



INFLUENCES OF SOME ENVIRONMENTAL FACTOR ON GROWTH AND SPORULATION OF SELECTED *Trichoderma* spp.

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ABSTRACT

Two strains of *Trichoderma harzianum* and one strain each of *Trichoderma viride*, *Trichoderma pseudokoningii* and *Trichoderma virens* were evaluated at different temperature and pH on culture medium in the laboratory. The *Trichoderma* spp., grow and sporulate well at 25°C. The best pH for growth and sporulation of all the strains were 5.5 and 6.5 respectively. Among the five strains tested, *Trichoderma harzianum* BHU was observed to sporulate and growth well in all the variable factors.

KEYWORDS: *Trichoderma*, Temperature, pH, Growth and Sporulation

The species of the genus *Trichoderma* have been reported as most potential biocontrol agents against several soil-borne phytopathogens (Lewis and Papavizas, 1991; Haran *et al.*, 1996a; 1996b; Elad, 2000; Hermosa *et al.*, 2000; Kredics, *et al.*, 2003; Joshi, *et al.*, 2010) due to their ability to successfully antagonise other fungi. Establishment of the *Trichoderma* and other biocontrol agents in the soil ecosystem has greatly affected by numerous biotic and abiotic factors (Dandurand and Knudsen, 1993; Eastburn and Butler, 1988a, b; Hubbard *et al.*, 1983; Knudsen and Bin 1990; Papavizas, 1985). These may have negative influence in the biocontrol efficacy of *Trichoderma* strains; therefore, it is important to collect information about the effects of environmental factors on the different activities of *Trichoderma* strains that possess biocontrol potential. It was reported that the production of metabolites from different show varying effects on pathogens (Henis, 1984; Rifai, 1969; Papavizas, 1985). The effect of environmental factors appeared to be a key parameter to manipulate for both growth and sporulation. Temperature is an important environmental factor affecting the disease suppression abilities of antagonistic microbes on the crops (Callan *et al.*, 1990; Mathre and Johnston, 1995; Upadhyay *et al.*, 1991), while the role of pH in conidial germination of *Trichoderma* species has already been reported (Baker, 1986; Papavizas 1985) has stated that different species of *Trichoderma* have their own ecological preferences. In the present paper the influences of environmental factor, temperature and pH on the growth and sporulation of selected *Trichoderma* spp., have been investigated.

MATERIALS AND METHODS

Source of Antagonist and Culture Medium

The pure culture of different strains of *Trichoderma* species were obtained from Laboratory of Applied Mycology and Plant Pathology, Department of Botany, BHU, Varanasi, where the culture were maintained from the collection centres of National Botanical Research Institute, Lucknow and Indian Institute of Vegetable Research, Varanasi. Local species/strains of *Trichoderma* were isolated from soils of various locations from and the around BHU Campus, Varanasi, on the *Trichoderma* selective medium (TSM) (Askew and Laing, 1993).

Effect of Temperature

The effect of different temperature on growth and sporulation of the selected. *Trichoderma* spp., was observed at 25, 30, 35 and 40°C. A five mm diameter of agar block was cut from the margin of actively growing culture of each *Trichoderma* strains and placed centrally in Petri dish (9 cm, diameter) containing 20 ml Potato Dextrose Agar (PDA) medium in triplicate and the plates were incubated at the above temperatures on BOD incubator. The observations were recorded after 4 days of incubation.

Effect of pH

Different pH levels were adjusted in PDA medium at 3.5, 4.5, 5.5, 6.5 and 7.5 Erlenmeyer flasks (500 ml) separately by adding 1 N HCl or 1 N NaOH solution as required. A 5 mm diameter of agar block was cut from the margin of actively growing culture of each *Trichoderma* strains and incubated centrally in Petri-dish

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contained 20 ml of PDA medium. The observations were recorded after 4 days of inoculation.

Sporulation Count

The estimation of spore production of the selected *Trichoderma* spp., on different temperature and pH were done using the method described by El-Abyad *et al.*, (1983). One square cm. block of the different strains of *Trichoderma* spp., was cut from the margin of the actively growing colony and transferred separately to a test tube containing 2.5 ml of sterile distilled water. The tube was shaken continuously for 5 minutes. The density of spores was then counted using a haemocytometer under high power (450) in a Nikon Trinocular Microscope (Model E-600).

RESULTS AND DISCUSSION

In the present investigation the effect of different environmental factors on the growth and sporulation of selected *Trichoderma* spp., were studied. Temperature and pH are probably the most important environmental parameters affecting the antagonistic activity of *Trichoderma* strains. Therefore, it was important to collect information on the effects of these factors on mycelial growth of the strains.

Mycelial growth of the *Trichoderma* spp., was recorded at the temperature range 25-40⁰ C. The optimal temperature for the growth was found to be 25⁰ C for *Trichoderma viride* 1, *Trichoderma pseudokoningii* NBRI and *Trichoderma virens* BHU, where as it was 30⁰ C *Trichoderma harzianum* BHU and *Trichoderma harzianum* IVRI (Figure 1). Optimum temperatures for growth differ among species of *Trichoderma* (Danielson and Davey, 1973b; Komatsu, 1976). The result of the effect of pH (Figure 2) on mycelial growth reveals that both strains of *Trichoderma harzianum* possessed capability of growing at variable pH. The examined strains were able to grow under a wide range of pH from 3.5 to 7.5 with maximal growth at pH 5.5. The result was in conformity with the observation of Jackson *et al.*, (1991) who found that maximum biomass production of isolates was at pH values between 4.6 and 6.8. The objective of the study was to identify the *Trichoderma* species / strains that can grow most rapidly in laboratory test at a wide range of temperature and pH so that they may be used in fields having variable environmental conditions.

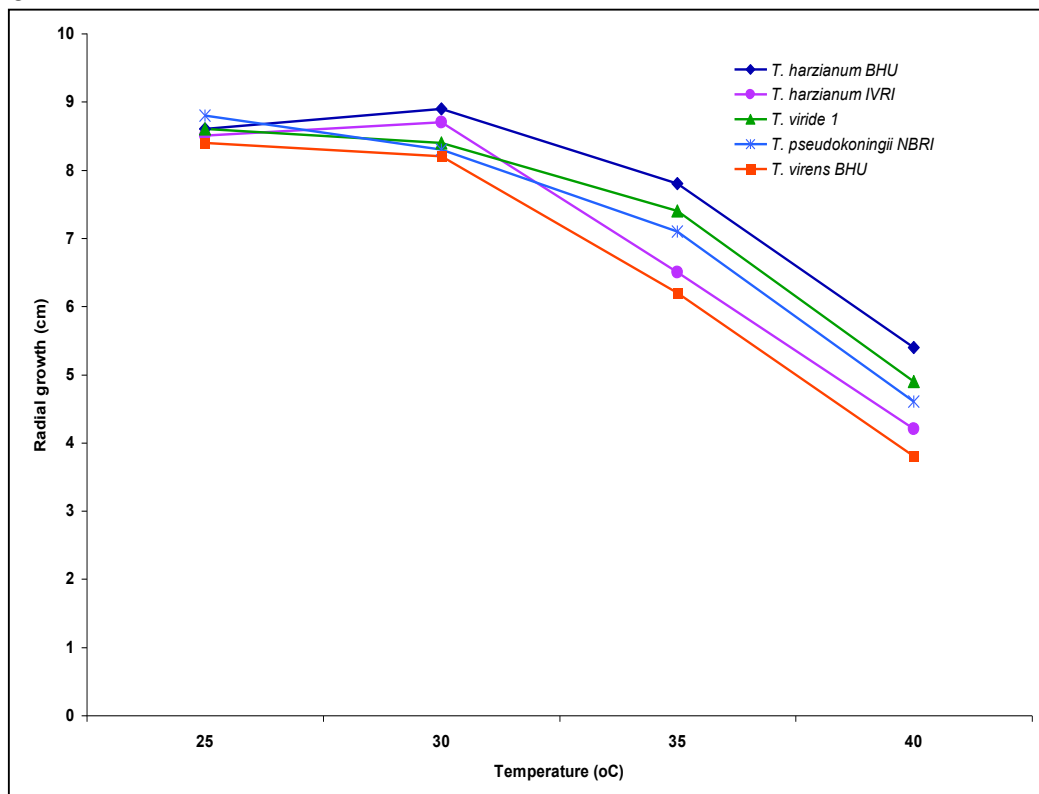


Figure 1: Effect of different temperature on growth of the selected *Trichoderma* species

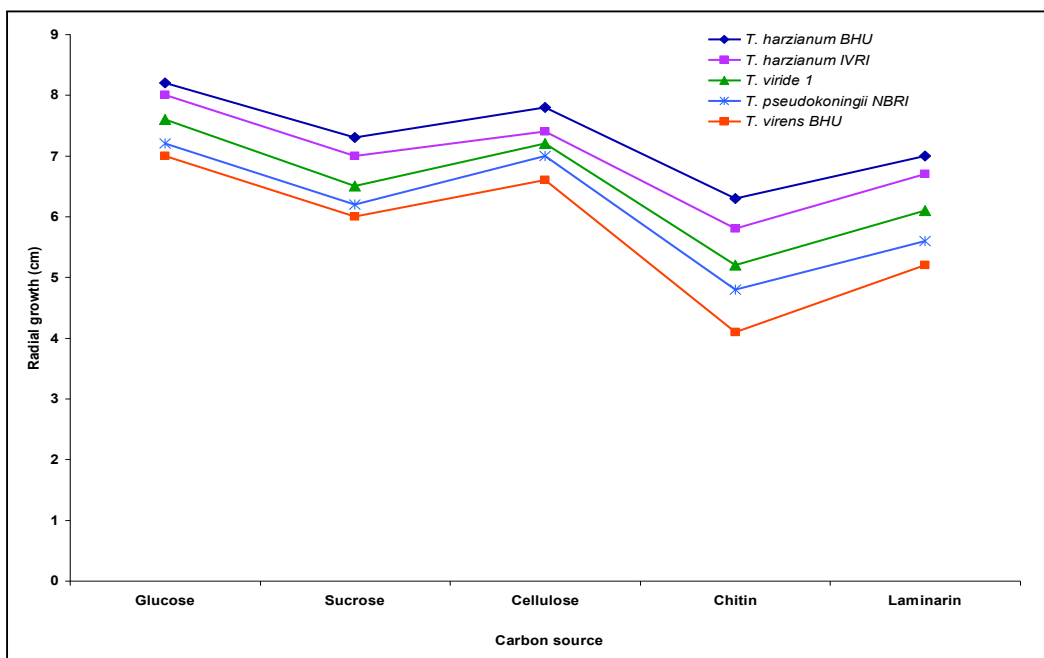


Figure 2: Effect of different pH on growth of the selected *Trichoderma* species

Table 1: Effect of different temperature (°C) on the sporulation of selected *Trichoderma* species

<i>Trichoderma</i> species	25	30	35	40
	Spores × 10 ⁵ per ml of culture			
<i>T. harzianum</i> BHU	296± 2.0	329± 2.1	261± 1.1	240± 4.0
<i>T. harzianum</i> IVRI	242± 2.1	284± 2.0	225± 1.1	208± 4.0
<i>T. viride</i> 1	219± 4.1	198± 4.0	157± 3.1	134± 3.1
<i>T. pseudokoningii</i> NBRI	239± 4.1	215± 4.0	185± 2.0	144± 2.1
<i>T. virens</i> BHU	214± 3.1	191± 2.1	157± 2.0	133± 1.1

*Values are average of three replicates ± SEM

Table 2: Effect of different pH on the sporulation of selected *Trichoderma* species

<i>Trichoderma</i> species	3.5	4.5	5.5	6.5	7.5
	Spores × 10 ⁵ per ml of culture				
<i>T. harzianum</i> BHU	114± 2.3	231± 4.5	289± 1.5	308± 3.2	234± 1.2
<i>T. harzianum</i> IVRI	108± 2.2	201± 3.3	222± 2.2	264± 2.4	198± 4.4
<i>T. viride</i> 1	98± 4.2	148± 3.2	184± 1.2	207± 2.5	143± 1.4
<i>T. pseudokoningii</i> NBRI	106± 4.3	155± 2.2	195± 3.3	226± 4.3	134± 2.5
<i>T. virens</i> BHU	93± 3.5	137± 2.4	189± 2.4	196± 1.2	123± 2.5

*Values are average of three replicates ± SEM

Although different strains showed different temperature requirement for growth and sporulation, but overall, it was found that all the strains, except both the strains of *Trichoderma harzianum* showed medium to good growth and sporulation at temperature around 25°C (Table 1). The relatively narrow range of temperature permitting reproduction suggest that this phase involve

some chemical and physical processes which are not necessary for vegetative growth and which are more exacting in their temperature pigment than are those which suffice for the vegetative phase (Hawker, 1966).

The pH of the medium greatly affected the sporulation of the *Trichoderma* spp. (Table 2). The optimum pH for all of the *Trichoderma* spp. was recorded

at 6.5. *Trichoderma* spp. significantly differed with each other in production of spores. The result was in conformity with Jackson *et al.*, (1991), who found that *Trichoderma* isolated produced optimum biomass at acidic pH range between 4.6 and 6.3. Das *et al.*, (1995) also reported that *Trichoderma harzianum* more effectively reduce sheath blight pathogen of rice in acidic soil (PH 6.8) but it also reduces the infection in neutral soil (PH 7) too. This acidic pH requirement of strain may be cause of natural good sporulating tendency of most fungi at pH range 5-6 (Lilly and Barnett, 1951).

ACKNOWLEDGEMENT

The author is thankful to Ex Prof. & HOD, Late Dr. Bharat Rai, Department of Botany, BHU, Varanasi for valuable guidance during the course of study.

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