

Original Research Article

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## Comparative Influence of Various Nutritional Media on Mycelial Growth and Cultural Characteristics of *Trichoderma harzianum*

Firdaus Benazir<sup>1\*</sup> and Reena Mohanka<sup>2</sup>

<sup>1</sup>Department of Botany,

<sup>2</sup>Department of Biochemistry, Patna University, Patna, Bihar, India

\*Corresponding author

### ABSTRACT

The agriculture sector has a vital challenge in reducing its reliance on chemical pesticides. Use of plant beneficial microorganisms is considered as one of the most attractive alternatives that makes a positive contribution to environmentally safe, sustainable agriculture. *Trichoderma* species have proved to be one of the promising bio-control agents for the management of plant diseases, and the search for novel species of the fungal antagonist is in progression. The aim of this research work is to isolate and evaluate *Trichoderma* species from rhizospheric soil on five different nutritional (culture) media PDA, SDA, MSDA, CDA, and TSM. The most effective culture media for linear growth and biomass production of *Trichoderma harzianum* was PDA, followed by CDA, SDA, MSDA and TSM. The highest linear growth (84mm) was found in Potato dextrose agar and the lowest linear growth (45.66 mm) was found in TSM. The maximum biomass (fresh and dry) was found in PDB (1541.7 mg/100 ml and 749 mg/100ml, respectively). The minimum biomass (fresh and dry) was observed on TSM (677.67 mg/100 ml and 365 mg/100ml, respectively). For isolation and growth studies of antagonistic fungi, as well as for various laboratory screenings, the selection of media is an important and critical factor. The present work focuses on the selection of the best supportive medium for the isolation, growth and biomass production of selected *Trichoderma harzianum* for future commercialization as bio-control agent.

#### Keywords

*Trichoderma*,  
biocontrol agent,  
biomass, antagonist,  
culture media

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### Introduction

Phytopathogenic fungi have become a devastating threat to food production and security worldwide. Global tendencies are shifting towards reducing the use of chemically synthesized pesticides, which cause a series of environmental problems, antimicrobial resistance, and human and animal health complications (Bondori *et al.*, 2019; Leiter *et*

*al.*, 2017). Biological control through antagonistic microbes has been a subject of research, resulting in a range of products as commercial biopesticides in the market. The beneficial organisms are termed “biocontrol agents” BCAs (Jensen *et al.*, 2016; Tronsmo *et al.*, 2020). Among all the BCAs, *Trichoderma* species are one of the most widely studied biocontrol agents (Papavizas, 1985; Harman *et al.*, 2004) for the management of plant diseases

caused by fungi, bacteria, and even nematodes (Khan *et al.*, 2011; Kumar *et al.*, 2017).

*Trichoderma* is a genus of free-living, fast-growing, filamentous symbionts as well as parasites of other fungi, with high population densities in soils worldwide (Harman *et al.*, 2004; Mustafa *et al.*, 2009).

Several *Trichoderma species* have long been recognized for their high reproductive ability, efficiently utilize nutrients, capacity to change the rhizosphere, aggressiveness against phytopathogenic fungi, and efficiency in improving plant growth and defense mechanisms (Schmoll *et al.*, 2010) by producing volatile and non-volatile compounds and enzymes (Woo *et al.*, 2007; Meyer, 2008).

Apart from this, many species of *Trichoderma* are widely exploited in various industries as sources of enzymes (cellulase, pectinase, amylase) (Kubicek *et al.*, 1998; Sekhar *et al.*, 2017) and antibiotics (Dennis *et al.*, 1971; Sivasithamparam *et al.*, 1998).

In recent times, *Trichoderma* isolates have emerged as an effective biocontrol agent against numerous plant pathogenic fungi (*viz.*, *Fusarium* sp., *Rhizoctonia* sp., *Alternaria* sp., *Pythium* sp.) of cultivated crop plants (Harman *et al.*, 2000; Sharma *et al.*, 2014).

There are specific features that the product must have in order to be produced commercially. To boost sporulation or conidia production and improve the yield economically, it is necessary to optimize the operational parameters by converting to less expensive or alternative substrates.

As a result, the current study is being conducted in an effort to provide a better nutritional medium for the *Trichoderma* species, which they need to retain their viability and biocontrol capacity.

And with constant research, it is not possible that in the near future, it might be able to take the place of chemical pesticides. The experiment was conducted

in the Microbiology laboratory, Department of Biochemistry, Patna University, Patna. For evaluation of various nutritional media on mycelial growth, biomass production, and cultural characteristics of selected *Trichoderma* species.

## Materials and Methods

### Collection of soil samples

The soil samples were collected from the rhizospheric area (10–15cm deep) of healthy plants near Patna, Bihar. Kept in sterile zip-lock bags; transported to the laboratory, and stored at 4<sup>0</sup>C until use.

### Isolation of fungi from soils

All the soil samples were inoculated on PDA plates followed by serial dilution. The plates were incubated for five days at 25±2 <sup>0</sup>C. After incubation, the suspected *Trichoderma* colonies were sub cultured.

### Preparation of different solid mycological media

Five different media were tested to evaluate the varying effects on mycelial growth and cultural characteristics of *Trichoderma* species.

**Potato Dextrose Agar (PDA)** – [Anonymous, 1968; Dhingra and Sinclair, 1985]

### Composition

Potato 200gm; Dextrose 20gm; Agar 15gm; DW 1000ml; Chloramphenicol, Streptomycin 0.05gm. 200gm of sliced potatoes were cooked in 400 ml of distilled water for 30 minutes or until softened, then strained through cheese cloth and 20gm of dextrose were added. Add 15gm of agar to 400ml of distilled water in a separate beaker and heat the solution until completely dissolved. Potato extracts, dextrose, and agar solutions were mixed together. The pH of the medium was adjusted to 5.6±0.2 and sterilized at 121<sup>0</sup> C for 15 min.

**Sabouraud Dextrose agar (SDA)** – [Rippon, 1988; Jonston and Booth, 1983]

### **Composition**

Dextrose 40gm; Peptone 10gm; Agar 15gm; DW1000ml; Chloramphenicol, Streptomycin 0.05gm. All the ingredients were dissolved in distilled water. The pH of the medium was adjusted to 5.6±0.2 and sterilized at 121<sup>0</sup>C for 15 min.

### **Modified SDA (MSDA)**

#### **Composition**

Dextrose 20gm; Peptone 5gm; Oatmeal 15gm; Agar 15gm; DW 1000ml; Chloramphenicol, Streptomycin 0.05gm. MSDA was prepared by using 20gm dextrose, 5gm oatmeal, 5gm peptone, and 15gm agar instead of 40gm dextrose and 10gm peptone.

All the ingredients were dissolved in distilled water. The pH of the medium was adjusted to 5.6±0.2 and sterilized at 121<sup>0</sup>C for 15 min.

**Czapek's Dox Agar (CDA)** – [Stevens1981]

#### **Composition**

Dextrose 30gm; Sodium nitrate 2gm; Dipotassium phosphate 1gm; Magnesium sulphate 0.5gm; Potassium chloride 0.5gm; Ferrous sulphate 0.01gm; Agar 20gm; DW1000ml; Streptomycin 0.05gm.

All the ingredients were dissolved in distilled water. The pH of the medium was adjusted to 5.6±0.2 and sterilized at 121<sup>0</sup>C for 15 min.

**Trichoderma specific medium (TSM)** – [Askew and Laing, 1993]

#### **Composition**

Glucose 3gm; Ammonium nitrate 1gm; Magnesium sulphate 0.20gm; Dipotassium phosphate 0.9gm; Potassium chloride 0.15gm; Metalaxyl 0.20gm;

Rose bengal 0.15gm; Agar 15gm; DW 1000ml; Chloramphenicol 0.25gm. All the ingredients were dissolved in distilled water except Rose Bengal and metalaxyl.

The pH of media was adjusted to 5.6±0.2 and sterilized at 121<sup>0</sup>C for 15 min. Rose Bengal and metalaxyl were added after cooling the media. All the media were poured (20ml) aseptically into each sterile petri plate, and three replications were made for each experiment.

### **Inoculation and incubation**

5mm mycelial discs of *Trichoderma* species were cut from the peripheral zone of a 5 day old culture and transferred to the center of each plate containing different media. All the plates were incubated at 25±2<sup>0</sup>C for 7 days.

### **Average Linear Growth Rate (ALGR) of *Trichoderma* species on different types of media**

The linear growth rate of *Trichoderma* species on different media was recorded from day 1 till full growth. An average linear growth rate (ALGR) was calculated by the following formula (Aneja, 1993; Elad, 1981).

$$(C_3 - C_0) / T = \text{ALGR (mm/day)}$$

Where C<sub>3</sub> = Colony diameter (mm) after 3 days of inoculation

C<sub>0</sub> = Initial colony diameter (mm) of inoculum

T = Time difference (3 days)

### **Cultural characteristics of *Trichoderma* species on different types of media**

After 7 days of incubation, cultural characteristics of *Trichoderma* species growing under different nutritional conditions were observed, such as colony front color, colony reverse color, margin, texture, and mycelial thickness.

### **Biomass estimation of *Trichoderma* species on different types of media**

*Trichoderma* species was grown in 250 ml Erlenmeyer flasks containing 100ml of broth media of potato dextrose broth (PDB), Sabouraud dextrose broth (SDB), Modified Sabouraud dextrose broth (MSDB), Czapek's dox broth (CDB), and *Trichoderma* specific broth (TSB) for measurement of fresh and dry weight. Five mm discs of five day old *Trichoderma* species were inoculated in all the broth media under aseptic conditions with three replications. The flasks were incubated inside a shaker incubator at 150 rpm to avoid colony formation and clumping on the surface of the medium for 14 days at  $25\pm 2^{\circ}\text{C}$  temperature.

After 14 days, the fungal mycelial of each flask was separated by filtration using Whatman No. 1 filter paper. The filtered fungal mycelial was kept on fresh pre-weighed filter paper and air dried at room temperature for 24 h. The fresh weight of fungal mycelial was then quantified (mg/100ml). After that, each fungal mycelial was placed in an oven at  $50^{\circ}\text{C}$  for 24 hours and the dry weight (mg/100ml) was determined.

### **Data Analysis**

The experimental design was completely randomized with three replications. The data was statistically analyzed by SPSS (Version 21) and the significance of the data was tested using Duncan's test,  $p = 0.05$ .

## **Results and Discussion**

### **Identification**

The temporary slides were prepared by lactophenol cotton blue staining, and the *Trichoderma* isolates were identified as *Trichoderma harzianum* with the help of standard mycological keys (Bissett, 1991; Rifai, 1969) and further confirmed by ITCC (Division of Plant Pathology ICAR-Indian Agricultural Research institute, New Delhi).

### **Linear growth of *Trichoderma harzianum* on different types of media**

The isolated *Trichoderma harzianum* was examined on five different nutritional media (PDA, SDA, MSDA, CDA and TSM). The maximum linear growth was compared after three days because the full growth of *Trichoderma harzianum* on PDA and CDA was observed on the fourth day of incubation. The linear growth and average linear growth rate (ALGR) per day were highest (3 days) on PDA (87mm and 27.31mm/d) respectively and the lowest was found on TSM (31mm and 8.66mm/d) respectively. Significant linear growth was observed on PDA, followed by CDA, SDA, MSDA, and TSM. The growth was found relatively slow on MSDA but faster than TSM. Fig. 1 and Table 1

### **Cultural characteristics of *Trichoderma harzianum* on different types of media**

Cultural characteristics of the *Trichoderma harzianum* are shown in Fig. 2 and Table 1. The effects of different media (PDA, SDA, MSDA, CDA, and TSM) on *Trichoderma harzianum* had varying cultural characteristics (colony front color, colony reverse color, texture, mycelial thickness, and margin). The colony front color was dark green on PDA and a yellowish green with a white center on SDA. While on the MSDA, a yellow center with a pale white border and on the CDA, light green colors as compared to PDA, was observed.

On the TSM, there were 2 irregular green rings. The colony texture was compact on PDA, MSDA, and CDA, whereas it was moderately compact on SDA and loose on TSM. The colony margin was regular except for TSM.

### **Biomass production of *Trichoderma harzianum* on different types of media**

The maximum biomass of *Trichoderma harzianum* fresh and dry weight was 1541.7 mg/100ml and 749 mg/100ml, respectively, found in PDB, followed by CDB, SDB, and MSDB.

In CDB, 1512.7 mg/100 ml of fresh weight and 625 mg/100 ml of dry weight were found. Whereas SDB produced 1391 mg/100 ml fresh weight and 643 mg/100 ml dry weight. The minimum biomass fresh and dry weight was 677.67 mg/100ml and 365mg/100ml, respectively, in TSM. And MSDA has found 885mg/100 ml fresh weight and 479mg/100 ml dry weight (Fig.3).

Selection of an effective culture medium is an important factor for isolation and growth studies of *Trichoderma* spp. (antagonistic fungi). The present finding shows that the tested *Trichoderma species* reveal significant variations in cultural characteristics, linear growth, and biomass production.

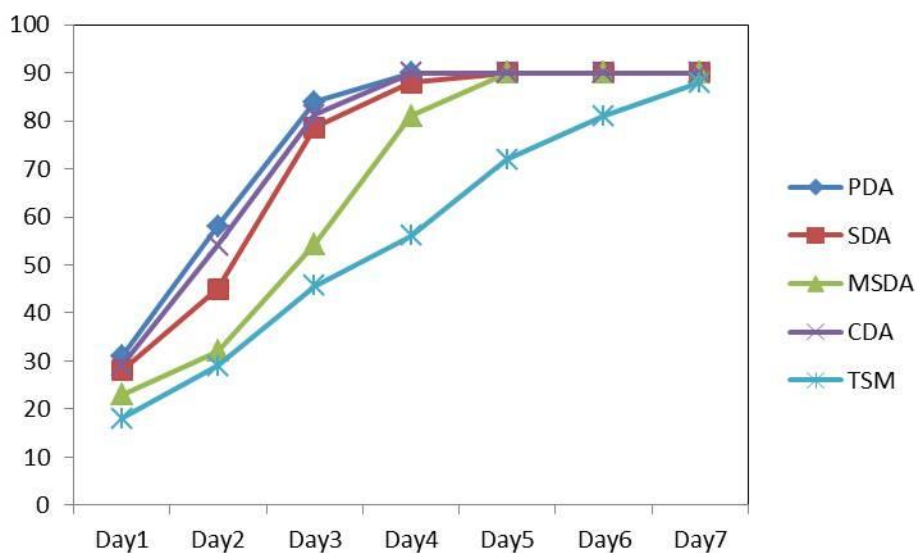
**Table.1** Cultural characteristic and ALGR/ Day of *Trichoderma harzianum* on different types of media

| Growth Media | Colony Front Color                          | Colony Reverse Color | Texture            | Colony Margin | Mycelial Thickness | ALGR / Day mm*           |
|--------------|---|----------------------|--------------------|---------------|--------------------|--------------------------|
| PDA          | Dark Green                                  | Transparent (cream)  | Compact            | Regular       | Moderately Thick   | 26.33± 0.69 <sup>a</sup> |
| SDA          | Yellowish Green with white center           | Orange yellow        | Moderately Compact | Regular       | Thick              | 24.55± 0.50 <sup>c</sup> |
| MSDA         | White surrounding with Yellow Centre        | Yellow               | Compact            | Regular       | Thick              | 16.44± 0.19 <sup>d</sup> |
| CDA          | light green, 1 ring at center               | Light Yellow         | Compact            | Regular       | Moderately Thick   | 25.44± 0.33 <sup>b</sup> |
| TSM          | White with Green Outline, 2 irregular rings | Almost colorless     | Loose              | Irregular     | Thin               | 13.55± 0.38 <sup>c</sup> |
| <b>CV</b>    |   |                      |                    |               |                    | <b>25.501</b>            |

\* Values are mean of three replication ± SD

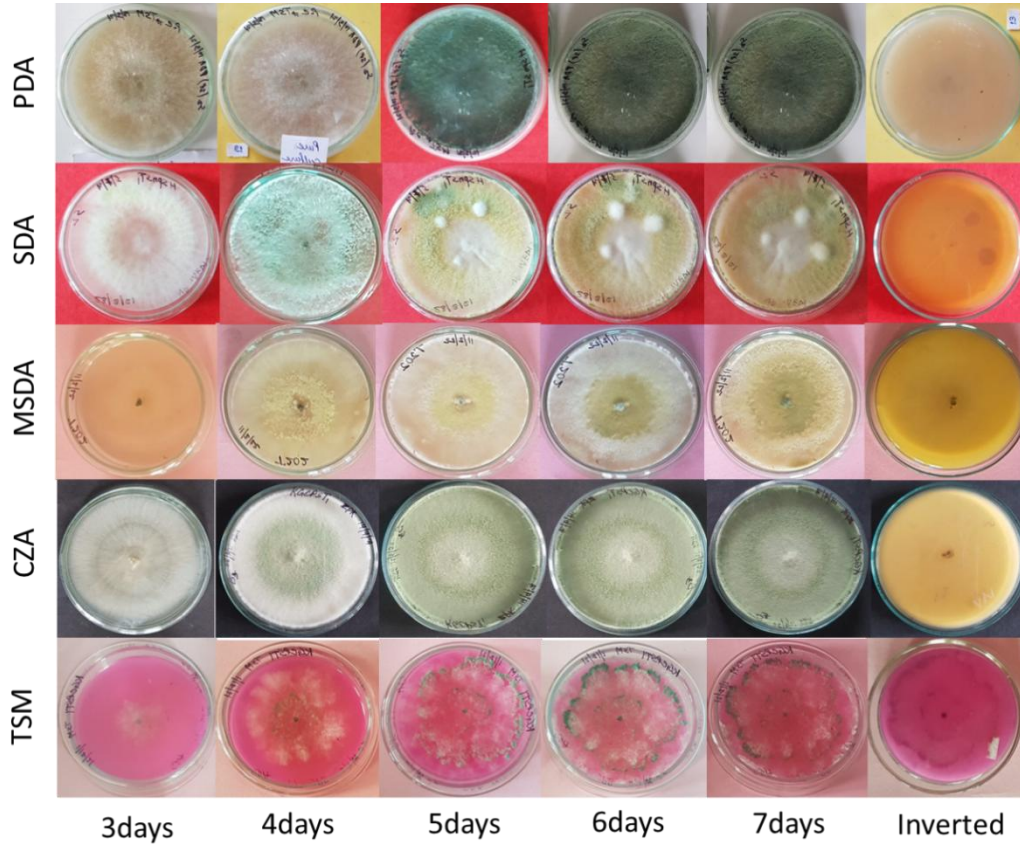
Similar alphabets in the superscript are not significantly different (p=0.05) using Duncan’s test

**Fig.1** Linear growth of *Trichoderma harzianum* from day 1 to day seven on five different types of media (PDA, SDA, MSDA, CDA, and TSM)

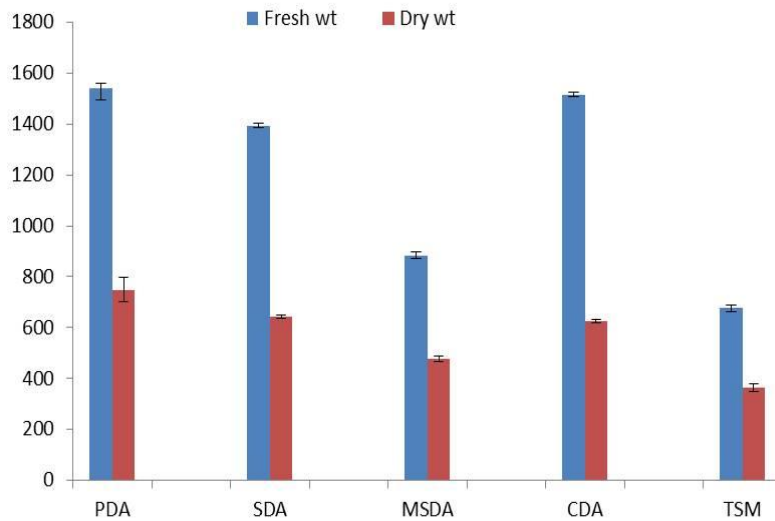




**Fig.2** Cultural characteristics of *Trichoderma harzianum* colony front view (3 days to 7 days) and colony reverse view on five different media PDA, SDA, MSDA, CDA, and TSM



**Fig.3** Biomass productions (fresh and dry weight) of *Trichoderma harzianum* on five different types of media (PDA, SDA, MSDA, CDA, and TSM)



Numerous studies have been conducted on the selection of the suitable medium for the effective isolation of prospective biocontrol agents. The cultural variations of *Trichoderma harzianum* were varied with respect to colony color, (front and reverse) colony texture, colony margin and mycelial thickness (Table.1 and Fig. 2).

The linear growth and growth rate per day were significantly different on the five tested media. On the third day of incubation, the highest linear growth (84mm) was found in Potato dextrose agar (PDA) and the lowest linear growth (45.6 mm) was found in TSM, followed by CDA, SDA and MSDA.

The full growth of *Trichoderma harzianum* on PDA and CDA was recorded on the fourth day of incubation. Whereas, average linear growth rate(ALGR) was statistically different on PDA and CDA. Mustafa *et al.*, (2009) evaluated the growth of *Trichoderma* spp. on five semi synthetic media, including PDA, and found that PDA was the best medium. According to Maheshwari *et al.*, 2000 the maximum growth of fungi, Potato dextrose was the most favorable. In the present study, the growth was very slow on TSM compared to other media for the tested *Trichoderma harzianum*. In terms of biomass (fresh and dry weight), the tested *Trichoderma harzianum* significantly varied (0.05). The maximum biomass production (fresh and dry weight) was found in PDB 1541.7 mg/100 ml, and 749 mg/100 ml and minimum biomass production (fresh and dry weight) was observed in TSM 677.67 mg/100 ml and 365 mg/100 ml.

Similarly, Jahan *et al.*, (2013) tested five culture media for growth of *T. harzianum* and discovered that PDA and PDB were more effective for linear growth and biomass production, respectively; water agar and water broth was least effective for both.

Vargas *et al.*, (2009) reported that PDAm (modified PDA) was the most effective (2.16 CFU), followed by PDA (1.70 CFU), while in the TSM (1.05 CFU).

Culture media is considered a useful tool for the

isolation and functional study of any beneficial soil microorganism. The tested *Trichoderma harzianum* was found to be capable of growing on all media with different growth rates and patterns. Among all the growth media tested in the present study, PDA was observed to be the excellent. PDA is the general medium most widely used in the isolation of fungi, having a complete nutritional basis Agrios (1988).

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### **Conflict of interest**

The authors declare that they have no conflict of interest.

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