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Impact of carbon & nitrogen sources on the *Verticillium lecanii and Metarhizium anisopliae*- Entomopathogenic fungi

Jitendra Mehta*, Neha Kaushal, Priya Sen, Dev Ratan Sharma, Maninder Singh Dhillon and Banwari Lal Mathuriya

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

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ABSTRACT

The in-vitro study of the mycelial growth of M. anisopliae and V. lecanii and the effect of different carbon and nitrogen sources on the entomopathogenic fungi is done with the avalibility of Czepeck Dox media. From this study, it was concluded that M. anisopliae and V. lecanii 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, M.anisopliae showed the high biomass product in Potassium Nitrate is 28.20g where as V. lecanii showed high growth of biomass product in Sodium Nitrate is 27.05g In carbon sources like Fructose, Lactose and Dextrose, M. anisopliae showed high growth of biomass product in Lactose is 21.86g where as V. lecanii showed high growth of biomass product in Fructose is 32.70g.

Key words: *M. anisopliae*, nutritients, germination, growth and development, *V. lecanii*.

INTRODUCTION

The use of the chemical pesticides is very important and due to this the entomopathogenic fungi is used as environment friendly alternative in the last decade and used at a fast speed [1]. The microorganisms are kept under consideration and these are used as biocontrol agents against insects and special emphasis is put on *Metarhizium anisopliae* because of its qualities like shelf life, mode of action, non toxic effects to environment, high host specificity and non persistence. It forms mycelium whish contains spores and these spores are called conidia and are used for characterization. Factors like germination rate, growth, speculation are the indicators of virulence [2, 3]. The growth and development of entomopathogenic fungi depends upon the nutrient factors [4]. Nutrients are the factors which provide energy for biosynthesis nad thus helps in growth and development of any microorganism [5]. The knowledge of nutritional requirements is the main need in the cultivation of microorganisms. The carbhydrates, proteins, lipids , nucleic acids are made up of macro elements like carbon, hydrogen, nitrogen, sulphur, phosphorus,and theseare involved in mechanisms like host pathogen interaction and self defence mechanisms. Carbon is the major component and the mlecules of carbon also contributes to oxygen and hydrogen. The effect of the nutrient sources on the growth and development of microorganisms is studiie by [6]. 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 [7]. 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. These elements are acquired by the organic sources containing carbon but inorganic sources are also needed [8]. The CN ratio has a much important effect on the mycelial growth and conidia formation of Colletotrichum trunctum [9, 10, 11]. The effect of growth nutrients on the viability and virulence of conidial spores are studied by [12, 13] studied the CN ratio effect on the growth, sporulation and biocontrol activity of Taloromyces flavus.The growth charactristics in addition to growth substances are useful in the tolerance selection studies.The prepration used fopr the growth, storage and transport of microorganism can be used in solid [14] or liquid form [15]. The media should have all the nutritional requirements for the growth of microorganisms. The study is done on fourteen isolates of *Metarhizium anisopliae* and seventeen isolates of *Verticellum lecanii* 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 fungal 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 subcultured plates of *M. anisopliae* and *V. lecanii* 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 *Verticellum lecanii* and *Metarhizium anisopliae* 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 *Verticellum lecanii* and *Metarhizium anisopliae*. 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 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 psi for 20 min.

The first Czepeck Dox 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 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 *Verticellum lecanii* and *Metarhizium anisopliae* 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, Czapeck Dox media with different concentration of constituents are used to study the impact of carbon and nitrogen sources on *M. anisopliae* and *V. lecanii.* The impacts of carbon and nitrogen sources are studied on the basis of the total biomass production of the fungi on *M. anisopliae* and *V. lecanii*. These results are shown in the Tables 1, 2 and Fig. 1, 2 for *V. lecanii* and Tables 3, 4 and Fig. 3, 4 for *M. anisopliae.*

Effect of carbon and nitrogen sources on the biomass production of entomopathogenic fungi *M. anisopliae* and *V. lecanii*

The Czepeck Dox media with nitrogen sources showed the high biomass product formation of *Verticellum lecanii* in sodium nitrate with 27.05g. It is followed by Potassium Nitrate which produces 24.70g of biomass product. In Ammonium Sulphate as constituent, the total biomass product formation is 21.69g. While *Metarhizium anisopliae* showed the highest biomass product formation in Potassium Nitrate with 28.20g. It is followed by Sodium Nitrate which produces 25.55g of biomass product. In Ammonium Sulphate as constituent, the total biomass product formation is 24.43g.

The Czepeck Dox media with carbon source showed the high biomass product formation of *Verticellum lecanii* in Fructose as the main constituent with 32.70g. It is followed by Lactose which produces 22.26g of biomass product. In Dextrose as constituent, the total biomass product formation is 26.05g. While *Metarhizium anisopliae* showed the highest biomass product formation in Lactose with 21.86g. It is followed by Fructose which produces 20.87g of biomass product. In Dextrose as constituent, the total biomass product formation is 20.68g.

Table 1: Total biomass production of *V. lecanii* as influenced by nitrogen source

Nitrogen Source	Total biomass content(g/250 ml)			Mean
	1g/l	1.5g/l	2g/l	
Sodium nitrate	29.06	32.66	19.43	27.05
Potassium nitrate	25.70	27.64	20.76	24.70
Ammonium sulphate	25.04	18.96	21.69	21.69

Table 2: Total biomass production of *V. lecanii* as influenced by carbon sources

Carbon Source	Total biomass content(g/250 ml)			Mean
	10g/l	20g/l	30g/l	
Fructose	35.21	35.42	27.48	32.70
Lactose	19.700	23.400	23.700	22.26
Dextrose	18.98	29.85	29.34	26.05

Table 3: Total biomass production of *M. anisopliae* as influenced by nitrogen sources

Nitrogen Source	Total biomass content(g/250 ml)			Mean
	1g/l	1.5g/l	2g/l	
Sodium nitrate	31.68	29.44	15.54	25.55
Potassium nitrate	30.88	28.34	25.39	28.20
Ammonium sulphate	20.14	21.18	31.98	24.43

Figure 1: Total biomass production of *Verticellum lecanii* as influenced by nitrogen sources

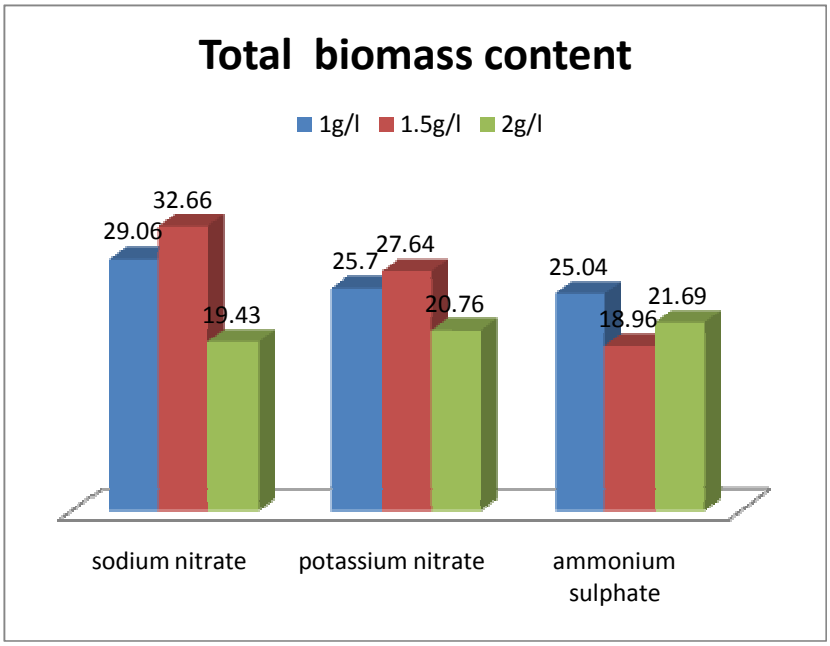

Figure 2: Total biomass production of *Verticellum lecanii* as influenced by carbon sources

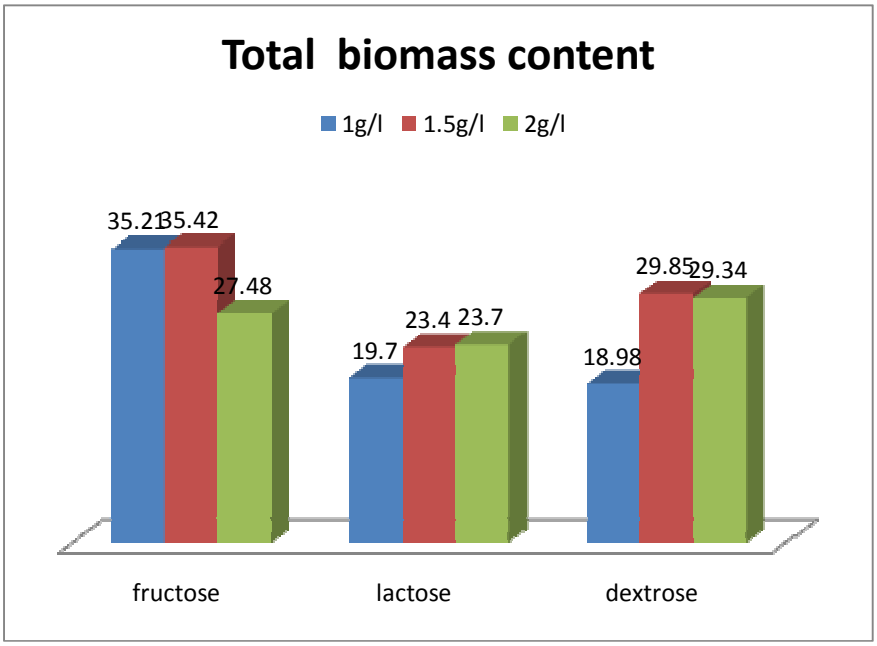

Figure 3: Total biomass production of *Metarhizium anisopliae* as influenced by nitrogen sources

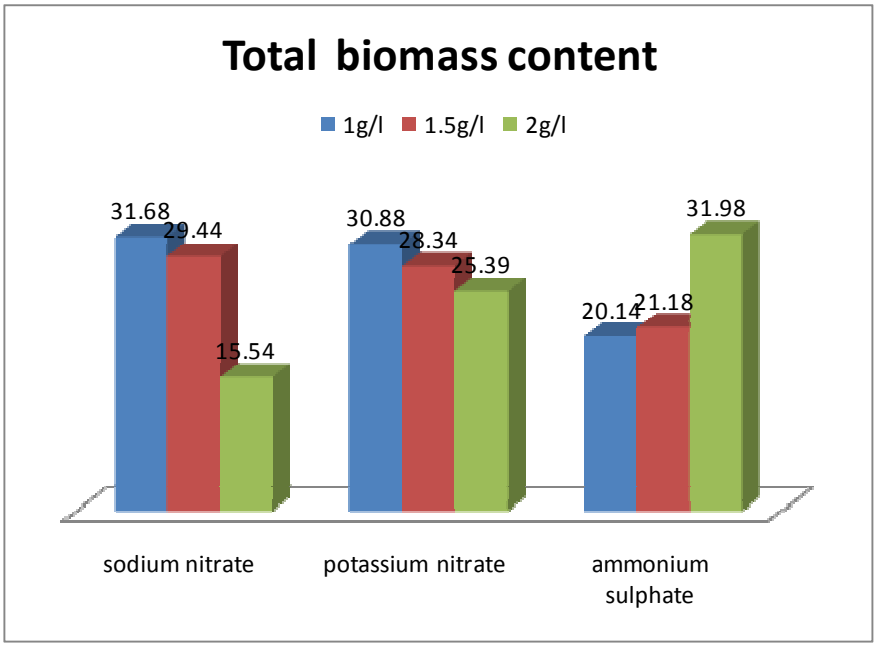

Figure 4: Total biomass production of *Metarhizium anisopliae* as influenced by carbon sources

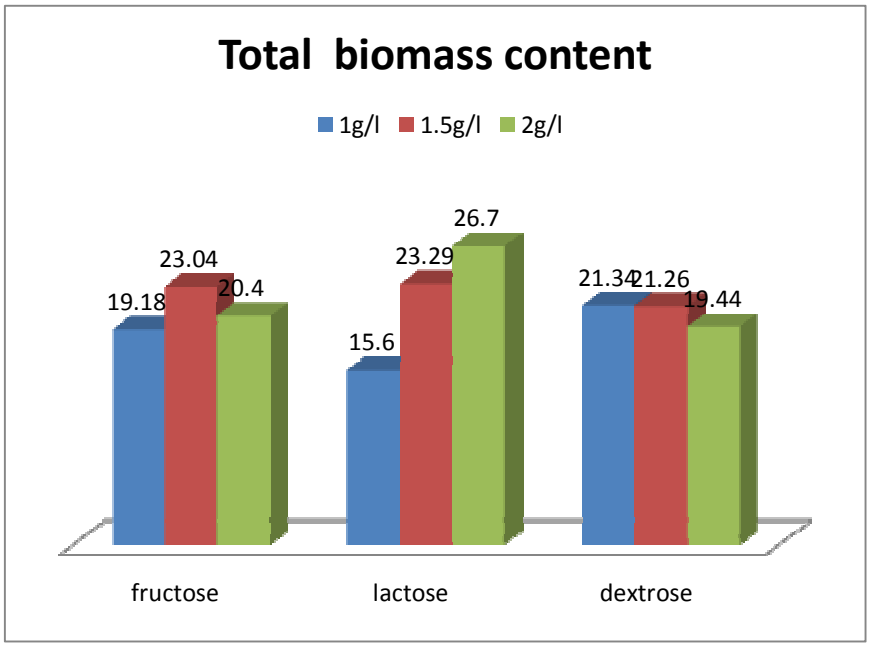

Table 4: Total biomass production of *M. anisopliae* as influenced by carbon sources

Carbon Source	Total biomass content(g/250 ml)			Mean
	10g/l	20g/l	30g/l	
Fructose	19.18	23.04	20.40	20.87
Lactose	15.60	23.29	26.70	21.86
Dextrose	21.34	21.26	19.44	20.68

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 field ever. In turn, Entomopathogenic fungi not only increase the fertility of soil, but also are eco friendly and do not affect the other beneficial microorganisms. The quantity of Entomopathogenic fungi to be used are also meager than that of chemical pesticides and are easy to apply. As all above methods we was concluded that comparatively pearl millet and carrot, yeast medium have a high growth rate for mass production of entomopathogenic (*Verticillium lecanii & Metarhizium anisopliae)* fungi which have a good role in biopesticide and agriculture production.

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