I.J.A.B.R, VOL. 3(3) 2013: 374-376 ISSN 2250 – 3560

INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

EFFECT OF DIFFERENT CARBON AND NITROGEN SOURCES ON THE BIOMASS OF *BEAUVERIA BASSIANA*

[a]Yadav Seema, [b]Tandan Neeraj & [a]Kumar Krishan
[a]Department of Biotechnology, Singhania University, Rajasthan - 333515, India
[b]Scientific and Applied Research Center, Meerut - 250001, India

ABSTRACT
The selective *Beauveria bassiana* isolate effects on different carbon and nitrogen sources with the basic YPD medium. Study of different concentration media 250 g/ml amount of medium with sucrose and dextrose in minimum value 15 g/L, and maximum value 30 g/L percent and four nitrogen as well as carbon sources viz., KNO_3, $NaNO_3$, $(NH4)_2SO_4$, NH_4NO_3 at 0.15 g/L, 0.20 g/L, 0.25 g/L and 3.0 g/L percent on the mycelia biomass of fungi, *Beauvaria bassiana*, were evaluated, independently. The results revealed that starch @ 35 g/L was the best carbon source which recorded maximum dry biomass of the fungus followed by sucrose (4.44 g/250 ml) and fructose (3.16 g/250 ml). Among nitrogen sources, KNO_3 (5.00 g/250 ml) was found to be best source followed by NH_4NO_3 (4.12 g/250 ml) at 0.4 per cent for multiplication of *Beauveria bassiana.*

KEYWORDS - *Beauveria bassiana*, Biomass, Carbon sources, N-Source, YPD

INTRODUCTION
The intensive use of chemical in agriculture has yielded fruitful results in terms of improving total production but over the years it adversely affected the environment. Over use of chemical fertilizers has seriously deteriorated the "Hidden Hunger" by increasing chemical load, it is toxic rather than benign to surrounding flora and fauna on which community depends for other needs**.** In view of these side effects, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times. Of the several microbial pathogens *viz.*, bacteria, fungi, viruses, protozoans and entomopathogenic nematodes reported, only a few have been studied systematically for their usefulness. Fungal biological control agents can be ineffective if conidia are unable to germinate because water availability is suboptimal (Doberski, 1981; Gillespie & Crawford, 1986; Hsiao *et al.*, 1992) if they have inadequate carbohydrate reserves (Lane *et al.*, 1991a; Harman *et al.*, 1991). One way to overcome these problems is to modify the carbohydrate content of the fungal inocula (Harman *et al.*, 1991; Hallsworth & Magan, 1994). A careful evaluation of these beneficial pathogens can leads to gainful exploitation in microbial control programmers (Burges, 1998). Entomogenous fungi are potentially the most versatile biocontrol agents, due to their wide host range that often results in natural epizootics. An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Nadeau *et al.*, 1996). Additionally, commercial considerations such as identification of existing or novel isolates, quality control of product and patent protection (Leathers *et al.*, 1993) would benefit the development of efficient process for large scale multiplication.

MATERIALS & METHOD
To study the growth pattern of *B. bassiana* on different carbon and nitrogen source on the basis of biomass produced by the fungi were evaluated by varying different carbon sources in the basic PDA medium with lactose, starch, fructose and dextrose at 1**,** 2, 3 and 4 percent, respectively. Sources at recommended dose (4%) in the basal media served as the check. Similarly, different nitrogenous sources viz., KNO_3, $NaNO_3$, $(NH_4)_2$ SO_4 and NH_4NO_3were evaluated at KNO_3, $NaNO_{3,}$ $(NH_4)_2$ SO4, NH_4NO_3 at 0.15, 0.20, 0.22 and 0.23 percent. Initially, the fungi were cultured on Sabouraud's Maltose Yeast Extract (SMAY) Agar plates for two weeks. The conidial suspension was prepared by shaking conidia from a 5 mm diameter agar plug into a test tube containing 10 ml sterile water mixed with 0.05 per cent Tween 80. The conidial suspension was mixed thoroughly by shaking at 80 rpm for 10 min and the conidial population was determined using Haemocytometer. Two hundred microlitre (0.2 ml) aliquot of the final suspension was inoculated into 250 ml broth containing different sources and levels of carbon and nitrogen. The flasks were aerated on a shaker at 90 rpm under room temperature for 8-9 days. After complete mycelia growth, the fungal mat was taken out and filtered through Whatman No. 1 filter paper, later dried in an oven at 60°C to constant weight and dry weight was recorded.

RESULTS & DISCUSSION
The ubiquitous natural occurrence of the species *B. bassiana*, its long history of use in biocontrol, carbon and nitrogen are the most vital nutrients required for growth and sporulation (Campbell *et al.*, 1983). Starch, among the carbon sources studied, supported the highest growth of *B. bassaina* (8.12 g/250 ml) followed by sucrose (7.0 g/250 ml) and fructose (5.73 g/250 ml). Lactose supported least growth with only 5.0 g/250 ml of dry biomass production

(Table 1 and Fig 1). In earlier studies, the most effective carbon source for sporulation of *Nomuraea ri1eyi* was reported to be dextrose (IM *et al.*, 1988) and maltose (Balardin and Loch, 1989). Edelstein *et al.* (2004) on the other hand found that media with potato extract induced higher growth rate. The interaction effect of carbon source and levels on biomass production of *B.bassiana* was maximum with starch at 30 g/l (8.12g/250ml).

As well as increases concentration contains medium up to 15 to 30g/L, mean biomass also increased, from 4.32g at 15g/L to 8.12g/L at 30g/L. Among the different nitrogen containing medium evaluated for production of *Beauveria bassiana* $KN0_3$ (4.72 up to 7.13g/250ml) was found to be best source followed by NH_4N0_3 (5.56 upto 6.70 g/250ml). However, when $NaN0_3$ was used as a nitrogen source, there was reduction in the growth (4.48g/250 ml). The optimum concentration of nitrogen was found to be 2.5g/L (7.13g/250ml) for all nitrogen source (Table 2 and Fig 2). Li and Holdon (1994) also found $KN0_3$ support better growth and sporulation of *Beauveria bassiana* than $(NH_4)_2 S0_4$. The NH_4 ions acidify the medium and the acidity up to certain level encourages the growth of fungi but beyond that limit, it is probably responsible for induction of sporulation as inferred by Rath *et al*, (1995), while characterizing *Beauveria bassiana* strain on different source of organic acids as carbon sources.

TABLE 1: Total biomass production of *Beauveria bassiana* on carbon containing growth medium.

Carbon source	Total dry biomass content (g/250 ml)			
	15 g/L	20 g/L	25 g/L	30 g/L
Lactose	5.0	5.15	4.42	4.00
Starch	5.42	5.00	5.00	4.44
Fructose	4.47	5.41	5.18	5.73
Sucrose	5.62	6.13	5.67	7.00
Dextrose	6.13	6.37	6.54	8.12

FIGURE 1. Total biomass production of *Beauveria bassiana* on carbon containing growth medium

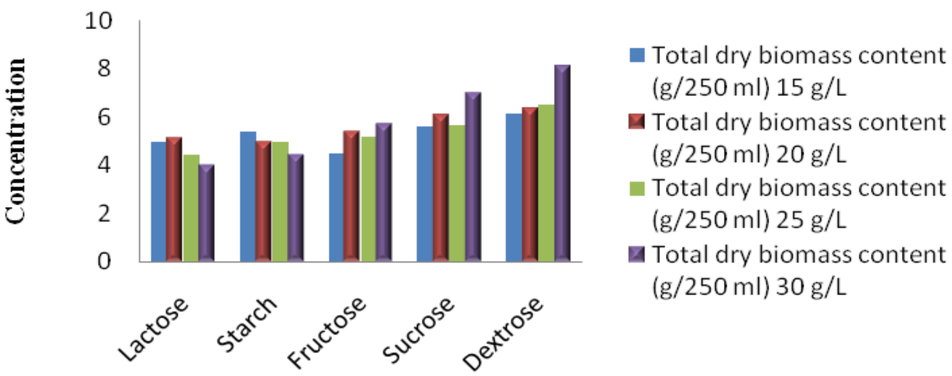

TABLE 2. Total biomass production of *Beauveria bassiana* on nitrogen containing growth medium.

Nitrogen source	Total dry biomass content (g/250 ml)			
	1.5 g/L	2.0 g/L	2.5 g/L	3.0 g/L
$NaNO_3$	4.48	5.90	5.16	4.79
KNO_3	4.72	6.15	7.13	6.98
$(NH_4)_2SO_4$	6.48	5.12	5.56	5.81
NH_4NO_3	5.00	5.12	5.89	6.82

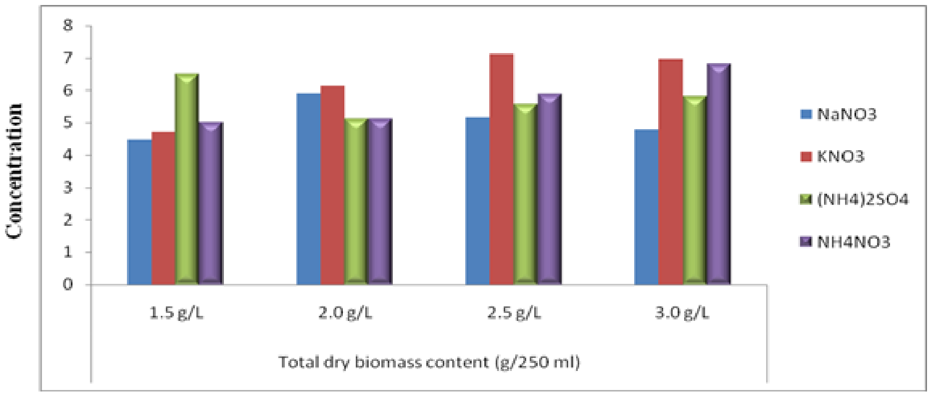

FIGURE 2. Total biomass production of *Beauveria bassiana* on nitrogen containing growth medium.

REFERENCES

Burges, H.D. (1998) Formulation of mycoinsecticides. In: Formulation of microbial biopesticides (Ed. H. D. Burges). Kluwer Academic Publishers Dordrecht, pp.131-185.

Balardin, R.S. & Loch, L.C. (1989) Semi-synthetic cultures media for N. riley mass production. Pesquisa Agropecuaria Brasileira, 24: 375-381.

Campbell, R. K., Eikenbary, Barnes, G.L., Artwright, B.O. & Eikenbary, R.D. (1983) Growth and sporulation of *Beauveria basiana* and *Metarhizium anisopliae* in a basal medium containing various carbohydrate sources. Journal of Invertebrate Pathology, 41: 117-121.

Doberski, L. W. (1981) Comparative laboratory studies on three pathogens of the elm bark beetle *Scol_yt~ss co/ytus:* effect of temperature and humidity on infection of *Beazlveria bassiana, Metarhi.yizlm anisopliae* and *Paecilomyces farinoszls. J Invertebr Pathol37,* 195-200.

Edelstein, J.D., Lecuona, R.E. & Trumper, E.V. (2004) Selection of culture media and in vitro assessment of temperature dependent development of Nomuraea rileyi. Biological Control, 33: 1-11.

I.M, D.J., Lee, M.H., Aguda, R.M. & Rombach, M.C. (1988) Effect of nutrients on pH on the growth and sporulation of four entomogenous hypomycetes fungi (Deuteromycotina).Korean Journal of Applied Entomology, 27: 41-46.

Gillespie, A. T. & Crawford, E. (1986) Effect of water activity on conidial germination and mycelial growth of *Beazlveria bussiana, Metarhixium anisopliae, Paecilomyces spp.* and 1 *"erticillium lecanii.* In *Fundamental and Applied Aspects of Invertebrate Pathology*. Edited byR. A. Samson, J. M. Vlak & D. Peters, p. 254. Wageningen: Society of Invertebrate Pathology.

Hallsworth, J. E. & Magan, N. (1994) Effects of KCl concentration on accumulation of acyclic sugar alcohols and trehalose in conidia of three entomopathogenic fungi. *L e t t -4ppl Microbiol* 18, 8-11.

Hsiao, W. F., Bidochka, M. 1. & Khachatourians, G. G. (1992) Effect of temperature and relative humidity on the virulence of the entomopathogenic fungus, *Verticillium lecanii,* toward the oat-bird aphid, *Rhopalosiphum padi* (Horn., Aphididae). *J Appl Entomolll4,* 484-4'10.

Harman, G. E., Jin, X., Stasz, T. E., Peruzzotti, G., Leopold, A. C. & Taylor, A. G. (1991) Production of conidial biomass of *Trichodermu barxianurn* for biological control. *Biol Control 1,* 23-28.

Li, D.P. & Holdom, D.G. (1994) effects of pesticides on growth and sporulation of *Metarhizium anisopliae* (Deuteromycotina Hyphomycetes). Journal of Invertebrate Pathology, 63: 209-211.

Leathers, T.D., Gupta, S.C. And Alexander, N.J. (1993) Mycopesticides: status, challenges and potential. Journal of Industrial Microbiology, 12: 69-75.

Lane, B. S., Trinci, A. P. 1. & Gillespie, A. T. (1991A) Endogenous reserves and survival of blastospores of *Beauveria bassiana* harvested from carbon- and nitrogen-limited batch cultures. *Mycol Res 95,* 821 828.

Lane, B. S.. Trinci, A. P. J. & Gillespie, A. T. (1991B) Influence of cultural conditions on the virulence of conidia and blastospores of *Beaweria bassiana* to the green leafhopper *Nephotettix virescens. Mycol Res 95,* 829-833.

Nadeau, M.P., Dunphy, G.B. And Boisvert, J.L., (1996), Development of Erynia conial (Zygomycetes Entomophthorales) on the cuticle of the adult black flies Simulium rostratum and Simulium decorum (Diptera Simuliidae). Journal of Invertebrate Pathology, 68: 50-58.

Rath A.C., Carr, C.J. And Graham, B.R. (1995) Characterization of *Metarrhizium anisopliae* strains by carbohydrate utilization (AP150CH). Journal of Invertebrate Pathology, 65: 152-161.