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

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**ABSTRACT**

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.

**Keywords** Biomass, *Trichoderma*, Carbon, Nitrogen, Media

**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 suppressing 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.

**Results and Discussion**

**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.

**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                  |
|------------------|----------------------------------------|------------------|----------------------------------------|
| T₁               | Control                                | T₉               | Peptone+ potassium nitrate             |
| T₂               | Peptone                                | T₁₀              | Peptone+ urea                          |
| T₃               | Glucose                                | T₁₁              | Glucose + ammonium sulphate            |
| T₄               | Mannitol                               | T₁₂              | Glucose + potassium nitrate            |
| T₅               | Ammonium sulphate                      | T₁₃              | Glucose+ urea                          |
| T₆               | Urea                                   | T₁₄              | Mannitol+ ammonium sulphate            |
| T₇               | Potassium nitrate                      | T₁₅              | Mannitol+ potassium nitrate            |
| T₈               | Peptone + ammonium sulphate            | T₁₆              | Mannitol+ urea                         |

**Table.2** Effect of C treatments on biomass production

| Treatments       | Biomass production |       |       |
|------------------|--------------------|-------|-------|
|                  | Fresh weight (g)   | Dry weight (g) |
| T₁-Control       | 4.3                | 0.9   |
| T₂-Peptone       | 17.4               | 6.6   |
| T₃-Glucose       | 14.5               | 3.8   |
| T₄-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) |
| T₅-Ammonium Sulphate | 14.9              | 5.1   |
| T₆-Urea          | 5.5                | 2.0   |
| T₇-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) |
| T8 - Peptone + Amm. Sul.     | 17.8               | 7.1        |
| T9 - Peptone + Pot. Nitrate  | 14.3               | 5.2        |
| T10 - Peptone + Urea         | 9.1                | 2.9        |
| T11 - Glucose + Amm. Sul.    | 15.2               | 5.5        |
| T12 - Glucose + Pot. Nitrate | 13.7               | 4.7        |
| T13 - Glucose + Urea         | 10.4               | 3.3        |
| T14 - Mannitol + Amm. Sul.   | 14.3               | 5.4        |
| T15 - Mannitol + Pot. Nitrate| 12.8               | 5.7        |
| T16 - 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 of Rai 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 (MacNlish, 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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