Evaluation of different carbon and nitrogen sources for better growth and sporulation of *T. harzianum* (Th14)

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An investigation was undertaken to evaluate the effectiveness of carbon and nitrogen sources on mycelial growth and sporulation of *Trichoderma harzianum* (Th14). Among the tested different carbon sources viz., jaggery, honey, sugar, dextrose and peptone, significantly maximum biomass and sporulation was observed in honey (1190 mg; 7.06×10⁸) followed by dextrose (1037 mg; 5.27×10⁸) and jaggery (992 mg; 5.50×10⁸) whereas among tested nitrogen sources viz., ammonium sulphate, sodium nitrate, potassium nitrate, urea, ammonium nitrate and calcium nitrate, significant maximum biomass was observed in ammonium sulphate (1035 mg) followed by sodium nitrate (965 mg), and ammonium nitrate (955 mg). However, in sporulation these nitrogen sources were at par with each other. The present result would be helpful in enhancing the conidia and biomass production of local strain and great importance when considering the production of *T. harzianum* for use as a biocontrol agent.

Key words: Carbon sources, nitrogen sources, biomass, sporulation, *Trichoderma harzianum*.

INTRODUCTION

Pathogens cause world-wide economically significant diseases in numerous agricultural, horticultural and ornamental crops. Most of the pathogens are difficult to control by conventional fungicides. Biocontrol represents an economical, environmentally friendly alternative to chemical pesticides for diseases produced by phytopathogens. *Trichoderma* strains have received particular attention as bio control agent of fungal plant pathogens. The effort for isolating and developing the indigenous bio control agents against various soil-borne plant pathogenic fungi continually conducted in several countries for alternative in reducing application of chemical pesticide (Said, 2007). There are some advantageous to apply indigenous bio control agents for solving local plant disease problems, such as naturally, they are available in the local plant rhizospheres, there is no obstacle for climate change and it gives opportunity for domestic income. In order to commercialize these bio control agents for biofungicide, suitable cultural methods and fermentation production systems should be developed, and also the optimal conditions for spore production should be determined.

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Several authors (Lewis and Papavizas, 1983, 1991; Harman et al., 1991; Agosin et al., 1997, Mehta et al., 2012 and Rajput et al., 2014, Rajput and Shahzad, 2015) have studied the effects of environmental factors such as carbon and nitrogen sources, carbon to nitrogen ratio (C/N ratio), pH, and temperature on spore production and spore viability of some strains of T. harzianum. But for new or local strains, such effects should be studied again, because each strain has its own characteristic responses for these factors, besides that, insufficient information is available on the influence of nutritional and culture conditions on the quantity of spore produced and spore viability for industrial purposes.

In general, commercial preparations of Trichoderma sp. for biological control consist of bulk produced conidia, which are the asexual reproductive units of this fungus. Bulk production of conidia typically relies on manipulation of nutrients to promote conidiation of many species of Trichoderma. Carbon and nitrogen sources are important parameter affecting growth and sporulation of Trichoderma. About half of the dry weight of the fungus cells consists of carbon, which gives an indication of the important role of carbon compounds like growth and development of fungal cell (Moore- Landecker, 1996). Our experiment was focused on the effects of different carbon and nitrogen sources on growth and sporulation of T. harzianum in in-vitro.

**MATERIALS AND METHODS**

An experiment was conducted in the Oilseed Pathology Lab, Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) India to study the effect of different carbon and nitrogen sources on growth and sporulation of T. harzianum (Th14). Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were prepared following the standard procedure (Anonymous, 1968). A stock preparation of media, pH of the medium was adjusted to 6.00 by adding N/10, HCl/ NaOH using pH meter. Prepared medium was sterilized in an autoclave at 121°C temperature for 20 min.

**Microorganism**

The Trichoderma harzianum isolate Th14 obtained from culture collections of Bio control laboratory, Department of Plant Pathology, GBPUA and T, Pantnagar for the present investigation. Trichoderma harzianum isolate Th14 have strong antagonistic property and could be exploited for biological product. The fungal antagonist was maintained on potato dextrose agar (PDA) slant and stored in refrigerator for further studies.

**Effect of carbon sources on growth and sporulation of T. harzianum**

Different carbon sources viz., jaggery, honey, sugar, dextrose and peptone at 1.0, 2.0 and 3.0% were added to potato broth (PB) medium without carbon sources the medium was autoclaved. Chloramphenicol at 500 ppm was added to sterilized stock media just before inoculation to inhibit bacterial growth. The flasks (250 ml capacity) containing 100 ml of each sterilized amended carbon medium were inoculated with 5 mm discs (2 No.) cut from 4 days old actively growing culture of T. harzianum (Th14). There were three replications kept for each treatment. The flasks were incubated at 27±1°C for 12 days.

**Effect of nitrogen sources on growth and sporulation of T. harzianum**

Different nitrogen sources viz., ammonium sulphate (NH₄)₂SO₄, sodium nitrate (NaNO₃), potassium nitrate (KNO₃), urea (NH₂)₂, ammonium nitrate (NH₄NO₃) and calcium nitrate Ca (NO₃)₂ were used separately at 0.1 and 0.3 per cent to see its effect on in -vivo growth and sporulation of T. harzianum by the methods as described above.

**Determination of mycelial biomass and sporulation**

After 12 days incubation (DAI) the fungal mycelial mat along with spores in each flask (100 ml broth) of each treatments were separated by Whatman paper No.1. The fungal bio biomass on the filter paper was dried at room temperature for 48 h. Then the fungal dry weight (mg/100 ml) was measured with electronic balance. The collected mycelial mat of each flask was air dried and makes it in fine powder. The prepared powder was properly mixed in their respective flask. One ml of this suspension, well shaken, was added to 9 ml of sterilized distilled water to make 10⁻¹ dilution. The procedure was repeated till the desired dilutions were obtained. The spore concentration was measured by using haemocytometer.

**Statistical concentration**

The experiment was conducted with completely randomized design (CRD) and the experimental data were statistical analyzed using STPR1. Data were subjected to analysis of variance and treatment means were compared by an appropriate Duncan’s multiple range test (P < 0.05) under SPSS 16.

**RESULTS AND DISCUSSION**

**Effect of carbon sources on growth and sporulation of T. harzianum**

The T. harzianum grew well and sporulate on all the carbon sources tested during the present investigation (Table 1). The biomass and sporulation increased with increasing concentrations in amended potato broth media. Biomass and sporulation were measured in (mg/100 ml) and (spores/ ml) respectively. Significant maximum biomass was observed at 3.0% concentration with all the carbon sources compare to 1.0 and 2.0% concentration. Among the different carbon sources, significantly maximum biomass and sporulation was observed in honey (1190 mg; 7.06×10⁸) followed by dextrose (1037 mg; 5.27×10⁸) and jaggery (992 mg; 5.50×10⁸) while minimum in peptone (723 mg; 4.04×10⁸) at 3.0% concentration.

In the present findings, first time honey was used and observed, it is the best carbon sources as compare to
other in biomass and sporulation of *T. harzianum*. There is no report use of honey as a carbon sources for the production of biomass and sporulation of *T. harzianum*. However, present findings are supported by earlier worker Prasad et al. (2002) who reported that jaggery (3%) and wheat flour (10%) enhance conidial yield of *T. harzianum*. Chattannavar et al. (2006) who reported maltose and dextrose as a best carbon source among glucose, galactose, maltose, sucrose, fructose, mannitol, and dextrose tested for growth and sporulation of *T. harzianum*; Sebran and Haida (2008) who reported that *Trichoderma* grow well in liquid medium amended with dextrose, glucose, fructose and sucrose as a carbon source with increased sporulation (1.6×10^6). Mehta et al. (2012) reported dextrose as carbon and ammonium sulfate as nitrogen source for better growth and sporulation of *T. viride*.

Fungi use a large number of organic compounds as a carbon sources and about half of the dry weight of the fungus cells consists of carbon, which gives an indication of the important role of carbon compounds within the cell (Moore- Landecker, 1996). In the present experiment honey showed best as a carbon source, the reason behind may be that honey is a complex carbon source, which have many organic compounds.

### Table 1. Effect of different carbon sources on mycelial growth and sporulation of *Trichoderma harzianum*.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Cultural characters</th>
<th>Spores/ ml* (×10^6)</th>
<th>Dry mycelial weight* (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaggery</td>
<td>Thick mycelial mat with dark green sporulation</td>
<td>3.54^d</td>
<td>6.79^d</td>
</tr>
<tr>
<td>Honey</td>
<td>Thick mycelial mat with very good dark green sporulation</td>
<td>4.81^d</td>
<td>7.06^d</td>
</tr>
<tr>
<td>Sugar</td>
<td>Thick mycelial mat with dark green sporulation</td>
<td>2.95^ab</td>
<td>4.37^ab</td>
</tr>
<tr>
<td>Dextrose</td>
<td>Thick mycelial mat with optimum sporulation</td>
<td>5.50^c</td>
<td>7.27^c</td>
</tr>
<tr>
<td>Peptone</td>
<td>Thin mycelial mat with slight sporulation</td>
<td>2.52</td>
<td>3.89</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td></td>
<td>4.04</td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>9.08</td>
<td></td>
</tr>
</tbody>
</table>

A- Carbon source, B- Concentration; *Mean of three replicates; Values in each vertical column followed by same letter do not differ significantly.

### Table 2. Effect of different nitrogen sources on mycelial growth and sporulation of *Trichoderma harzianum*.

<table>
<thead>
<tr>
<th>Nitrogen source</th>
<th>Cultural characters</th>
<th>Spores/ ml* (×10^6)</th>
<th>Dry mycelial weight* (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH₄)₂SO₄</td>
<td>Thick mycelial mat with very dark green sporulation</td>
<td>9.06^d</td>
<td>10.12^d</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>Thick mycelial mat with very dark green sporulation</td>
<td>8.73^d</td>
<td>9.77^d</td>
</tr>
<tr>
<td>KNO₃</td>
<td>Thin mycelial mat with optimum sporulation</td>
<td>6.06^d</td>
<td>6.79^d</td>
</tr>
<tr>
<td>Urea</td>
<td>Thin mycelial mat and with slight sporulation</td>
<td>8.75^d</td>
<td>10.04^d</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>Thick mycelial mat with good dark green sporulation</td>
<td>7.81^c</td>
<td></td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>Thick mycelial mat with good dark green sporulation</td>
<td>8.29^c</td>
<td></td>
</tr>
<tr>
<td>PDB</td>
<td>Thin mycelial mat with optimum sporulation</td>
<td>5.09</td>
<td>5.13</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td></td>
<td>0.53</td>
<td>0.28</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>2.85</td>
<td>5.65</td>
</tr>
</tbody>
</table>

A- Nitrogen source, B-Concentration.*Mean of three replicates; Values in each vertical column followed by same letter do not differ significantly.

Effect of nitrogen sources on growth and sporulation of *T. harzianum*

Increasing concentrations of nitrogen sources viz., ammonium sulphate, sodium nitrate, potassium nitrate, urea, ammonium nitrate, and calcium nitrate showed positive correlation with the biomass and sporulation of *T. harzianum* (Table 2). Significant maximum biomass and sporulation was observed at 0.3% concentration with all
the nitrogen sources compare to 0.1%. Biomass and sporulation were measured in (mg/100 ml) and (spores/ml) respectively. At 0.3% significant maximum biomass was observed in ammonium sulphate, (1035 mg) followed by sodium nitrate (965 mg) and ammonium nitrate (955mg) and was at par with each other. However, in sporulation these nitrogen sources were at par with each other at 0.3% concentration. Almost similar trends were observed at 0.1%. Minimum biomass in calcium nitrate (766 and 796 mg) and sporulation in urea (6.06×10^5; 6.79×10^5) was observed. 

The present findings are in accordance with the findings of Taweil et al. (2009) who reported ammonium sulphate as nitrogen source for better growth and sporulation of Trichoderma; Abiodun et al. (2012) who reported that growth and sporulation of Trichoderma was greatly favored with the media amended with NaN_3, peptone and NH_4SO_4 as a nitrogen source. Shiesole and Mishra (2014) reported that ammonium sulphate was the most suitable nitrogen source in term of sporulation among the tested nitrogen sources such as urea, potassium nitrate, ammonium chloride, ammonium nitrate and sodium nitrate.

**Conclusion**

In conclusion, this study showed that Trichoderma harzianum (Th14) has the ability to use a variety of carbon and nitrogen sources for good biomass and spore production at different levels. The biomass and sporulation was best with honey as carbon source and ammonium sulphate as nitrogen source and this is of great importance when considering the production of T. harzianum for use as a bio control agent.

**Conflict of Interests**

The authors have not declared any conflict of interests.

**ACKNOWLEDGMENTS**

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