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Variation in spore size of *Trichoderma* and *Gliocladium* in different mass multiplication media

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In the present investigation the effect of different media on the spore shape of *Trichoderma harzianum* and *Gliocladium vireus* was studied. It was observed that the phialospore and chlamydospore of *T. harzianum* and *G. virens* exhibited wide variation in shape and size under different media.

Key words : *Trichoderma harzianum, Gliocladium virens*, media, phialospore, chlamydospore

INTRODUCTION

Trichoderma and *Gliocladium* are the two well known genera for biological control of various soil borne plant pathogens (Papavizas, 1985). Two types of spores are produced by these species viz. phialospores (conidia) and chlamydospores. The shape and size of these two spore types are an useful characters for differentiation of these genera at species level (Hornby, 1990). The chlamydospores has another importance to prolong the survival of the fungus both *in vivo* and *in vitro.* (Caldwell, 1958 ; Lewis and Papavizas, 1983 ; 1984). Usually inexpensive and commonly available agricultural wastes are used for mass multiplication of *Trichoderma* and *Gliocladium* to make the biocontrol economically feasible (Sangeetha and Jeyarajan, 1993 ; Prasad and Rangeshwaran, 2000). Several mass multiplication media have been used successfully for these antagonists (Papavizas and Lewis, 1981 ; Elad *et al.*, 1981 ; Gangadharan and Jeyarajan, 1990 ; Harman *et al.*, 1991) ; but their effect on spore morphology is not well understood. Therefore, this work has been conducted to get a clean idea about the variation in spore shape in different media.

MATERIALS AND METHODS

Various locally available organic materials like well decomposed farm tard manure (F. Y. M.), rice bran (RB), wheat bran (WB), maize meal (MM) etc. with or without supplementing calcium sulphate ($CaSO_4$), ammonium tartarate (AT) and molasses (Papavizas *et al.*, 1984) were prepared for the isolation of *Trichoderma* and *Gliocladium* and purified in potato dextrose agar (PDA) medium.

Various treatments were arranged like T_1 = RB, T_2 = WB, T_3 = MM, T_4 = RB + AT, T_5 = WB + AT, T_6 = MM + AT, T_7 = FYM, T_8 = FYM + Mol., T_9 = FYM + Mol. + At, T_{10} = FYM + Mol + $CaSO_4$, T_{11} = FYM + Mol + $CaSO_4$ + AT. Some of the treatments were supplemented with $CaSO_4$ @ 3g/kg to bring down the pH to acidic level and some other were ammended with molasses (2%) as additinal supplement nutrient and incubated for 10 days to stabilize the nutritional properties. The moisture level was adjusted to 50-60%. The organic media supplemented with various ammendments were sterilised in polypropylene bags and incubated with 6.0 mm diameter mycelial plug of the selected antagonists (*Trichoderma harzianum* and *Gliocladium virens*) taken from the periphery of actively growing 6 days old cultures. The bags were incubated in BOD incubator at 28±1°C for a period of 21 days under 12 hr alternate light and dark conditions.

Slides were prepared from mass multiplication bag and stained with 0.1% lactophenol cotton blue. Spores were measured by a standardized light microscope with the magnification of 100x.

RESULTS AND DISCUSSION

Measurement of phialospores

The results on the Table 1 and Table 2 represented

the micrometric measurements (both length and breadth) of conidia of *Trichoderma harzianum* and *Gliocladium virens* grown on different organic substrates. A scan of the results clearly showed that the phialospores in this experiment could be classified into different morphological shapes like spherical to oval. In gereral, the range of phialospore measurement was higher in *G. virens* than *T. harzianum.* Depending upon the medium phialospores of *Trichoderma* varied significantly both in their length and breadth producing largest spores in FYM containing different additives. But no such variation was observed in case of *Gliocladium.*

Table 1 : Measurements of phialospores of *Trichoderma harzianum* on different substrates.

Treatments	Substrates	Range (µm)		Mean (µm)		L : B ratio
		Length	Breadth	Length	Breadth	
T_1	RB	1.7-3.4	1.7-3.4	2.52	2.01	1.26 : 1
T_2	WB	1.7-3.4	1.7-2.89	2.62	2.25	1.15 : 1
T_3	MM	1.7-3.4	1.7-3.4	2.51	2.18	1.15 : 1
T_4	RB+AT	1.7-3.4	1.7-3.4	2.81	2.38	1.18 : 1
T_5	WB+AT	1.7-3.4	1.7-3.4	2.68	2.31	1.15 : 1
T_6	MM+AT	1.7-3.4	1.7-2.89	2.60	2.26	1.15 : 1
T_7	FYM	1.7-3.4	1.7-3.4	2.54	2.33	1.09 : 1
T_8	FYM+Mol.	1.7-3.4	1.7-3.4	3.15	2.87	1.10 : 1
T_9	FYM+Mol.+AT	2.55-4.55	2.55-4.25	3.23	3.10	1.04 : 1
T_{10}	FYM+Mol.+$CaSO_4$	2.55-4.55	2.55-4.25	3.45	3.26	1.06 : 1
T_{11}	FYM+Mol.+$CaSO_4$+AT	2.55-4.55	2.55-3.4	3.23	2.97	1.09 : 1
	CD(P=0.01)			0.51	0.42	

* Based on the average of 50 observations in different fields.

Table 2 : Measurements of phialospores of *Gliocladium virens* on different substrates.

Treatments	Substrates	Range (µm)		Mean (µm)		L : B ratio
		Length	Breadth	Length	Breadth	
T_1	RB	3.4-5.1	1.7-5.1	4.25	3.49	1.22 : 1
T_2	WB	3.74-5.44	3.06-4.08	4.17	3.33	1.25 : 1
T_3	MM	3.4-5.1	2.6-5.1	4.31	3.75	1.15 : 1
T_4	RB+AT	3.4-5.1	1.7-5.1	4.04	3.31	1.22 : 1
T_5	WB+AT	3.74-5.44	3.06-4.08	4.21	3.34	1.26 : 1
T_6	MM+AT	3.4-5.1	2.6-5.1	4.07	3.37	1.16 : 1
T_7	FYM	3.4-5.1	2.6-5.1	3.95	3.29	1.20 : 1
T_8	FYM+Mol.	3.4-5.1	2.6-4.25	4.29	3.44	1.25 : 1
T_9	FYM+Mol.+AT	2.55-5.1	2.55-4.25	3.82	3.31	1.15 : 1
T_{10}	FYM+Mol.+$CaSO_4$	2.55-5.1	2.55-4.25	4.56	3.70	1.23 : 1
T_{11}	FYM+Mol.+$CaSO_4$+AT	2.55-5.1	2.55-4.25	3.99	3.35	1.19 : 1
	CD(P=0.01)			0.54	0.49	

* Based on the average of 50 observations in different fields.

Measurement of chlamydospores

The chlamydospores produced by *T. harzianum* on different substrates either singly or in combination of different annulments like molasses, $CaSO_4$ and ammonium tartarate followed a similar trend (Table 3). In some of the treatments the chlamydospores were almost round (T_3, T_6, T_7, T_8, T_9,) while in others (T_1, T_2, T_4, T_5, T_{10}, T_{11},) they were elliptical to ovoid. The smallest chlamydospore was produced in T_5 and largest one in T_6 with the dimension of 7.43 × 5.89 µm and 11.42 × 10.11 µm respectively.

Table 3 : Measurements of chlamydospores of *Trichoderma harzianum* on different substrates.

Treatments	Substrates	Range (µm)		Mean (µm)		L : B ratio
		Length	Breadth	Length	Breadth	
T_1	RB	5.1-11.9	5.1-10.3	8.04	6.89	1.17 : 1
T_2	WB	5.1-12.8	3.4-10.2	7.56	6.06	1.25 : 1
T_3	MM	5.95-11.9	5.1-10.2	11.07	9.91	1.12 : 1
T_4	RB+AT	5.1-12.8	5.1-10.3	7.94	6.68	1.19 : 1
T_5	WB+AT	5.1-12.8	3.4-10.2	7.42	5.89	1.26 : 1
T_6	MM+AT	6.1-13.5	5.1-11.5	11.42	10.11	1.13 : 1
T_7	FYM	5.1-15.3	5.1-11.9	10.74	9.42	1.14 : 1
T_8	FYM+Mol.	5.1-15.3	5.1-11.9	10.65	9.34	1.14 : 1
T_9	FYM+Mol.+AT	5.1-15.3	3.4-11.9	9.56	8.45	1.13 : 1
T_{10}	FYM+Mol.+$CaSO_4$	5.1-15.3	5.1-15.3	9.86	7.95	1.24 : 1
T_{11}	FYM+Mol.+$CaSO_4$+AT	5.1-15.3	5.1-15.3	9.86	8.03	1.23 : 1
	CD(P=0.01)			2.08	1.99	

* Based on the average of 50 observations in different fields.

Table 4 : Measurements of chlamydospores of *Gliocladium virens* on different substrates.

Treatments	Substrates	Range (µm)		Mean (µm)		L : B ratio
		Length	Breadth	Length	Breadth	
T_1	RB	6.8-17.0	6.8-13.6	10.965	9.180	1.19 : 1
T_2	WB	5.1-10.2	3.4-8.5	8.967	6.579	1.36 : 1
T_3	MM	6.8-17.2	6.8-8.5	8.840	8.060	1.10 : 1
T_4	RB+AT	6.8-17.0	5.1-13.6	9.521	8.137	1.17 : 1
T_5	WB+AT	5.1-10.2	3.4-8.5	8.993	6.918	1.30 : 1
T_6	MM+AT	6.8-17.5	6.8-10.2	9.235	8.246	1.12 : 1
T_7	FYM	5.1-11.9	5.1-10.2	8.390	7.425	1.13 : 1
T_8	FYM+Mol.	5.1-11.9	3.4-10.2	9.457	8.153	1.16 : 1
T_9	FYM+Mol.+AT	5.1-11.9	3.4-10.2	8.245	7.183	1.15 : 1
T_{10}	FYM+Mol.+$CaSO_4$	5.1-10.2	3.4-8.5	7.967	6.928	1.15 : 1
T_{11}	FYM+Mol.+$CaSO_4$+AT	5.1-11.9	3.4-10.2	8.330	7.395	1.13 : 1
	CD(P=0.01)			2.141	1.823	

* Based on the average of 50 observations in different fields.

Similarly in case of *G. virens* (Table 4) the chlamydospores produced were either elliptical or spherical with the smallest one having dimension of 8.245 × 7.183 µm and largest one was 10.96 × 9.18 µm.

There was no conclusive differences between two genera for chlamydospore size as it was found in case of phialospores. Variations in spores measurement in different mass multiplication media was so wide that morphological study sometimes may create confusion. The nutritional status of different media like pH, C : N ratio, available minerals etc. might have played the major role in such variation. Ammonium tartarate is known to induce chlamydospore production in media. All media produced chlamydospores with or without ammonium tartarate, but no speical role was found out in the size of chlamydospores. However, the importance of spore size on its viability can be an important subject for future study.

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