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EFFECT OF DIFFERENT MEDIA AND pH ON GROWTH AND SPORULATION OF DIFFERENT NATIVE *TRICHODERMA SPP*

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ABSTRACT
Different native *Trichoderma* isolated from agricultural soils were tested *in vitro* for suitable media, their pH levels tolerance and sporulation. The colony growth diameter and sporulation of fungi were greatly influenced by the different type of growth medium and pH. Keeping this back ground in mind the present work was undertaken.Variation in mycelial growth and fungal sporulation was observed at different media tested. On the basis of microscopic observation and growth characteristics,two native T Pun and T Pun2 isolates were tentatively identified as *Trichodermavirens* and *Trichodermaharzianum*, respectively. Colony radial growth and sporulation of native *Trichoderma* isolates was found to be excellent on Potato Dextrose Agar followed by Oat meal agar medium at optimal environmental conditions. All the *Trichoderma* isolates can grow at pH range between 5 and 8.Isolates T Pun2 and T Pun showed growth at all the pH, but the combination of growth (75.77, 70.87) and sporulation (7.85×10^{7}, 6.0×10^{7}) was found to be best at pH 6 respectively. Whereas, least growth (57.53, 63.23) of T Pun2 and T Pun isolates were found at pH8 and minimum sporulation (1.8×10^{7}, 2.87×10^{7}) were also observed in TSM medium respectively.

INTRODUCTION

The growth of microorganisms in a synthetic medium is influenced by a number of physical and chemical factors. The nutrient composition of a culture medium plays a most important role in microbial growth (Tortora and Funk, 1995). Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction (Singh and Sharma, 2014). In laboratory, a wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of definite culture medium, temperature, light, water availability and adjacent atmospheric gas mixture (Kuhn and Ghannoum, 2003, Kumara and Rawal, 2008).An excellent mycelial growth and sporulation of *A. alternate* was obtained on PDA medium followed by Czapek's medium (Rajashree R. Pawar *et al*., 2014).

The abiotic factors deteriorated the antagonistic properties of *Trichoderma*, against the phytopathogenic fungi (Dluzniewska, 2003). In India, there is great diversity in soil characteristics especially with respect to soil pH. Antagonistic mycoflora are subjected to variation in soil characteristics, soil temperature, pH, rainfall pattern, affecting their adaptability to a specific environment and edaphic conditions. The changing ecological conditions greatly influence the growth, population, and physiological and biochemical status of a fungus and thereby alter its competitive ability. Lewis and Papavizas (1991) reported the profound influence of extrinsic factors on the activity of biocontrol agents. For maximum utilization of antagonistic potential of an organism, it is necessary that the environment to which a prospective antagonist is added should be studied carefully and modified accordingly to assist the establishment and proliferation of antagonists. The effects of various minerals, growth factors, carbon and nitrogen source, pH, and temperature on antibiotic production of biocontrol strains of *Ps. fluorescens* in defined liquid media have been examined Slininger *et al*., 1992; 1995; Dewangan *et al*. 2014).Physical factor such as soil moisture and pH influence the activity of bio control agents; unfavorable temperature may be an even more important limiting factor. The pH of the soil affects enormously the bio control system. Also, fungi are sensitive to extreme temperature, which either lower their viability or are lethal (Panasenko, 1967; Kumar, *et al.,* 2014). Further the effect of temperature, heavy metals, water relations, even the pesticides and pH have influence on mycelial growth of phytopathogenic fungi as well as biocontrol agents. As in all microorganisms even in *Trichoderma*, the exterior factors alter its morphological characteristics as well as physiological functions (Kollie*t al.,* 2012). While among the all microorganisms even in *Trichoderma*, the external factors change its morphological characteristics as well as physiological functions. Among these factors, pH is perhaps the most important environmental parameter affecting the mycoparasitic performance of *Trichoderma* strains (Kredics, *et al.,* 2004). A particular value of pH is compulsory to note the maximum growth where these biocontrol agents can be multiplied and pathogen can be controlled. The studies on the variation of pH by different workers publicized that *Trichoderma* isolates showed optimum growth and sporulation rate at diverse pH values ranging from 2 to 7 (Bandyopadhyay, *et al.,* 2003; Begoude,

et al., 2007). Several researchers has been concluded that, *Trichoderma spp*. have antagonistic and biologically control potential against a wide range of soil borne microorganisms. (Hanson and Howell, 2004; Bajwa *et al*., 2004). As a result, for exploiting the optimal antagonistic potential of *Trichoderma* which is to be applied as biocontrol agent the consequence of pH on their mycelial growth should be tested. With these perspectives, an investigation was undertaken to study the influence of different culture media and pH on the mycelial growth, colony characters and sporulation patterns of fungi isolated from the soil.

MATERIALS AND METHODS

Collection of the soil samples

For isolation of *Trichoderma* spp., soil samples from different crop rhizosphere, usually rich in well decomposed organic matter were collected. The soils were taken after removing 1-2 cm top soil to discard undecomposed organic matters. The isolates of *Trichoderma spp.,* and their sources are enlisted below in Table 1

Isolation of *Trichoderma* spp

Four native *Trichoderma* isolates were isolated from soil of different ecological niches, using soil dilution plate method (Waskman and Fred, 1922) on TSM, (Elad, *et al*., 1981; Modified by Saha and Pan, 1997). The collected soil samples were air dried, ground to powder using mortar and pestle. 10g of powdered soil sample was mixed with 90mL of sterile distilled water to prepare 10^{-1} dilution. This suspension was used for serial dilution up to 10^{-5}. One ml of the suspension from 10^{-3}, 10^{-4} and 10^{-5} dilution was plated separately on 20mL of modified TSM previously poured in sterile Petri plates. The suspension was distributed uniformly on medium surface by horizontal shaking and incubated at 28 ± 1ºC for seven days. The green colonies of the antagonist isolates usually appeared 4 to 5 days after incubation. Each colony was studied separately under microscope using 0.1% lacto phenol-cotton blue strain (0.1g cotton blue was mixed with 100mL of standard lacto phenol preparation) and compared with Rifai's (1969) monograph on *Trichoderma*, Nagamani *et al*. (2006) Handbook of soil fungi and Bisset's (1991) a revision of the genus *Trichoderma*. The shape, size, aggregation of phialospores and phialides were considered as main criteria for identification. On the basis of microscopic observation and growth characteristic native T pun and T pun2 isolates were tentatively as *Trichoderma virens* and *Trichoderma harzianum*, respectively.

Effect of different cultural media

Different agar media namely

Potato dextrose agar (PDA, Riker and Riker, 1936), Oat meal agar (OMA, Johnson and Curl, 1972) and *Trichoderma* specific medium (TSM, synthetic medium) were prepared separately and used to evaluate the mycelial growth and sporulation of the native *Trichoderma* isolates. The six millimetres discs of isolated fungi were inoculated in the middle separately in the respective media. All the treatments were carried out in triplicates and incubated at 28 ± 1^0C temperature for 90 hours. The degree of sporulation of fungi was determined according to Standard Methods (Sharma and Sharma, 2011).

Effect of pH on the growth and sporulation

Four native *Trichoderma* isolates were assessed for growth and sporulation on liquid potato dextrose agar (PDA) medium. . It was made initially by without adding agar to the medium. Then the medium was dispensed in five conical flasks, each flask containing 100 ml medium. When the medium was cooled up to room temperature, it was taken and pH of each flask was adjusted by adding 1(N) HCl or 1(N) NaOH solution as required. The pH of the medium was set at pH 5, 6, 7 and 8 in four flasks. Then, for 100ml medium, 2 g agar was added to each flask and the medium in the flasks was sterilized. Then, pouring was done in the petriplates. The entire four different pH containing medium was poured into different plates. All the treatments were carried out in triplicates. Then isolated *Trichoderma* were inoculated in the each plate and these plates were kept in incubator at 25 ± 1ºC for four days. After four days, the observations were taken.

Statistical Analysis

The data was subjected to analysis of variance (ANOVA) and significance of variance was presented at 5% level using INDOSTAT windows version.

RESULTS AND DISCUSSION

Effect of different media on growth and sporulation of different *Trichoderma* spp.

Different isolates of *Trichodermaspp*. showed different growth patterns in different media. Here we find the medium in which the growth and sporulation of the isolate is best. As different media have different impact on the isolates our purpose is to find out the best media suitable for different isolates of *Trichoderma* spp. Three different media tested were potato dextrose agar (PDA), oat meal agar medium (OMA, non-synthetic medium) and *Trichoderma* specific medium (TSM, synthetic medium).After 90 hrs of inoculation, it was found that all the isolates showed full growth in the plates (that is 9 cm growth) in both the non-synthetic medium, but less growth was recorded on *Trichoderma* specific medium. From the data presented in Table 2, it is evident that the highest mycelial growth were recorded in potato dextrose medium as 83.33 mm, 90.00 and 82.66 mm of T Pun, T Pun2 and Tmnp

Table 1: **Crop rhizosphere and sources for different native isolates of *Trichoderma* spp**

Isolate	Sources	location
T.Pun	Turmeric	Isolated from Cultivated land, U.B.K.V.
T.Pun2	Potato field	do
T.mnp	-	Collected from B.C.K.V, Mohanpur
Thdl	-	Collected from Delhi

Table 2: Variation in growth of native isolates of *Trichoderma* spp. in different media at different time intervals.

Media	Isolate	Growth (mm) 24 hrs	36 hrs	48 hrs	90 hrs
Oat meal agar (OMA)	T Pun	26.66	51.88	81.66	90.00
	T Pun2	33.33	61.88	90.00	90.00
	T mnp	27.99	51.77	74.22	90.00
	Thdl	26.66	41.44	61.99	90.00
Trichoderma specific medium (TSM)	T Pun	11.99	17.88	77.88	70.00
	T Pun2	13.99	24.55	54.66	90.00
	T mnp	11.33	18.77	48.66	90.00
	Thdl	8.99	13.66	25.66	54.00
Potato Dextrose Agar (PDA)	T Pun	33.83	56.85	83.22	90.00
	T Pun2	35.66	64.50	90.00	90.00
	T mnp	30.55	56.16	82.66	90.00
	Thdl	30.33	52.61	72.61	90.00
For isolate	SEm±	0.42	0.45	0.36	0.17
	CD (P=0.05)	1.23	1.32	1.06	0.51
For media	SEm±	0.36	0.39	0.31	0.15
	CD (P=0.05)	1.06	1.15	0.92	0.44
For isolate × media	SEm±	0.72	0.78	0.62	0.30
	CD (P=0.05)	2.13	2.29	1.83	0.87

Table 3: Effect of different media on sporulation of different *Trichodermaspp*

Isolates	Sporulation on PDA	Sporulation on OMA	Sporulation onTSM
T Pun	6.0×10^7	2.8×10^7	1.8×10^7
T Pun2	7.85×10^7	5.8×10^7	2.85×10^7
Thdl	4.85×10^7	5.4×10^7	2.55×10^7
T mnp	6.55×10^7	3.0×10^7	1.25×10^7

Table 4: Effect of pH on growth of *Trichoderma* spp.

Isolate	Growth rate of *Trichoderma* isolates at different pH 5.0	6.0	7.0	8.0
T Pun	58.56	60.83	56.90	41.77
T Pun 2	73.73	75.77	61.90	57.53
T mnp	73.67	70.87	68.10	63.23
Thdl	51.73	56.20	53.43	52.0
For Isolate	SEm±			1.16
	CD (P=0.05)			4.74
For pH	SEm±			1.16
	CD (P=0.05)			4.74
For pH×Isolate	SEm±			2.33
	CD (P=0.05)			9.4

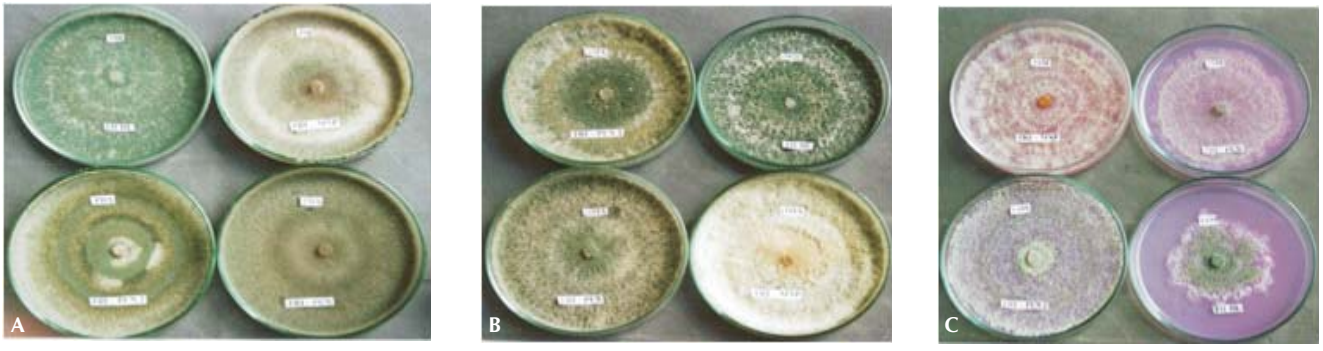

Plate 1: Effect of different media (A) PDA, (B) OMA, (C) TSM on growth and sporulation of different *Trichoderma* spp

respectively followed by Oat meal agar (OMA) and *Trichoderma* specific medium (TSM). Perusal of the data (Table 3) indicated that PDA medium was the most suitable for sporulation of native *Trichoderma* spp. Maximum sporulation of T Pun, T Pun2 and Tmnp (6.0×10^7, $7.85\times10^7$and 6.55×10^7 respectively) was observed on PDA

Table 5: Effect of pH on sporulation of *Trichoderma* spp.

Isolate	Colony Forming Unit at different pH Sporulation at different pH 5.0	6.0	7.0	8.0
T Pun 2	4.25×10^7	5.0×10^7	3.0×10^7	2.25×10^7
T Pun	3.42×10^7	4.0×10^7	2.1×10^7	1.6×10^7
T mnp	3.15×10^7	2.78×10^7	1.95×10^7	1.4×10^7
Thdl	2.27×10^7	3.5×10^7	2.25×10^7	1.0×10^7

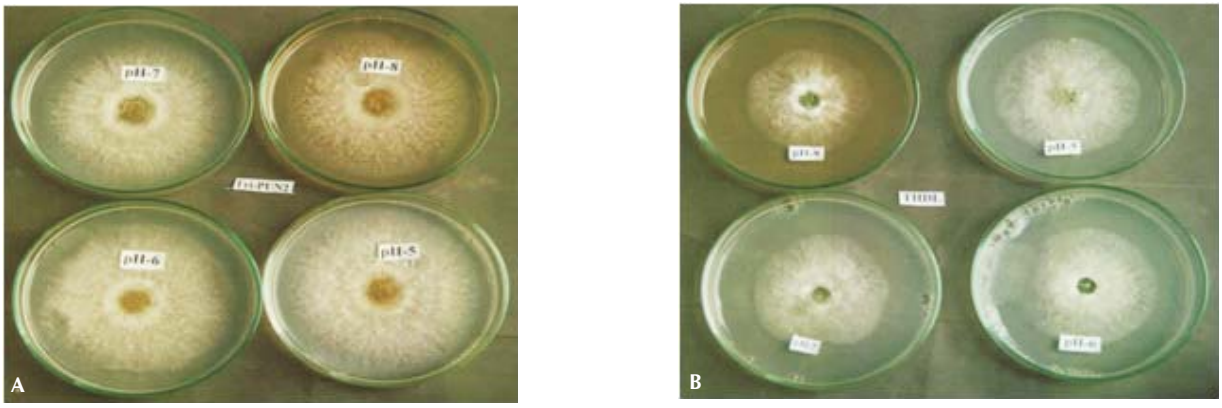

Plate 2: Effect of different pH (5, 6, 7, 8) on growth and sporulation of different native *Trichoderma* spp. (A) Tpun2 (B) Thdl.

medium after 4 days of inoculation, whereas maximum sporulation of Thdl (5.4×10^7)recorded on OMA medium (Plate 1). These results were conformed to the findings of Samuels *et al.,* 1998 and Gupta, *et al*., 2003, Singh, *et al*., 2011. Kumar and Singh, 2008. In another study, Kumar *et al*. (2008) also found that Potato dextrose broth medium to be the best medium for the growth of *Sclerotium rolfsii*.

On the contrary, less growth and sporulation of T Pun and Thdl were recorded on TSM. This may be due to the fact that the presence of some unknown substances in the natural medium (PDA & OMA) but not in the synthetic medium (TSM) which induced /influenced the growth and sporulation of *Trichoderma* spp.

Effect of pH on growth and sporulation of *Trichoderma* spp.

Similar to other physiological parameters, pH also plays a significant role in the growth and sporulation. The optimum pH for the growth and sporulation of native *Trichoderma* isolates was responded differently to various pH levels (5.0, 6.0, 7.0 and 8.0) were maintained on potato dextrose media in three replicates as shown in the Table 4 and 5.From the data presented in Table 4 indicated that all the four isolates of *Trichoderma spp*. could grow at a pH range between 5 and 8. T Pun2 isolate also showed growth at all the pH. But the combination of growth and sporulation was found to be best at pH 6 (Plate 2). The growth and sporulation of T Pun and Thdl isolates were also found to be best at pH 6.This result were in accordance with the findings of Singh, *et al*. (1998), who showed that *Trichoderma harzianum* (MTR 35 isolate) showed maximum population density at pH 6.5.Tmnp isolate also showed highest growth at a pH range between 5 and 8. But the combination of growth and sporulation was found to be best at pH 5. Similar results also found by Jackson, *et al*. (1991), who recorded that *Trichoderma* isolates produced optimum biomass at acidic pH ranges between 4.6 and 6.3. Again Bandyopadhyay, *et al*. (2003) also reported that *Trichoderma* grow best at acidic to neutral pH range (pH 5 to 7).Thus from the present experiment we can conclude that native *Trichoderma* isolates grow best at acidic pH range of 5 to 6. This low pH requirement maybe due to the cause that most fungi have the tendency to sporulate well at low pH range and so the native isolates of *Trichoderma* showed best growth and sporulation at pH 5 to 6. Kunming (2004), also confirms the above results where pH range 5-8 and optimally at pH 5-6 has a significant influence on growth and sporulation on *T. atroviride*.

However, Singh, *et al*. (2011), examined the potential application value of *Trichoderma atroviride* strains in culture media on different pH and favourable growth was seen at pH range 7-7.5 has a significant influence on growth and sporulation.

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