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Influence of environmental factors and pesticides on biocontrol potential of *Trichoderma harzianum*

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Abstract

Environmental factors especially abiotic factors and pesticides influence the efficacy of *Trichoderma* spp. At 25^{0}C temperature spore germination of *T. harzianum* was significantly higher after 12 h and 24 h which further improved after 24 h of incubation. However, there was no effect on germ tube length at 20, 25 and 30^{0}C. No spore germination was found at 10^{0}C and 15^{0}C and the mycelial growth was also less. A combination of 30 per cent soil moisture and of pH 5.0 were found to be the most suitable for the mycelial growth of *T. harzianum*. Herbicide Alachlor adversely affected the growth of bioagent whereas pendimethalin was found less toxic at 0.01 per cent concentration. Fungicides Carbendazim and Mancozeb both were found toxic to bioagent but Captaf was comparatively less toxic to the bioagent. Biopesticide, Wanis was most toxic to the growth of *T. harzianum* at all concentrations tested, but Neemgold was found comparatively less toxic. Insecticides chlorpyriphos and phorate were found toxic to the bioagent even at the lowest concentration (0.01%).

Key words: *Trichoderma* spp., biocontrol, environmental factors.

Introduction

It is very essential to consider the pesticides being used and the environmental factors which influence the biocontrol efficacy of *Trichoderma*, while planning its application for the biological control (Kredics *et al.*, 2003). Temperature (Mukherjee *et al.*, 1997; Singh *et al.*, 2002), temperature and pH (Jackson *et al.*, 1991) and combination of soil moisture and temperature (Zaidi and Singh, 2004) have been reported to be important abiotic factors influencing the efficacy of bioagents. Dry conditions may occur even in normally less dry soils as a result of normal drying between rains. On the other hand, biocontrol agents may be needed against plant pathogens in dry soils. Water potential is known to influence the mycelial growth and secretion of enzymes by bioagents. *Trichoderma* and *Gliocladium* spp. both prefer acidic conditions (pH 5.5-6.5) for their growth but pH has no effect on sporulation (Pandey *et al.*, 1977). The present investigation highlights the effect of abiotic environmental factors and common pesticides on the biocontrol efficacy of *Trichoderma* spp.

Material and Methods

Testing antagonistic activity

Antagonistic activity of *T. harzianum* was tested against various soilborne pathogens, *viz., Fusarium oxysporum* f. sp. *pisi, Sclerotinia minor, Sclerotium rolfsii, Rhizoctonia solani* and *Pythium* sp. on potato dextrose agar (PDA) medium using dual culture technique (Huang and Hoes, 1976). The cultures were incubated 23 ± 1^{0}C. Each treatment was replicated thrice. The linear growth of the bioagent and the soilborne pathogens from the centre of the plate was recorded after the control plates were completely covered by the pathogen. Growth inhibition of pathogen by bioagent over control was calculated by using the standard formula (Vincent, 1947).

Effect of temperature

Growth characteristics of the fungus *T. harzianum* were evaluated under controlled environmental conditions. The sporulating ability, germ tube length and mycelial growth of the *T. harzianum* at different temperatures, *viz.*, 10, 15, 20, 25 and 30^{0}C were studied on PDA.

Spore germination

A three mm diameter mycelial agar disc was placed in the centre of Petriplate containing PDA and incubated at 25^{0}C to assess spore

germination. The spore suspension was made by pouring 15ml sterilized water in Petriplates and transferring the suspension to test tubes. Ten fold dilutions of the spore suspension were made to the extent that spores could be easily seen on the slides. Spore suspension was then dispensed with micro pipette on to clean micro slides and evaluated for germination at 10,15,20,25 and 30^{0}C. Observations on spore germination were recorded after 12 and 24 h. The spore was considered to be germinated when the germ tube length exceeded the maximum width of spore.

Germ tube length

Germ tube length was measured with the help of ocular micrometer that was calibrated with the help of stage micrometer.

Mycelial growth

Three mm diameter mycelial agar disc was placed in the centre of Petriplates containing PDA and incubated at temperatures, *viz.*, 10, 15, 20, 25 and 30 ± 1^{0}C to assess mycelial growth of the test fungus after 5 days.

Effect of soil moisture on T. harzianum: A known weight of oven-dried soil was taken and water was added to it to bring the soil moisture to desired level i.e. 10, 15, 20, 30 and 50 per cent. The soil samples were kept in pots and total weight (pot + soil + moist) was recorded. The moisture level was maintained by adding water equivalent to lost, through evaporation as determined by weighing. The soil was inoculated with 510-mg/kg inoculum of bioagent multiplied on wheat bran at 22 ± 1^{0}C up to 30 days.

The moisture content of the soil was determined by the following formula:

$$SM = \frac{FW - DW}{DW} \times 100$$

FW = Fresh weight of soil
DW = Dry weight of soil
SM = Soil moisture

Influence of pH on T. harzianum

The effect of pH on *T. harzianum* was studied, by adjusting the pH levels of the medium (PDA) to 4.0, 5.0, 6.0, 7.0 and 8.0. The pH of the medium was adjusted by adding either 1N HCl or 1N NaOH depending upon the requirement. Mycelial agar disc (3mm dia) of *T. harzianum* was placed in the centre of the Petriplate and mycelial growth was recorded after 5 days of incubation at 25 ± 1^{0}C. Each pH level was replicated thrice.

Effect of pesticides on Trichoderma spp.

Pesticides tested included two herbicides pendimethalin [N- (1-ethylpropyl)-2,6- dinitro – 3, 4 - xylidine] and alachlor [2 – chloro - 2', 6' - diethyl-*N*-methoxymethylacetanilide]), three fungicides, carbendazim [Methyl benzimidazol-2-ylcarbamate], mancozeb [manganese ethylenebis (dithiocarbamate) (Polymeric)] and captaf ([N- (trichloromethylthio) cyclohex – 4 - ene-1, 2 - dicarboximide]) two Neem based biopesticides (Wanis and Neemgold) and two insecticides (chlorpyriphos [O, O-diethyl O-3, 5,6-trichloro-2-pyridyl phosphorothioate] and phorate [O,O-diethyl S-ethylthiomethyl phosphorodithioate]). The effect of above pesticides was studied on spore germination and mycelial growth of test antagonist using poisoned food technique.

Spore germination

Spores of *T. harzianum* obtained from 10 days old cultures shaked in distilled water for uniform concentration. Slide germination method (Anon., 1948) was employed for evaluating the effect of above said pesticides. Pesticide suspensions were made double the strength of the concentrations of 0.01, 0.05 and 0.1 per cent in 15 ml distilled water. On the micro slide, the two concentrations along with spore suspension were dispensed with micro pipette and incubated at 25± 1^{0}C. Spore germination was recorded under microscope (10x) after 2, 4, 8, 16 and 24 h of incubation.

Mycelial growth

All the pesticides were evaluated at three concentrations, viz., 0.01, 0.05 and 0.1 per cent against *T. harzianum* using poisoned food technique. Double strength of PDA was prepared in distilled water and sterilized at 1.5 kg cm^{-2} pressure for 15 minutes. Equal amounts of both PDA and pesticide concentrations were then mixed aseptically. Twenty ml of the mixture was poured in each sterilized Petriplate (90 mm diameter) under aseptic conditions. In control sterilized distilled water replaced the pesticidal suspension in the double strength medium. The solidified medium (PDA + pesticide) was then inoculated with mycelial agar disc (3 mm diameter) cut from 5 days old culture of *T. harzianum* grown on PDA. The inoculated Petri plates were incubated at 25 ± 1^{0}C and the colony diameter was recorded in each treatment after the control plates were completely covered with the fungus. Each treatment was replicated thrice.

The per cent inhibition of the mycelial growth was calculated.

Results and Discussion

Effect of temperature on spore germination, germ tube length and mycelial growth

Spore germination after 12 and 24 h of incubation at 25°C was invariably more than other temperatures (Table 1). After 12 h of incubation, the per cent spore germination was 23.96 per cent which increased to 47.96 per cent after 24 h of incubation. At 20°C temperature after 12 h of incubation, there was 16.43 per cent spore germination that increased to 37.76 per cent after 24 h incubation. However, at 30°C temperature the spore germination was drastically reduced (6.63 %) after 12 h which slightly increased to 12.76 per cent after 24 h. There was no spore germination at 10 and 15°C temperatures. Germ tube length ranged between 3 to 3.5 mm at 20 - 30°C after 24 h incubation.

At 25°C, mycelial growth was also maximum (48.66 mm) followed by 32.66 mm at 20°C, 22.00 mm at 30°C, 12.33 mm at 15°C and lowest mycelial growth of 6.66 mm was found at 10°C.This indicated that the optimum temperature for mycelial growth of *T. harzianum* is 25°C (Table 1).

Effect of soil moisture on T.harzianum

Results on the effect of different soil moistures on multiplication of *T. harzianum* revealed that the population of this bioagent increased up to 20th day at 10 per cent soil moisture (Table 2) However, in other moisture regimes the population of the bioagent increased up to 15th day and then started decreasing. Thirty per cent soil moisture was found best for bioagent population to build up followed by 20 per cent whereas, 50 per cent soil moisture was inhibitory for the build up of bioagent population.

Effect of pH on Trichoderma harzianum at 25°C

It is clear from the results reported herein that pH 5 is found most favorable for the growth of *T. harzianum.* Per cent mycelial growth at pH 5 was 90.00 per cent followed by 86.66 per cent at pH 4, 77.66 per cent at pH 6, 73.00 per cent at pH 7 and there was no mycelial growth at pH 8. This indicates that *T.harzianum* thrives well in acidic soils.

Table 1. Effect of temperature on spore germination, germ tube length and mycelial growth

Temperature (°C)	Spore germination** (%)		Germ tube length after 24 h (mm)	Mycelial growth (mm dia.)***
	After 12 h	After 24 h		
10	0.00 (1.00)	0.00 (1.00)	-	6.66
15	0.00 (1.00)	0.00 (1.00)	-	12.33
20	16.43 (4.16)	37.76 (6.22)	3	32.66
25	23.96 (4.92)	47.96 (6.93)	3.25	48.66
30	6.63 (2.71)	12.76 (3.69)	3	22.00
CD (P =0.05)	1.02	0.97		3.98

*Average of three replications, **Figures in parentheses are square root transformed values
***Figures in parentheses are arc sine transformed values

Table 2. Effect of soil moisture on population dynamics of *Trichoderma harzianum* at 25°C

% soil moisture/ Days	Colony count (cfu x 10^6/g soil)*			
	5	15	20	30
10	6.66	9.33	16.66	9.00
15	11.66	21.33	12.33	4.00
20	13.33	23.66	18.00	6.33
30	17.66	26.00	23.66	12.33
50	4.33	3.00	1.00	00.00
CD (P =0.05)	3.21	8.13	2.57	12.47

Initial population = 5 x 10^5, *Average of three replications

T.harzianum is a promising candidate for the biological control of plant pathogenic fungi. Efficacy of antagonistic *Trichoderma* strains for biological control is influenced by the environmental factors, especially abiotic environmental factors. It was found that at 25°C after 12 h and 24 h, spore germination was significantly high which further improved after 24 h of incubation. However, germ tube length was almost same at 20, 25 and 30°C. Whereas there was no spore germination and the mycelial growth was also least at 10°C and 15°C. Maximum mycelial growth was found at 25°C. Kredics *et al.* (2003) also reported that most of the *Trichoderma* strains are mesophillic and cannot survive at low temperature. Effect of soil moisture levels tested on *T. harzianum* at 25°C revealed that, 30 per cent moisture was found to be optimum, and at this level maximum population of antagonist was retained up to 30 days of incubation followed by 20 per cent moisture. Devi (2005) observed 35 per cent soil moisture optimum and observed up to 60 days. Zaidi and Singh (2004) observed 30 per cent moisture to be optimum for the multiplication of *T. harzianum*. Maximum mycelial growth of *T. harzianum* was observed at pH 5 followed by at pH 4. Jackson *et al.* (1991) reported that optimum biomass production occurred between 20 and 30°C and at pH ranges between 4.6 and 6.8. *Trichoderma* spp. are known to be favoured by acidic environment (Dewan and Sivasithamparam, 1988, Harman, 1991). Jeong *et al.* (1997) observed better rhizosphere competence of *T. harzianum* and *G. virens* in soil at pH 5.0 and 6.0, respectively.

Evaluation of pesticides against T. harzianum Poisoned food technique

Two fungicides (carbendazim, mancozeb), two insecticides (chlorpyriphos and phorate) showed 100 per cent mycelial inhibition at the two/ three concentrations followed by herbicide alachlor which inhibited 96.93, 90.50 and 43 per cent at 0.1, 0.05 and 0.01 per cent respectively (Table 3). Neem based biopesticide, Wanis also showed strong toxicity (88.63 %) at 0.1 per cent concentration. Herbicide pendimethalin and biopesticide Neemgold were comparatively less toxic.

Table 3. Effect of pesticides on the mycelial growth of *Trichoderma harzianum* by poisoned food technique

Treatments	Per cent inhibition in colony diameter*		
	0.1% conc.	0.05% conc.	0.01% conc.
Pendimethalin	72.03 (58.12)	36.93 (37.39)	12.00 (20.17)
Alachlor	96.93 (80.35)	90.50 (72.21)	43.00 (40.90)
Carbendazim	-	100.00 (89.96)	100.00 (89.96)
Mancozeb	-	100.00 (89.96)	100.00 (89.96)
Captaf	-	56.16 (48.56)	18.80 (25.62)
Wanis	88.63 (70.32)	85.60 (67.73)	84.46 (66.86)
Neemgold	60.33 (50.97)	27.13 (31.32)	18.80 (25.62)
Chlorpyriphos	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Phorate	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
CD (P = 0.05)	4.76	4.82	4.67

*Average of three replications, Figures in parentheses are arc sine transformed values, - Not tested

Effect of pesticides on the conidial germination T. harzianum

Among fungicides; carbendazim was most toxic and did not allow spore germination at 0.05 per cent or more concentration each after 24 h of incubation whereas mancozeb and captaf completely inhibited spore germination at 0.1 per cent (Table 4).The insecticide; chlorpyriphos resulted zero per cent conidial germination at 0.1 per cent whereas, herbicide pendimethalin resulted 7.33 per cent germination at 0.1 per cent concentration. Phorate at 0.05 per cent concentration resulted 9.43 per cent conidial germination, where as mancozeb, chlorpyriphos, Neemgold, pendimethalin resulted 11, 11.66, 11.66 and 11.73 per cent conidial germination, respectively and were statistically at par with each other. After 24 h of incubation, conidial germination increased to 15 per cent with chlorpyriphos followed by phorate

Table 4. Effect of pesticides on spore germination of *Trichoderma harzianum*

Treatments	Spore germination (%) at concentrations					
	0.1 %**	0.05%**	0.01%*	0.1 %**	0.05%**	0.01%*
	After 12 h			After 24 h		
Pendimethalin	7.33	11.73	14.40	14.66	26.86	28.83
	(2.64)	(3.51)	(22.26)	(3.52)	(5.23)	(32.36)
Alachlor	13.33	21.66	18.33	15.00	33.33	36.66
	((3.73)	(4.71)	(25.18)	(3.96)	(5.76)	(37.18)
Carbendazim	-	0.00	32.43	-	0.00	34.33
		(1.00)	(34.52)		(1.00)	(22.20)
Mancozeb	-	11.00	16.66	-	25.33	25.00
		(3.43)	(23.66)		(5.06)	(29.67)
Captaf	-	16.66	15.53	-	23.86	21.06
		(4.16)	(23.00)		(4.96)	(27.24)
Wanis	17.76	15.00	28.86	26.63	26.66	35.66
	(4.31)	(3.96)	(31.93)	(5.16)	(5.18)	(36.28)
Neemgold	8.33	11.66	12.00	16.66	16.66	18.00
	(2.77)	(3.54)	(19.75)	(3.71)	(4.19)	(24.95)
Chlorpyriphos	0.00	11.66	11.33	0.00	15.00	21.76
	(1.00)	(3.19)	(18.83)	(1.00)	(3.56)	(27.34)
Phorate	11.63	9.43	21.33	16.10	16.10	24.00
	(3.51)	(3.18)	(27.33)	(4.12)	(4.12)	(29.18)
CD (P =0.05)	(1.73)	(1.47)	NS	NS	(1.81)	NS

*Figures in parentheses are arc sine transformed values
** Figures in parentheses are square root transformed values

(16.10 %), Neemgold (16.66 %), captaf (23.86 %), mancozeb (25.33 %), Wanis (26.66 %), pendimethalin (26.86 %) and alachlor (33.33 %). However, at 0.01 per cent concentration after 12 and 24h treatments did not differ significantly.

Mukhopadhyaya *et al.* (1992) reported the insensitivity of *Trichoderma* and *Gliocladium* species to carboxin and captan. Sharma and Mishra (1995) reported that captan showed comparatively low inhibition of *T. harzianum* than thiram. Jayaraj and Ramabadran (1996) reported the influence on the mycelial growth, sporulation and cellulase production of *T. harzianum*. The insecticides phorate and carbofuran caused maximum reduction of mycelial biomass and also affected sporulation. Mclean *et al.* (2001) reported that *T. harzianum* was least sensitive to proxymidone and captan and most sensitive to mancozeb, tebuconazole and thiram. Glasshouse pot trial further confirmed that *T. harzianum* was sensitive to mancozeb but tolerant to captan. Upadhyay *et al.* (2004) reported that Bavistin and Kribenomyl completely inhibited the growth and sporulation of *T. viride in vitro*. Devi (2005) reported that Wanis completely inhibited the growth of all the bioagents whereas Neemgold and Neemazal caused 49.1 to 100 per cent inhibition at 0.5 per cent and 12.6 – 65.3 per cent inhibition at 0.1 per cent concentration but captan showed less toxicity to all the bioagents. Bavistin resulted in higher inhibition of bioagents followed by Thiram. So may be concluded that bioagent, *T. harzianum* can be integrated with pesticides like captaf, pendimethalin, Neemgold etc., at lower concentration (0.01%), which are not or very less toxic to the bioagent especially for mycelial growth which will reduce the use of pesticides.

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