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Impact of Carbon & Nitrogen Sources on the *Trichoderma viride* (Biofungicide) *and Beauveria bassiana* (entomopathogenic fungi)

Jitendra Mehta*, Madhulika Jakhetia, Sudha Choudhary, Jishan Mirza, Dilip Sharma, Pawan Khatri, Priyanka Gupta, Meenu M. Nair

Microbiology & Pathology Division, Vital Biotech Research Institute
University of Kota, Kota- 324009 (Rajasthan) India.

ABSTRACT

The in-vitro study of the mycelial growth and sporulation of T.viride and B.bassiana and the effect of different carbon and nitrogen sources on the Biofungicide *and entomopathogenic fungi is done with the availability of Czepeck Dox Broth media. From this study, it was concluded that T.viride and B.bassiana showed higher growth potentials on almost all the nutrient sources studied, the nitrogen sources used in Czepeck Dox media are Sodium Nitrate, Potassium Nitrate and Ammonium Sulphate. In these nitrogen sources, T.viride showed the high biomass product in Ammonium Sulphate that is 25.68g where as B.bassiana showed high growth of biomass product in Potassium Nitrate which is 29.96g. In carbon sources like Fructose, Lactose and Dextrose, T.viride showed high growth of biomass product in Dextrose which is 25.15g where as B.bassiana showed high growth of biomass product in Fructose as 24.16g.*

Key words: *T.viride*, nutrients, germination, Czepeck Dox Broth, *B.bassiana*.

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 pathogens 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 considered as a potential control strategy in recent years and search for these biological 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 [1, 2, 3, 4].

Biopesticides based on bacteria, viruses, entomopathogenic fungi and nematodes are often considerable scope as plant protection agents against several insects [5]. Use of entomopathogenic fungi as biological control agents for insect species has increased the global attention during the last few decades. The mycoinsecticide based on *Beauveria bassiana* (Balsamo) Vaillemin [6, 7], *Paecilomyces fumosoroseus* (Wize) Brown and Smith [8, 9] and *Verticillium lecanii* (Zimm.) Viegas [10] have been used to control various insect pests. Production of adequate quantities of a good quality inoculum is an essential component of the biocontrol programme. The production of entomopathogens may be taken up by the following methods based on the quantity of the product desired: 1)

relatively small quantities of the inoculum for laboratory experimentation and field-testing during the development of mycopesticide and 2) development of a basic production system for large-scale production by following the labour intensive and economically viable methods for relatively small size markets. China [11] and America [11, 12] supply fungal pathogens by this method in sufficient quantities for niche markets in their immediate area. Development of simple and reliable production system follows the basic multiplication procedures of submerged liquid fermentation for the production of blastospores, which are short lived, and hydrophilic [13] or solid state fermentation [14] for the production of aerial conidia. However, the most viable mass production technologies include making use of a diphasic strategy in which the fungal inoculum is produced in liquid culture, which is further utilized for inoculating the solid substrate(s) for conidia production [15].

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 defence mechanisms. Carbon is the major component and the molecules of carbon also contribute to oxygen and hydrogen. The effect of the nutrient sources on the growth and development of microorganisms is studied by [16]. The fungus which is used and the media components used are responsible for the mycelial growth and spore yield. Although the saprophytic fungi utilize a range of nutrient sources but for the mass production and commercialization, simple and cheap media needed [17]. For the full growth of microorganisms, the macro elements like carbon, hydrogen, oxygen, sulphur, phosphorus and nitrogen are required which are the components of carbohydrates, nucleic acids and proteins The growth characteristics in addition to growth substances are useful in the tolerance selection studies. The preparation used for the growth, storage and transport of microorganism can be used in solid [18] or liquid form [19]. The media should have all the nutritional requirements for the growth of microorganisms. The study is done on fourteen isolates of *Trichoderma viride* and seventeen isolates of *Beauveria bassiana* and the effect of nutrient sources on the growth, sporulation, and conidial germination are examined in different media containing carbon and nitrogen sources in ratio. The media which supports the best growth is very effective for the low cost production of Entomopathogenic fungi. The main objective of this work has been the development of low cost method for the propagation of the fungi which yields high inoculums levels and thus results in high mass production.

MATERIALS AND METHODS

Entomopathogenic and Biofungicide culture

The cadavers of the insects that appeared to be infected by fungi were collected during survey and brought to the laboratory and pathogens were isolated on specific media. To isolate the fungi, mycosed insects collected from the fields were surface sterilized with 5 per cent sodium hypochlorite and then rinsed with sterile water several times. In a sterile petridish, the diseased specimens were crushed and a small portion of infected part was transferred to a culture plate containing selective medium and kept under constant Observation for the growth and development of microorganisms. After 5 days of incubation, the organisms were sub-cultured for purification. Slants of each culture were prepared from purified culture and microscopic observations such as morphological characters of mycelium and conidia.

Maintenance of culture

A loopful of inocula from sub cultured plates of *T.viride* and *B.bassiana* were transferred to Potato Dextrose Agar (PDA) slants and maintained as pure culture.

For laboratory studies, the fungus was cultured on PDA medium. The medium was sterilized at 15 psi for 20 min at 121 ºC in autoclave, poured to sterilized plates, cooled and inoculated with pure culture of the fungus under aseptic conditions. The plates were then incubated at room temperature (25±2°C) for ten days. After complete sporulation, conidia from the medium were harvested by washing them thoroughly with sterilized water containing Tween-80 (0.2%) for immediate use. Otherwise, spores were harvested with the help of a small sterile metal spatula. Harvested conidia were air dried under laminar air flow and stored in a small air tight screw cap vials (10 cm with 2.5 cm diameter) in refrigerator at 4°C before using for further studies. Colony forming units (cfu) were estimated by plating technique. Suspension of spores was made using distilled water with Tween-80 (0.2%) and filtered through a double layered muslin cloth. Spore count was made using a double rolled Neubauer's haemocytometer after necessary serial dilutions under phase contrast microscope. From the stock solution, further dilutions were made to obtain the required concentrations for further studies.

Culture media

Culture media representing disparate carbon and nitrogen sources and ratios were used in the study of the entomopathogenic fungi. This media is used for the growth of culture of desired microorganisms. First of all, the desired microorganisms are selected and screened from MTCC, Chandigarh. From this culture collection centre, the pure culture of desired microorganisms *Trichoderma viride* and *Beauveria bassiana* are obtained.

Then the mother plate of desired microorganisms has to be formed. For this Potato Dextrose Agar (PDA) medium is prepared. Then the mother plates are prepared. The slants of the desired microorganisms are also prepared. Then we examined the macroscopic and microscopic features of *Trichoderma viride* and *Beauveria bassiana*. After this the different media are prepared with different concentrations of different constituents. The media which is used to study the impact of carbon and nitrogen sources on entomopathogenic fungi is Czepeck Dox Broth Medium with different concentrations of different constituents. The pH of all the media was maintained at 7.0 and was sterilized at 121°C at 15 lbs for 20 min.

The first Czepeck Dox Broth Medium is prepared in which the main constituent is sodium nitrate. The sodium nitrate is used in concentration of 1g, 1.5g, and 2g per liter in different conical flasks respectively. The second Czepeck Dox Broth Medium is prepared with main constituent with potassium nitrate with concentration 1g, 1.5g, 2g per liter respectively. The third Czepeck Dox Medium prepared to study the impact of nitrogen sources consist of ammonium sulphate as the main constituent in concentration of 1g, 1.5g, and 2g respectively.

Then the fourth Czepeck Dox Medium is prepared to study the impact of carbon source with main constituent lactose in concentration of 10g, 20g, and 30g per liter. The fifth Czepeck Dox Medium is prepared with fructose as major constituent in concentration of 10g, 20g, and 30g per liter. The sixth media is prepared with dextrose as main constituent with same range of concentrations of 10g, 20g, and 30g per liter.

After the media preparation is done, the inoculation has to be performed. The pure cultures of *Trichoderma viride* and *Beauveria bassiana* are then inoculated in different concentrations of media prepared and allow them to incubate for 3 weeks. The growth of fungus starts in media and the results are examined after every week of the inoculation. The same process of examining is repeated in second and third week. After three weeks are finished, the filtration of the media with biomass of fungus is done and the weight of the biomass is measured to study the impact of nitrogen and carbon sources on the entomopathogenic fungi.

RESULTS AND DISCUSSION

In the present study, Czepeck Dox Broth media with different concentration of constituents are used to study the impact of carbon and nitrogen sources on *Trichoderma viride* and *Beauveria bassiana*. The impacts of carbon and nitrogen sources are studied on the basis of the total biomass production of the fungi on *T. viride* and *B. bassiana*. These results are shown in the Tables 1, 2 and Fig. 1, 2 for *T. viride* and Tables 3, 4 and Fig. 3, 4 for *B. bassiana*.

Effect of carbon and nitrogen sources on the biomass production of *T. viride* (Biofungicide) and *B. bassiana* (entomopathogenic fungi)

The Czepeck Dox media with nitrogen sources showed the high biomass product formation of *Trichoderma viride* in ammonium sulphate with 25.68g. It is followed by Potassium Nitrate which produces 23.34g of biomass product. In sodium nitrate as constituent, the total biomass product formation is 22.20g. While *Beauveria bassiana* showed the highest biomass product formation in Sodium Nitrate with 31.43g. It is followed by Potassium Nitrate which produces 29.96g of biomass product. In Ammonium Sulphate as constituent, the total biomass product formation is 27.50g.

The Czepeck Dox media with carbon source showed the high biomass product formation of *Trichoderma viride* in Dextrose as the main constituent with 25.15g. It is followed by Fructose which produces 24.99g of biomass product. In Lactose as constituent, the total biomass product formation is 20.24g. While *Beauveria bassiana* showed the highest biomass product formation in Fructose with 24.16g. It is followed by Dextrose which produces 21.53g of biomass product. In Lactose as constituent, the total biomass product formation is 19.76g.

Table 1: Total biomass production of *T.viride* as influenced by nitrogen source

Nitrogen Source	Total biomass content(g/250 ml)			Mean
	1g/l	1.5g/l	2g/l	
Sodium nitrate	9.13	8.75	12.96	22.20
Potassium nitrate	10.19	9.79	10.09	23.34
Ammonium sulphate	10.64	11.33	11.13	25.68

Table 2: Total biomass production of *T.viride* as influenced by carbon sources

Carbon Source	Total biomass content(g/250 ml)			Mean
	10g/l	20g/l	30g/l	
Fructose	10.74	10.79	10.40	24.99
Lactose	8.41	8.90	8.80	20.24
Dextrose	10.17	11.15	11.49	25.15

Table 3: Total biomass production of *B. bassiana* as influenced by nitrogen sources

Nitrogen Source	Total biomass content(g/250 ml)			Mean
	1g/l	1.5g/l	2g/l	
Sodium nitrate	13.30	13.10	15.10	31.43
Potassium nitrate	13.60	12.80	10.70	29.96
Ammonium sulphate	12.00	11.70	11.60	27.50

Table 4: Total biomass production of *B. bassiana* as influenced by carbon sources

Carbon Source	Total biomass content(g/250 ml)			Mean
	10g/l	20g/l	30g/l	
Fructose	9.20	12.20	8.30	24.16
Lactose	9.10	8.60	6.20	19.76
Dextrose	8.20	9.50	11.50	21.53

Figure 1: Total biomass production of *Trichoderma viride* as influenced by nitrogen sources

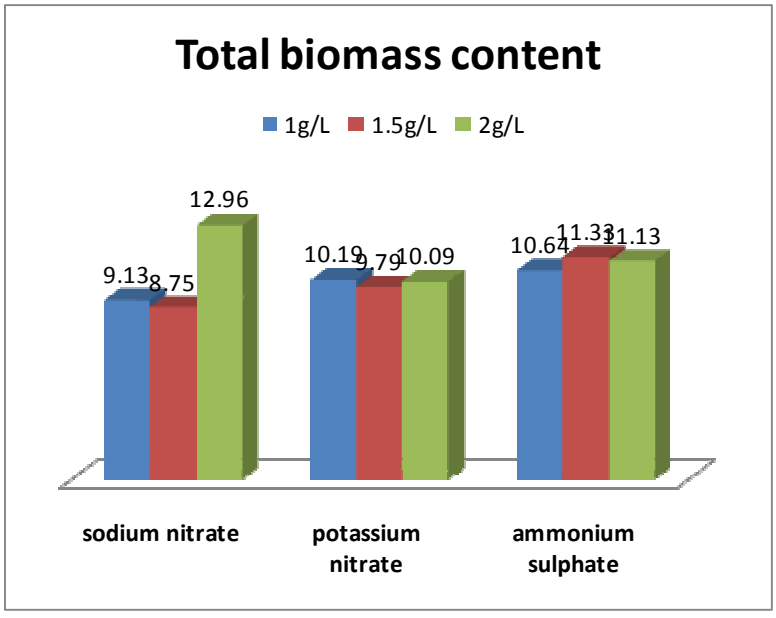


Figure 2: Total biomass production of *Trichoderma viride* as influenced by carbon sources

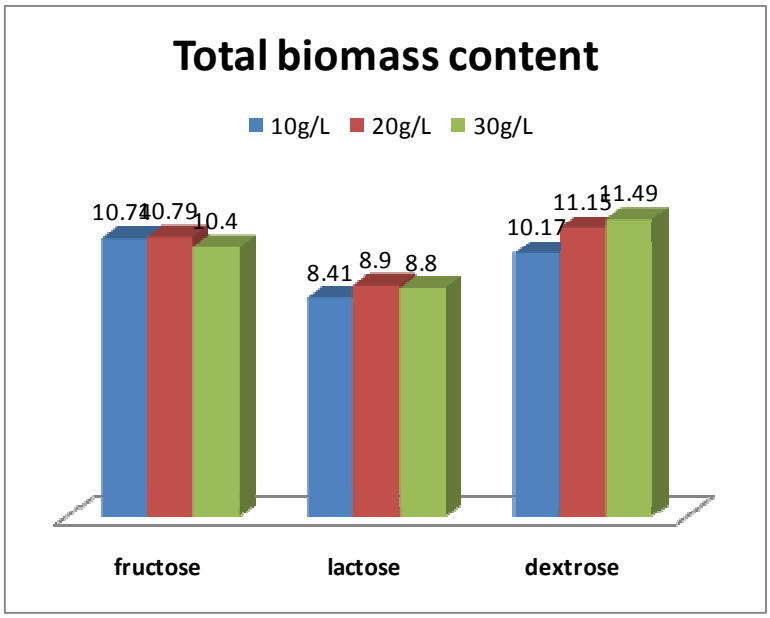


Figure 3: Total biomass production of *Beauveria bassiana* as influenced by nitrogen sources

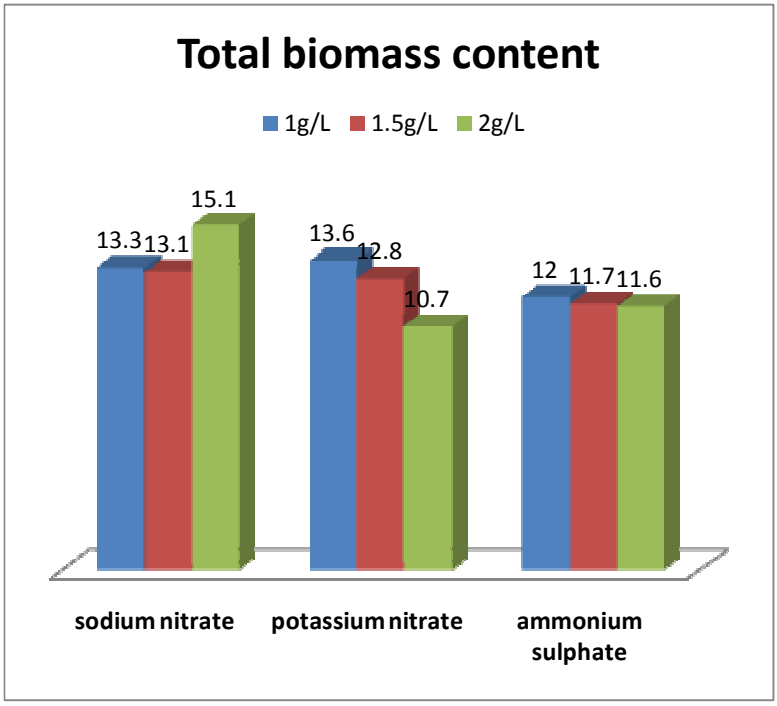
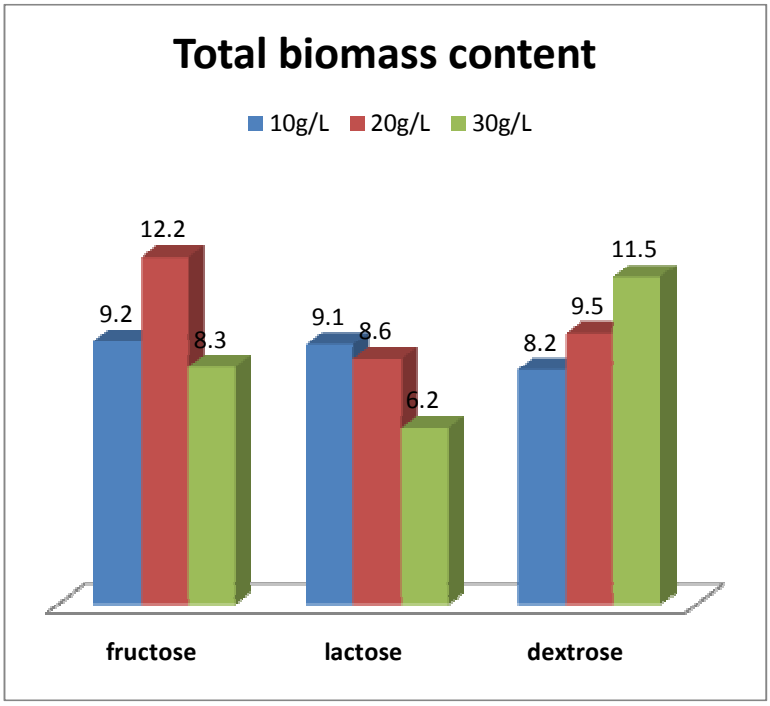


Figure 4: Total biomass production of *Beauveria bassiana* as influenced by carbon sources



CONCLUSION

After the green revolution, the use of chemical pesticides have not only decreased the overall fertility of soil, but also polluted the environment. These have been proven beneficial for crop only once but devastating for the field forever. In turn, Biofungicide and Entomopathogenic fungi not only increase the fertility of soil, but also are eco-friendly, non hazardous and do not affect the other beneficial microorganisms. The quantity of both Biofungus and Entomopathogenic fungi to be used are also meager than that of chemical pesticides and are easy to apply. As from all above methods we have concluded that as compared to pearl millet and carrot, yeast medium have a high growth rate for mass production of Biofungicide (*Trichoderma viride*) and entomopathogenic (*Beauveria bassiana*) fungi which have a good role in biopesticide and agriculture production.

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