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Original Article

# MASS CULTIVATION OF TRICHODERMA HARZIANUM USING AGRICULTURAL WASTE AS A SUBSTRATE FOR THE MANAGEMENT OF DAMPING OFF DISEASE AND GROWTH PROMOTION IN CHILLI PLANTS (CAPSICUM ANNUUM L.)

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**ABSTRACT**

**Objectives:** The main objective of this study is to cultivate *Trichoderma harizanum* using agricultural waste as a substrate for the management of damping of disease and growth promotion in Chilli plants.

**Methods:** *T. harizanum* was isolated from MCRC Main Agricultural Research Station, Chennai, and identified through microscopic observation by using standard keys. *F. oxysporum*, *R. solani*, and *A.alternata* were bought from University of Madras, Guindy Campus Chennai. The antagonistic activity of *T. harizanum* against pathogenic fungi responsible for damping off disease i.e., *Fusarium oxysporum*, *R. solani,* and *Alternaria alternate* were studied in dual culture method. *T. harizanum* was formulated with different agricultural wastes i.e., Sugarcane baggase, vermicompost, paddy straw and talcum powder. The efficacy of the different *T.harizanum* inoculum was tested at green house level by pot culture study using Chilli seedlings.

**Results:** The growth and sporulation of *T.harizanum* was faster in sugarcane baggase followed by vemicompost, talcum powder and paddy straw. The well *T. harizanum* grown agricultural substrate directly applied in to the soil for different treatment. After 7th week control plant showed more number of leaves (96%), maximum plant height (75%), maximum number of buds (12%) and fruits formation (10%) was observed in sugarcane baggase alone treatment and talcum powder alone treatment respectively.

**Conclusion:** Sugarcane baggase and talcum powder formulated *T. harizanum* can be optional not only for the control of damping off disease and also to augment the growth and yield regulation in chilli plants.

**Keywords:** Biocontrol, *R. solani,* Sorghum seeds.

## INTRODUCTION

The green revolution has led to intensified agriculture to meet the ever increasing demands for food and fiber, which is practiced at great cost to the environment, resulting in continuous damage of natural ecosystems, ground water and food-stuff pollution and other environmental degradation. Indiscriminate use of chemical pesticides and fertilizers in modern agriculture has resulted in the development of several problems such as pesticide resistance in pests, resurgence of target and non-target pests, destruction of beneficial organisms like honey bees, and chemical residues in food, feed and fodder. *T. harzianum* is a well-organized biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds [1, 2]. *R. solani* causing damping-off disease of seedlings as well as root and stem rot in young transplants is a major soil-borne pathogen of chilli (*Capsicum annuum* L.). Although some chemicals are known to control *R. solani*, they are not effective always. Furthermore, being a vegetable crop, using chemicals for disease control is probably not advisable in view of the residue problems. Biocontrol of plant pathogens using antagonistic fungi and bacteria, therefore, assumes significance. Among the antagonistic fungi, *T. harzianum* has shown promise as a biocontrol agent of *R. solani* in chilli (Bunker and Mathur, 2001). However, *T. harizanum* treated chilli plants showed more plant height (75 %), number of leaves (60 %), buds (10 %) and fruits (6 %) than control. Hence we concluded that, *T. harizanum* can be recommended not only for the control of damping off disease in chilli plants and also to enhance the growth of the plants [3]. Biological control is based upon the natural enemies of harmful organisms, usually bacteria or fungi. These natural enemies are multiplied by manufacturers and sold as ready-to-use control products. Growers can use biological control to replace (part of) their chemical control. *R. solani* is the main causal agent of damping-off disease of seedlings as well as root and stem rot in young transplants of several plant species [4].

Damping off occurs, when seeds or young seedlings are attacked by these pathogens. Seeds attacked by these fungi usually fail to germinate. Seedlings can be damaged in two ways: the roots may not cause the seedling to wilt and die quickly, or the seedling may be attacked on the stem at the ground line, causing the seedling to collapse. In Mexico, 75% of chili pepper producers point to "damping-off" or root rotas the most important disease during seedling phase [5] and it is present in 88 of each 100 seedling plots causing losses from 1 to 15% [6] As a result, producers tend to use more seeds than they normally would, as a means of compensating the losses caused by this disease. In India, Chilli is a major crop and widely used by people of India. During monsoon season the seedlings are attacked by *R. solani* so that the number of plants in the field becomes less leading to yield loss. Hence the damping off disease in Chilli plants should be controlled effectively to reduce seedling damage.

## MATERIALS AND METHODS

### Isolation of *Trichoderma harizanum*

The soil samples were made from five cm depth near the root zone of chilli plants grown at MCRC Main Agricultural Research Station, Chennai. They were pooled and representative sample was drawn. The bio-agents were isolated from the representative sample by following the serial dilution plate technique $10^{-2}$ and $10^{-4}$ were obtained and used for isolation of fungal bio-agents. 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 27 ± 10C for seven days for the developments of fungal colonies. The colonies with characteristic growth of *Trichoderma* spp were observed under the microscope and growth from such colonies was subcultured on agar slants.

**Plant Pathogens**

*F. oxysporum*, *R. solani*, and *A.alternata* were kindly donated by Dr. N. Mathivanan, CAS in Botany, University of Madras, Guindy Campus Chennai.

**Antagonostic activity of *T.harizanum against R. solani, F. oxysporum and A.alternata*:**

The antagonistic activity of *T. harizanum* against *Fusarium oxysporum*, *R. solani,* and *Alternaria alternate* were studied in dual culture method. PDA plates were inoculated with a 5 mm disc from five-day-old cultures of the plant pathogens in the centre of the plate. Three 5 mm disc of the *T. harzianum* isolate was placed on the periphery of the petriplate on the same day for *R. solani* and for the other 2 pathogens, the *T.harizanum* was palced after 2 days of placing the plant pathogens and incubated at room temperature for six days.

***T. harizanum* Culture in LSF Medium:**

To prepare the initial inoculum, *T.harizanum* 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 were checked and the medium in which the conidial formation was earlier selected for further studies.

**Cultivation of *T.harizanum***

**(i). Preparation of Sorghum seeds and initial inoculum of *T.harizanum*:**

100 g of sorghum seeds were boiled up to 20 to 25 minutes to soften grains and cook about 25%, drain water and spread sorghum seeds to cool down to decrease the moisture content. 2g 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 aseptically inoculated with the *T.harizanum* mats grown in liquid culture and incubated at room temperature for 5-7 days.

**(a). Cultivation using baggase**

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

**(b). Cultivation using Compost**

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

**(c). Cultivation using Paddy straw**

10 grams of paddy straw was used for the cultivation of *T.harizanum.* The culture of *T.harizanum* on paddy straw was carried out as per the above protocol using sugarcane bagassse. It was used for the pot culture experiments.

**Green house study**

The efficacy of the different *T.harizanum* inoculum was tested at green house level by pot culture study using Chilli seedlings. 3 kg garden soil mixed with vermin 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 *T.harizanum* ( Sugarcane baggase grown)
3. *R. solani* and *T.harizanum* ( vermicompost grown)
4. *R.solani* and *T.harizanum* ( Paddy straw grown)
5. *R. solani* and *T.harizanum* ( Talc formulation)
6. *T.harizanum* ( Sugarcane baggase grown)
7. *T.harizanum* ( vermicompost grown)
8. *T.harizanum* ( Paddy Straw grown)
9. *T.harizanum* ( Talc formulation)
10. Control (No treatment)

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

**Inoculation of pathogen to chilli seedlings and Treatment of plants with *T. harizanum*:**

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.*T.harizanum* 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.

**Observations**

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 period, and number of fruits were observed.

**RESULTS AND DISCUSSION**

**Isolation of *Trichoderma harizanum***

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 green 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 *Trichoderma harizanum* .The isolate was sub cultured and stored in PDA slants at -20ºC.

**Antagonostic activity of *T.harizanum against R. solani, F. oxysporum and A.alternata*:**

*T. harizanum was* evaluated for its antagonistic activity against the above fungi in Petri dishes containing PDA medium. *T. harizanum* inhibited the growth of all the pathogens. On the 3rd day the inhibition was seen clear and the following days, the biocontrol agent, *T. harizanum* colonised over the pathogens. As *F. oxysporum* and *A.alternata* are slow growers, on 7th day *T. harizanum* was found to grow over the pathogens.

***R. solani.***

***A.aternata.***

***F. oxysporum.***

**Fig. 1: Antagonistic activity of *T.harizanum* against *R. solani, Fusarium oxysporum* and *A.aternata*.**

[7] also reported similar observations on hyphal interactions and between the bioagent, *T. harzianum* and *Alternaria solani.* That occurred by different means such as growing in contact, complete colonization and coiling around the hyphae of the pathogen, direct penetration and formation of appressorium-like structures. The interaction between *T. harzianum* and conidia of *A. solani* resulted in malformations and changes in spore shape, growing in contact and attaching spores and formation of node-like structure between two successive conidia.

In this study, *T.harizanum* was observed to overgrow the pathogens after 7 days of incubation.

**Cultivation of *T.harizanum***

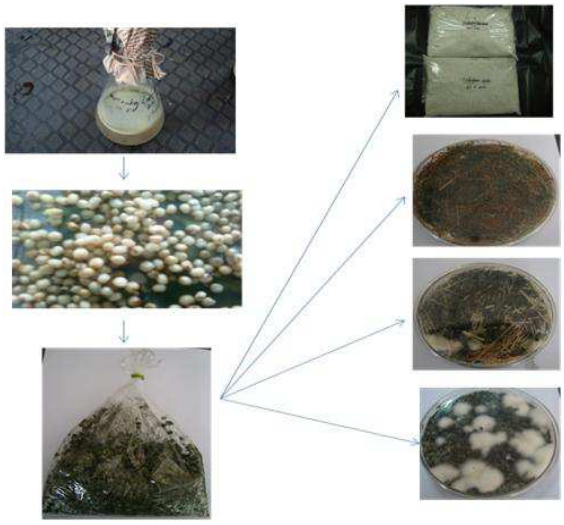
The fungal mat formed in the mineral salts medium with whey medium was used to the inoculate sorghum seeds and spore biomass was prepared in boiled sorghum seeds. (Fig.3). The fungal mycelium slowly colonized the sorghum seeds and after 7 days sthe entire bag was fully of green colour growth with mycelium and spores on the seeds.

**Large scale cultivation of *T. harizanum*:**

The inoculum developed on the sorghum seeds were used to inoculate large scale culture of *T. harizanum*. In this study, agricultural wastes like paddy straw and sugarcane baggase were used. As these two substrates are available in plenty in villages, farmers can use these waste for development of bio control agent.

Also we have used vermin compost which is rich in organic matter that supports the growth of *T.harizanum.* The substrates were inoculated with cultured sorghum seeds. The growth and sporulation of *T.harizanum* was faster in sugarcane baggase followed by compost and then on paddy straw.

**The process of cultivation of biocontrol agent, *T.harizanum***



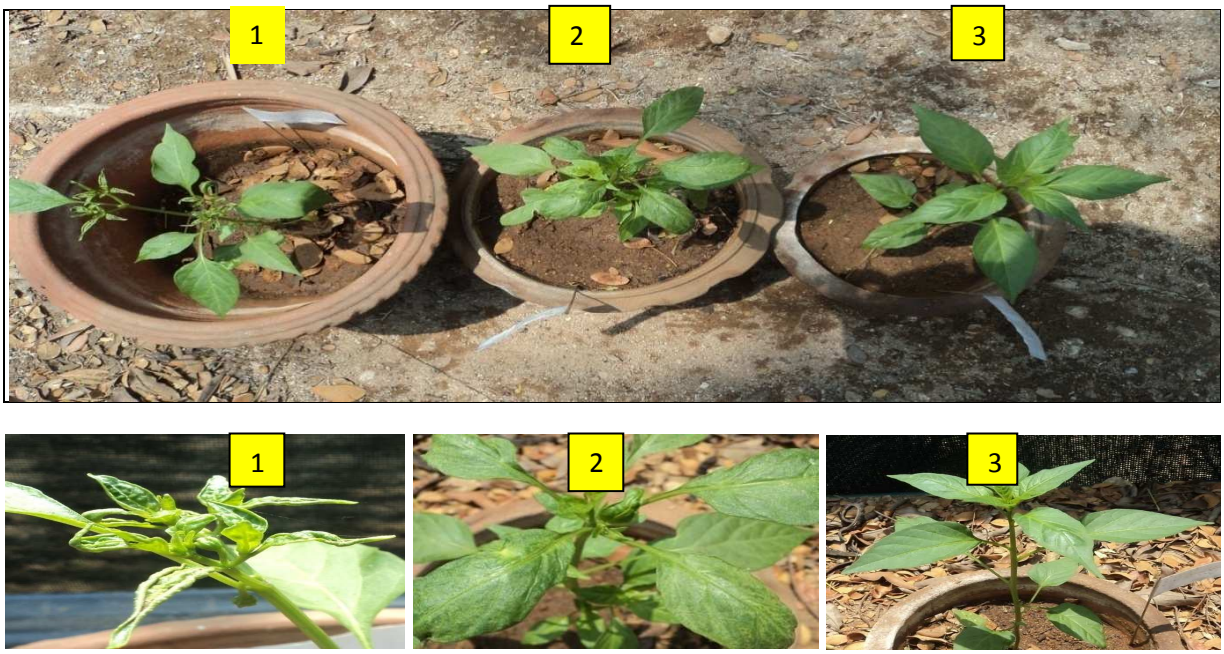
There are two major methods of inoculum production of *Trichoderma* spp. viz., solid state fermentation and liquid state fermentation. In solid fermentation, the fungus is grown on various cereal grains, agricultural wastes and by products. The solid state production is highly labour intensive and fit for cottage industry. These products are used mainly for direct soil application in nurseries /main fields to suppress the soil-borne inoculums. 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.

[8] suggested different organic media like neem cake, coir pith, farmyard manure, and decomposed coffee pulp for its multiplication. Addition of *T. harzianum* into organic media like neem cake, coir pith, farmyard manure and decomposed coffee pulp causes an immediate increase in the population up to 3 day. Increase in the total population density of *T. harzianum* in the media suggests that competition by native fungi in non-sterile carrier media was not a limiting factor in colonization of the media by the antagonist. Soil amended with organic materials like neem cake, coir compost, farmyard manure and *Gliricidia* leaves showed better growth and survival of antagonist than in soil alone. The carrier materials like neem cake, coir pith, farmyard manure and decomposed coffee pulp serve as nutrient additives to the crop in addition to inoculum production they support. [9] Reported that the pre-boiled sorghum grains, coir pith + neem cake (1:1), cow dung + neem cake (1:1) + wheat flour (10%) maintained high populations of *T. harzianum* and *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.harizanum.* However, colonization and sporulation was quick in sugarcane bagasse.

**Green house study:**

Table 1 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 *Trichoderma* spp in the unautoclaved soil. Also, in the other treatments where the plants were treated with both pathogen and *T. harizanum*, 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 bio control agent, the younger leaves were healthy.




**1 – Control** **2 - *R. solani*;** **3 - *T.harizanum***

**Fig. 2: Morphology of younger leaves of Chilli plant in different treatments:**

The results showed that the average plant height of 75 cm in plants treated with bio control agent grown in sugarcane baggase which was followed by plants treated with Talc formulation and compost (72 cm height**)**. However, the plant height was 50 cm in the case of control and 57 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* (Table.1). However, it was noted that the flowers withered later. But in *T. harizanum* treated plants all the flowers yielded fruits.

These results show that *T. harizanum* 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 *T.harizanum* treated plants, only after getting the final yield it can be concluded on the use of this strain as a plant growth promoter. [10] Reported that some strains of *Trichoderma spp.* to promote the shoot fresh weight and some were found to be inhibitory at 1.0% level, further studied the shoot growth and flowering in Petunia,

**Table 1: Effect of *T.harizanum* in the growth parameters of Chilli plant**

| Treatment period | 1st week | | | | 2nd week | | | | 3rd week | | | | 4th week | | | | 5th week | | | | 6th week | | | | 7th week | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | L | H | B | F | L | H | B | F | L | H | B | F | L | H | B | F | L | H | B | F | L | H | B | F | L | H | B | F |
| S B | 6 | 15 | \0 | 0 | 7 | 18 | 0 | 0 | 12 | 24 | 0 | 0 | 24 | 38 | 0 | 0 | 32 | 45 | 2 | 0 | 45 | 02 | 3 | 3 | 60 | 75 | 1 | 6 |
| S B + *R.solani* | 4 | 15 | 0 | 0 | 6 | 18 | 0 | 0 | 12 | 23 | 0 | 0 | 23 | 32 | 0 | 0 | 34 | 40 | 0 | 0 | 44 | 08 | 2 | 3 | 58 | 55 | 4 | 5 |
| Talcum | 5 | 15 | 0 | 0 | 8 | 19 | 0 | 0 | 14 | 24 | 0 | 0 | 22 | 32 | 0 | 0 | 30 | 44 | 4 | 0 | 45 | 00 | 8 | 4 | 72 | 72 | 1 | 7 |
| Talc + *R. solani* | 6 | 15 | 0 | 0 | 7 | 16 | 0 | 0 | 12 | 22 | 0 | 0 | 18 | 30 | 0 | 0 | 23 | 38 | 2 | 0 | 40 | 20 | 4 | 2 | 59 | 57 | 8 | 1 |
| P S | 3 | 15 | 0 | 0 | 4 | 16 | 0 | 0 | 82 | 20 | 0 | 0 | 14 | 30 | 0 | 0 | 23 | 32 | 2 | 0 | 24 | 43 | 3 | 1 | 32 | 45 | 6 | 1 |
| P S + *R. solani* | 5 | 15 | 0 | 0 | 5 | 19 | 0 | 0 | 80 | 00 | 0 | 0 | 00 | 00 | 0 | 0 | 00 | 00 | 0 | 0 | 00 | 00 | 0 | 0 | 0 | 00 | 0 | 0 |
| CT | 5 | 15 | 0 | 0 | 8 | 19 | 0 | 0 | 12 | 23 | 0 | 0 | 16 | 30 | 0 | 0 | 33 | 49 | 4 | 0 | 45 | 20 | 6 | 0 | 73 | 72 | 1 | 2 |
| CT + *R. solani* | 4 | 15 | 0 | 0 | 6 | 18 | 0 | 0 | 12 | 20 | 0 | 0 | 18 | 35 | 0 | 0 | 26 | 45 | 2 | 0 | 38 | 55 | 4 | 0 | 58 | 62 | 6 | 2 |
| *R. solani* | 5 | 15 | 0 | 0 | 7 | 17 | 0 | 0 | 12 | 20 | 0 | 0 | 16 | 29 | 2 | 0 | 28 | 42 | 4 | 3 | 35 | 20 | 8 | 2 | 71 | 51 | 1 | 1 |
| Control | 5 | 15 | 0 | 0 | 7 | 19 | 0 | 0 | 16 | 24 | 0 | 0 | 23 | 28 | 3 | 0 | 41 | 36 | 6 | 4 | 54 | 10 | 3 | 10 | 50 | 13 | 3 |

SB – *T.harizanum* grown in Sugarcane Baggase ; PS- *T.harizanum* grown in Paddy Straw ; CT- *T.harizanum* grown in Compost

**Marigold and verbena in Average of these replicates**

Detail and reported that depending on concentration, each strain was able to increase either number of flowers, weight of flowers, shoot fresh weight or shoot dry weight, with only one strain applied at 1% w/v showing increase values in all these parameters studied. Yet another work by these authors report that variety of strains of *Trichoderma* cultured on a molasses medium added to peat / sand

potting compost at 1% and 0·1% dry wt. When lettuce (*Lactuca sativa* L) seeds were planted in the compost, some strains of the fungus prompted seedling emergence and produced larger plants, while other strains had an inhibitory effect on those processes [11, 12] postulated that when plants are grown under optimal conditions, expression of growth promotion is unlikely, whereas under suboptimal conditions, enhanced growth can be achieved. Observations on field grown lettuce support this hypothesis, with increased growth of plants treated with *T. longipile* 6Sr4. In this situation, plants were grown under suboptimal conditions due to the presence of minor pathogens (e.g. *Sclerotinia minor*). Likewise, [13] reported a growth promotion effect of *Trichoderma* in sweetcorn. When low-medium vigour plants were treated, but not when high vigour plants were treated. From these reports, it can be concluded that the *T. harizanum* strain used in this study has to be tested again with different dosages for studying the growth promotional activity of plants.

## CONCLUSION

There were 9 treatments including control. The *T.harizanum* protected the seedlings from damping off disease. The growth promotion attributes like height, plant vigour, fruit formation were found good in the plants treated with *T.harizanum* grown in sugarcane baggase and talcum powder treated pots. A simple easy and cost effective method to mass culture the biocontrol agent *T.harizanum* by using sugarcane bagasse and talcum powder formulation.

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