dilution factor}$$

#### \*CFU-Colony forming Unit

#### Contribution (Subha *et al.* 2013)

The number of colonies per plate in 1 g of soil was calculated. The percent contribution of each isolate was calculated by using the following formula:

$$\% \text{ Contribution} = \frac{\text{Total No. of CFU of an individual species}}{\text{Total No. of CFU of all species}} \times 100$$

#### \*CFU-Colony forming Unit

## RESULTS AND DISCUSSIONS

### Isolation of Soil fungi

Diversity refers to the variability of life forms which can be among plants, animals and microorganisms. Fungi are important components of biodiversity which has major role in global ecological processes. The soil fungal

diversity in the paddy field was analyzed by soil dilution method during the course of study from October 2016–April 2017. In the present study 64 fungal colonies belonging to 8 fungal genera were isolated from the paddy fields of Suryamaninagar. 8 fungal genera including 2 unidentified spp. were isolated from the selected site (Table 1). They were *Aspergillus flavus*, *Penicillium chrysogenum*, *Fusarium oxysporum*, *Cladosporium cladosporioides*, *Trichoderma viride*, *Curvularia lunata*, unidentified sp1 and unidentified sp2 were found. It was observed that maximum percentage contribution showed by *Aspergillus flavus* (32.81%), followed by *Penicillium chrysogenum* (18.75%) *Cladosporium cladosporioides* (10.93%), *Fusarium oxysporum* (10.93%), *Trichoderma viride* (7.81%), *Curvularia lunata* (6.25%) and unidentified sp1 (3.12%). On the contrary, lowest percentage contribution (1.56%) was observed for unidentified sp2, (Table 1, Fig.3). Similar study was done in different parts of India where *Aspergillus* sp and *Penicillium* sp were dominant fungal genera (Kumar *et al.*, 2015) in Uttar Pradesh. Our results were supported by Sharma (2010) and Sharma *et al.*, (2011) from soil of Darjeeling and Lachung, Kavaka.

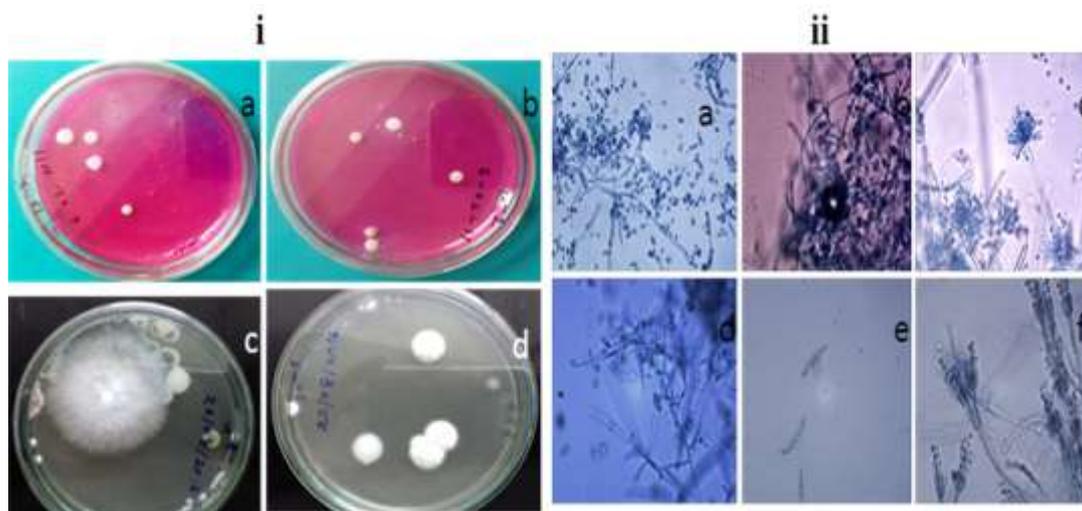


Fig 2: Isolated fungal colonies in plates and microscopic photo, i. Fungal colonies in plates, ii. Microscopic photo, a. Unidentified1, b. Unidentified 2 c. *Aspergillus flavus*, d. *Trichoderma viride*, e. *Fusarium oxysporum* and f. *Penicillium chrysogenum*.

Table1: Isolated fungal genera with percent contribution.

Fungal genera	Contribution (%)
<i>Aspergillus flavus</i>	32.81
<i>Penicillium chrysogenum</i>	18.75
<i>Fusarium oxysporum</i>	10.93
<i>Cladosporium cladosporioides</i>	10.93
<i>Trichoderma viride</i>	7.81
<i>Curvularia lunata</i>	6.25
Unidentified sp1	3.12
Unidentified sp2	1.56
Sterile Hyphae-White colony	7.81

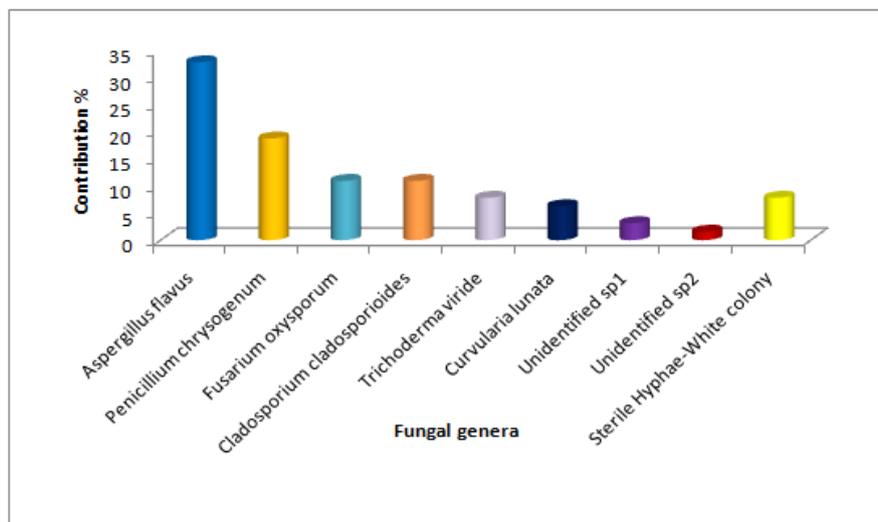


Fig 3: Showing the total fungi isolated from the soil.

### Seasonal variation of Soil mycoflora

During the study period *Aspergillus flavus* and *Penicillium chrysogenum* were found in both the seasons in post monsoon and winter. *Fusarium oxysporum* was isolated from post monsoon soil only but *Cladosporium cladosporioides*, *Trichoderma viride*, *Curvularia lunata*, unidentified sp1 and unidentified sp2, were isolated from winter season only (Table 2). Similar study done by Kaushal and Singh, 2013 from their study diversity and seasonal variation of soil fungi isolated from Lake.

Table 2: Fungi isolation from soil seasonally.

Fungal genera	Post monsoon	Winter
<i>Aspergillus flavus</i>	+	+
<i>Penicillium chrysogenum</i>	+	+
<i>Fusarium oxysporum</i>	+	-
<i>Cladosporium cladosporioides</i>	-	+
<i>Trichoderma viride</i>	-	+
<i>Curvularia lunata</i>	-	+
Unidentified sp1	-	+
Unidentified sp2	-	+
Sterile Hyphae-White colony	+	+

(+) Present, (-) Absent

Table 3: Physical characteristics of soil.

Crop field	pH	Electrical conductivity (mV)	Moisture content (%)	Temperature ( $^{\circ}$ C)
Paddy	6.026 $\pm$ 0.003	45.00 $\pm$ 0.005	37.76 $\pm$ 0.007	29.6 $\pm$ 0.017

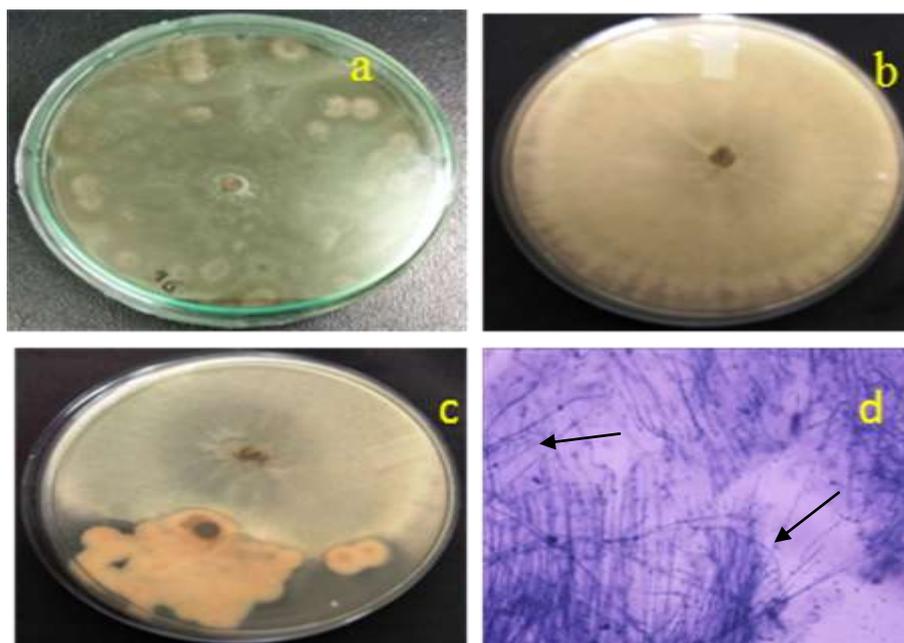
### In vitro Antagonistic activity

In dual culture both the fungi grow simultaneously without any hinderance with each other upto 4 days but 5<sup>th</sup> day onwards it was observed that the mycelia of *Penicillium chrysogenum* was unable to penetrate the *Trichoderma viride* mycelia. The results obtained showed that percent inhibition of mycelial growth was 55.67% against *Penicillium chrysogenum* (Fig. 4). After 7 days onwards it was found that the hyphal tips of

### Soil Characteristics

Various physical properties of soil were measured of the paddy field. During the sampling period the pH of the soil was 6.026 $\pm$ 0.003, moisture content 37.76 $\pm$ 0.005%, electrical conductivity 45.00  $\pm$ 0.007 mV and temperature 29.6 $\pm$ 0.017 $^{\circ}$ C were recorded. The Soil pH, organic content and water are the main factors affecting the fungal population and diversity (Yu *et al.*, 2007). It has been reported that the density of fungal population occurred during the monsoon (rainy) season when the soil moisture was significantly high. Deka *et al.*, (1984) has reported that environmental factors such as pH, moisture, temperature, organic carbon, organic nitrogen play an important role in the distribution of mycoflora.

*Penicillium chrysogenum* become disorganised and disassociated. Finally, the hyphal tips of *Penicillium* were decomposed and get distorted without any further growth (Fig.4).



**Fig 4: Antagonism of *Trichoderma viride* against *Penicillium chrysogenum* and microscopic hyphal appearance after interaction. a, Control of *Penicillium chrysogenum* b, Control of *Trichoderma viride* and c, Antagonism against *Penicillium chrysogenum*, e, Microscopic hyphal appearance of *Penicillium chrysogenum* after interaction with *Trichoderma viride*.**

Etabarian (2006) reported *Trichoderma viride* prevent growth of the colony area of *Macrophomina phaseoli* by 19.2% and 34.9% using the dual culture and cellophane methods respectively. Henis *et al.*, (1983) reported that the different isolates of *Trichoderma* parasitized *S. rolfisii* with varying percentages of inhibition. Dharmaputra *et al.*, (1994) tested two isolates of *T. harzianum* and one isolate of *T. viride* against *Ganoderma* spp. and found that all isolates inhibited the mycelial growth of the pathogen, but *T. harzianum* showed the best performance. Most other studies also reported that *T. viride* and *T. harzianum* were the most effective growth inhibitors of plant pathogens (Poddar *et al.*, 2004; Lane & Bowen 2005; Dubey *et al.*, 2007; Hajieghrari *et al.* 2008).

In this study, the interaction between *Trichoderma viride* and *Penicillium chrysogenum* revealed *Trichoderma* sp. penetrated the *Penicillium* sp. through aplanospores without coiling around it. Similar result was found by Monteiro *et al.*, 2010 against *Fusarium* sp.

#### CONCLUSION

This study is an effort to understand the soil microbial diversity in the paddy fields at Suryamaninagar as soil microflora. *Trichoderma viride* is the promising bio-controlling agent to avert the development of several soil born pathogenic fungi. Potentiality of *Trichoderma viride* was evaluated against soil borne *Penicillium chrysogenum* by dual culture techniques and was found to be more efficient in controlling the growth of the tested pathogen. The presence of antagonistic fungus, *Trichoderma viride*, in the soil thus seems to be

exclusive in nature for keeping the population of other detrimental fungi under check.

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#### CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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