Research Paper :

Mass multiplication and shelf-life of *Metarhizium anisopliae* and *Beauveria bassiana* in solid and liquid formulations

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SUMMARY

In present investigation, *Metarhizium anisopliae* and *Beauveria bassiana* were produced in bulk quantity by using liquid fermentation technology where in Sabouraud dextrose yeast broth was used as medium. The maximum fermentation biomass of *M. anisopliae* was obtained after 96 hours of fermentation. The maximum fermentation biomass of *M. anisopliae* was obtained after 72 hours of fermentation. Soya lecithin, and neem oil were used for liquid formulation. Formulated final products were stored at 30^{0}C to study shelf-life of these products. Soya lecithin and neem oil formulations retained shelf-life for 300 days, while vermicompost, de-oiled castor cake and farmyard manure formulations retained shelf-life for 200,190 and 160 days, respectively as compared to gypsum and talc powder where the cfu/g declined by 110 days after storage. The results of present research indicate that solid formulation in vermicompost and liquid formulation in soya lecithin and neem oil supported the growth of both the biocontrol agents during storage thus increasing the count over storage, which is a major advantage for the marketing of these biocontrol agents.

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Economically important agricultural, horticultural and ornamental crop plants are attacked by various insects and pests, resulting in billions of dollars in cumulative crop losses. Currently, the most widely used control measure for suppressing these pests is the use of pesticides. The indiscriminate use of chemicals created serious problems by causing health hazards. Blindness, asthma, cancer, skin disorders, enlargement of liver, neural malfunction and to some extent even psychological problems. Widespread knowledge of groundwater contamination, together with the fact that pesticides are among the most exclusively used synthetic chemicals in agriculture worldwide (Peterson and Highley, 1993). However, problems encountered, such as development of pest resistance to chemicals. The chemical method developed to control too has its own limitations such as high capital investment, non-remunerative, poor availability, selectivity, temporary effect, efficacy affected by physico-chemicals and biological factors, pollution of food and feeds, health hazards, environmental pollution, etc. Considering these limitations biological control is an important approach in this direction. Pest biologic control is being considered as an important part of integrated pest management (IPM), which is a more ecological friendly strategy than conventional chemical pest control (Naylor and Erhich, 1997). The mycoinsecticides based on deuteromycetous fungi such as *M. anisopliae* (Agarwal, 1990), *B. bassiana* (Sandhu *et al.*, 2001), *N. rileyi* (Tang *et al.*, 1999) have been reported to be useful to control insect pests. Mass production of biocontrol agents using low cost technology is the logic requirement for successful exploitation of biocontrol agents. Similarly, the production process must result in biomass with excellent shelf-life even under adverse storage conditions. The combination of formulation, application and the selection of the strain is one of the key steps for field trials. Use of different oil based formulations for mycoinsecticides has been extensively studied (Lomer and Lomer, 2001). It has been suggested that oil formulation can prevent

conidial desiccation, and improve adhesion of conidia to the hydrophobic surface of insect cuticle (Inyang *et al.*, 2000; Vimala Devi and Prasad, 1996). The inoculum formulated in oils increased the efficacy of biocontrol agent and prolonged viability of conidia (Daoust and Roberts, 1983). Therefore, efforts were made to produce *M. anisopliae*, and *B. bassiana* in bulk quantity using low cost technology and shelf-life of these two entomopathogenic fungi in solid and liquid formulations.

Generally, biocontrol agents are mass multiplied using solid fermentation technology. Some of the disadvantages of solid fermentation method are 1.high volume of the substrate required, 2.contamination during fermentation and 3.long time required for fermentation. On the contrary, liquid fermentation will help in the mass multiplication of microorganisms under axenic conditions within a short time. The objectives of this research are mass multiplication of *M. anisopliae* and *B. bassiana* using liquid fermentation technology and Evaluation shelf of life of final product in solid and liquid formulations.

MATERIALS AND METHODS

Location of study:

The large-scale production of *B. bassiana* and *M. anisopliae* was carried out in Department of Plant Pathology, Directorate of Groundnut Research, Junagadh, Gujarat, during 2008. For mass multiplication of *B. bassiana* and *M. anisopliae,* fermentor (Model BIOFLOW-2000) with a working volume of 10 liters was used.

Entomopathogenic fungi:

B. bassiana and *M. anisopliae* were isolated from *Helicoverpa armigera* and *Spodoptera litura* collected from cotton and soybean crops. These biocontrol agents were maintained on Potato dextrose agar slants at 4 ºC. These two entomopathogenic fungi were used for the large scale production.

Culture media:

The mass production was carried out in five different broths *viz.,* Potato dextrose broth, Glucose nitrate broth, Maltose peptone broth, Molasses–yeast extract broth and Sabouraud dextrose yeast broth in fermentor. To reduce the production cost, yeast extract was successfully replaced by soy flour in Sabouraud dextrose yeast broth.

Initial inoculum for inoculation of fermenter:

M.anisopliae and *B. bassiana* were grown on Potato dextrose agar medium for seven days. For whole culture preparation, spores were removed from agar surface by scraping with sterile spatula after adding distilled water. One ml of this inoculum was added to 100 ml of medium in 250 ml Erlmenmeyer flask. The flasks were incubated at 28^0 C for 72h. The entire contents of the flasks were churned using a blender and one litre of initial inoculum was added to 9 liters of fermentor medium.

Mass multiplication of *M. anisopliae* and *B. bassiana:*

Five broths *viz.,* Potato dextrose broth, Glucose nitrate broth, Maltose peptone broth, Sabouraud dextrose broth and Molasses yeast extract broth were tested for mass production of *M.anisopliae* and *B. bassiana* in fermentor. Different parameters such as temperature, dissolved oxygen, pH, stirring rate, fermentation duration and foam control which influence the growth of the biocontrol agent during fermentation were studied for maximum biomass production. After a series of trials, suitable broth and optimum conditions for fermentation were standardized.

Formulation development and shelf-life of final product:

The fermentor biomass of *M.anisopliae* and *B. bassiana* was formulated in solid and liquid carrier. For solid formulation vermicompost, de-oiled castor cake, well-decomposed farmyard manure, gypsum and talc power were used. Soya lecithin and neem oil were used for liquid formulation. Formulated final products were stored at 30ºC to study shelf-life of these products. Upon harvest, the biomass was mixed with fine powder of these five carriers in 1:10 proportion and kept for three days under shade for drying. The final solid formulated products contained 10% moisture, were packed in polythene bags @ 500 g/ pack and then stored at 30ºC for 10 months. For liquid formulations soya lecithin and neem oil was used as career with 0.1% Tween 20. Three liquid formulations such as :

– Soya lecithin + conidial suspension 10^7 /ml + 0.1% Tween 20

– Neem oil + conidial suspension 10^7 /ml + 0.1% Tween 20

– The conidial suspension (10^7) with 0.1% Tween 20 and without any carrier served as control.

Shelf-life of final products in solid formulation:

Viability of formulated product in solid formulation was tested for 10 months. The final products in solid formulated containing 10% moisture were packed in

polythene bags @ 500 g/ pack and then stored at 30ºC for 10 months. Samples from these packets were drawn at 15 days intervals and tested for viability of the formulated product. Populations of these biocontrol agents were tested taking 100mg of the product and diluted to 10^6. One hundred mg biomass was taken in 1ml sterile water and mixed thoroughly by vortex mixture and allowed to stand. Then from the supernatant, serial dilution was prepared up to 10^6. From the 10^6 dilutions, 100 µl of suspension was taken and spread uniformly on Petridishes containing Potato dextrose agar medium using a spreader without disturbing surface of the medium. Five replications were maintained for each formulation. The Petriplates were incubated at 28^0C for two days and numbers of colonies were counted.

Shelf-life of final products in liquid formulation:

Viability of formulated product in liquid formulation was tested for 10 months. The final products were packed in plastic bottles @ 500ml/ bottle and then stored at 30^0C for 10 months. Samples from these bottles were drawn at 15 days intervals and tested for viability. Populations of these biocontrol agents were tested taking 100µl of the product and diluted to 10^6. This 100µl sample was taken in 1000µl sterile water and mixed thoroughly by vortex mixture and serial dilution was prepared up to 10^6. From the 10^6 dilution, 100 µl of suspension was taken and spread uniformly on Petridishes containing Potato dextrose agar medium using a spreader without disturbing surface of the medium. Five replications were maintained for each formulation. The Petriplates were incubated at 28ºC for two days and numbers of colonies were counted.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Mass multiplication of *M.anisopliae* and *B. bassiana:*

Potato dextrose broth, Glucose nitrate broth, Maltose peptone broth, Sabouraud dextrose broth and Molasses–yeast extract broth were tested for mass production of *M. anisopliae* and *B. bassiana* in fermentor. Among these, Sabouraud dextrose yeast broth was found to be suitable for the maximum growth of *M.anisopliae* and *B. bassiana*. Sabouraud dextrose yeast broth (1 % dextrose, 0.2 % peptone, and 1% yeast extract) was used as medium. To reduce the production cost, yeast extract was successfully replaced by soy flour. Dry weight of biomass of *M.anisopliae* and *B. bassiana* was obtained after 96 hours and 72 hours, respectively in molasses yeast extract broth showed greatest mass of fermentation (Fig. 1).

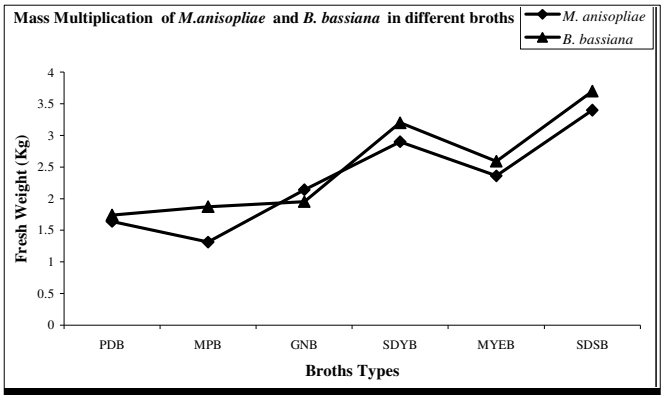

Fig. 1 : Mass production of *M.anisopliae* and *B. bassiana* in six broths (PDB-Potato dextrose broth, MPB-Maltose peptone broth, GNB-Glucose nitrate broth, SDB-Sabouraud dextrose yeast broth, MYEB-Molasses yeast extract broth and SDSB- Sabouraud dextrose soya flour broth)

Shelf-life of *M. anisopliae* and *B. bassiana:*

Among the liquid formulations tested, soya lecithin and neem oil formulations retained shelf-life for 300 days, while solid formulation such as vermicompost, de-oiled castor cake and farmyard manure formulations retained shelf-life for 210,190 and 160 days, respectively as compared to gypsum and talc powder where the cfu/g declined by 90 days after storage. A fermenter biomass in liquid formulations of soya lecithin and neem oil had longer growth and survival rate as compared to solid formulations. Growth of *M.anisopliae* and *B. bassiana* continued in liquid formulation up to 210 and retained good viability for 300 days. There after, a reduction in cfu was recorded (Fig. 2 and 3). Among the solid formulations, vermicompost was found to be most suitable for growth and viability of *M.anisopliae* and *B.*

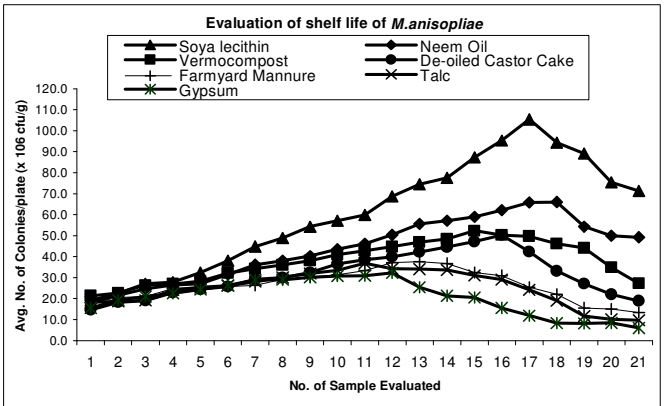

Fig. 2 : Evaluation of shelf-life of *M.anisopliae* in seven different formulations

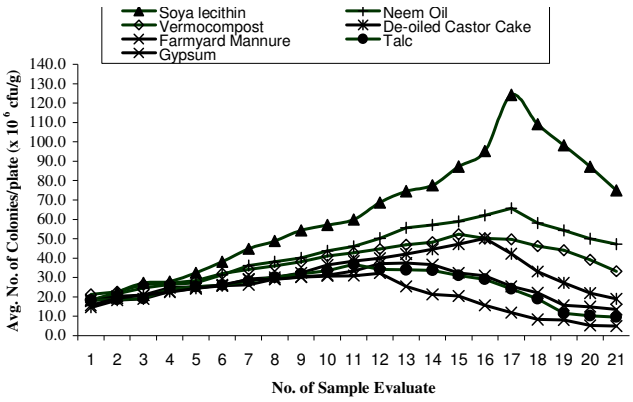

Fig. 3 : Evaluation of shelf-life of *B. bassiana* in seven different formulations

bassiana during storage. De-oiled castor cake, and farmyard manure based formulations retained better viability than talc and gypsum based formulations. Talc and gypsum based formulations had poor growth and shorter shelf-life. Maximum growth and higher survival rate during storage in soya lecithin, neem oil and vermicompost formulation is a major advantage for the marketing of these biocontrol agents on commercial level.

Use of different oil based formulations for mycoinsecticides has been extensively studied (Lomer and Lomer, 2001). It has been suggested that oil formulation can prevent conidial desiccation, and improve adhesion of conidia to the hydrophobic surface of insect cuticle (Inyang *et al.*, 2000; Vimala Devi and Prasad, 1996). Inyang *et al.* (2000) reported that sunflower oil/ Shellsol T formulations enhanced the infectivity of *M. anisopliae* for mustard beetle, *Phaedon cochleariae*. Similar observations were reported by Ibrahim *et al.* (1999) for *M. anisopliae*. Daoust and Roberts (1983) reported that the inoculum formulated in oils increased the efficacy of pathogen and prolonged viability of conidia. However, further research is required on large scale biomass production of entomopathogenic fungi, viability of final products in different formulations under laboratory and field conditions and their efficacy in the eco-friendly management of insect pests.

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