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Effect of temperature on growth and antagonistic activity of *Trichoderma* spp. against *Macrophomina phaseolina*

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ABSTRACT: **Studies were carried out on the influence of temperature on growth, survival and antagonistic performance of various strains of *Trichoderma* spp. against groundnut dry root-rot pathogen *Macrophomina phaseolina* (Tassi.) Goid. Different isolates of *Trichoderma viride, T. harzianum, T. longibrachiatum, T. hamatum, T. koningii* and *T. pseudokoningii* were employed at various temperatures, *viz.*, 15, 20, 25, 30, 35, 40 and 45°C for studying its cultural behaviour and antagonistic ability. Increased growth of the pathogen (*M. phaseolina*), and growth, sporulation and biomass production of the fungal antagonist (*Trichoderma* spp.) were observed between 25 and 35°C. Antagonistic activity of *Trichoderma* spp. against *M. phaseolina* was decreased with increase in temperature except for *T. pseudokoningii*, which showed maximum inhibition at 35°C. Survival of *Trichoderma* spp. on seed coat was maximum at lower temperature (15°C) than at higher temperature (35°C). At all the temperature regimes *T. harzianum* strain Th-5 has shown higher suppression of the root-rot pathogen, better growth and survival than strains of other species.**

KEY WORDS: *M. phaseolina*, Temperature, *Trichoderma* spp.

INTRODUCTION

Root rot disease of groundnut caused by *Macrophomina phaseolina* is the major constraint in different parts of the country. It is a wide spread soil and root inhabiting pathogen which infects more than 500 species of plants in tropical and sub tropical countries (Ghaffar, 1988). The severity of the disease is felt more in areas where the crop is cultivated under rainfed conditions. Usually the disease occurs in the later part of the crop (flowering/maturity phase) during which the crop experiences the drought and high temperature (Misra and Ghervade, 1983). Due to uneconomic nature of fungicides for rainfed crop and non-availability of effective fungicide for several soil-borne pathogens, biological control has been recognised as the feasible approach to plant disease management.

Different factors such as soil type, temperature, saprophytic survival of the pathogen/ antagonist, etc. are responsible for the success of introduced biocontrol strains in the field. Temperature is one of the major factors, which alters the antagonistic potential of the biocontrol agents against the disease. Previous studies indicated the influence of temperature on growth and antagonistic activity of *Trichoderma* spp. against

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various pathogens (Knudsen *et al.*, 1991; Elad and Krishner, 1993). However, no information is available regarding the influence of temperature on the activity of different *Trichoderma* spp. against *Macrophomina phaseolina*.

MATERIALS AND METHODS

Pathogen

The groundnut pathogen, *Macrophomina phaseolina* (Tassi.) Goid. was isolated from roots of infected groundnut plants showing typical dry root-rot symptoms by tissue-segment method on potato dextrose agar (PDA) medium. Axenic culture of the pathogen was obtained by single hyphal tip method and maintained in PDA slants. As described above different isolates of pathogen were isolated in the samples collected from surveyed areas. After isolation they were purified and tested for pathogenicity. The isolate showing high degree of pathogenicity was selected and used for the study.

Effect of temperature on pathogen

Effect of temperature on the growth of *M. phaseolina* was studied by inoculating 9 mm discs of actively growing pathogen culture in Petri-dishes with PDA. The Petri-dishes were incubated at different temperatures, *viz.*, 15, 20, 25, 30, 35, 40 and 45°C in a BOD incubator. For each treatment three replications were maintained. After 72 hours the colony growth and sclerotial production in 9 mm discs were recorded.

Antagonist

Trichoderma spp., *viz.*, *T. viride* (Tv-4, Tv-6), *T. harzianum* (Th-1, Th-5), *T. longibrachiatum, T. hamatum, T. koningii* and *T. pseudokoningii* were obtained from culture collections of the Department of Plant Pathology, Center for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore and used in this study.

Effect of temperature on *Trichoderma* growth and sporulation

The inoculated Petri-dishes with all the eight *Trichoderma* spp. isolates were subjected to the same temperature regimes as followed for *M. phaseolina*. After 72 hours the colony growth was measured and expressed in mm. The plates were kept under further observation to record the time taken for sporulation, which was assessed based on the appearance of green colour.

Effect of temperature on biomass production of *Trichoderma*

The flasks containing Yeast-molasses medium were inoculated with 9 mm discs of 3 day-old culture of different *Trichoderma* spp. and incubated at various temperatures as mentioned above. After 14 days the contents were filtered through Whatman No. 42 filter paper. The biomass was dried at 45°C for 24h and the values were expressed in mg. The treatments were replicated thrice.

Effect of temperature on antagonism

Antagonistic activity of different *Trichoderma* spp. against *M. phaseolina* at 25, 30 and 35°C was tested by dual culture technique (Dennis and Webster, 1971). The inoculated Petri-dishes were incubated up to 120 hours at above-mentioned temperatures. The growth of *M. phaseolina, Trichoderma* and inhibition zone was measured in mm. Three replications were maintained for each treatment in completely randomized experimental design. The temperatures were chosen where the best growth of pathogen and antagonist were observed.

Effect of temperature on survival of *Trichoderma* by seed treatment

Different *Trichoderma* spp. were grown in molasses yeast medium (Papavizas *et al.*, 1984), formulated in talc powder (Jeyarajan *et al.*, 1994) and used @ 4g/ Kg of seeds. Pelleted seeds with different species of *Trichoderma* in talc formulation were kept at different temperatures, *viz.*, 15, 25 and 35°C. Suitable control without any treatment and three replications were maintained at each temperature. From each treatment 10g seed lots were drawn on 0, 30, and 60 days after treatment and shaken with 90 ml of sterile distilled water. The number of colony forming units (cfu) of each

Trichoderma sp. was estimated by dilution plate method using *Trichoderma* special medium (Elad and Chet, 1983). The values were expressed as cfu x 10^3/g of seed.

RESULTS AND DISCUSSION

Influence of temperature on *M. phaseolina*

Pathogen growth and sclerotial production increased with increase in temperature from 20 to 30°C (Table 1). No growth was observed at 15 and 45°C. Maximum pathogen growth and sclerotial production was obtained between 30 and 35°C. Similarly various workers reported optimum temperature between 30 and 35°C for growth and sclerotial formation (Ratnoo and Bhatnagar, 1991).

Table 1. Effect of temperature on mycelial growth and sclerotial production of *M. phaseolina*

Temperature (°C)	Colony dia (mm) 72 h	Sclerotia/9mm disc 72h
20	36.7 (1.57)	0.0 (0.00)
25	77.0 (1.89)	7.0 (0.88)
30	90.0 (1.96)	122.0 (2.08)
35	90.0 (1.96)	149.0 (2.17)
40	83.0 (1.92)	160.5 (2.20)
CD (P=0.05)	0.04	0.12

Figures in parentheses are log-transformed values.

Influence of temperature on *Trichoderma* spp.

Results revealed that *Trichoderma* growth and activity were influenced by temperature and the response varied with species. At 35°C most of the antagonistic isolates showed decline in growth and this was still reduced at 40°C. Results also indicated that none of the *Trichoderma* spp. isolates grow and sporulate at extremes of 15 and 45°C. Almost all *Trichoderma* spp. showed better growth, sporulation and biomass production between 25 and 35°C (Table 2, 3 & 4). However, *T. Koningii* and *T. hamatum* exhibited significantly higher growth between 20 and 30°C and *T. pseudokoningii* between 30 and 40°C (Table 4). Previous reports also indicated that the optimal growth temperature for *T. koningii* and *T. harzianum* was 25 to 30°C (Hadar *et al.*, 1984).

Influence of temperature on *Trichoderma* antagonism

Antagonistic activity of *Trichoderma* spp. decreased with increase in temperature from 25 to 35°C except for *T. pseudokoningii*, which exhibited increased activity with increase in temperature up to 35°C. However all *Trichoderma* spp. significantly inhibited the growth of *M. phaseolina*. The amount of reduction in pathogen growth ranged from 40 to 72, 44 to 67 and 33 to 67 per cent at 25, 30 and 35°C, respectively. At 25 and 30°C *T. harzianum*-5 exhibited maximum inhibition (Table 5). It supports the findings of Harlapur *et al.* (1988) who reported that maximum activity of *T. viride* and *T. harzianum* exhibited at 30°C at which *Sclerotium rolfsii* was active. Knudsen *et al.* (1991) also reported that incidence of sclerotial colonization of *Sclerotinia sclerotiorum* by *T. harzianum* was higher at 25°C than at 15°C.

Influence of Temperature on *Trichoderma* survival

Experimental results revealed that survival ability of all *Trichoderma* spp. was maximum at cooler temperature (15°C) than at higher temperature (35°C), while *T. pseudokoningii* alone maintained higher population at 35°C. Overall population reduction at 35°C was 6.5 per cent compared to 15°C.

The reduction in population 60 DAT was 7.7, 11.0 and 19.5 per cent at 15, 25 and 35°C respectively. At all temperatures, Tv-6, Th-1 and Th-5 maintained higher population than other species (Table 6). Storage at 5°C was beneficial for retaining viability and biocontrol efficacy of *T. harzianum* (Lewis *et al.*, 1991; Dandurand and Knudsen, 1993).

The results of the study clearly established that the fungal pathogen is able to grow and produce sclerotia at 40°C. While all the antagonistic isolates except *T. pseudokoningii* failed to grow at 40°C or show a reduced growth indicating that at higher

Table 2. Effect of temperature on mycelial growth of *Trichoderma* spp.

Trichoderma spp.	Colony diameter (mm) 72 h					
	20° C	25° C	30° C	35° C	40° C	Mean
T. viride (4)	24(1.40)	38(1.59)	77(1.89)	64(1.81)	35(1.55)	34.1(1.18)
T. *viride* (6)	31(1.50)	90(1.96)	90(1.96)	90(1.96)	68(1.84)	52.7(1.32)
T. harzianum (1)	37(1.58)	90(1.96)	90(1.96)	90(1.96)	45(1.66)	50.3(1.30)
T. harzianum (5)	37(1.58)	90(1.96)	90(1.96)	90(1.96)	45(1.66)	50.3(1.30)
T. longibrachiatum	22(1.37)	35(1.55)	78(1.90)	81(1.91)	58(1.77)	38.8(1.21)
T. hamatum	29(1.48)	38(1.59)	68(1.84)	58(1.77)	38(1.59)	33.2(1.18)
T. koningii	44(1.65)	78(1.90)	90(1.96)	45(1.66)	28(1.47)	40.8(1.23)
T. pseudokoningii	19(1.30)	35(1.55)	67(1.83)	85(1.93)	90(1.96)	42.2(1.22)
Mean	30.5(1.48)	61.9(1.76)	80.9(1.91)	75.4(1.87)	51.0(1.69)	
CD (P=0.05) = Species - 0.02 Soil types - 0.02 Species x Soil types - 0.06						

Figures in parentheses are log-transformed values.

Table 3. Effect of temperature on sporulation of *Trichoderma* spp.

Trichoderma spp.	Days for sporulation				
	20° C	25° C	30° C	35° C	40° C
T. viride (4)	7	4	4	3	5
T. viride (6)	7	3	3	3	4
T. harzianum (1)	6	3	3	3	5
T. harzianum (5)	6	3	3	3	5
T. longibrachiatum	8	4	4	3	4
T. hamatum	7	5	4	5	6
T. koningii	5	3	3	5	6
T. pseudokoningii	9	5	4	4	3

Table 4. Effect of temperature on biomass production of *Trichoderma* spp.

Trichoderma spp.	Dry weight of biomass (mg)					
	20° C	25° C	30° C	35° C	40° C	Mean
T. viride (4)	242(2.38)	332(2.52)	302(2.48)	263(2.42)	182(2.26)	217.4 (1.77)
T. viride (6)	198(2.30)	317(2.50)	364(2.56)	362(2.56)	282 (2.45)	243.4(1.80)
T. harzianum (1)	287(2.46)	432(2.64)	473(2.67)	405(2.61)	245(2.38)	199.1(1.74)
T. harzianum (5)	275(2.44)	422(2.63)	401(2.60)	375(2.57)	232(2.36)	180.0(1.70)
T. longibrachiatum	200(2.30)	325(2.51)	362(2.56)	299(2.48)	208(2.32)	263.1(1.82)
T. hamatum	195(2.29)	295(2.47)	320(2.51)	299(2.48)	152(2.18)	188.6(1.72)
T. koningii	273(2.45)	415(2.62)	371(2.57)	332 (2.52)	200(2.30)	227.3(1.79)
T. pseudokoningii	178(2.25)	308(2.49)	387(2.59)	382(2.58)	320(2.51)	225.1(1.77)
Mean	231(2.36)	355.6(2.55)	372.5(2.57)	339.5(2.53)	227.5(2.34)	
CD (P=0.05) = Species - 0.02 Temperature - 0.02Species x Temperature						

Figures in parentheses are log-transformed values.

Table 5. Effect of temperature on antagonistic activity of *Trichoderma* spp.

Trichoderma spp.	Colony diameter (mm) at temperature (°C)								Inhibition zone (mm)			
	M. phaseolina				*Trichoderma*							
	25	30	35	Mean	25	30	35	Mean	25	30	35	Mean
T. viride (4)	25	38	40	34.3	60	48	45	51.0	5	4	5	4.7
T. viride (6)	30	35	35	33.3	55	51	52	52.7	5	4	3	4.0
T. harzianum (1)	30	36	40	35.3	54	49	47	50.0	6	5	3	4.7
T. harzianum (5)	25	30	45	33.3	60	54	42	52.0	5	6	3	4.7
T. longibrachiatum	40	35	35	36.7	46	51	53	50.0	4	4	2	3.3
T. hamatum	45	50	60	51.7	42	37	28	35.7	3	3	2	2.7
T. koningii	25	32	55	37.3	61	53	33	49.0	4	5	2	3.7
T. pseudokoningii	50	48	30	42.8	38	39	56	44.1	2	3	4	3.0
Mean	33.8	38.0	42.5		52.0	47.7	44.5		4.3	4.3	3.0	

		M. phaseolina	*Trichoderma* spp.	Inhibition zone
CD (P=0.05)	Species	3.6	3.1	1.0
	Temperature	2.2	1.9	0.6
	Species x Temperature	6.3	5.4	1.8

Table 6. Effect of temperature on the survival of spores of *Trichoderma* spp.

Trichoderma spp.	0 DAT	cfu x 10^3/g of seed 60 DAT			
		15° C	25° C	35° C	Mean
T. viride (4)	62(1.79)	57(1.76)	55(1.74)	47(1.67)	57.7(1.76)
T. viride (6)	71(1.84)	64(1.83)	62(1.79)	56(1.82)	66.1(1.82)
T. harzianum (1)	72(1.86)	66(1.82)	65(1.81)	58(1.76)	67.8(1.83)
T. harzianum (5)	68(1.83)	62(1.76)	60(1.78)	54(1.73)	64.1(1.81)
T. longibrachiatum	61(1.79)	55(1.74)	53(1.72)	54(1.73)	57.3(1.76)
T. hamatum	58(1.76)	51(1.71)	50(1.70)	42(1.62)	52.7(1.72)
T. koningii	60(1.77)	63(1.80)	58(1.76)	44(1.64)	58.2(1.76)
T. pseudokoningii	59(1.77)	50(1.70)	49(1.69)	56(1.75)	54.8(1.74)
Mean		58.8(1.77)	56.5(1.75)	51.3(1.71)	

CD (P=0.05) Species - 0.01
Temperature - 0.01
Species X Temperature - 0.01

DAT - Days after treatment
Figures in parentheses are log-transformed values.

temperature regimes the pathogen may take upper hand and the introduced biocontrol agent may not survive. Above results also showed that the *T. pseudokoningii* was able to grow and effectively inhibit the pathogen growth at higher temperature regimes. The same isolate of *T. pseudokoningii* can be utilized at field level to find out its field efficacy against groundnut root rot pathogen under rained / dry conditions.

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