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Trichodermaviride: Isolation, Characterization, Cultivation and Its Application as Effective Biocontrol Agent

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Abstract:

The genus*Trichoderma* consists of anamorphic fungi isolated primarily from soil and decomposing organic matter, with teleomorphs, belonging to the ascomycete genus Hypocrea. They can work against fungal phytopathogens through mechanism such as mycoparasitism, competing for nutrients and space, modifying environmental conditions and antibiosis and plant defensive mechanisms. *Trichodermaviride* have also been reported to produce a plethora of secondary metabolites showing antimicrobial activity. *Trichodermaviride* have been widely used in agriculture as biocontrol agents and inoculants to provide plant growth promotion. The molecular mechanism supporting this highly desirable beneficial effect of plant growth promotion are not fully clarified and include improvement of nutrient availability and uptake for the plant. Many *Trichoderma* strains colonize plant roots of dicots and monocots.

Keywords **—*Trichodermaviride*, Biocontrol, Antagonist, Ecosystem, Growth Promotor.**

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I. INTRODUCTION

Trichodermaviride are free living fungi commonly widespread in soil and root ecosystem. Recent discoveries show them as opportunistic, avirulent plant symbionts as well as parasites of other fungi. Some strains establish robust and long lasting colonization of roots by entering into the first epidermal layers. Root colonization frequently results in enhancing of growth and development, crop productivity or induction of resistance to abiotic and biotic factors.

They are involved in fundamental activities that ensure the stability and productivity of both agricultural and natural ecosystem. The presence of *Trichoderma* in plants involves an induction of resistance, often localized or systemic. Effective *Trichoderma* strains are able to induce a stronger response in the plant compared to pathogen triggered immunity.

The aim of the present study is to select beneficial fungi belonging to *Trichoderma* genus, to be add as soil inoculants, in order to develop an innovative, economical and suitable substrate alternative of biopesticides or biofertilizers. The activity involved the selection of *Trichodermaviride* isolates for their ability to grow in the roots, as endophytes, or in the rhizosphere, to protect plants against plant pathogens or to act as plant growth promoters.

II. METHODS AND MATERIALS

A. Isolation of Fungus

The soil samples were taken from 5 cm depth near the root zone of chilli plants grown in Nawkhala, Nagbhidtaluka, Chandrapur district and isolation was made through Dilution Plate Technique. They were pooled and representative sample was drawn. The bioagents were isolated from the representative sample by following the serial dilution plate technique 10^{-3}were obtained and used for isolation of fungal bioagents. 1 ml of suspension from respective dilution was transferred aseptically into a petri plates containing the medium separately. The plates were rotated manually for uniform distribution and the suspension in the medium is allowed to solidify. The plates were incubated at 25°C for seven days for the developments of fungal colonies. The colonies with characteristics growth of *Trichodermaviride* were observed under the microscope by staining with lactophenol cotton blue stain and growth from such colonies was subcultured on agar slants.

B. DemonstrationofIAAProduction

Fungus culture were grown in PDB amended with tryptophan (5 mm). Culture were centrifuged at 10,000 rpm for 20 minutes. 2 ml of supernatant was mixed with 2 drops of ortho phosphoric acid and 4 ml of salkowski reagent. Tubes were incubation at room temperature for 23 minutes. The intensity of pink colour was read at 540 nm spectrophotometrically.

Table:
Demonstration of IAA Production by *Trichodermaviride*

Sr. No.	Sample	O.D. at 540 nm
1.	PDB 1-A2, Col-1	0.56
2.	PDB, S-L	0.68
3.	S-A, Col-2	0.75

C. Ammonification

Prepare PDB that contains an organic nitrogen substrate which is most suitable. Inoculate the PDB by individual cultures of fungus. Inoculate the broth for 24 hours and add 0.5 gm soil in each tube. Add nesslers reagent to the tubes. The presence of ammonia is indicative of ammonification which is detected by the yellow to brown precipited after adding nesslers reagent.

D. AntagonisticandMycoparasiticActivity

Trichodermaviride, resulting to be the most interesting against antagonistic tests against *Rhizoctoniasolani*. Antibiosis and mycoparasitism were evaluated on PDA (Potato Dextrose Agar, 39 gl-1, Difco). PDA disks of 6 mm diameter, cut from the edge of actively growing colony of each antagonist and pathogen, were placed at the opposite sides (at 4.5 cm each other) on PDA plates.

Plates were incubated at 24 °C with 12 h/12 h darkness/light cycles. Radii of each pathogen approaching and not approaching the colony of antagonists and the distance between the two fungi were measured on PDA three times a day until the two colonies came in contact. Values were used to create growth curves (sigma plot 10) and radial growth data were submitted to analysis of variance of regression in order to compare the slope and the elevation of curves in presence/absense of the antagonist, assuming $P < 0.05$ as a significant level. Mycoparasitism was evaluated on PDA, after 14 days, overgrowth and sporulation of the antagonists on pathogens colonies were assessed. Interactionzones and overlapping regions for each antagonist/pathogen combination were analysed bymicroscopic investigations and coilings and short loops around the host hyphae were recorded.

E. Cultivation

To prepare the initial inoculum, *T.viride* was cultured in different media like Potato Dextrose Broth (PDB), mineral salts medium with either whey or corn steep liquor or biogas slurry. The green conidial formation and the time of conidial formation was earlier selected for further studies.

100 gm of sorghum seeds were boiled up to 20 to 25 minutes to soften grains and cooked about 25%, drain water and spread sorghum seeds to cool down to decrease the moisture content. 2 gm of calcium carbonate was added per 100 gm of parboiled semi dried sorghum seeds to remove the excess moisture and transferred to autoclavable polypropylene bags and autoclaved at 121°C at 15 minutes. After cooling, the sorghum seeds were was aseptically inoculated with the *Trichoderma* mats grown in liquid culture and incubated at room temperature for 5-7 days.

10 gm of the sugarcane bagasse was sterilized in a large petri plates with optimized moisture content of 60%, inoculated with the sorghum seeds cultured with *Trichodermaviride* and incubated under room temperature for 10 days. When the bagasse is completely colonized by *Trichodermaviride* and conidia formed, it was used for the pot culture experiments.

10 gm of vermi compost prepared at MCRC was used for the cultivation of *Trichodermaviride*. The culture of *Trichodermaviride* on compost was carried out as per the above protocol using sugarcane bagasse. It was used for the pot culture experiments.

10 gm of paddy straw was used for the cultivation of *Trichodermaviride*. The culture of *Trichodermaviride* on paddy straw was carried out as per the above protocol using sugarcane bagasse. It was used for the pot culture experiments.

F. GreenHouseStudy

The efficacy of the different *Trichodermaviride* inoculum was tested at green house level by pot culture study using chilli seedlings. 3 kg garden soil mixed with vermi compost was filled in mud pots. 15 day old chilli seedlings were transplanted. The following were the different treatments.

1. *R. solani* alone
2. *R. solani* and *Trichodermaviride* (Talc formulation)
3. *Trichodermaviride* (Talc formulation)
4. Control (No treatment)

Each treatment had three replications (three pots-one plant / pot). The pots were watered every day.

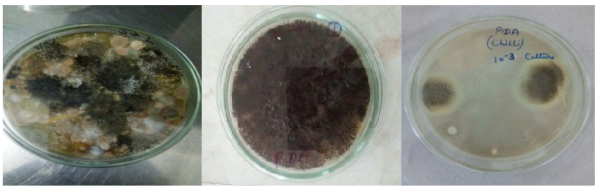
G. InoculationofPathogenandTreatmentofChilliSeedlings

7 days old culture of *R. solani* in PDB was used for inoculation. 12 ml of culture having mycelia was used for inoculation and mixed with rhizosphere soil. *Trichodermaviride* grown in different agro-waste or formulation was included into the soil by digging out the soil around the root zone and mixed well with soil. All the treatments and control plant were compared by observing the rate of infection and other biometric parameters namely shoot length, number of leaves, number of flowers, flowering peroid and number of fruits were observed.

III. RESULT AND DISCUSSION

A. IsolationofFungus

A number of colonies were observed in PDA plate after 3-5 days. When the serially diluted samples were plated on PDA media. Colony that produced brown colour conidia was picked, observed under microscope by staining with lactophenol cotton blue stain. The microscopic analysis of the mycelium with spore revealed that the isolate was *Trichodermaviride*. The isolate was sub-cultured and stored in PDA slants at 20°C.

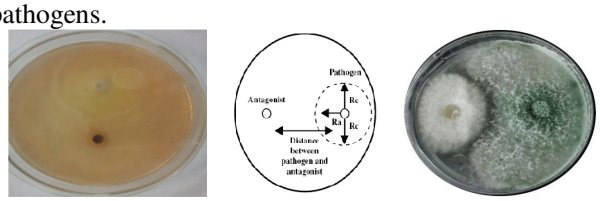


(a) Plate 1 **(b) Plate 2** **(c) Plate 3**

Figure 1: (a)Pure plate culture of fungus isolated from soil of chilli plant (b) Subculture of fungus isolated from plate 1 (c) Subculture of fungus isolated from plate 2

B. Antagonistic and Mycoparasitic Activity

Trichodermaviride was evaluated for its antagonistic activity against the above fungi in petri dishes containing PDA medium. *Trichodermaviride* inhibited the growth of all the pathogens. On the third day the inhibition was seen clear and the following days, the biocontrol agent, *Trichodermaviride* colonised over the pathogens. As *R. solani* are slow grower, on seventh day *Trichodermaviride* was found to grow over the pathogens.



(a) **(b)** **(c)**

Figure 2: (a) Antagonistic activity against *R. solani* (b) Confrontation plate for antagonistic tests (c) Mycoparasitic activity against *R. solani*

C. Cultivation

The fungal mat formed in the mineral salts medium with whey medium was used to the inoculate sorghum seeds and spore biomass was prepared in boiled sorghum seeds. The fungal mycelium slowly colonized the sorghum seeds and after 7 days the entire bag was fully of green colour growth with mycelium and spores on the seeds.

The inoculum developed on the sorghum seeds were used to inoculate large scale culture of *Trichodermaviride*. In this study, agricultural wastes like paddy straw and sugarcane bagasse were used. As these two substrates are available in plenty in villages, farmers can use these waste for development of biocontrol agent. Also we have used vermi compost which is rich in organic matter that supports the growth of *Trichodermaviride*. The substrates were inoculated with cultured sorghum seeds. The growth and sporulation of *Trichodermaviride* was faster in sugarcane bagasse followed by compost and then on paddy straw.

There are two major methods of inoculum production of *Trichodermaviride* viz., solid state fermentation and liquid state fermentation. In solid fermentation, the fungus is grown on various cereal grains, agricultural wastes and by products. In liquid state fermentation, *Trichoderma* is grown in inexpensive media like molasses and yeast medium in deep tanks on a commercial scale. Biomass from the liquid fermentation can be made into different formulations like dusts, granules, pellets, wettable powders. Suggested different organic media or the carrier materials like been cake, coir pith, farmyard manure and decomposed coffee pulp causes an immediate increase in the population up to 3 day and serves as nutrient additives to the crop in addition to inoculum production they support. Soil amended with organic materials like been cake, coir compost, farmyard manure and Gliricidia leaves showed better growth and survival of antagonist than in soil alone. Reported that the pre-boiled sorghum grains, coir pith + been cake (1:1), cow dung + neem cake (1:1) + wheat flour (10%) maintained high populations of *T.viride* within 10 days of inoculation. Likewise in our study, biomass and sporulation was good in sorghum seeds and in other substrates used for growing *T.viride*. However, colonization and sporulation was quick in sugarcane bagasse.

D. Green House Study

Study shows the observations on the different treatments of the chilli plant. There was no *R. solani* incidence observed in the *R. solani* alone treatment. This could be

due to residual *Trichodermaviride* in the unautoclaved soil. Also, in the other treatments where the plants were treated with both pathogen and *Trichodermaviride*, there was no disease developed. However, it was observed that the plants that received the biocontrol agent had a good vigour. The younger leaves developing at the meristem were infected and curled in control plants, and not much affected in the plants that received both pathogen and biocontrol agent. Conversely, in the plants that received only biocontrol agent, the younger leaves were healthy.

These result show that *Trichodermaviride* has protective effect from pathogens and increase the height of the plants. Though the branches and leaves were less and the plants witnessed delayed flowering in *Trichodermaviride* treated plants, only after getting the final yield it can be concluded on the use of this strain as a plant growth promoter.

The result showed that the average plant height of 70 cm in plants treated with biocontrol agent grown in sugarcane bagasse which was followed by plants treated with talc formulation and compost (67 cm height). However, the plant height was 45 cm in the case of control and 52 cm in *R. solani* treated plants. Even though plant height was higher, the number of leaves and buds were more in the control plants and the plants treated with the pathogen, *R. solani*. However, it was noted that the flowers withered later. But in *Trichodermaviride* treated plants all the flowers yielded fruits.

1. Control **2. *R. solani*3.*T. viride***

Figure 3: Morphology of younger leaves of chilli plant in different treatments

IV. CONCLUSION

The *Trichodermaviride* protected the seedlings from damping off disease. The growth promotion attributes like height, plant vigour, fruit formation were found good in the plants treated pots. A simple easy and cost effective method to mass culture the biocontrol agent *Trichodermaviride* by using sugarcane bagasse and talcum powder formulation.

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