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Some Physiological Studies on Trichoderma Harzianum Rifai

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ABSTRACT

Among eight liquid media Brown's medium and Elliott's medium supported maximum mycelial growth of *T. harzianum*. A pH range of 4.0 to 6.0 was found most suitable for the growth. Maximum germination of spores occurred at 35°C exposure for 5 min while it failed to germinate after exposure at 55°C for 5 min.

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INTRODUCTION

Trichoderma harzianum Rifai, a common soil inhabitant, is well recognized for its antagonistic activity against important soil-borne plant pathogens. Information on the growth and sporulation of *T. harzianum* and factors affecting it *in vitro* can be useful in developing the technology for large scale production of the bio-agent. Although some *in vitro* studies have been taken up on this antagonist, more information is required. Therefore, studies were undertaken on the effect of liquid media and H-ion concentration on the mycelial growth and viability of spores of *T. harzianum* at different temperature.

MATERIALS AND METHODS

Liquid media : Eight liquid viz. Richard's solution, Czapek's, Elliott's, Asthana and Hawker's, Brown's medium, sucrose nitrate, potato dextrose broth and glucose broth were tried. The pH of each medium was adjusted to 6.0 before autoclaving. Twenty ml of each liquid medium was taken separately in 100 ml Erlenmeyer flasks, sterilized at 15 lbs p.s.i. for 20 min and inoculated with mycelial disk (5 mm) from 7 day old culture of *T. harzianum* (Anand isolate) grown on potato dextrose agar (PDA). After 15 days of incubation at 28°C, mycelial mat was collected on Whatman filter paper (No. 44), dried at 60°C and weighed.

H-ion concentration : Since Brown's medium was found best it was used at different pH levels viz. 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. Flasks containing 20 ml of sterilized Brown's medium at respective pH values were inoculated and incubated as in liquid media studies.

Exposure of conidia to different temperature : Five ml spore suspension of *T. harzianum* was taken in sterile test tubes

and kept in thermostatically controlled water bath cum shaker at different temperature of 35, 40, 45, 50 and 55°C for 5, 15, 30, 45 and 60 min. After exposure to various temperatures and time, one ml of spore suspension was poured onto 2% agar medium (4 petri plates in each treatment). The petri plates were examined under microscope for germination of the spores after 36 hrs of incubation at 28°C. At least 400 spores were counted in each replication.

RESULTS AND DISCUSSION

Effect of liquid media on the growth of *T. harzianum*

Significant differences in dry mycelial weight of *T. harzianum* was observed in each liquid medium (Table-1). Maximum (183.75 mg) mycelial weight was recorded in case of Brown's medium which did not differ with Alliott's medium (170.00 mg). Sucrose nitrate medium was next best but did not differ significantly from Asthana and Hawker's medium and potato dextrose broth. Richard's solution, glucose broth and Czapek's solution were found poor for the growth of *T. harzianum*. The differences in dry mycelial weight in various liquid media may be due to differences in the composition of the medium. Brown's and Elliott's medium contain L-asparagina and magnesium sulphate which might be responsible for supporting better growth of *T. harzianum*. These results are similar to those of Shukla and Mishra (1970) who reported that growth of *T. viride* was significantly increased when magnesium sulphate was added to the medium. Sesan (1981) observed that *T. viride* grew best on media containing peptone, mine acids, DL-asparagine and ammonium salts.

TABLE 1

Effect of liquid media on the mycelial growth of *T. harzianum*.

Liquid medium	*Dry mycelial weight in mg**
Richard's solution	115.00
Czapek's solution	126.25
Sucrose nitrate medium	158.75
Elliott's medium	170.00
Asthana and Hawker's medium	153.75
Brown's medium	183.75
Potato dextrose broth	141.25
Glucose broth	118.75
S.Em.	6.89
C.D. (0.05)	20.11
C.V. (%)	9.44

* Average of four replications.

Effect of H-ion concentration on the growth of *T. harzianum* :

Dry mycelial weight of *T. harzianum* was maximum at pH 4.5 with no significant difference at pH 4.0. However, with increasing H-ion concentrations, there was gradual decline in dry mycelial weight except at pH 5.5 and 6.0 which supported equal growth (190 mg). Brian and Homming (1950) reported that pH 4.0 to 6.0 was the best for the spore production of *T. viride*. Chet and Baker (1980) concluded that acidic condition enhanced the growth of *T. harzianum*.

TABLE 2

Effect of H-ion concentrations on the mycelial growth of *T. harzianum*.

pH	*Dry mycelial weight in mg**
4.0	220
4.5	245
5.0	210
5.5	190
6.0	190
6.5	170
7.0	155
7.5	130
8.0	105
S.E.m.	8.69
C.D. (0.05)	25.21
C.V. (%)	9.68

*Average of four replications.

Effect of different temperature on the viability of spores of *T. harzianum* :

Maximum spore germination was observed at an exposure for 5 min. at 55°C. More than 60% spores germinated upto 45° at 30 min. exposure and thereafter there was decline in germination at subsequently higher temperature and time of exposure. Spores failed to germinate when exposed to 55°C for even 5 min. Hence it can be concluded that *T. harzianum* spores can not survive exposure to higher tempertures. Danielson and Davey (1973) found that maximum temperature favourable for the growth and sporulation of this bioagent was in the range of 30-38°C.

TABLE 3

Effect of temperature and time of exposure on the viability of *T. harzianum* spores.

Time (minute).	*Spore germination (%)* Temp. °C*				
	35°	*40°*	*45°*	*50°*	*55°*
5	87.1	70.5	66.5	31.8	0.0
15	86.0	66.6	62.5	28.6	0.0
30	82.4	62.6	60.3	22.3	0.0
45	71.7	44.0	40.8	12.0	0.0
60	58.5	32.2	22.7	9.7	0.0

	S. Em.	C. D.	(0.06)	C. V. (%)
Temp. (T)	3.0		8.58	22.82
Time (H)	3.35		9.59	
T X H	6.70		NS	

*Average of 4 replications.

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