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Evaluations of different culture media for mass production of *Beauveria bassiana*

SS Shinde[1], NS Dale[2]
[1] Associate Professor, Department of Zoology, Vivekananad Arts Sardar Dalip Singh Commerce and Science College, Samarth Nagar, Aurangabad, Maharashtra, India
[2] Research Scholar, Department of Zoology, Vivekananad Arts Sardar Dalip Singh Commerce and Science College, Samarth Nagar, Aurangabad, Maharashtra, India

Abstract
Among different six media, Potato dextrose yeast broth emerged as a best medium for mass production of *B. bassiana* and recorded highest 10.50 gms /50ml of mat formation at 7 days after inoculation. At 14 days after inoculation it shows 13.41 gms/50ml of mat formation while after 21 days after inoculation shows highest growth of mat of 15.67 gms/50ml. Potato dextrose broth as the second best medium which produced 9.37 gms/50ml of mat formation at 7 days after inoculation. At 14 days after inoculation it shows 11.21 gms/50ml of mat formation where as at 21 days after inoculation shows 13.11 gm/50ml of mat formation.

Keywords: *Beauveria bassiana*, culture media, potato dextrose, production

1. Introduction
B.bassiana the white muscardine fungus belongs to Division: Eumycota, subdivision: Deuteromycotina, class: Deuteromycetes, order: Moniliales and family: Magnoliaceae, Genus. Beauveria and species Bassiana. It is globally occurring soil born mycelia fungus. It produces conidiophores (exospores) and a low molecular weight toxin. These are beauvericin, bassianin, beauveriolides, bassianolide and tenellin. It also produces antibiotic oosporein and degrading enzymes. A fungus, unlike insect bacteria and viruses does not require ingestion for infection in the host. The infection of insect by entomopathogenic fungi occurs following germination of conidia or blastopores on the cuticle and its penetration through the integument (Clarkson *et al.* 1998) [1]. In several cases infection through mouth parts, anal orifice, digestive and genital tracts have also been reported. (Ferron, 1978) [2]. Fungal pathogenesis begins with adhesion of conidia to the cuticle of host, their germination, penetration in cuticle through germ tube. Finally, fungus develops inside the body of the host which results in the death of the host insect. The death of the host insect may result due to physical blockage of the gut, tracheal circulatory system, non-availability of nutrients, invasions of organs by fungus and toxicosis due to toxins produced by *B.bassiana* under suitable environmental conditions, death is followed by external sporulation of fungus (Moore and prior, 1996) [6].
Hall (1954) [4] Propagated *B.bassiana* in artificial medium. Rombach *et al.* (1988) [8] reported that maximum growth of *B.bassiana* was possible in liquid culture containing sucrose and yeast extract at 3.5 percent each. However, production of maximum conidia (4.62×10^6 conidia mg^{-1}) was recorded in medium containing 2 percent maltose alone with 0.75 percent yeast extract. They also reported that production of dry mycelia may be a practical method for mass production of *B.bassiana* for use on rice. Hegedus *et al.* (1990) [5] reported that better conidia Production of *B.bassiana* on N-acetyl-D-glucosamine than on media like yeast extract-Peptone-glucose, glucose with ammonium salt or N-acetyl-D-galactose-amine.
Geetha and Balaraman (2001) [3] reported that SDB + PEG 2% media produced higher biomass (102 mg/ml) and blastospore yield ($4x10^8 ml^{-1}$) of *B.bassiana.* Sharma *et al.* (2002) observed the highest conidial count in isolates of *Beauveria* Spp, when grown on molasses yeast broth. All the grain media supported good sporulation however, bajra, sorghum and maize grains for *M. anisopliae* and cowpea grains among grain media supported good sporulation of for *B.bassiana* and *B.brongniartii*. Among the agar media, molasses yeast and sabourauds media were suitable for sporulation in all the three entomopathogens. Siwach and Jaipal (2004) [10] found that *B.bassiana* Sporulated poorly in press mud, but the incorporation of dextrose significantly enhanced conidial production in this medium.
Potato dextrose agar medium is most commonly used media for growth and sporulation of various fungi is also proved to be the most productive media for growth of *B.bassiana* (Nirmala *et al.* 2005) [7]. Safavi *et al.* (2007) [9] reported that yeast extract and peptones was found ideal for growth spore yield and germination. The present investigation was carried out to study the best culture media for mass production of *Beauveria bassiana.*

2. Material and Methods
The experiment was laid out in CRD with six treatments and five replications. The fungus *B.bassiana* was grown on test media like malt extract dextrose broth, malt extract peptone broth, Sabourauds dextrose broth yeast extract, potato dextrose yeast broth and potato dextrose broth for finding out the most suitable medium for mass multiplication of *B.bassiana*. The 750 ml sized glass Saline bottles each containing 50ml medium were autoclaved under 15 lbs pressure at 121^0c for 15 minutes and inoculated in laminar flow with 1ml inoculums / bottle using disproven Syringe

and stacked for 21 days at 28 $\pm 2^0$c temperature. Noting of weight of empty bottles, Bottles with media and fungal mat weight after 7, 14 and 21 days are noted and mean of five replications was calculated for six treatments and the media which gives highest fungal mat is used for mass production of *B.bassiana.*

3. Result and Discussion

The six media (Table1) were evaluated for suitability of medium for mass production of *B.bassiana*. The growth of *Beauveria bassiana* was calculated on the basis of actual mat weight in grams at 7, 14 and 21 days after inoculation. It was 6.04 to 10.83 grams, 6.35 to 13.41 gram and 6.95 to 15.67 grams at 7, 14 and 21 days after inoculation. At 7 days after inoculation Sabourauds maltose broth + yeast extract recorded highest (10.83g) mat formation. However it was at par with sabourauds dextrose broth + yeast broth (10.64gm). It was followed by potato dextrose yeast broth (10.50 gm) and potato dextrose broth (9.37 gm). The lowest mat weight was recorded on malt extract peptone broth (6.04gms) and malt extract dextrose broth (6.30gms).

Table 1: Suitability of medium for mass production of *B.bassiana.*

Treatment	Media	Mat weight (gms)7 DAI	Mat weight (gm) 14 DAI	Mat weight (gm) 21 DAI
1.	Malt extract dextrose broth	6.30	6.95	8.26
2.	Malt extract peptone broth	6.04	6.35	6.95
3.	Sabourauds dextrose broth +yeast extract	10.64	11.02	11.29
4.	Sabourauds maltose broth +yeast extract	10.83	10.94	11.24
5.	Potato dextrose broth	9.37	11.21	13.11
6.	Potato dextrose yeast broth	10.50	13.41	15.67

DAI-Days after inoculation

At 14 days after inoculation potato dextrose yeast broth recorded highest (13.41 gms) mat formation. It was followed by potato dextrose broth (11.21gms) and sabourauds dextrose broth + yeast extract (11.02gms) sabourauds maltose broth +yeast extract shows 10.94 gms mat growth. The lowest growth was recorded on malt extract peptone broth (6.35gms) and malt extract dextrose broth (6.95gms.)

At 21 days after inoculation potato dextrose yeast broth recorded highest (15.67gms) mat formation. However it was at par with potato dextrose broth shows (13.11gms) Sabourauds dextrose broth +yeast extract shows 11.29gms, whereas sabourauds maltose broth +yeast extract shows 11.24gm. Malt extract dextrose broth recorded 8.26 gms and the lowest growth 6.95 gms were observed on malt extract peptone broth.

In the present study potato dextrose yeast broth (PDYB) emerged as the best medium for culturing *B.bassiana*. Dextrose was stimulatory for the growth and significantly enhanced conidial production (Siwach and Jaipal 2004) [10] and higher biomass production (Nirmala *et al.* 2005) [7]. Rombach *et al.* (1988a) [8] reported that maximum growth of *B.bassiana* was possible in liquid culture containing sucrose and yeast extract at 3.5 percent each.

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