INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 7 | ISSUE 2 | OCTOBER, 2014 | 424-428 ● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/7.2/424-428

Study of different temperature levels on radial growth and dry mycelial weight of *Trichoderma* spp. isolated from red gram based conservation agriculture ecosystem

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Received : 15.03.2014
Revised : 25.08.2014
Accepted : 06.09.2014

KEY **W**ORDS :
Trichoderma spp., Radial growth, Dry mycelia, Temperature

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ABSTRACT

Different temperatures and pH were taken to observe the radial growth and dry mycelial weight of *Trichoderma* spp. The radial growth of *Trichoderma* was maximum for all the four species *i.e., T. harzianum, T. viride, T. hamatum* and *T. virens* (90, 82, 91.3 and 85mm) at 30°C, where it was minimum in four species at 40°C (30.70, 35, 22.53 and 20 mm), respectively and dry mycelium of *T. harzianum* (1.05 mg), *T. viride* (1.83 mg), *T. hamatum* (2.42 mg) and *T. virens* (0.82 mg) were maximum at 25°C whereas, the radial growth of four isolates were maximum at pH 6 (90, 88, 92 and 91mm) in *T. harzianum, T. viride, T. hamatum* and *T. virens*, respectively. At neutral pH, radial growth of *T. harzianum* (89 mm), *T. viride* (91 mm), *T. hamatum* (89 mm) and *T. virens* (87 mm) was minimum and dry mycelium weight of *T. harzianum* (729 mg), *T. viride* (1639.67 mg), *T. hamatum* (798 mg) and *T. virens* (583.67 mg) were maximum at pH 8. It was minimum at pH 4 for *T. harzianum* (120 mg), *T. viride* (257 mg), *T. hamatum* (154 mg) and *T. virens* (262.67 mg).

How to view point the article : Prabhavathi, N.M., Amaresh, Y.S., Naik, M.K., Mallesh, S.B. and Kuchanur, P.H. (2014). Study of different temperature levels on radial growth and dry mycelial weight of *Trichoderma* spp. isolated from red gram based conservation agriculture ecosystem. *J. Plant Protec.*, **7**(2) : 424-428.

INTRODUCTION

Indian agriculture is entering into a new phase. The major research and development efforts in the green revolution have focused on enhancing productivity of selected food grains and few other crops. Over the past three decades or so, internationally rapid strides have been made to evolve and spread resource conservation technologies like zero and reduced tillage systems better management of crop residues and planting systems, which enhance conservation of water and nutrients (Hobbs and Gupta, 2004). According to current estimates globally, CA systems are being adopted in some 80 m ha, largely in the rain fed areas and that the area under CA is increasing rapidly (FAO, 2010).

Trichoderma strains of great importance as bio control strains should have better stress tolerance levels than the plant pathogens against which they are going to be used for biological control. The abiotic factors deteriorate the antagonistic properties of *Trichoderma*, against the

phytopathogenic fungi. Besides the effect of temperature, heavy metals, water relations, even the pesticides and pH influence on mycelial growth of phytopathogenic fungi as well as biocontrol agents. As in all micro-organisms even in *Trichoderma*, the external factors modify its morphological characteristics as well as physiological functions. The studies on the variation of temperature by different workers revealed that *Trichoderma* isolates showed optimum growth and sporulation rate at different temperature values ranging from 15 to 40°C as reported by Sarojini *et al.* (2012). In this study different isolates of *Trichoderma* spp. were isolated and tested against different temperatures and also recorded dry mycelial weight So, for exploiting the optimal antagonistic potential of *Trichoderma* which is to be applied as biocontrol agent (BCA), the effect of temperature on their mycelial growth should be tested. Hence, an investigation was undertaken to study.

MATERIAL AND METHODS

Samples were collected from Conservation Agriculture of redgram ecosystem at College of Agriculture, Bheemarayanagudi experimental plot. Soil samples were collected from zero tillage with mulch, zero tillage without mulch, raised bed with mulch, raised bed without mulch and farmers practice blocks from rhizosphere of red gram. Bio agents from these soil samples were isolated using serial dilution method (Dennis and Webster, 1971). One gram of soil sample was taken separately and suspended in 9 ml of sterile distilled water and stir red well to get 1:10 dilution (10^{-l}), one ml of this was transferred to test tube containing 9 ml of sterile distil water to get 1:100 (10^{-2}) dilution. Likewise the dilution of the sample was prepared about 10^{-4} and 10^{-7} aseptically and pipetted out into each sterile Petri plate separately to which a quantity of 15 -20 ml of sterilized and cooled *Trichoderma* specific medium was poured and gently rotated in clock wise and anti clock wise direction to let the suspension distributed uniformly in the medium.The plates were incubated at 28 ± 1°C for 5 days and colonies were observed.

Effect of temperature on different *Trichoderma* spp. :

The growth of isolates of *Trichoderma* was tested at 15, 20, 25, 30, 35 and 40°C. Twenty ml of Potato dextrose broth was dispensed and sterilized in 100 ml conical flask. Each flask was inoculated with 5 mm disc of fungus and incubated for three weeks at different temperature levels as mentioned above. Each treatment was replicated thrice. After incubation period, the dry mycelal weight was recorded.

Effect of hydrogen ion concentration on different *Trichoderma* spp. :

Potato dextrose agar media was used as the basal medium. The pH of the media was adjusted using 0.1 N alkali (NaOH) or 0.1 N acid (HCl). According to the schedule of Vogel (1951), the medium was buffered to already adjusted pH by adding 50 ml of disodium hydrogen phosphate citric acid buffer for required pH. The different hydrogen ion concentrations used were 4, 5, 6, 7, 8, 9 and 10.

PDA was prepared and the pH was adjusted as described earlier. After that the sterilized medium was poured into Petri plate and then inoculated with 5 mm culture disc. Plates were incubated five days at 28±1°C. Each treatment was replicated thrice. The radial growth of mycelia and sporulation was recorded. The results were analyzed statistically.

Statistical analysis :

The data were subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

Effect of different temperature levels on radial growth and dry mycelial weight of *Trichoderma* spp. :

In this study, different temperatures such as 15, 20, 25, 30, 35 and 40°C were taken to observe the radial growth and

Table 1 : Effect of different temperature levels on radial growth of different *Trichoderma* spp.

Temperatures (°C)	Radial growth (mm)			
	T. harzianum	*T. viride*	*T. hamatum*	*T. virens*
15	32.25	70.93	44.17	55.03
20	35.30	60.33	52.20	40.00
25	60.03	65.00	39.00	54.67
30	90.00	82.00	91.33	85.00
35	69.00	67.33	61.00	67.00
40	30.70	35.00	22.53	20.00
S.E. ±	0.764	0.613	0.589	0.634
C.D. (P=0.01)	3.301	2.649	2.543	2.739

dry mycelial weight of *Trichoderma* spp. *viz.*, *T. harzianum, T. viride, T. hamatum* and *T. virens*. The radial growth of *Trichoderma* was maximum for all the four species *i.e.*, *T. harzianum, T. viride, T. hamatum* and *T. virens* (90, 82, 91.33 and 85 mm) at 30°C, where it was minimum in four species at 40°C (30.70, 35, 22.53 and 20 mm), respectively. The radial growth of *Trichoderma* species was reduced in 15 and 20°C temperature. Dry mycelial weight of *T. harzianum* (1.05 mg), *T. viride* (1.83 mg), *T. hamatum* (2.42 mg) and *T. virens* (0.90 mg) were maximum at 25°C. Above and below 25°C the dry mycelial weight was reduced (Table 1, 2 and Plate 1).

The results were similar to those obtained by Goldfarb *et al.* (1989) where in *Trichoderma* spp. were tested for linear growth rates at 5 temperature levels (5-25°C). Optimum biomass was produced at temperature between 20-30°C by Jackson (1973). The isolates *T. viride, T. polysporum* and *T. harzianum* were most antagonistic. The isolates *T. viride* and *T. harzianum* were more antagonistic at 20°C and *T. viride* was found to produce maximum xylanase at pH 5.5 and 30°C (Sashi *et al.,* 2008).

Effect of different pH levels on radial growth and dry mycelia weight of *Trichoderma* spp. :

Different pH such as 4, 5, 6, 7, 8, 9 and 10 are taken to record the radial growth and dry mycelial weight of

Table 2 : Effect of different temperature levels on dry mycelial weight of different *Trichoderma* spp.

Temperatures (°C)	Dry mycelial weight (mg)			
	T. harzianum	*T. viride*	*T. hamatum*	*T. virens*
15	0.790	0.90	1.50	0.740
20	0.930	1.710	1.810	0.820
25	1.050	1.830	2.420	0.900
30	0.961	1.540	2.090	0.830
35	0.815	1.680	1.710	0.910
40	0.450	0.920	0.810	0.400
S.E. ±	0.011	0.014	0.0480	0.008
C.D. (P=0.01)	0.047	0.058	0.209	0.036

Table 3 : Effect of different pH levels on radial growth of *Trichoderma* spp.

pH levels	Radial growth (mm)			
	T. harzianum	*T. viride*	*T. hamatum*	*T. virens*
4	70.20	67.57	65.00	62.00
5	75.00	77.00	71.00	69.33
6	89.00	91.00	89.00	87.00
7	90.00	88.00	92.00	91.00
8	66.00	68.00	61.20	57.00
9	49.20	59.00	45.00	38.00
10	36.00	38.00	39.00	33.00
S.E. ±	0.660	0.546	0.677	0.667
C.D. (P=0.01)	2.780	2.299	2.848	2.807

Table 4 : Effect of different pH levels on dry mycelia weight of different *Trichoderma* spp.

pH levels	Dry mycelia weight (mg)			
	T. harzianum	*T. viride*	*T. hamatum*	*T. virens*
4	0.120	0.257	0.154	0.262
5	0.148	0.811	0.410	0.581
6	0.521	0.798	0.519	0.823
7	0.663	0.103	0.769	0.112
8	0.729	0.163	0.798	0.583
9	0.485	0.431	0.456	0.101
10	0.246	0.258	0.278	0.388
S.E. ±	0.891	0.664	0.690	0.845
C.D. (P=0.01)	0.375	0.279	0.290	0.355

Plate 1 : Effect of PH levels on dry mycelial weight of *Tricho derma* spp.

Trichoderma spp *viz., T. harzianum, T. viride, T. hamatum* and *T. virens*. The radial growth of four isolates were maximum at pH 6 (90, 88, 92 and 91mm) in *T. harzianum, T. viride, T. hamatum* and *T. virens*, respectively. At neutral pH, radial growth of *T. harzianum* (89 mm), *T. viride* (91 mm), *T. hamatum* (89 mm) and *T. virens* (87 mm) was minimum, whereas, alkali pH supported less radial growth of all the *Trichoderma* isolates. Dry mycelia weight of *T. harzianum* (0.729 mg), *T. viride* (1.639mg), *T. hamatum* (798 mg) and *T. virens* (0.583.67 mg) were maximum at pH 8. It was minimum at pH 4 for *T. harzianum* (0.120 mg), *T. viride* (0.257 mg), *T. hamatum* (0.154 mg) and *T. virens* (2.62 mg), respectevely (Table 3 and 4).

Longa *et al*. (2007) observed that *Trichoderma* strains were able to grow in a wide range of pH from 2 to 6 with an optimum at 4.00. Jackson (1973) found that optimum biomass production of three *Trichoderma* isolates occurred at pH range between 4.6 to 6.89. The *Trichoderma* strains were found to be able to display their activities under wide range of pH levels (Kredics *et al*., 2004). Bhattiprolu (2008) studied the growth of *T. virens* can grow at pH range of 4-9 but optimum pH varied from 5-6. The different strains of *Trichoderma* could grow at pH range between 5-9. Strain M-7 of *Trichoderma* showed best growth and sporulation at pH 8. This strain may be the nature of alkaline soil and this because adaptive to high pH condition and grow well in the alkaline pH (Bandyopadhyaya *et al*., 2002 and Jackson 1973).

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