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## Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L.

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### ABSTRACT

Several isolates of *Trichoderma viride*, isolated from different region of Allahabad district were selected for antagonistic screening against fungal pathogens such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Alternaria alternata*, *Fusarium solani* and *Colletotrichum capsici* of Moong bean (*Vigna radiata*). The isolate Tr 8 showed 70%, 68.2%, 70%, 73.3%, 69.3% and 70.1% growth inhibition against *R. solani*, *S. rolfsii*, *M. phaseolina*, *A. alternata*, *F. solani* and *C. capsici* respectively. The cell free culture filtrate of *T. viride* Tr 8 showed 61.5, 58.32, 63.45, 62.62% radial growth at 10% concentration against *R. solani*, *S. rolfsii*, *M. phaseolina*, *C. capsici* respectively. While, 20% concentration observed 100% mycelial growth inhibition. Thus, the *Trichoderma viride* isolate Tr 8 could be further exploited for commercial scale up under localized climatic conditions.

**Key words:** Antagonistic activity, Fungal pathogens, *Trichoderma viride*, *Vigna radiata* L.,

### INTRODUCTION

Moong bean [*Vigna radiata* (L.) Wilczek] is one of the most important pulse crops. It is grown in almost all parts of the country. It is an excellent source of high quality protein and consumed in different ways as dal, halwa, snack and so many other preparations. Ascorbic acid (Vitamin C) is synthesized in sprouted seeds of moong bean with increment in riboflavin and thiamine. Since moong bean is a leguminous crop, it has the capacity to fix atmospheric nitrogen through symbiotic nitrogen fixation. It is also used as green manure crop. Being a short duration crop it also provides an excellent green fodder to the animals.

Several fungal plant pathogens such as *S. rolfsii*, *A. alternata*, *R. solani*, *M. phaseolina*, *C. capsici* are involved in causing serious damage at any stage of the Moong bean therefore, it is very difficult to manage these pathogens by the use of chemicals or through breeding for disease resistance. This led to indiscriminate use of synthetic pesticides and the emergence of several social, environmental, healths, economic and new pest problems. Additionally, consumers are becoming gradually more concerned about chemical pollution of the environment and pesticide residues in food and there is an increasing demand for products coming from sustainable agriculture and eco-farming. Thus both, customers and industries, are highly interested in finding alternative methods of disease control.

Biological control is a promising tool to maintain current level of agricultural production while reducing the release of polluting chemical pesticides to the environment. It is a complex process made up from several successive steps generally initiated by a remote sensing of host which stimulates directed growth; subsequently contact is made between fungal antagonist and host (pathogen) surface.

*Trichoderma* is filamentous fungi the species of which were previously considered to be culture contaminants. *Trichoderma* is a very versatile mold: a nuisance for people, a useful fungus for industry and biocontrol and a bane to other fungi. *Trichoderma* spp. is present in nearly all soils and other diverse habitats. In soil, they frequently are the most prevalent culturable fungi. *Trichoderma* species now should be added to the growing list of emerging filamentous fungal pathogens [1].

In recent years, considerable success has been achieved by the use of fungal bioagent. But so far there is lack of feasible and effective formulation of bioagents to exploit it commercially. The present investigation was undertaken to explore the possibility of making and use of different formulation of *T. viride* Pers., which can devising an effective delivery system with ease to apply, practicable, feasible and economically viable for improving the disease control potential of *T. viride* Pers., *Trichoderma* species are common inhabitant of rhizosphere and contribute to control of many soil borne plant diseases caused by fungi [2]. Apart from biological control, in many cases increased plant growth response was also noted after application of *Trichoderma* in greenhouse or field trials [3,4,5].

## MATERIAL AND METHODS

The present investigation was conducted in Biological Product Laboratory, Department of Botany, University of Allahabad.

### Collection of Soil Sample

The soil samples were collected in different localities of Allahabad and adjoining areas. Soil samples (50g each) were collected from four corners and the center of the field from various places. The samples were collected from top 2-5cm depth of soil. The five samples were mixed to make a composite sample (250g). The composite soil samples were collected from a particular field in the brown paper bag labeled separately.

**Isolation and Identification of *Trichoderma* sp.**

One gram of the soil sample was taken and added to 1ml of sterilized distilled water to make a dilution of  $10^{-1}$ . This suspension was then subjected to serial dilutions and a dilution of  $10^{-5}$  was attained. One milliliter of each dilution viz.,  $10^{-3}$  to  $10^{-4}$  was poured on to *Trichoderma* Specific Medium (TSM) [6] and purified by single spore method. They were identified on the basis of their morphological characters [7]. Cultures were identified according to conidiophore, shape of the phialides and emergence of phialophores and phialospores. The purified and identified cultures of *Trichoderma* spp. were maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for further use.

**Collection of diseased specimens**

The diseased samples were collected in different localities of Allahabad and adjoining areas. Random sampling technique has been applied in the fungal collection procedure. The diseased plants of moong bean (*Vigna radiata* L.) were collected in polythene bags and brought to the Biological Product Laboratory, Department of Botany, University of Allahabad for isolation of pathogens.

**Screening of *Trichoderma viride* isolates against plant pathogenic fungi using dual culture method**

Out of 17 isolates of *Trichoderma* sp. 4 isolates were identified as *Trichoderma viride* from different soil samples. These isolates were tested for antagonism against broad range of common plant pathogen and further studies were done for selected pathogens, by using dual culture techniques as developed by Morton and Stroube [8]. The mycelial bits of 5 mm diameter of *Trichoderma viride* strain and pathogen were placed opposite to each other on petri plates containing sterilized PDA. The plates were run in triplicates with one control set maintained without inoculating the *Trichoderma viride* isolates. The plates were incubated at  $27 \pm 1^{\circ}\text{C}$  for one week. The growth of Pathogen tested against all the isolates of *Trichoderma viride*. The data were recorded regularly on the growth of the pathogen and *Trichoderma viride* isolates. Percentage of mycelial growth inhibition was calculated according to the formula:

$$\text{MGI}\% = (\text{dc} - \text{dt}) \times 100/\text{dc}$$

Where, dc= fungal colony diameter in control sets, dt= fungal colony diameter in treatment sets.

**Extraction extracellular metabolites of *Trichoderma viride* Tr 8**

The effect of extracellular metabolites of selected isolates of *Trichoderma* on the radial growth of pathogen was determined by the addition of cell free culture filtrates (CCF) on agar medium. For this 50 ml of potato dextrose broth was inoculated with 1 ml of spore suspension ( $10^5$ cfu/ml) of the selected isolate. *Trichoderma viride* Tr 8 cells were harvested from stationary phase culture after 10 days. The culture filtrates were centrifuged at 10,000 rpm at 4°C. The culture filtrate was sterilized by passing through a 0.2  $\mu\text{m}$  filter. The filters were added to pre cooled potato dextrose agar medium at final concentration of 0, 5, 10, 15, and 20 % (v/v) before pouring into petri plates. Each plate were inoculated with 5 mm mycelia disc of the pathogen cut with a sterile cork borer (5mm dia.) from the advancing margins of plate culture grown on PDA. The inoculated plates were incubated at  $27 \pm 1^{\circ}\text{C}$ . The colony diameter in each concentration was

recorded. The pathogen inoculated on PDA medium without any culture filtrate served as control (Fig. 1).

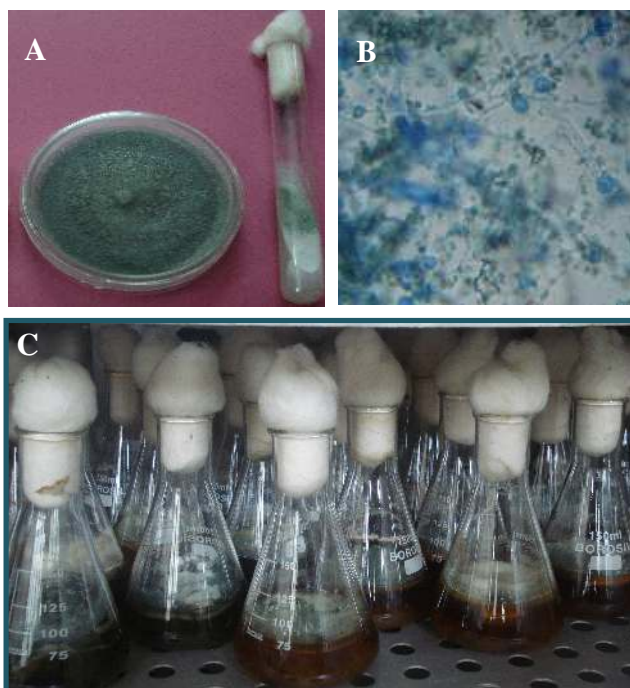
## RESULTS AND DISCUSSION

### Isolation and Identification of *Trichoderma* isolates

Out of 17 isolates of *Trichoderma* sp. 4 isolates were identified as *Trichoderma viride* from different soil samples which were collected from different places and different crops (Table 1). These isolates were tested for antagonism against plant pathogens causing disease in *Vigna radiata* L.

**Table 1** Identified isolates of *Trichoderma viride* from different places of Allahabad

S. No.	<i>Trichoderma</i> isolates	Identified as
1.	Tr.3	<i>T. viride</i>
2.	Tr.8	<i>T. viride</i>
3.	Tr.12	<i>T. viride</i>
4.	Tr.14	<i>T. viride</i>



**Fig. 1** Cultural characteristic of *Trichoderma viride* isolates Tr 8 (A) *Trichoderma viride* isolates Tr 8 in solid medium (B) Microscopic view of *Trichoderma viride* isolates Tr 8 (C) *Trichoderma viride* isolates Tr 8 in liquid medium

### Cultural Characteristic of *Trichoderma viride*

Colonies fast growing (5-9cm) conidiation forming compact tufts or more effuse, glaucous to dark bluish-green (Fig.1). Reverse typically uncolored, less often pale yellowish. Odour usually distinctly aromatic, as of coconut. Conidiophores usually not extensively branched and having a relatively loose arrangement, branches most often paired, or single or 3-verticillate, often

appearing flexuous. Phialides frequently paired, or arising singly or 3-verticillate, narrowly lageniform, 8-14µm X 2.4-3.0µm. Conidal globose to ellipsoidal, usually conspicuously warted, bluish-green to dark green, 4.0-4.8 X 3.5-4.0µm *Trichoderma viride* (J.Miller, Giddens & Foster) von Arx, Beih.) [7].

### Survey of diseased specimens and isolation of pathogens

The diseased samples of moong bean (*Vigna radiata* (L) R. Wilczek) were observed in different localities of Allahabad and adjoining areas. From these samples seed and seedling rot, root rot and leaf blight, *Macrophomina* blight, *Alternaria* leaf spot and anthracnose were observed. The maximum disease incidence was recorded as seed and seedling rot, *Alternaria* leaf spot, root rot and leaf blight, and anthracnose respectively. The pathogens Isolated from these samples are *Alternaria alternata*, *Colletotrichum capsici* *Rhizotonia solani*, *Macrophomina phaseolina*, *Pythium aphanidermatum*, *Sclerotium rolfsii* etc.

From the surveys it was concluded that *Alternaria alternata*, *Colletotrichum capsici* *Rhizotonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii* are the common pathogens of moong bean plants and associated with a number of diseases of moong bean crop, therefore selected for studies.

### Antagonistic activity of *Trichoderma viride* isolates using dual culture method against fungal pathogens of moong bean

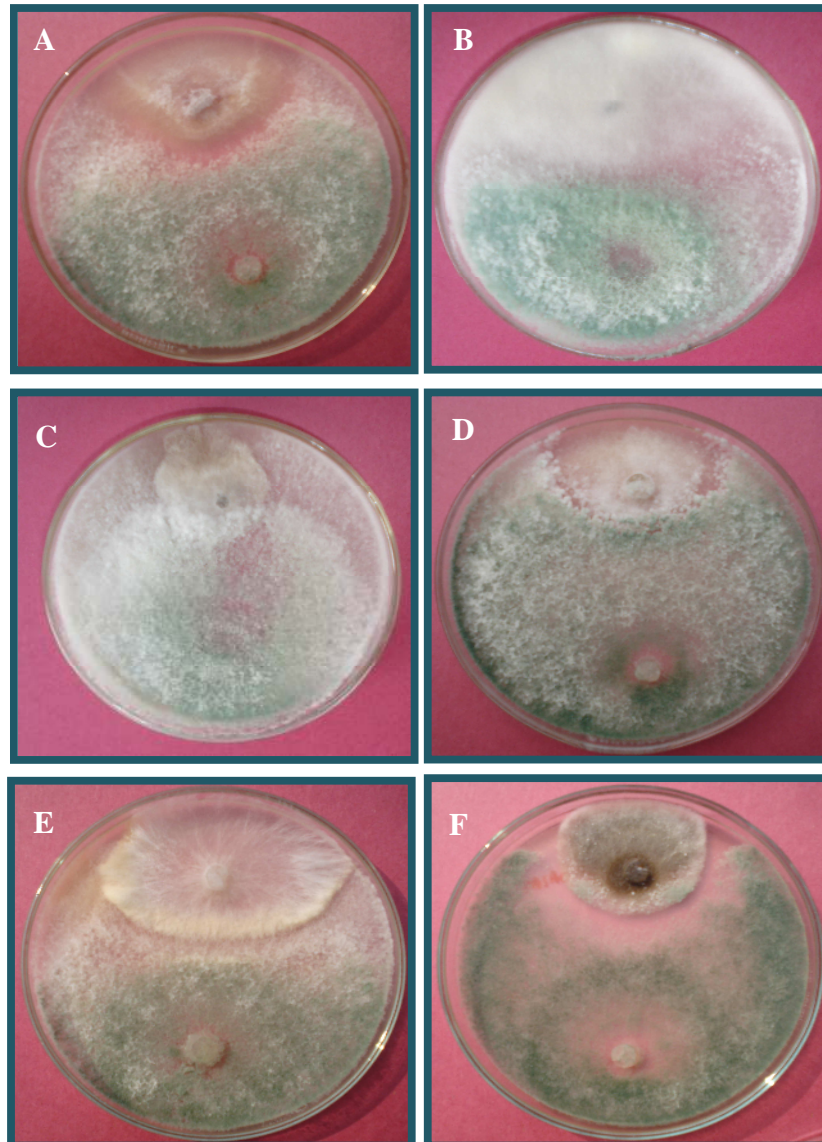
In the present study four *Trichoderma viride* isolates, isolated from different region of Allahabad district were selected for screening against fungal pathogens such as *Rhizoctonia solani*, *Sclerotium rolfsii* *Macrophomina phaseolina*, *Alternaria alternata*, *Fusarium solani* and *Colletotrichum capsici* of moong bean.

**Table 2** Antagonistic activity of various isolates of *Trichoderma viride* against different fungal plant pathogens of Moong bean (*Vigna radiata*)

S. No.	Isolates of <i>Trichoderma viride</i>	% Growth inhibition of various fungal plant pathogens by dual culture method					
		<i>R. solani</i>	<i>S. rolfsii</i>	<i>M. phaseolina</i>	<i>A. alternata</i>	<i>F. solani</i>	<i>C. capsici</i>
1.	Tr.3	61.25	57.50	52.86	67.50	62.50	66.25
2.	Tr.8	70.00	68.24	70.00	73.33	69.32	70.14
3.	Tr.12	67.50	58.75	61.25	66.25	65.00	61.25
4.	Tr.14	60.00	56.25	62.50	63.75	57.50	62.50

Each value is mean of three replicates

However, the isolate Tr 8 showed excellent antagonistic activity against fungal plant pathogens causing disease in Moong bean (*Vigna radiata*) was identified as *Trichoderma viride*. In dual culture a clear zone of inhibition was observed exhibiting antibiosis between pathogen and antagonist. It was observed that Tr 8 (*T. viride*) reduced the growth of *R. solani* by 70.00%, *S. rolfsii* by 68.24%, *M. phaseolina* by 70.0%, *A. alternata* by 73.33%, *F. solani* by 69.32% and *C. capsici* by 70.14% (Table 2; Fig. 2 and 3). Chet *et al.*, [2] reported that *Trichoderma* species are common inhabitant of rhizosphere and contribute to control of many soil borne plant diseases caused by fungi. *Trichoderma viride* and *T. harzianum* were reported by several workers as the best antagonists for growth inhibition of several soil and seed borne plant pathogens [9,10,11].



**Fig. 2** Antagonistic activity of *Trichoderma viride* isolates Tr 8 against various fungal plant pathogens of Moong bean (*Vigna radiata*) (A) *F. solani* + *T. viride* Tr 8, (B) *M. phaseolina* + *T. viride* Tr 8, (C) *R. solani* + *T. viride* Tr 8, (D) *C. capsici* + *T. viride* Tr 8, (E) *S. rolfsii* + *T. viride* Tr 8, (F) *A. alternata* + *T. viride* Tr 8

#### **Antifungal activity of extracellular metabolites of *T. viride* Tr 8**

In our study, the effect of cell free culture filtrate of selected *Trichoderma* isolate (*T. viride*) on different common fungal pathogens (*R. solani*, *S. rolfsii*, *M. phaseolina*, *C. capsici*) of moong bean are presented in Table 3.

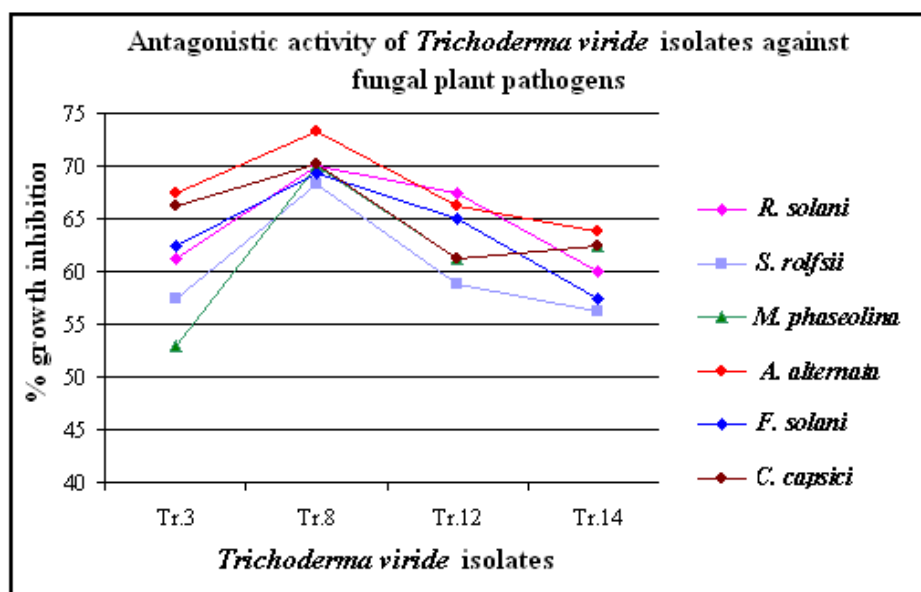


Fig. 3 Graphical representation of antagonistic activity of different isolates of *Trichoderma viride* against fungal pathogens of Moong bean (*Vigna radiata*)

Table 3 Effect of cell free culture filtrate of *Trichoderma viride* Tr 8 on growth of different common fungal pathogens of Moong bean

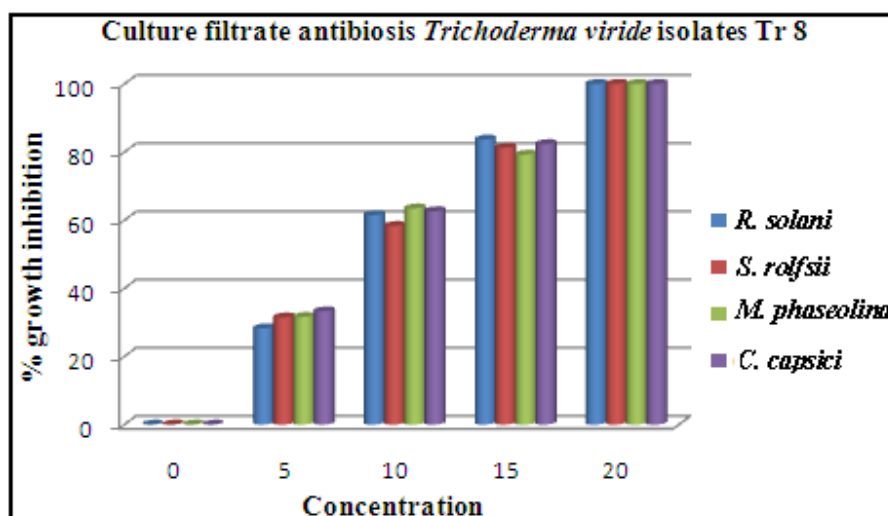
S. No.	Concentration of <i>Trichoderma viride</i> (Tr 8) metabolites	% growth inhibition of various fungal plant pathogens			
		<i>R. solani</i>	<i>S. rolfsii</i>	<i>M. phaseolina</i>	<i>C. capsici</i>
1.	0	0	0	0	0
2.	5	28.25	31.45	31.6	33.25
3.	10	61.5	58.32	63.45	62.62
4.	15	83.67	81.25	79.25	82.33
5.	20	100	100	100	100

Each value is mean of five replicates

The results indicate that cell free culture filtrate of *T. viride* reduced the radial growth of *R. solani*, *S. rolfsii*, *M. phaseolina*, *C. capsici* 100% at 20% concentration, whereas at 15% concentration 83.67, 81.25, 79.25 and 82.33% reduction of radial growth were observed respectively, at 10% concentration 61.5, 58.32, 63.45, 62.62% reduction observed and at 5% concentration 28.25, 31.45, 31.60, 33.25% reduction were observed. The maximum inhibitions in mycelial growth (100%) of pathogens were observed at 20% concentration of *T. viride* cell free culture filtrate (Fig. 4).

However, biocontrol agents are found to be managing the disease effectively as well as they are ecologically sound proof [12,13]. Nothing in biological control should be thought of as impossible at the present state our technology [14,15]. It is now widely recognized that use of ecofriendly biopesticide is a distinct possibility for the future and can be successfully exploited in modern agriculture especially within the framework of integrated pest management system without affecting our precious ecosystem [16,17,18].

Fig. 4 Graphical representation of cell free culture filtrate of *Trichoderma viride* (Tr 8) on growth of different common fungal pathogens of moong bean



### CONCLUSION

Biological control is a promising tool to maintain current level of agricultural production while reducing the release of polluting chemical pesticides to the environment. It is a complex process made up from several successive steps generally initiated by a remote sensing of host which stimulates directed growth, subsequently contact is made between fungal antagonist and host (pathogen) surface. This step provides the specific recognition event involving hydrophobic interaction and interaction between complementary molecules present on both host and parasite. Antagonistic interactions of *Trichoderma viride* isolate Tr 8 showed excellent activity against various plant pathogens causing disease in Moong bean (*Vigna radiata*) Thus, the *Trichoderma viride* isolate Tr 8 could be further exploited for commercial scale up under localized climatic conditions.

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