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Evaluation Of The Growth Performance Of *Trichoderma harzianum* (Rifai.) On Different Culture Media

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Abstract: *To evaluate the growth of Trichoderma harzianum on different culture media namely, potato dextrose agar, modified potato dextrose agar, water agar, carrot agar and cornmeal agar. Linear growth was recorded at 24 hours intervals after inoculation and average growth rates were calculated. Fresh weight and dry weight were also recorded. The highest linear growth, fresh weight and dry weight were found in potato dextrose agar and lowest in water agar. The highest values were followed by modified potato dextrose agar which was statistically similar to carrot agar which was differed and followed by cornmeal agar.*

Key word*: growth performance, Trichoderma harzianum, different culture media*

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I. Introduction

Biological control involves the use of biological organisms to control pathogens or diseases. The microbial inoculants as biocontrol agents are effective and attractive alternatives to prevent the deficiencies brought about by the exclusive reliance on chemicals (Nakkeeran *et al.*, 2002). Members of *Trichoderma* particularly *T. harzianum* are promising biological control agents (bioprotectants) against plant diseases. *Trichoderma* are free-living fungi and common in soil and root ecosystems. They are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi (Harman *et al*., 2004). These filamentous fungi are very wide spread in nature, with high population densities in soils and plant litters. They are saprophytic, quickly growing and easy to culture and they can produce large amount of conidia with long shelflife. *T. harzianum* is environmentally safe to control plant pathogen compared to any other pesticides. Farmers can easily use this antagonistic pathogen commercially to increase their yield of crop and decrease the using cost of pesticides (http://thiqaruni.org/engpdf9/42.pdf). Among the different bio-control agents so far identified, species of *Trichoderma* are the most effective in reducing disease incidence of various crops.

Biomass used for biological control must be inexpensive to produce. It should be capable of being dried with retention of a high level of germinable propagules, be insensitive to environmental fluctuations (e.g., temperature and humidity) and possess a long shelf life. Studies were conducted for the use of various culture media for growth of *T. harzianum* (Elad *et al*. 1981 and Harman *et al*. 1990; 1991).The minimal media Czapek Dox and Richard's medium supported a high level of conidial production of *T. harzianum*, but that overall yields were low. The addition of V8 juice to these media increased yields by 8- to 16-fold, but only 1 to 10% of the conidia produced was viable after vacuum drying (G.E. Harman *et al*., 1991).

To use this antagonist (*T. harzianum*) commercially, it is necessary to produce the maximum biomass with least economic cost. So, it is important to search suitable and cheap media for growth of *T. harzianum.* Considering the above facts, the present investigation was undertaken to evaluate the growth performance of *T. harzianum* on different culture media

II. Materials And Methods

An experiment was conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, to evaluate the performance of different media for growth of *T. harzianum.*

An isolate of *T. harzianum* was collected from the preserved isolates of Bangladesh Agricultural research Institute (BARI), Joydebpur, Gazipur.

PDA was prepared following the standard procedure (Anonymous, 1968). After preparation of media, pH of the medium was adjusted to 6.00 by adding 1% HCl using pH meter. After melting the prepared medium was sterilized in an autoclave at 121°C temperature for 20 minutes.

For the Multiplication of *T. harzianum*

PDA medium was poured in sterilized petridishes, 20ml in each. A 5mm block of the 3 days old pure culture of *T. harzianum* was placed upside down at the center of each plate. The block was cut with the help of a

flame sterilized cork borer (5mm diameter). The inoculated petridishes were kept in the growth chamber at 27±2ºC temperature. All the works were done under aseptic condition.

For the Preservation of *T. harzianum*

Sterilized PDA medium were poured into sterilized test tubes, 10ml in each. Then the test tubes were sterilized in an autoclave at 121°C temperature for 20 minutes. After autoclaving, slanting of test tube was done at 45° angles to increase the surface area of the medium in the test tube. 7 days old fungal hyphae with the help of a inoculation niddle were placed in the test tube. After inoculation test tubes were kept in the growth chamber at 27±2ºC temperature.

Preparation of Different Media

Potato Dextrose Agar (PDA)

PDA was prepared following the standard procedure (Anonymous, 1968) and the pH was adjusted to 6.00 using pH meter with the help of 1% HCl. The medium was then sterilized in an autoclave at 121°C temperature for 20 minutes.

Modified Potato Dextrose Agar (MPDA)

Modified PDA was prepared by using 125gm potato and 15gm dextrose instead of 200g potato and 20g dextrose respectively. The pH of the medium was adjusted to 6.00 using pH meter with the help of 1% HCl. The medium was then sterilized in an autoclave at 121°C temperature for 20 minutes.

Water Agar (WA)

Water agar was prepared following the standard procedure (Anonymous, 1968). 1000ml distilled water was boiled at first. After boiling it was poured into a beaker. The beaker is then placed on a heater with magnetic stirrer to mix 15g agar and then adjusted to 1000ml by distilled water. The pH of the medium was adjusted to 6.00 using pH meter with the help of 1% HCl. The medium was then sterilized in an autoclave at 121°C temperature for 20 minutes.

Carrot agar (CA)

400g carrot was cut into slice and boiled in 1000ml distilled water. After boiling it was sieved into a beaker. The beaker is then placed on a heater with magnetic stirrer to mix 20g agar and then adjusted to 1000ml by distilled water. The pH of the medium was adjusted to 6.00 using pH meter with the help of 1% HCl. The medium was then sterilized in an autoclave at 121°C temperature for 20 minutes.

Cornmeal agar (CMA)

20g cornmeal was weighted on an electric balance and mixed with 1000ml boiling water slowly. After mixing, it was sieved into a beaker. The beaker is then placed on a heater with magnetic stirrer to mix 15g agar and then adjusted to 1000ml by distilled water. The pH of the medium was adjusted to 6.00 using pH meter with the help of 1% HCl. The medium was then sterilized in an autoclave at 121°C temperature for 20 minutes.

Pouring of Media

In all cases of media 20ml medium was poured in each petridish. where as five plates for each medium.

Inoculation and Incubation

Advanced hyphae of 3 days old culture was used for inoculation. A 5mm block of the mycelium was cut with flame sterilized cork borer (5mm). The mycelial blocks were taken from the edge of the colony. Each mycelial block was placed upside down at the centre of each plate. Five replicated plates were used for each medium. The inoculated petridishes were kept in the growth chamber at room temperature (27±2°C) and 90% relative humidity (RH) until the mycelia touch the edge of petridishes.

Measurement of Average Linear Growth Rate (ALGR) of *T. harzianum* on Different Growth Media

After 3 days of incubation, linear growth (mm) of *T. harzianum* was recorded. Linear growth measured by averaging three diameters taken from each colony.

Average linear growth rate was measured by the following formula (Aneja, 1993 and Elad *et al.*, 1981).

$$ALGR\ (mm/day) = (C3-C0)/3$$

Where C3= Colony diameter after 3 days of inoculation

C0= Initial colony diameter of inoculation

Colony Characteristics of *T. harzianum*

After 4 days of incubation, different colony characters such as surface, color, margin, texture and hyphal thickness was observed visually in each different media.

Measurement of Fresh Weight and Dry Weight of *T. harzianum* on different media

For the measurement of fresh weight and dry weight of *T. harzianum* was grown in 100ml of potato dextrose broth (PDB), modified potato dextrose broth (MPDB), water broth (WB), carrot broth (CB), cornmeal broth (CMB) in 250ml conical flask. These media were inoculated with 5mm block of 3 days old culture of *T. harzianum* grown on PDA. The inoculated conical flasks were kept in the growth chamber at 27±2°C temperature and 90% RH. The conical flasks were shaked at 24 hours of interval till 14 days of inoculation to inhibit colony formation on the surface of growth. After 14 days inoculation the fungal mass in each conical flask was separated by filtration using Whatman paper No. 1. The filtrates were discarded and the fungal biomass on the filter paper was air dried at room temperature for 24 hours. Then the fresh weight (mg/100ml) was measured with electric balance. The fungal biomass of all treatments was oven dried at 70°C temperature for 72 hours. Then the oven dried fungal biomass was weighted (mg/100ml) to have the dry weight.

Experimental Design and Data Analysis

Experimental Design was Completely Randomized Design (CRD) with five replications **and** data was analyzed **statistically** using MSTAT-C computer program and means were compared following Duncan's Multiple Range Test (DMRT).

III. Result and Discussion

Average Linear Growth Rate (ALGR) of *T. harzianum* on Different Culture Media

Average linear growth of *T. harzianum* on different tested media was varied significantly (p=0.01) (Table 1). The growth rate was highest in PDA (22.86 mm/day) and lowest in water agar (13.18 mm/day). Higher linear growth on PDA was followed by MPDA medium (20.29 mm/day) and also differed significantly. Linear growth on MPDA was statistically similar with the growth on CA medium (18.95 mm/day). Linear growth on CMA medium (16.53 mm/day) varied significantly from the linear growth on CA and WA medium.

Table 1. Average linear growth rate on different culture media

Growth medium	Average linear growth rate (mm/day)
Potato dextrose agar	22.86 a
Modified potato dextrose agar	20.29 b
Carrot agar	18.95 b
Cornmeal agar	16.53 c
Water agar	13.18 d
CV (%)	3.38%
Level of significance	0.01

Azher Mustafa and co-workers (2009) studied the growth of *Trichoderma spp.* on five semi synthetic media including PDA and found PDA as the best medium. Das and co-workers (1997) also studied growth of *Trichoderma spp.* on PDA and four other natural media and found wheat bran as the best.

Linear Growth of *T. harzianum* on Different Culture Media

From the initial day of inoculation to 3 days after inoculation the growth of *T.* harzianum on different culture media is shown in figure 1. At the first day the highest growth was found in potato dextrose agar and the lowest was obtained from water agar medium. In the 2nd day the maximum growth was obtained in potato dextrose agar and minimum growth was obtained in water agar medium. At the 3rd day the maximum growth was found in potato dextrose agar medium and minimum growth was found in water agar medium. Growth of *T. harzianum* was consistent on all media from first day to third day except MPDA. Growth of *T. harzianum* on MPDA was above to WA in the first day but it was next to PDA in the third day.

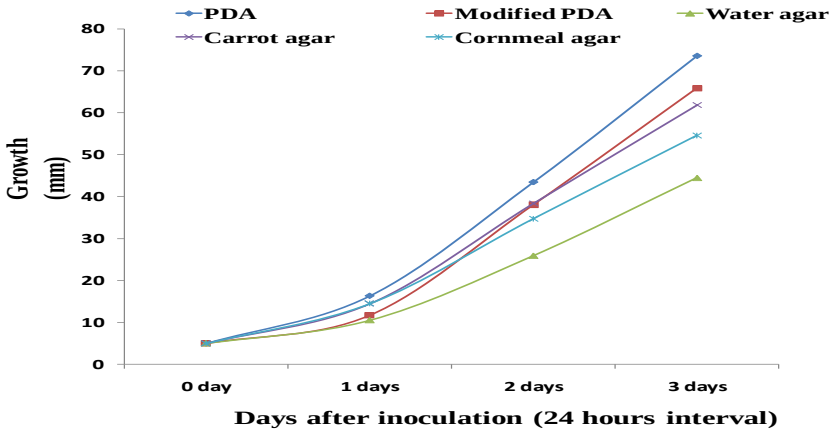

Figure 1: Linear Growth of *T. harzianum* from initial days of inoculation to 3 days after inoculation

Colony Characteristics of *T. harzianum* on Different Culture Media

Colony characteristics of *T. harzianum* on different culture media is shown on table 2. There exists no difference in color of the upper and lower surfaces of *T. harzianum* in all of the five media (plate 1, plate 2, plate 3, plate 4, and plate 5). The colony margin of all the media was regular. A few variations were observed in colony texture, moderately compact colony texture was found in potato dextrose agar (Plate 1) while loose and puffy colony texture was found in modified potato dextrose agar and cornmeal agar medium (Plate 2, plate 5). Water agar medium had a loose colony texture (Plate 5), whereas compact colony texture was found in carrot agar medium (Plate 3). In the case of hyphal thickness some variations were observed, thick hyphal thickness was found in the case of potato dextrose agar medium (Plate 1) and moderately thick hyphal thickness was found in the case of modified potato dextrose agar, carrot agar and cornmeal agar medium (Plate 2, plate 3, plate 4) and very thin hyphal thickness was found in water agar medium (Plate 5).

Table 2. Colony Characteristics of *T. harzianum* on different media

Growth Media	Surface	Color	Margin	Texture	Hyphal thickness
Potato dextrose agar (PDA)	Upper	White	Regular	Moderately compact	Thick
	Lower	White			
Modified Potato dextrose agar (MPDA)	Upper	White	Regular	Loose, puffy	Moderately thick
	Lower	White			
Carrot agar (CA)	Upper	White	Regular	Compact	Moderately thick
	Lower	White			
Cornmeal agar (CMA)	Upper	White	Regular	Loose, puffy	Moderately thick
	Lower	White			
Water agar (WA)	Upper	White	Regular	Loose	Very thin
	Lower	White			

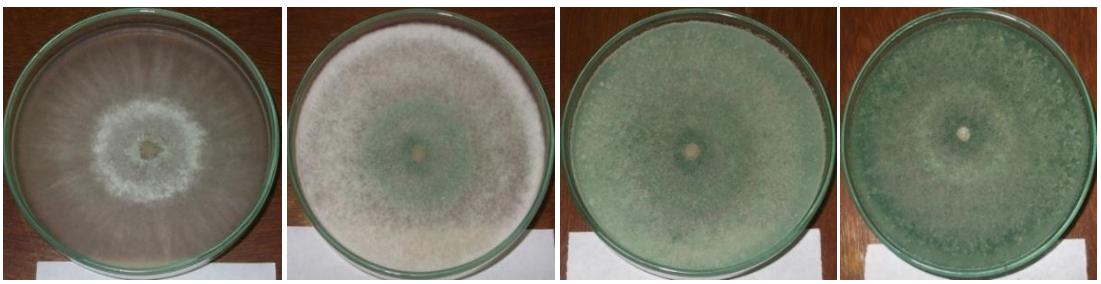

Plate 1: Images of *T. harzianum* in PDA at 24hours of interval after 3 days of inoculation

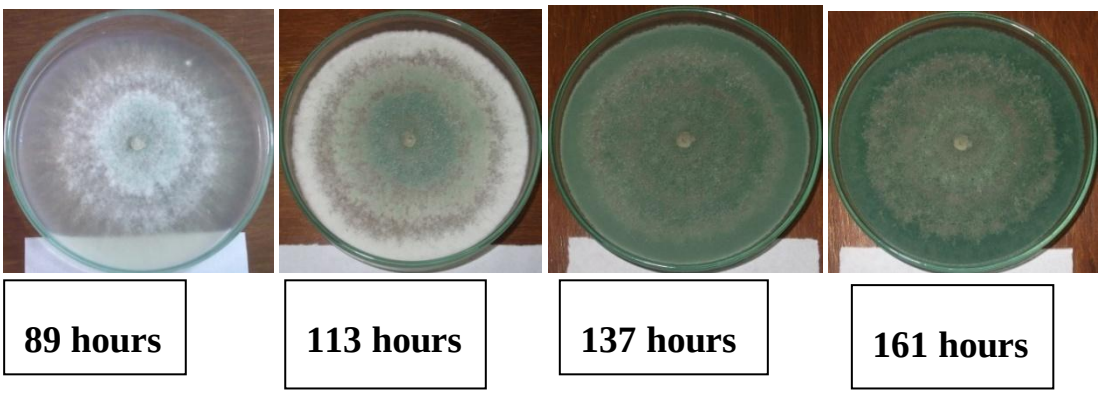

Plate 2: Images of *T. harzianum* in MPDA at 24hours of interval after 3 days of inoculation

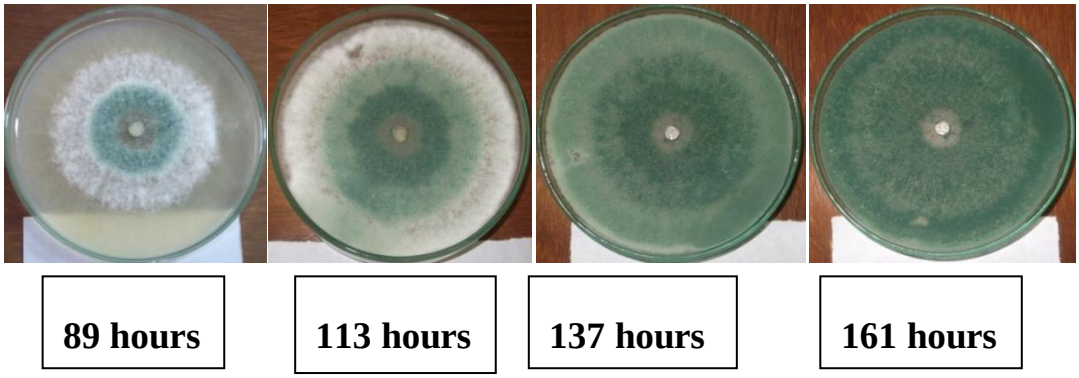

Plate 3: Images of *T. harzianum* in CA at 24hours of interval after 3 days of inoculation

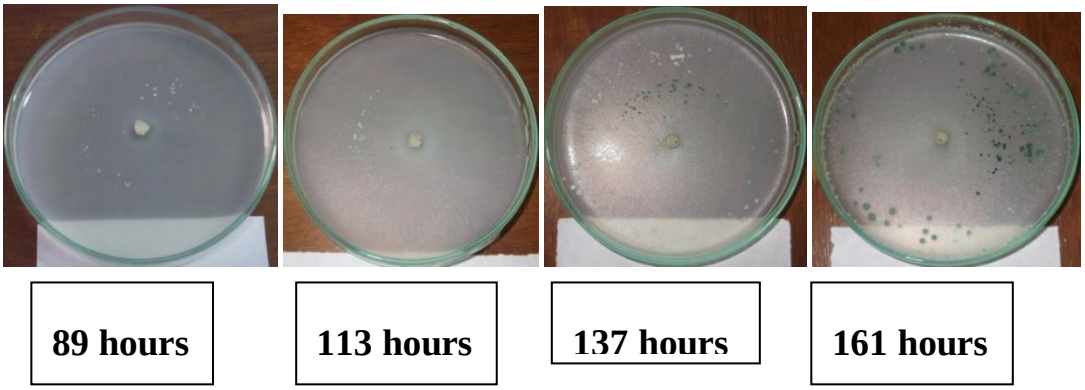

Plate 4: Images of *T. harzianum* in CMA at 24hours of interval after 3 days of inoculation

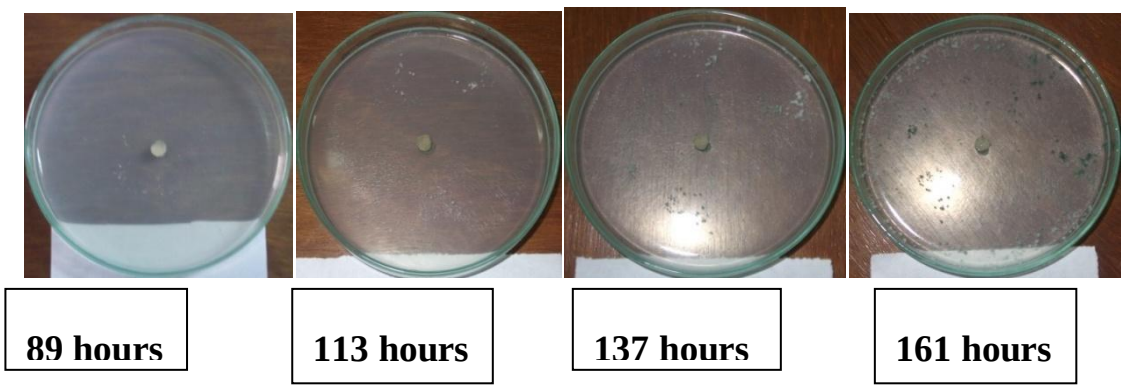

Plate 5: Images of *T. harzianum* in WA at 24hours of interval after 3 days of inoculation

Biomass of *T. harzianum* on five Culture Media

Fresh weight

Fresh weight of *T. harzianum* on different tested media varied significantly (p=0.01) (Table 3).The fresh weight was highest in PDB (637.00 mg) and lowest in WA (6.60 mg). Highest fresh weight on PDB was followed by MPDB (484.20 mg) and also differed significantly. Fresh weight on MPDB was statistically similar with the weight on CMB (469.00 mg).Fresh weight on CB (251.20 mg) varied significantly from the fresh on CMB and WB.

Dry weight

Dry weight of *T. harzianum* on different tested media varied significantly (p=0.01) (Table 3).The dry weight was highest in PDB (627.00 mg) and lowest in water agar (3.00 mg). Highest fresh weight on PDB was followed by MPDB (476.00 mg) and also differed significantly. Fresh weight on MPDB was statistically similar with the weight on CMB (465.40 mg).Fresh weight on CB (242.60 mg) varied significantly from the fresh on CMB and WB.

Table 3. Fresh Weight and Dry Weight of *T. harzianum* on different media

Growth medium	Fresh Weight (mg)	Dry Weight (mg)
Potato dextrose broth	637.00 a	627.00 a
Modified potato dextrose broth	484.20 b	476.00 b
Cornmeal broth	469.00 b	465.40 b
Carrot broth	251.20 c	242.60 c
Water broth	6.60 d	3.00 d
CV (%)	2.00	1.79
Level of signicance	0.01	0.01

Azher Mustafa and co-workers (2009) studied the biomass of *Trichoderma spp.* on five semi synthetic media including PDA and found PDA as the best medium. Das and co-workers (1997) also studied biomass of *Trichoderma spp.* on PDA and four other natural media and found wheat bran as the best.

Fresh weight and dry weight of *T. harzianum* on different media were found consistent to linear growth of the fungus except cornmeal broth and carrot broth media. Linear growth was higher in carrot agar than cornmeal agar but fresh weight and dry weight were higher in cornmeal agar than carrot agar. In cornmeal agar texture was puffy indicating less linear growth but more aerial growth that might corresponds to the more biomass.

IV. Conclusion

Potato dextrose agar was found more effective and water agar was found less effective for average linear growth rate of *T. harzianum* and Maximum biomass of *T. harzianum* was produced in potato dextrose broth and the minimum was produced in water broth.

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