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Biomass Production of *Trichoderma viride* as Influenced by Carbon and Nitrogen Sources

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A B S T R A C T

The biomass production of *Trichoderma* species is greatly influenced by the type of nutritional sources besides environmental factors. To evaluate the influence of nutrient sources on the biomass production of *T. viride* various carbon sources such as peptone, mannitol, glucose and nitrogen sources such as ammonium sulphate, urea and potassium nitrate were used in this study. Among the tested carbon sources, significantly highest biomass production was observed in peptone supplemented media followed by glucose and mannitol. In case of nitrogen sources, the most favoured was ammonium sulphate yielding the maximum biomass. Urea did not appear to show any positive effects on the biomass production. Combined effect of peptone with ammonium sulphate proved to be significant. Based on the present study it can be stated that *Trichoderma* behaves differently to different carbon and nitrogen sources, which can be used as a tool in investigating their ecological niche and nutritional requirements.

Introduction

Popularly known as a biocontrol agent, *Trichoderma* is gaining importance over the years owing to its eco-friendly properties where the harms caused by chemical fertilizers have been a concerned. These groups of fungus provide an active role in supressing diseases which could have otherwise limit the productivity of crops. It has been reported that around 90% of pathogenic fungi controlled are with the application of different strains of *Trichoderma*. *Trichoderma* species are ubiquitous and can be found in all kinds of habitat. Its potential to reduce disease incidence can however be intercepted by environmental or physiological conditions (Papavizas, 1985). Knowledge of such factors is necessary, one of which could be the nutritional requirements in terms of carbon and nitrogen sources. Carbon and nitrogen are equally important to *Trichoderma* species as they are to any microorganisms.

About half of the dry weight of the fungus cells is said to have composed of carbon, making it more likely of its importance to fungal growth (Moore- Landecker, 1996). Multiple findings on the effect of environment factors such as pH and temperature, growth factors, minerals on the biomass production of *Trichoderma* have been investigated (Harman *et al.,* 1991; Jayaswal *et al.*, 2003; Gao *et al.*, 2007; Mehta *et al*., 2012; Rajput *et al.*, 2014, Rajput and Shahzad, 2015; Roy *et al.*, 2015; Rai and Tewari, 2016). Having a thorough background on its nutritional requirements can also serve as a boost to mass production of *Trichoderma*. The present study conducted highlights the influence of different sources of carbon and nitrogen on the growth and sporulation of *Trichoderma viride*.

Materials and Methods

Cultures and media preparation

Cultures of *Trichoderma viride* present in Microbes Research and Production Centre, JNKVV, Jabalpur (MP) was used for the study. Potato dextrose broth was used as the culture media with the composition of 20 g dextrose, 200 g peeled potato in a litre of distilled water. The media was autoclaved at 15 psi for 30 minutes and isolates of *Trichoderma* were inoculated into the broth medium and kept to observe their growth. A treatment combination of 16 consisting of different carbon and nitrogen sources were investigated. The different treatment combinations are taken as followed (Table 1).

Carbon and nitrogen sources

Peptone, glucose, mannitol, ammonium sulphate, potassium nitrate and urea were used as carbon and nitrogen sources in this study. All these carbon and nitrogen sources were added @ 0.2% and 1% concentration respectively in sets of conical flask of 500ml containing the prepared potato dextrose media. The flask where neither carbon nor nitrogen sources was added was taken as control. The flasks were then inoculated with 1 ml of growing cultures of *Trichoderma viride* and cultured for 7 days in room temperature after which the growth and sporulation of *T. viride* was recorded and the effect of different carbon and nitrogen sources as well as their combined effect were analysed.

Biomass production of *T. viride*on different C and N sources

After 7 days of incubation the fungal mycelial mat from each flask were harvested and filtered through What man no 1. The filtrates were discarded and fresh mycelial weight (g/400 ml) for each treatment was recorded, dried at room temperature for 24 hours and dry weight was taken subsequently.

Results and Discussion

Effects of carbon sources on the biomass production

Biomass production of *T. viride* responded differently to different carbon sources used viz., peptone, glucose and mannitol. Media supplemented with peptone gave the maximum biomass production amongst all carbon sources with fresh and dry mycelial mat weight value of 17.4 g and 6.6 g respectively, followed by glucose and mannitol (Table 2 and Fig. 1).

Effects of nitrogen sources on the biomass production

The suitability of various nitrogen sources in enhancing biomass production of *T. viride*was also evaluated. The significantly highest biomass production was recorded on ammonium sulphate amended media. Treatment with urea showed a weak response (Table 3 and Fig. 2).

Combined effects of carbon and nitrogen sources on the biomass production

The combined use of carbon and nitrogen sources showed positive results on the biomass production of *T. viride*. Treatment combination of peptone with ammonium sulphate acted positively in producing the highest biomass yield. Glucose with ammonium sulphate ranked in close with significantly high response compared to the rest of the treatments. However, peptone with urea proved to be a poor combination with the least value of fresh as well as dry mycelial weight (Table 4).

Table.1 Treatment details

Treatment Number	Treatment combination	Treatment Number	Treatment combination
T_1	Control	T_9	Peptone+ potassium nitrate
T_2	Peptone	T_{10}	Peptone+ urea
T_3	Glucose	T_{11}	Glucose + ammonium sulphate
T_4	Mannitol	T_{12}	Glucose + potassium nitrate
T_5	Ammonium sulphate	T_{13}	Glucose+ urea
T_6	Urea	T_{14}	Mannitol+ ammonium sulphate
T_7	Potassium nitrate	T_{15}	Mannitol+ potassium nitrate
T_8	Peptone + ammonium sulphate	T_{16}	Mannitol+ urea

Table.2 Effect of C treatments on biomass production

Treatments	Biomass production	
	Fresh weight (g)	Dry weight (g)
T_1-Control	4.3	0.9
T_2-Peptone	17.4	6.6
T_3-Glucose	14.5	3.8
T_4-Mannitol	8.2	2.9
Mean	*11.1*	*3.6*

Table.3 Effect of N treatments on biomass production

Treatments	Biomass production	
	Fresh weight (g)	Dry weight (g)
T_5-Ammonium Sulphate	14.9	5.1
T_6-Urea	5.5	2.0
T_7-Potassium Nitrate	12.0	5.4
Mean	*10.8*	*4.2*

Table.4 Combined effect of C and N treatments on biomass production

Treatments	Biomass production	
	Fresh weight (g)	Dry weight (g)
T_8-Peptone+Amm. Sul.	17.8	7.1
T_9-Peptone+Pot. Nitrate	14.3	5.2
T_{10}-Peptone+Urea	9.1	2.9
T_{11}-Glucose+Amm. Sul.	15.2	5.5
T_{12}-Glucose+Pot. Nitrate	13.7	4.7
T_{13}-Glucose+Urea	10.4	3.3
T_{14}-Mannitol+Amm. Sul.	14.3	5.4
T_{15}-Mannitol+Pot. Nitrate	12.8	5.7
T_{16}-Mannitol+Urea	9.6	3.4
Mean	*13.0*	*4.8*

Fig.1 Biomass production of *Trichoderma viride* as influenced by carbon sources

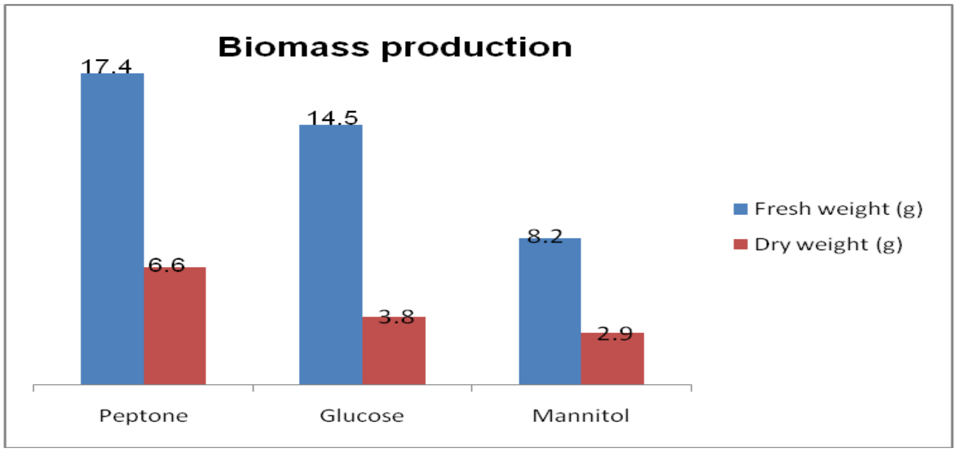
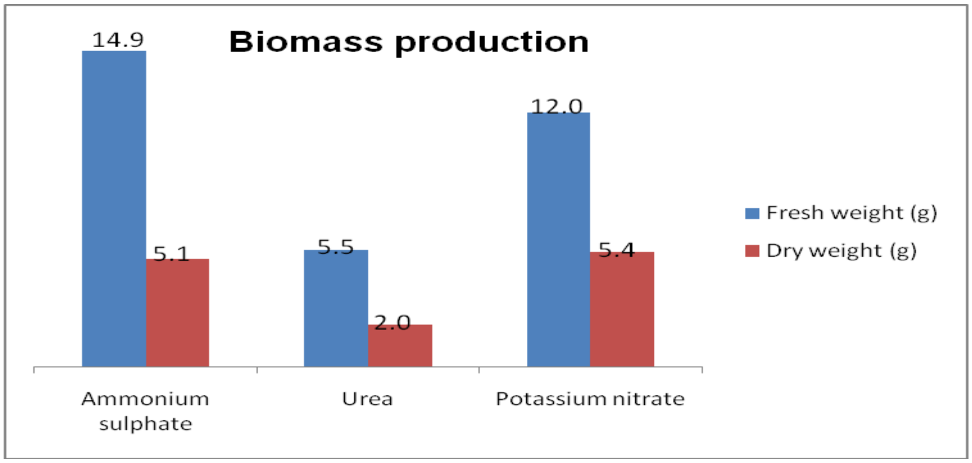


Fig.2 Biomass production of *Trichoderma viride* as influenced by nitrogen sources



The objective of supplementing media with additional carbon and nitrogen sources is a basis in understanding the nutritional requirements of *Trichoderma* species, their reaction to growth, sporulation and shelf life, which are all likely related to their ecological behaviour. Besides that it can also serve as a platform in expanding mass production of *Trichoderma*. Our results showed that peptone was a superior carbon source compared to glucose and mannitol. These could possibly be linked to its structure as being a mixture of peptides and amino acids which contains water soluble vitamins (Cochrane, 1958). This was in conformity with Jayaswal *et al.,* (2003) whose studies declared peptone to be the best carbon source in maximizing growth of *Trichoderma*. Reports by Cochrane, (1958) also stated that peptone was the most favoured carbon source. Contradictorily, others have reported glucose or sucrose to be the best carbon source (Rajput *et al.,* 2014; Abdullah *et al.,* 2005). Monga (2001) picked sucrose and glucose to have superior effects on the biomass production as well as sporulation.

It is noteworthy to state that the ammonium forms of nitrogen showed better result when used as nitrogen source as compared to other forms in the growth, sporulation, biomass production, shelf life of *Trichoderma*, indicating that *Trichoderma* species prefer ammonium forms of nitrogen. This remarkable finding could prove to be a great tool since the use of *Trichoderma* in biocontrol and as biofertilizer is increasingly becoming popular. The present investigation revealed that among all nitrogen sources supplemented media, ammonium sulphate appeared as the best. Our results are in accordance with the findings ofRai and Tewari, (2016) who also reported that the growth and sporulation of *Trichoderma* was most favoured by ammonium sulphate. It can be explained that the uptake of ammonium forms of nitrogen liberates acids and lower the pH (MacNIsh, 1988). *Trichoderma* species are known to have better growth under acidic condition. Therefore lowering of pH with the uptake of ammonium sulphate could serve as a reason for better growth and biomass production of *Trichoderma* species when treated with ammonium as nitrogen source (Nicholas, 1965).Similarly, Mehta *et al.,* (2012) also reported that among the various nitrogen sources used ammonium sulphate showed the highest biomass production. Urea did not do any justice in maximizing biomass production of T. viride. The same was reported by Jayaswal *et al.,* (2003) whose work also indicated the insignificant response by urea.

In case of combined effect, peptone with ammonium sulphate gave the highest biomass production. This could be possibly be due to their combined role in releasing water soluble vitamins and lowering the pH making the condition favourable for the growth and biomass production of *Trichoderma* as given in the statement above.

The results of the present study concluded showed that culture media for *T. viride* added with carbon and nitrogen sources proved to be suitable in enhancing biomass production which has a direct bearing on the growth and sporulation of *Trichoderma* species. It can be concluded that the biomass production was best with peptone as carbon source and ammonium sulphate as nitrogen source. Further investigation, however, is to be instigated since different *Trichoderma* species respond differently to added nutrient and in different concentrations.

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References

Abdullah, F., Nagappan, J. and Sebran, N.H. 2005. Biomass production of *Trichoderma harzianum* (Rifai) in palm oil mill effluents (Pome). *International Journal of Biology and Biotechnology.*, 2(3): 571-575.

Cochrane, V.W. 1958. Physiology of fungi. Publ. John Wiley and Sons.Inc.NY. pp45-49.

Gao, L., Sun, M.H., Liu, X.Z. and Che, Y.S. 2007. Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. *Mycological Research.*, 111(1): 87-92.

Harman, G.E., Jin, X., Stasz, T.E., Peruzzotti, G., Leopold, A.C. and Taylor, A.G. 1991. Production of conidial biomass of *Trichoderma harzianum* for biological control. *Biological Control.*,1:23-28.

Jayaswal, R.K., Singh, R. and Lee, Y.S. 2003. Influence of physiological and environmental factors on growth and sporulation of an antagonistic strain of *Trichoderma viride* RSR 7. *Mycobiology*.,31(1): 36-41.

MacNish, G.C. 1988. Changes in take-all (*Gaeumannomyces graminis* var. *tritici*), rhizoctonia root rot (*Rhizoctonia solani*) and soil pH in continuous wheat with annual application of nitrogenous fertilizer in Western Australia. *Australian Journal of Experimental Agriculture*.,28: 333-341.

Mehta, J., Jakhetia, M., Choudhary, S., Mirza, J., Sharma, D., Khatri, P., Gupta, P. and Nair, M.M. 2012.Impact of carbon & nitrogen sources on the *Trichoderma viride* (biofungicide) and *Beauveria bassiana* (entomopathogenic fungi). *European Journal of Experimental Biology.*, 2(6): 2061-2067.

Monga, D. 2001. Effect of carbon and nitrogen sources on spore germination, biomass production and antifungal metabolites by species of *Trichoderma* and *Gliocladium*. *Indian Phytopathology.*, 54(4): 435-43.

Moore- Landecker, E. 1996. Fundamentals of the fungi. Fourth Edition, Prentice-Hall Inc. USA. P 574.

Nicholas, D.J.D. 1965. Utilization of inorganic nitrogen compounds and amino acids by fungi, pp. 349-376. *In* Ainsworth, G. C. Sussman, A. S. (eds.). The fungi.Vol. 2, Academic Press, New York.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium* against pests. *Journal of Mycology and Plant Pathology*.,33(2):195-199.

Rai, D. and Tewari, A.K. 2016. Evaluation of different carbon and nitrogen sources for better growth and sporulation of *T. harzainum* (Th14). *Journal of Agricultural Biotechnology and Sustainable Development.*,8(8): 67-70.

Rajput, A.Q. and Shahzad, S. 2014. Effect of different substrates and carbon and nitrogen sources on growth and shelf life of *Trichoderma pseudokoningii*. *International Journal of Agriculture and Biology*.,16(5): 893-898.

Rajput, A.Q., Khanzada, M.A. and Shahzad, S. 2014. Effect of different substrates and carbon and nitrogen sources on growth and shelf life of *Trichoderma pseudokoningii. International Journal of Agriculture andBiology.*, 16(5): 893-898.

Roy, M.K., Hembram, S. and Debnath, A. 2015.Effect of different media and pH on growth and sporulation of different native *Trichodema* spp. *The Bioscan.*, 10(4): 1833-1837.

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