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Research Notes

Effect of carbon and nitrogen sources on the biomass and spor production of antagonists

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Trichoderma and *Gliocladium* are saprophytic soil fungi, which have been identified as potential biocontrol agents of many important plant diseases. However, the biocontrol treatments with these fungi often give inconsistent and erratic results. This may be due to lack of information concerning the nutritional requirements for the germination and growth. Hence, there is need to define the nutritional requirments of individual biocontrol agents in laboratory culture. Hence this study was undertaken in the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore during 1996-1997 to find the effect of some nutrient factors on the biomass and spore production by the 5 fungal isolates, *T.viride, T.harzianum, T.koningii, T.pseudokoningii* and *Gliocladium virens*.

A basal medium based on Czapek dox solution (Dhingra and Sinclair, 1986) was prepared by mixing appropriate amounts of individual chemical solutions which had been autoclaved (15 min at 121.6°) separately.

The carbon sources *viz.* glucose, glycerol and sucrose were added to the basal medium at the rate of 40g l^{-1} (providing approximately 16g C atoms/lit) as this quantity allowed both biomass and spore production. The nitrogen sources *viz.* ammonium chloride and potassium nitrate was added at the rate of 2g l^{-1} of basal medium providing approximately 0.31 and 0.52g N atoms l^{-1} respectively.

One gram of *T.viride* and *T.harzianum* powder was taken and dissolved in 9ml of sterile distilled water to get 10^{-1} dilution. Likewise serial dilutions were made upto 10^{-6} concentration, which contained 2x105 spores ml^{-1}. Then 1ml of each conidial suspension was inoculated separately into Erlenmeyer flask containing 30ml of medium and incubated for 7 days at 25°C. Three replications were maintained for each of carbon and nitroge sources for each antagonist. After 7 days, th mycelial mat was harvested on pre-weighe filter paper (What man No.1) and dried a room temperature for 48h and the weight wa recorded.

Spore production was also recorded. Fo this the spore along with the medium wa mixed thoroughly and 1ml of the spore suspensio was transferred to 9ml sterile distilled wat and taken upto 10^{-6} dilution and the spo count was made with haemocytometer and th number of spores were recorded for each treatmen separately for carbon and nitrogen sources.

The present study revealed that *T.viride, T.harzianum* and *T.pseudokoningii* recorded the highest mycelial dry weight of 0.36, 0.26 and 0.35g 30 ml^{-1} respectively with sucrose as the carbon source. *T.koningii* and *Gliocladium virens* recorded the highest mycelial growth of 0.38g 30ml^{-1}, 0.53g 30 ml^{-1} respectively. The present finding was in accordance with previous studies on the C-nutrition of *Trichoderma* spp. were capable of utilizing variety of compounds as a sole source of carbon (Danielson and Davey, 1973).

The production of abundant spores was noticed in *T.harzianum, T.koningii* and *T.viride* with all the carbon sources, when compared to other antagonists. However, *T.pseudokoningii* recorded only a slight increase in the spore production when glucose and sucrose were used as carbon source and *G.virens* produced spores only when glucose was used as carbon source (Table 2). It may be suggested that *T.viride, T.harzianum* and *T.koningii* required atleast any one of the carbon source for its spore production irrespective of the mycelial production with other carbon sources.

able 1. Effect of carbon sources on the biomass production of antagonists

arbon ource	Biomass on 7th day (g 30 ml^{-1})					
	T. viride	*T. harzianum*	*T. koningii*	*T. pseudokoningii*	*G. virens*	Pooled mean
one	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
lucose	0.24 (0.09)	0.23 (0.09)	0.29 (0.11)	0.31 (0.11)	0.21 (0.08)	0.25 (0.09)
lycerol	0.05 (0.02)	0.25 (0.09)	0.38 (0.14)	0.00 (0.00)	0.53 (0.18)	0.24 (0.08)
ucrose	0.36 (0.13)	0.26 (0.10)	0.35 (0.12)	0.35 (0.13)	0.28 (0.10)	0.32 (0.12)
ooled ean	0.16 (0.06)	0.18 (0.07)	0.25 (0.09)	0.16 (0.06)	0.26 (0.09)	-

D (P=0.05) Carbon source (0.008)
Antagonists (0.009)
Interaction (0.019)

Figures in the parentheses indicate mean transformed values)

able 2. Effect of carbon sources on the spore production of antagonists

arbon ource	Number of spores (10^6 ml^{-1})					
	T. viride	*T. harzianum*	*T. koningii*	*T. pseudokoningii*	*G. virens*	Pooled mean
one	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
lucose	10.33 (1.05)	90.33 (1.96)	50.33 (1.71)	4.00 (0.69)	3.66 (0.66)	31.73 (1.21)
lycerol	7.00 (0.90)	85.00 (1.93)	70.00 (1.85)	0.00 (0.00)	0.00 (0.00)	32.40 (0.93)
ucrose	10.33 (1.05)	35.33 (1.56)	60.33 (1.78)	3.33 (0.63)	0.00 (0.00)	21.86 (1.00)
ooled ean	691 (0.75)	52.66 (1.36)	45.16 (1.33)	1.83 (0.33)	0.92 (0.16)	-

D (P=0.05) Carbon source (0.02)
Antagonists (0.02)
Interaction (0.05)

Figures in the parentheses indicate mean transformed values)

The studies on the nitrogen sources showed iat *T.viride* and *G.virens* produced the maximum iycelial dry weight with ammonium chloride Table 3). Similar trends were reported by)anielson and Davey (1973) that the growth of *Trichoderma* spp. on NH_4^+N was consistently superior compared to the growth on NO_3^-N. The mycelial weight of *Trichoderma* spp. and *G.virens* was consistently and found superior compared to the growth on NO_3^-N. The mycelial weight of *Trichoderma* spp. increased in media supplemented with 2 g l^{-1} N supplied as NH_4CL and $NaNO_3$ (Watanabe *et al.* 1987). In *T.pseudokoningii*, mycelial dry weight increased when potassium nitrate was supplied as the nitrogen source (Table 3). The result was supported by Hackskaylo *et al.* (1954). Ward and Henry (1961) reported that tests in non buffered media have indicated that NO_3^- was better source of N than NH_4^+. Considering the spore production of antagonists with nitrogen sources, *T.viride* and *T.koningii* produced more spores with NH_4 as the nitrogen source, whereas *T.pseudokoningii* and *G.virens* produced spores with potassium nitrate as N source (Table 4). It may be inferred that the antagonists are essential for spore production.

References

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Table 3. Effect of nitrogen sources on the biomass production of antagonists

Nitrogen source	Biomass on 7th day (g 30 ml^{-1})					
	T. viride	*T. harzianum*	*T. koningii*	*T. pseudokoningii*	*G. virens*	Pooled mea
None	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ammonium chloride	0.04 (0.01)	0.00 (0.00)	0.00 (0.00)	0.07 (0.02)	0.10 (0.04)	0.04 (0.01)
Potassium nitrate	0.02 (0.01)	0.00 (0.00)	0.00 (0.00)	0.09 (0.03)	0.00 (0.00)	0.02 (0.01)
Pooled mean	0.02 (0.01)	0.00 (0.00)	0.00 (0.00)	0.05 (0.02)	0.03 (0.01)	-

CD (P=0.05) Nitrogen source (0.001)
Antagonists (0.001)
Interaction (0.002)

(Figures in the parentheses indicate mean transformed values)

Table 4. Effect of nitrogen sources on the spore production of antagonists

Nitrogen source	Number of spores (10^6 ml^{-1})					
	T. viride	*T. harzianum*	*T. koningii*	*T. pseudokoningii*	*G. virens*	Pooled mea
None	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ammonium chloride	11.00 (1.07)	0.00 (0.00)	4.33 (0.00)	0.00 (0.00)	0.00 (0.00)	3.06 (0.35)
Potassium nitrate	3.67 (0.66)	0.00 (0.00)	2.67 (0.56)	6.00 (0.84)	2.67 (0.56)	3.00 (0.52)
Pooled mean	4.88 (0.58)	0.00 (0.00)	2.33 (0.42	2.00 (0.28)	0.88 (0.18)	

CD (P=0.05) Nitrogen source (0.03)
Antagonists (0.04)
Interaction (0.07)

(Figures in the parentheses indicate mean transformed values)

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