Original Research Article

# Exploring the beneficial properties of Trichoderma viride and development of economic medium for its mass production

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## Abstract

*Trichoderma viride* is a soil fungus which can colonize plant parts and avoids the multiplication of plant pathogens, various studies revealed that *Trichoderma viride* can be a promising bio control agent against various plant pathogenic fungi, so it can replace the chemical antifungal agents and avoid excessive use of hazardous chemicals contributing to soil infertility and environmental pollution. *Trichoderma viride* can grow on a range of substrates and has various other novel properties to make it an effective tool in sustainable agriculture, Hence an attempt has been made here to investigate various beneficial properties of *Trichoderma viride* and to develop an economic growth medium from agro waste. This study showed that *Trichoderma viride* can be used not only as a bio pesticide but also as a bio fertilizer and even as an enzyme producer, bio surfactant. Despite of such novel properties it can be produced by using cheaper agricultural wastes like pulses or rice bran, and fruit peals. Fruit peals found to be the best substrate for *Trichoderma viride*

**Key Words:** *Trichoderma viride*, bio pesticide, mass production, beneficial properties.

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## INTRODUCTION

Major concerns in worldwide cultivation are plant diseases causing a loss of millions of dollars. so there is an urgent need of plant disease management to ensure constant supply of food for growing population. The extensive use of chemical pesticides have adverse effect on environment and leads to dominance of the species which are resistant to chemicals(Kumar and Gupta, 2012).this affects species diversity which can be dangerous to environment and ultimately to human beings. In this situation, it is necessary to replace chemicals with bio control agents. Various plant Pathogenic fungi can be killed by antagonistic action of a beneficial fungal species *Trichoderma*. *Trichoderma* also have been exploited as growth promoting agent (Sanjeev Kumar, *et al* 2014) Trichoderma species are free living fungi found in soil and root ecosystem. They produce a variety of compounds which provide local or systemic resistance to plants**..** (N.Ranasingh., A.saurabh and M.Nedunchezhiyan,2006).Various species of this genus are avirulent and opportunistic plant symbionts (Herman, G.E *et al*, 2004**).**

**Trichoderma Viride:** *Trichoderma viride* is most widely known for its potential to control plant diseases (Kolombet *et al*. 2001, Eziashi *et al*., 2010, Eslaminejad Parizi *et al*. 2012). *T. viride* was found an efficient agent to control the *R. solani* toxin activity against the same disease (Sriram *et al*., 2000).It can utilize a variety of substrates including grains (E.Esposito and M. da Silva, 1998). Hence a study has been undertaken to explore

various properties of *T viride* and development of cheaper media for its mass production.

## MATERIALS AND METHODS

Pure culture of T*richoderma viridae* (NCIM-1346), *Aspergillus awamori* (NCIM-861), *A. Pallunans* (NCIM-1049), *A.niger*(NCIM-596) ,*A.brassiliensis* (ATCC 16404), *Fusarium oxysporium* (NCIM-1350), *Penicillum notatum* (NCIM-741), were procured from NCIM cultures available in Department of Microbiology, Dayanand Science College, Latur. And activated on Potato Dextrose agar slants before use.

**Biomass production of *T. Viridae*:** For the comparison of biomass production of *T. viridae*, synthetic medium (Yeast Extract Glucose broth) and agricultural waste media (rice medium, pulses medium, fruit medium) were prepared.

**Preparation of inoculum:** The inoculum for biomass production of *T. viridae* was prepared by suspending spore of *T. viridae* in 100 ml of sterile D/W

**Preparation of media for biomass production of *T.viride* .**

**Synthetic medium:** 250 ml of Yeast extract glucose broth (glucose- 15gm, potassium phosphate monobasic- 3.18g, potassium phosphate dibasic- 5.2g, magnesium sulfate- 0.12 g, yeast extract – 0.5g, ammonium chloride- 0.54g, d/w-1000 ml,pH 5.5,)sterilized at $121^0$C for 15 minutes was inoculated with 10 ml inoculum of *T viridae* and incubated at $28^o$C till sporulation started, observed for further growth.

**Agricultural waste media: Rice medium** (rice processing waste-20g. in 500 ml of D/W), **pulses medium** (pulses processing industry waste-20g in 500ml of D/W) 20g. of rice and pulses each were taken in separate flasks and were boiled. After complete boiling rice and pulses were filtered in separate flasks and the volume of medium was made up to 500ml using distilled water, $P^H$ was adjusted to 5.5 . Both media were autoclaved at $121^o$c for 20 min. after cooling to room temperature 25 ml inoculum of *T. viridae* was inoculated and the flasks were incubated at $28^0$c till sporulation started and was observed for further growth.

**Fruit waste medium:** For fruit waste medium 5g. peel of each banana, pomegranate, apple and orange were ground collectively and added to 500 ml of distilled water., pH was adjusted to 5.5.The medium was autoclaved at $121^o$c for 15 min, after cooling to room temperature 10 ml inoculum of *T. Viridae* was inoculated and the flask was incubated at $28^0$c till sporulation started and observed for further growth.

**Extraction of biomass of *T. viridae*:** After maximum biomass production in the medium, for extraction of biomass of *T. viridae*, the content in the medium was filtered through pre-weighed Whatman filter paper. The residue on the filter paper was wet weighed and further dried to take dry weight. The filtrate in the flask was centrifuged at 10000 rpm for 15 min and supernatant was collected in sterile flasks and was further used as extract.(Mrudula Khandelwal, Sakshi Datta, Rajesh Kumar, 2012).

**Evaluation of various properties of *T viride* Phosphate solublization:** For the preparation of Pikovyaska agar medium (glucose-10g.tricalcium phosphate 5g., ammonium sulfate-0.5g., potassium chloride- 0.2g., magnesium sulfate-0.1g., manganese sulfate-trace, ferrous sulfate- trace, yeast extract-0.5g., agar agar- 20 g., Distilled water-1000ml) all components except tricalcium phosphate were added in 700 ml of D/W,pH was adjusted to 5.5, tricalcium phosphate was separately added in 300 ml distilled water. both flasks were autoclaved at $121^o$c for 20 min. After cooling upto $50^o$c, the components in both flasks were mixed and poured onto the sterile Petri plates. The plates were spot inoculated with *T. viridae* and were incubated at $28^o$c for 24-48 hrs. The plates were observed for the clear zone around the colony. (D. L. Rudresh, M. K. Shivprasad and R. D. Prasad,2005)

**Bio-surfactant activity of *T viride*:** E24 assay was performed to check bio-surfactant activity of *T.viridae*, two sets of tubes were prepared, one as 'control' and another as 'test'. In control tubes 5 ml of D/W and 2 ml of crude oil were added . In 'test' tubes, 5 ml of fruit extract and 2 ml of crude oil were added in each tube. Levels of water and respective oil were measured. All tubes were kept at room temperature for 24 hrs and again levels were measured.(M.M. Sidkey, H. F. Mohammad, N. I. Eikhouly 2016) E24 value was calculated using following formula:-

$$E\ 24\% = \frac{\text{total height of the emulsified layer}}{\text{total height of solution}} \times 100$$

**Antagonistic activy of *T viride***: The antagonism of *T. viridae* was examined by performing dual culture technique on PDA(potato peeled -20 g, dextrose- 20g, agar-20 g, d/w- 1000 ml pH 5.5 sterilized at$121^0$Cfor 15 min) plates. For that the sterile filter paper discs (Himedia, SD64) soaked in spore suspensions of *T. viridae* and test fungi (*A.awamori, A. pallunans, A. niger, A. brassiliensis, Fusarium oxysporium,*) were placed at 2 cm away from periphery of the PDA plates. All the plates were incubated at $28^o$C for 4 days. (M. A. Rahman, M. F. Alam 2009).

**Antifungal assay of *T viride* culture filtrates:** The antifungal efficacy of *T. viridae* was examined by using disc diffusion method. For that spore suspension of test fungi (*A awamori, A. pallunans, F oxysporium and P notatum*) were spread on Muller Hington agar ( beef extract- 2 g, acid hydrosylate of casein- 17.50 g, starch –

1.50g, agar- 20 g, D/w- 1000 ml) plates of pH 5.5. The discs of respective culture filtrates were placed on plates. As a control, distilled water disc was also placed on same plate. All the plates were incubated at 28°c for 24 hrs (M. A. Rahman, M. F. Alam 2009). Amphoterecin B 5mcg disc was used as positive control.

**Lignolytic assay of *T. viridae*:** Lignolytic fungal medium (dipotassium hydrogen phosphate-1g., potassium chloride-0.5 g., lignin- 25 g., agar- 20 g., d/w-1000 ml, pH-5.4) agar plates were prepared by sterilizing medium at 121°c for 20 min. The plates were spot inoculated with culture of *T. viridae*. All the plates were incubated at 28°c for 3 days. (A. M. Deshmukh 1997)

## RESULTS AND DISCUSSION

In present study, several naturally available substance are tested for biomass production in which we got different results as follows.

**Biomass production of T viride**

**Table 1:** Growth of T viride on various media

| | Appearance of sporulation -Days | Dry weight of Biomas safter 12days- g/500ml medium |
|---|---|---|
| 1 | 3 | 10.4 |
| 2 | 9 | 8.08 |
| 3 | 6 | 14.2 |
| 4 | 3 | 15.2 |

N.B.1.Synthetic medium, 2. Rice waste, 3.Pulses processing waste, 4. Fruit peels

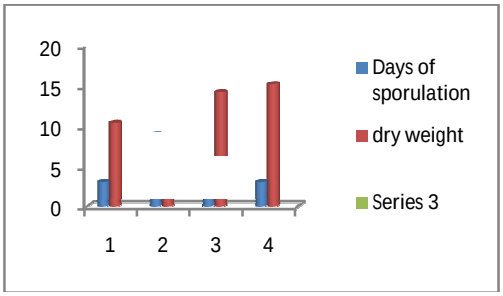


**Figure 1:** Growth of T viride on varios media

N.B.1.Synthetic medium, 2. Rice waste, 3.Pulses processing waste, 4. Fruit peels

**Figure 2:** Growth of T viride on varios media

**Dry weights of fungal mat on various media**

**Figure 3:**

N.B. Dry weight = weight of filter paper+dried biomass- weight of filter paper Evaluation of various properties of *T viride* Phosphate solubilization assay:

**Figure 4:**
Pikovyaska agar plate incubated at $28^0$C for 24hrs

**Table 2:** Phosphate solubilization assay

| A-mm | B-mm | B-A-mm |
|---|---|---|
| 104 | 114 | 10 |

N.B. A-colony diameter of *T.viride* B- Zone of solubiliztion (B-A )- zone of solubilization excluding growth Antagonisic activy of *T viride*:

**Figure 5:** Dual culture technique

N.B. PDA plate incubated at $28^0$C, for 72 hours showing Inhibition of test fungi due to inasive growth of T viride.
2.4 Antifungal activity of culture filtrates of T viride.

**Table 3:** Antifungal activity of culture filtrates of T viride

| Test Fungi | Zones of inhibition in mm including disc diameter 6mm | | | | |
|---|---|---|---|---|---|
| | R | F | P | G | Ab |
| *A.awamori* | 14 | Nil | 11 | 11 | 8 |
| *A.pullunans* | 15 | 11 | 15 | Nil | 13 |
| *F.oxysporium* | 11 | 16 | 14 | Nil | 11 |
| *P. notatum* | 11 | 11 | 11 | Nil | 15 |

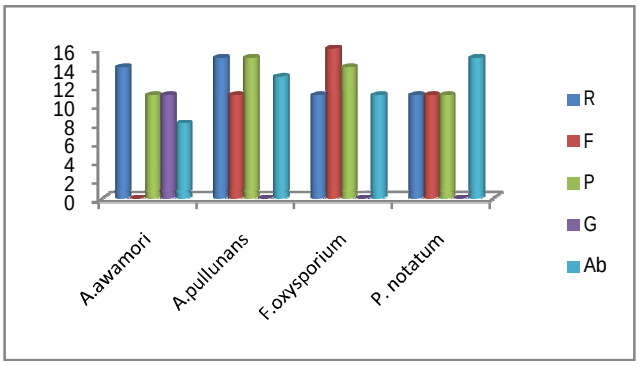


**Figure 6:** Graph- Antifungal activity of culture filtrates of T viride

N.B. R- culture filtrate of rice medium, F- culture filtrate of fruit peel medium, P- culture filtrate of pulses medium, G- culture filtrate of of glucose yeast extract broth, Ab- disc of antibiotic amohotericin B 5mcg

**Figure 7:** Antifungal activity of culture filtrates of T viride

N.B. Muller Hington agar plates incubated at $28^0$C for 48 hrs.
Biosurfactant activity of culture filtrate of *T viride* in fruit medium: Photo plate 6-E 24.

**Figure8:**

**Table 4:** E24 value of culture filtrate of *Tviride* in fruit medium:

| Sample | Height of emulsified layer | | Total height of solution | |
|---|---|---|---|---|
| | 0hr | 24 hr | 0hr | 24 hr |
| Control | 5 mm | 5 mm | 22 mm | 22 mm |
| test | 5 mm | 15 mm | 22 mm | 22 mm |

**biosurfactant activity of T. viridae –**

E24% = total height of the emulsified layer ×100
total height of solution

E24% = 15 ×100 / 22

E24% =68.1%

**Lignin utilization assay of *T viride***


**Figure 9:**

N.B growth of *T virideon* lignin agar at $28^0$C for 72 hrs.

## DISCUSSION

Most of the previous studies report that *Tviride* is effective against Rhizoctonia solani (Lo 1997) and various other plant pathogenic fungi, it is used as a biocontrol agent for various plant diseases (Sarma *et al*.(2014).therefore we studied its antifungal activity against various plant pathogenic fungi *A. pallunans*(NCIM-1049), *A.niger* (NCIM-596), *A.brassiliensis* (ATCC 6404), *Fusarium oxysporium* (NCIM-1350), *Penicillum notatum*(NCIM-741), and we found promising results against all the test fungi. This study also gave a simple and economic medium for mass production of *T viride* which is prepared from fruit peels which are not only the kitchen waste daily produced by dwellings but also it is produced by large scale and small scale agro industries, in this regard our study may help in organic waste management to some extent. We also found that *T viride* can be a good biosurfactant with E24 value 68.1%, this result is optimistic for oil pollution management. In another study we found that *T viride* can effectively utilize lignin as sole source of carbon which is hardly metabolized by other organisms. also it has considerable phosphate solubilzing activity with zone of solubilisation 10 mm

## CONCLUSIONS

Hence it is concluded that fruit peels which are the kitchen and agro waste can be the best economic medium for large scale biomass production of *T viride*. It is also concluded that T viride shows various beneficial properties like phosphate solublization and Lignolytic activity, These properties make *T viride* an important tool for green revolution and sustainable agriculture..

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