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Comprehensive evaluation of *Trichoderma harzianum* and *Trichoderma viride* on different culture media & at different temperature and pH

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Abstract

Five different culture media namely, Potato dextrose agar, Potato carrot agar, Carrot agar, Czapek dox agar and Vegetable juice, were evaluated for enhanced growth performance of *Trichoderma harzianum* as well as *Trichoderma viride*. For both species, the highest radial growth were found in Potato dextrose agar (PDA) as 90.0 mm and lowest in Vegetable juice (V₈) as 50.0 mm and 65.5 mm for *T. harzianum* and *T. viride* respectively. Then *Trichoderma* spp. was grown on PDA at various temperature i.e. at 15 °C, 25 °C, 30 °C, 35 °C and also at various pH levels viz; 5, 6, 7, 8 and 9. For both *Trichoderma* spp. the highest radial growth was obtained at 25 °C and lowest at 15 °C and 45 °C while mycelial weight was highest at pH 6 as 1.13g and at pH 5 as 0.63g for *T. harzianum* and *T. viride* respectively.

Keywords: *Trichoderma harzianum*, *Trichoderma viride*, culture media, temperature, pH

1. Introduction

Trichoderma are free-living fungi, common in soil and root ecosystems. They are opportunistic, avirulent plant symbionts, as well as parasites of other fungi (Harman *et al.*, 2004) [7]. Members of *Trichoderma* sp. particularly *T. harzianum* and *T. viride* are promising biological control agents (bioprotectants) against plant diseases. Farmers can easily use this antagonistic pathogen commercially to increase crop yield and decrease the cost of pesticides (<http://thiqaruni.org/engpdf9/42.pdf>). 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) [6, 8, 9]. 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) [9].

To use these antagonist (*T. harzianum* and *T. viride*) 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. Temperatures, soil moisture and soil type are among the important factors which can affect the efficacy of a biocontrol agent (Spadaro & Gullino, 2005). Samuels *et al.* investigated mycelium growth of various *Trichoderma* green moulds isolates and demonstrated that only *T. harzianum* grew well and sporulated at the temperature of 35 °C, whereas *T. atroviride* displayed slower growth under these conditions. According to Magan and Lacey and Plaza *et al.* temperature are the principal abiotic parameters determining the germination and growth potential of micro-organism propagules

Considering the above facts, the present investigation was undertaken to evaluate the growth performance of *T. harzianum* and *T. viride* on different culture media as well as the effect of temperature and pH on the mycelia growth.

2. Material and methods

An experiment was conducted in the Bio-control Laboratory of Plant Pathology Discipline, Chandra Shekhar Azad University Of Agriculture And Technology, Kanpur, U.P, to evaluate the performance of different media as well as to study the effect of factors like temperature and pH on growth of *T. harzianum* and *T. viride*. An isolate of *T. harzianum* and *T. viride* was collected from the preserved isolates of Bio-control laboratory.

2.1 To evaluate the growth performance of *Trichoderma* spp. on different culture media

Five culture media namely, Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), Carrot Agar (CA), Czapek’s Dox Agar medium (CDA) and Vegetable juice Agar medium (V₈) were prepared. The media were prepared following the standard procedure. The prepared medium was sterilized in an autoclave at 121 °C temperature for 20 minutes. In all cases of media, 20ml medium was poured in each petridish. Six plates of each medium were poured (Three for *T. harzianum* and three for *T. viride*). Advanced hyphae of 10 days old culture was used for inoculation. A bit 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. Three replicated plates were used for each medium (three replication of *T. harzianum* and *T. viride*). 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.

2.1.1 Measurement of average linear growth rate (ALGR) of *Trichoderma* spp. on different growth media

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

Eq. (1) Average linear growth rate was measured by the following formula (Aneja, 1993 and Elad *et al.*, 1981) [6].

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

Where C3= Colony diameter after 3 days of inoculation
C0= Initial colony diameter of inoculation

Eq.(2) Average radial growth rate was measured by the following formula :-

$$R_{avg} = \frac{R_1+R_2+R_3}{3}$$

$$R_x = \frac{d_1+d_2}{2}$$

Where d₁= first diameter
d₂=second diameter
R_x=Radial growth of replication “x”

2.2 Effect of temperature on the growth of *Trichoderma* spp. on PDA as culture media

Influence of temperature on the linear hyphal growth of *Trichoderma* spp. was studied *in-vitro* on PDA (15ml) in 90mm Petri plates adjusted for each temperature. The fungal growth was observed by placing a bit (5mm) of pure culture at the centre of the Petri plates with the help of sterile cork borer and three replications were taken for each treatment. The cultures were incubated at 15, 25, 30, 35 and 45°C in BOD incubator and mycelial growth of *Trichoderma* sp. was recorded at every 24 hours up to 7 days.

2.3 Effect of pH on the growth of *Trichoderma* spp. on Potato dextrose broth as culture media

Potato dextrose broth was prepared following the standard procedure. The pH of broth was adjusted as 5.0, 6.0, 7.0, 8.0 and 9.0 by adding N/10 HCl or NaOH using pH meter. One

hundred mL broth of each pH was poured in 250 mL flasks and sterilized in an autoclave at 121 °C temperature for 30 minutes. A bit of the 10 days old pure culture of *Trichoderma* sp. was placed upside down in the flask under aseptic conditions. The inoculated flasks were kept in a BOD incubator at 25±2°C temperature. All treatments were replicated thrice. After 15 days of incubation mycelial mat was harvested from the flask by collecting the culture filtrate through sterilized filter paper (Whatman Filter Paper No. 4). The harvested mycelium was kept in hot air oven at 35°C for 48 hours. The biomass was calculated by obtaining the dry weight of mycelium using oven dry method.

3. Result and discussion

3.1 Growth performance on different culture media

3.1.1 *T. harzianum*

The growth rate was highest in PDA (90.0 mm) followed by PCA (80.0 mm) and CDA (75.5 mm). Least mycelial growth was recorded in V₈ Juice agar as 50.0 mm at 168 h of incubation. Radial growth of *T. harzianum* on CA (55.5) medium is statistically at par with V₈ Juice Agar.

3.1.2 *T. viride*

The growth rate was highest in PDA (90.0 mm) and lowest in V₈ Juice Agar (65.5 mm). Maximum radial growth was recorded on PDA followed by PCA and CDA as 85.5 and 82.5 mm respectively. Carrot Agar media supported medium growth as 70.0 mm at 168 hr of incubation which is statistically at par with V₈ Juice Agar.

Table 1: Average linear growth rate on different culture media

Culture Media	ALGR (mm/day)	
	<i>T. harzianum</i>	<i>T. viride</i>
PCA	17.50	20.43
CA	13.60	17.47
PDA	19.83	23.27
CDA	12.77	16.63
V ₈	10.97	14.40
SE	1.23	1.09
CD@5%	2.74	2.44

* Mean of three replications

3.2 Effect of temperature and pH on growth of *Trichoderma* spp.

3.2.1 *Trichoderma harzianum*

Mycelial growth of *T. harzianum* on PDA media at different temperature varied significantly. The growth rate was highest at 25 °C as 90.0 mm and lowest at 15 °C and 45 °C as 21.3 and 20.0 mm respectively. Statistically, maximum growth was obtained at 25 °C followed by 30 °C and 35 °C while minimum at 15 and 45 °C.

Dry weight of *T. harzianum* on Potato dextrose broth at different pH varied significantly. Mycelial weight was highest at pH 6 as 1.13 g followed by 5 and 7. Mycelial dry weight was lowest at pH 9 as 0.79 g. statistically, pH 6 and 8 were similar while, pH 7 and 5 were at par with each other.

3.2.2 *Trichoderma viride*

Mycelial growth of *T. viride* on PDA media at different temperature varied significantly. The growth rate was highest at 25 °C as 90.0 mm and lowest at 15 °C and 45 °C as 15.5 and 15.8 mm respectively. Mycelial growth of *T. viride* at 30 °C and 35 °C was medium with 85.5 and 80.0mm after 168h of incubation. Statistically, maximum growth was obtained at

25 °C followed by 30 °C and 35 °C while minimum at 15 and 45 °C which were at par with each other.

Dry weight of *T. viride* on Potato dextrose broth at different pH varied significantly. Mycelial weight was highest at pH 5 as 0.63 g followed by pH 6. Mycelial dry weight was lowest at pH 9 as 0.31 g. statistically, pH7 and 8 were at par while, pH 6 was different from all other pH as 0.54g

Table 2: Mycelial growth rate of *Trichoderma* spp. at different temperatures

Temperature	ALGR(mm/day)	
	<i>T. harzianum</i>	<i>T. viride</i>
15 °C	7.83	6.92
25 °C	21.30	22.12
30 °C	20.03	16.62
35 °C	18.23	19.50
45 °C	5.40	4.25
SE	0.072	1.07
CD@5%	0.16	2.38

* Mean of three replications

Table 3: Mycelial dry weight of *Trichoderma* spp. at different pH

Treatments	Mycelial dry weight (g)*	
	<i>T. harzianum</i>	<i>T. viride</i>
pH5	0.84	0.63
pH6	1.13	0.54
pH7	0.86	0.49
pH8	0.81	0.46
pH9	0.79	0.31
SE	0.08	0.04
CD@5%	0.17	0.09

* Mean of three replications

4. Conclusion

In the present study of the growth performance of *Trichoderma harzianum* and *Trichoderma viride* on different culture media, for both species, the highest radial growth were found in Potato Dextrose Agar and lowest in vegetable juice. While the evaluation of the growth performance of *Trichoderma harzianum* and *Trichoderma viride* at different temperatures, for both species, the highest radial growth were obtained at 25 °C. And for pH, in case of *Trichoderma harzianum*, the dry weight was highest at pH6 and lowest at pH9. While in case of *Trichoderma viride* the dry weight was highest at pH5 and lowest at pH9.

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