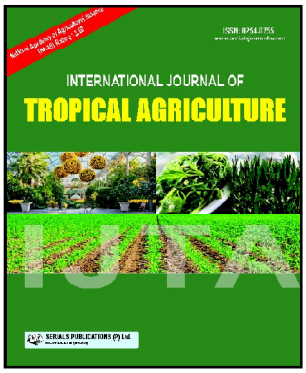


INTERNATIONAL JOURNAL OF TROPICAL AGRICULTURE

ISSN : 0254-8755

available at http: www.serialsjournal.com



Volume 35 • Number 4 • 2017

# Impact of Different Carbon Sources on Mutants of *Trichoderma*.

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***Abstract:*** Out of seventeen isolates of *Trichoderma*, T1 and T7 isolates were selected for the development of mutants on the basis of highest antagonistic efficacy against *Sclerotium rolfsii* and *Macrophomina phaseolina* pathogen of groundnut and mungbean respectively. Conidial suspension with concentration of $10^7$ cfu/ml was treated with ethylmethyl sulfonate with five concentrations @ 50, 75, 100, 125 and 150 µl/ml for 30 and 60 minutes. Selected single cell colonies were again treated with 0.1 and 0.2% colchicine. On the basis of highest antagonism 5 mutants were selected for estimation of the mycelial growth and sporulation of *Trichoderma* by using different carbon sources was done. From this study, it was observed that three mutants of *Trichoderma* ($M_6$, $M_{12}$ and $M_{10}$) showed higher mycelial growth and mycelial dry weight potential on fructose, whereas mannitol was found efficient to support maximum sporulation.

***Key words:*** Trichoderma, mutants, Czapeck Dox Broth, nutrients

## INTRODUCTION

Soil borne pathogens have a broad host range and persist for longer periods in soil by resistant resting structures. Chemical control of soil borne pathogen provides certain degree of control but at the same time have adverse effects on environment affecting the beneficial soil microorganisms. Therefore biological control of plant pathogens has been consider as a potential control strategy in recent years and search for these biocontrol agents is increasing. *Trichoderma* is the most commonly used fungal biological control agent and have long been known

as effective antagonists against plant pathogenic fungi (Papavizas, 1985, Kumar and Mukerji, 1996). The knowledge of nutritional requirements is the main need in the cultivation of microorganisms using any cultural technique. The carbohydrates, proteins, lipids, nucleic acids are made up of macro elements like carbon, hydrogen, nitrogen, sulphur, phosphorus and these are involved in mechanisms like host pathogen interaction and self defense mechanisms. Carbon is a major component and the molecules of carbon also contribute to oxygen and hydrogen.

## MATERIALS AND METHODS

Conidiospores of 8 days old cultures were collected and incubated in 0.2M phosphate buffer of 8.0 $P^H$ containing 50,75,100,125 and 150 il ethyl methane sulfonate (Sigma) per ml phosphate buffer for 30 and 60 min with the spore concentration of $10^7$ cfu/ml placed on YMG agar medium. The plates were incubated at 28± $2^0$C for two days. The colonies developed from single spore were selected and were inoculated in conical flasks containing 50ml Natick medium and incubated at 28±2 $^0$C with shaking at 120rpm for 18hr. The conidia were treated with 0.1 and 0.2% (w/v) colchicine (Loba-Chemic ind) and incubated at $28^0$C with shaking at 200rpm for 7 days. The 0.1 ml of dilution of the conidia were plated on medium containing 0.1% (v/v) Triton X-100 and incubated at $28^0$C for six days. Accordingly among two mother cultures $M_1$ and $M_7$, five and eight mutants possessing high antagonistic activity were selected for further studies.

Effect of various carbon sources was assessed altering the amount of carbon source (sucrose) in Czapek's Dox broth medium. Substitution of sources was made on molecular weight basis in the basal medium.The amount of nitrogen source (2.0 g) was kept constant and carbon source was changed. Sucrose was substituted with other carbon sources like dextrose, fructose, cellulose and mannitol on molecular weight basis. The observations were recorded on the basis of radial mycelial growth, mycelial dry weight and sporulation (number of spores per ml).

### Radial mycelial growth

Czapek's-Dox agar medium with different carbon sources were inoculated with the respective *Trichoderma* mutants by keeping 6mm disc in the plate. These plates were incubated at room temperature and radial mycelial growth was recorded on 3$^{rd}$, 5$^{th}$, and 7$^{th}$ day.

### Mycelial dry weight

Czapek's-Dox broth with different carbon and nitrogen sources were inoculated with respective *Trichoderma* individually. The culture was incubated for 10 days at room temperature and filtered through Whatman paper no. 40. The mycelial mat was air dried and kept in oven far 60 $^0$C for 1 hour. The oven dried weight of the mycelium of individual *Trichoderma* were noted separately.

### Estimation of spores

Haemocytometer was cleaned with alcohol and placed 0.1ml of well suspended spore suspension at the centre and covered with cover glass. The preparation was allowed to stand for 2 min before counting so that spores settle to the bottom of the square. Five squares were chosen randomly and the spores inside the square were counted. The spore load was calculated.

## RESULTS AND DISCUSSION

The radial mycelial growth of the five selected *Trichoderma* mutants on various carbon sources was studied by growing the *Trichoderma* mutants on Czapek's Dox medium, which was used as basal medium. The experiment was conducted to study the ability of different mutants to utilize carbon as essential element from different carbon soruces as its utilization depends on enzyme system.

## Mycelial growth

Significant differences were observed among the different manosaccharides and diasaccharides utilized by the mutants of *Trichoderma*. Fructose was found to support the maximum growth of three mutants viz, $M_6$, $M_{12}$ and $M_{10,}$ and it was between the range of 77.78 to 88.89 mm at 7th DAI whereas cellulose supported to $M_6$ and $M_{15}$ i.e. 90.00 and 83.67 mm radial mycelial growth. Dextrose supported minimum radial mycelial growth of three mutants, whereas $M_{11}$ and $M_{10}$ exhibited minimum growth on sucrose and cellulose respectively (Table 1).Other sources of carbon indicate the variation among the mutants. After seventh day of incubation mannitol was preferred for their growth and multiplication by all the five test mutants as a next best source.

## Mycelial dry weight

Among the various carbon sources fructose was found to support maximum mycelial weight of two mutants of *Trichoderma* viz. $M_{10}$ and $M_{12}$ and it was 0.48 and 0.67g respectively where as dextrose preferred by $M_6$ and $M_{15}$ and it was in the range of 0.34 to 0.83 g. The second best carbon source was mannitol for all the five isolates (Table 1).

## Spore count

A carbon source mannitol was found efficient to support maximum sporulation of $M_{11}$ and $M_{15}$ and it was 138.4 x $10^6$ and 98.23 x $10^6$/ml, sucrose for $M_6$ (53.33), dextrose and cellulose for $M_{10}$ and $M_{12}$ and it was 65.36 and 125.25 x $10^6$/ml respectively (Table 2). It indicates that every mutant had an ability to utilize energy from varied types of carbohydrates. Though, the large preference was not achieved by the mutants but it is an indication that diversity exists among the mutants of *Trichoderma* on the basis of biosynthesis of carbon sources.

These findings are on the line of results of Danielson and Davey (1973) reported that ammonium nitrate was best nitrogen source for three species of *Trichoderma*. The present results corrborates the work carried out by Sharma and Mishra (1995) in respect to *Trichoderma harzianum*. Prasad and Rangeshwaran (2000) reported sucrose as a carbon source increases biomass production in

**Table 1**
**Effect of carbon sources on the radial mycelial growth and mycelial dry weight of *Trichoderma* mutants**

| *Mutant* | *Sucrose* | | *Dextrose* | | *Fructose* | | *Cellulose* | | *Mannitol* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Radial mycelial growth (mm)* | *Mycelial dry weight (g)* | *Radial mycelial growth (mm)* | *Mycelial dry weight (g)* | *Radial mycelial growth (mm)* | *Mycelial dry weight (g)* | *Radial mycelial growth (mm)* | *Mycelial dry weight (g)* | *Radial mycelial growth (mm)* | *Mycelial dry weight (g)* |
| $M_6$ | 88.56 | 0.13 | 77.55 | 0.78 | 88.89 | 0.45 | 90.00 | 0.09 | 89.44 | 0.14 |
| $M_{10}$ | 72.56 | 0.21 | 77.78 | 0.49 | 81.11 | 0.67 | 70.00 | 0.09 | 80.00 | 0.21 |
| $M_{11}$ | 70.00 | 0.18 | 59.89 | 0.34 | 77.78 | 0.34 | 73.89 | 0.18 | 77.56 | 0.21 |
| $M_{12}$ | 63.22 | 0.08 | 76.33 | 0.34 | 85.56 | 0.48 | 80.78 | 0.19 | 82.67 | 0.19 |
| $M_{15}$ | 75.00 | 0.19 | 50.33 | 0.83 | 79.44 | 0.52 | 83.67 | 0.18 | 81.44 | 0.22 |
| "F" test | Sig. | Sig | Sig. | N.S | Sig. | N.S | Sig. | Sig | N.S | N.S |
| SE(m) ± | 2.74 | 0.02 | 3.80 | 0.13 | 1.57 | 7.99 | 1.58 | 1.06 | 1.95 | 3.59 |
| CD(P=0.01) | 11.50 | 0.07 | 15.98 | | 6.60 | | 6.62 | 4.44 | | |

**Table 2**
**Effect of carbon sources on the sporulation of *Trichoderma* mutants ( 7th Day after inoculation)**

| *Mutant* | *Spore count 10⁶* | | | | |
|---|---|---|---|---|---|
| | *Sucrose* | *Dextrose* | *Fructose* | *Cellulose* | *Mannitol* |
| $M_6$ | 53.33 | 41.87 | 42.11 | 19.18 | 15.33 |
| $M_{10}$ | 45.16 | 65.36 | 51.26 | 49.61 | 59.90 |
| $M_{11}$ | 48.08 | 90.94 | 115.46 | 103.41 | 138.41 |
| $M_{12}$ | 19.35 | 15.84 | 15.92 | 125.25 | 20.88 |
| $M_{15}$ | 97.83 | 10.98 | 71.00 | 73.01 | 98.23 |
| "F" test | Sig. | Sig. | Sig. | Sig. | Sig. |
| SE(m) ± | 2.02 | 0.63 | 4.51 | 5.06 | 2.45 |
| CD (P=0.01) | 8.50 | 2.65 | 18.96 | 22.28 | 10.29 |

*Trichoderma harzianum*. Lalitha *et al.,(*2012) studied the combination effect of supplementary carbon and nitrogen sources in the production of amylases by *T. viride*, in solid state fermentation using corn cob residue as the substrate and observed that potential of solid state systems is immense when the combination of fructose and sodium nitrate as supplementary carbon and nitrogen sources. Jitendra Mehta *et al*., (2012) in *in-vitro* study, observed that *T. viride* showed higher biomass product in dextrose as a carbon source that is 25.15g. Mostafa *et al.,* (2012) recorded that nitrate potassium, as a best nitrogen source for *T. harzianum* isolate,T8, T14 while nitrate ammonium for T7, whereas regarding carbon sources arabinose was best carbon source for T7, fructose for T14 and mannitol for T8.

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