International Research Journal of Biological Sciences _____ *I. Res. J. Biological Sci.*
Vol. **1(4),** 12-16, August **(2012)**

Biomass Production of Entomopathogenic Fungi using various Agro Products in Kota Region, India

Mehta Jitendra*, Dhaker Jayraj Kiran, Kavia Ambika, Sen Priya, Kaushal Neha and Datta Sakshi
Microbiology and Pathology Division, Vital Biotech Research Institute, University of Kota, Kota- 324009, Rajasthan, INDIA

Available online at: www.isca.in
Received 8th June 2012, revised 15th June 2012, accepted 18th June 2012

Abstract

Entomopathogenic fungi like Verticillium lecanii and Metarhizium anisopliae were cultured in different media to produce highest biomass of fungus. In this, the agricultural products and organic products are used for the total biomass production in Kota district. The different media used for the production of biomass are vegetables, cereals, pulses, rice washed water, boiled rice water, saw dust, fruits etc. For the production of biomass of fungi grains media, organic media and non-synthetic media have been used. The maximum biomass production of Metarhizium anisopliae was observed in yeast extract media (36.96 gm in 250 ml). The maximum biomass production of Verticillium lecanii was also observed in yeast extract media (30.82 gm in 250ml).

Keywords: Entomopathogenic fungi, grain medium, non synthetic medium and organic medium, biomass production.

Introduction

The conventional chemical pesticides has not only enhanced the food production, but also adversely affected the environment and non-target organisms. Due to the side-effects of chemical pesticides, the sustainable crop production through eco-friendly pest management is essentially required in recent scenario. Among the several micro-organisms viz., bacteria, fungi, virus, protozoans and entomopathogenic nematodes, a few have been systematically studied for their effective beneficial characteristics. The systematic study of these beneficial micro-organisms can lead to gainful exploitation in microbial control programmes[1].

Entomopathogenic fungi are often reported as causing high levels of epizootics in nature and are the most versatile biological control agents, and are environmentally safe. An attractive feature of these fungi is that the virulence caused by contact and the action is through penetration[2]. These fungi subsume a heterogenous group of over 100 genera with approximately 750 species, notified from different insects. Many of these are proved to be highly potential in pest management. The most considerable fungal species are *Metarhizium* spp., *Beauveria* spp., *Nomuraea rileyi, Verticillium lecanii* and *Hirsutella* spp. In 1883, Metchnikoff commenced mass culturing of fungus and carried out the first experiment with two beetle pests. *Metarhizium anisopliae* (Metchnikoff) Sorokin is the second most widely exploited entomopathogenic fungus in biocontrol trials. It is known to attack over 200 species of insects belonging to orders Coleoptera, Dermoptera, Homoptera, Lepidoptera and Orthoptera[3].

Verticillium lecanii (zimm.) is widely called the "white holo" and it causes mycosis in a number of insects of orders Homoptera, Coleoptera and Lepidoptera.

For the simple and reliable production of blastospores which are short lived, and hydrophilic, the basic multiplication procedures of submerged liquid fermentation are followed[4]. Solid state fermentation for the production of aerial conidia[5]. However, the most viable mass production techniques include use of a di-phasic strategy in which fungal inoculum is produced in liquid culture, which is further used for inoculation of solid substrates for conidia production[6].

Present study was carried out to evaluate grains such as rice, wheat, pulses and maize at different temperatures, and naturally available solid media such as carrot, ladyfinger, jack seeds, rice husk, and saw dust for the biomass production of *Metarhizium anisopliae* and *Verticiilium lecanii.*

Material 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. Preliminary identification of fungi was made with the help of the Atlas of entomopathogenic fungi[7].

Maintenance of culture: A loopful of inoculum 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 30 min 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 (26±2°C) for ten days. After complete sporulation, conidia from the medium were harvested by washing them thoroughly with sterilized water containing Tween-20 (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-20 (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.

Grain Medium: Three grains viz rice, wheat and pulses were used for estimating the biomass of *Metarhizium anisopliae* and *Verticillium lecanii* at 25 ˚C. 20 g of each grain was washed well and boiled in distilled water for 1 hr. and then mesh properly and filter it, now makeup 1 liter with distilled water. Now these grain mediums were packed separately in individual 500 ml conical flask for *M. anisopliae* and *V. lecanii.* They were plugged with cotton wool and autoclaved at 15 psi for 1hr at 121˚C. After cooling, 1 gm of the fungal culture was inoculated into each flask, separately. All these procedures were done under laminar air flow chamber. They were incubated in BOD incubator at 25˚C for 3 weeks. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the culture and to break the mycelial mat.

After 14 days of incubation, the mycelial mat appears again in flasks. Now it was grow well for 21 days. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth and then taken biomass in each grain medium.

Non Synthetic Medium: Three non synthetic medium viz saw dust, rice brawn and carrot were used for estimating the biomass of *Metarhizium anisopliae* and *Verticillium lecanii* at 25˚C. 20 g of each grain was washed well and boiled in distilled water for 1 hr. and then mesh properly and filter it, now makeup 1 liter with distilled water. Now this non synthetic medium was packed separately in individual 500 ml conical flask for *M. anisopliae* and *V. lecanii.* They were plugged with cotton wool and autoclaved at 15 psi for 1hr at 121˚C. After cooling, 1 gm of the fungal culture was inoculated into each flask, separately. All these procedures were done under laminar air flow chamber. They were incubated in BOD incubator at 25˚C for 3 weeks. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the culture and to break the mycelial mat. After 14 days of incubation, the mycelial mat appears again in flasks. Now it was grow well for 21 days. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth and then taken biomass in each non synthetic medium.

Organic Medium: Three organic medium viz yeast, potato dextrose and jaggery were used for estimating the biomass of *Metarhizium anisopliae* and *Verticillium lecanii* at 25˚C. 20 g of each grain was washed well and boiled in distilled water for 1 hr. and then mesh properly and filter it, now makeup 1 liter with distilled water. Now this organic medium was packed separately in individual 500 ml conical flask for *M. anisopliae* and *V. lecanii.* They were plugged with cotton wool and autoclaved at 15 psi for 1hr at 121˚C. After cooling, 1 gm of the fungal culture was inoculated into each flask, separately. All these procedures were done under laminar air flow chamber. They were incubated in BOD incubator at 25˚C for 3 weeks. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the culture and to break the mycelial mat. After 14 days of incubation, the mycelial mat appears again in flasks. Now it was grow well for 21 days. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth and then taken biomass in each organic medium.

Results and Discussion

In the present study, several naturally available substrates of liquid media were tested for mass multiplication of *M. anisopliae* and *V. lecanii*. The success of microbial control of insect pests depends not only on the isolation, characterization and pathogenicity, but also on the successful mass production of the microbial agents in the laboratory. Large-scale availability of the pathogen is a primary requirement in the bio-control programme. For a successful integrated pest management programme, the agents like the entomopathogenic fungi should be amenable to easy and cheap mass multiplication.

Mycopathogens infect insects through cuticle and are therefore, the principle pathogens among sucking insects which cannot ingest other pathogens that infect through the gut wall. More than 750 species of entomopathogenic fungi have been described, of which only ten species have been extensively exploited. Current research efforts were directed at selecting native entomopathogenic fungi, characterizing them assessing their virulence and developing a formulation for them. The results obtained are discussed herein.

Grain Medium: Among the grain media, pulses medium produced significantly higher 26.50 gm and 20.70 gm per 250 ml of biomass production was recorded in *V. lecanii, M. anisopliae* respectively and also observed similar findings in

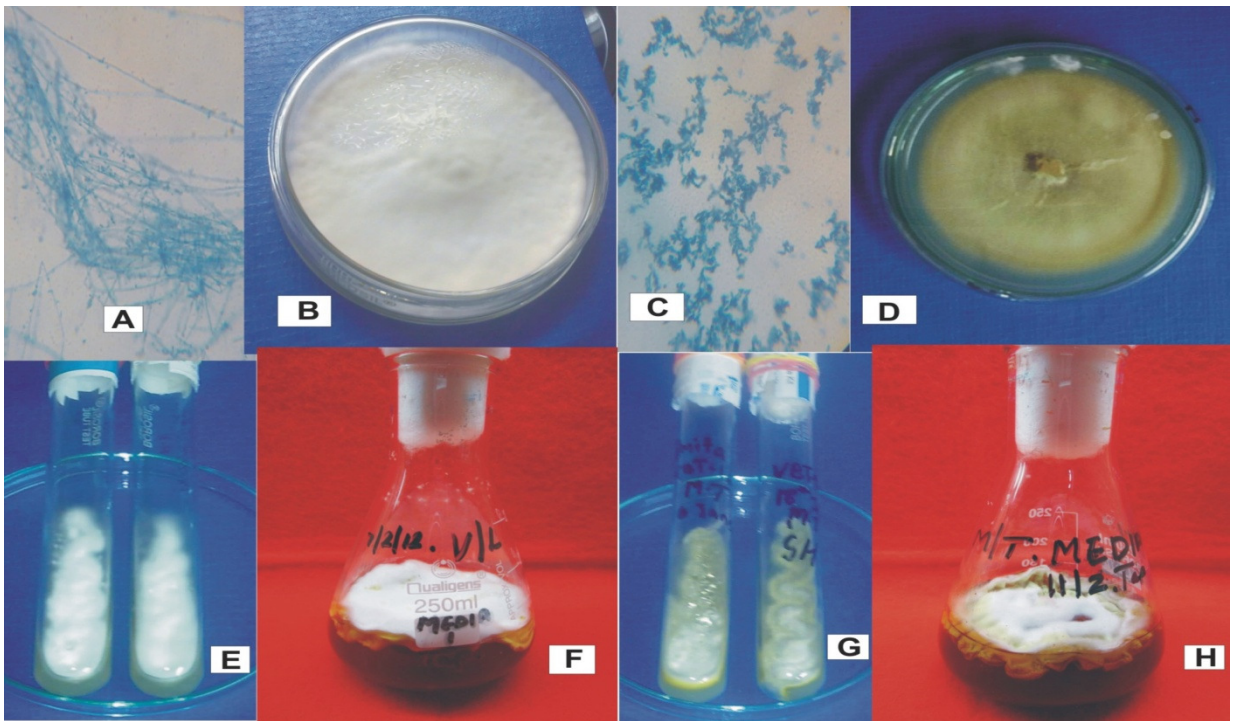
M. anisopliae. Abundance of minerals in the pulses medium may enhance the growth of fungi[8]. Rice and wheat medium also supported the growth of both the tested fungi.

Non Synthetic Medium: Among the non synthetic medium, carrot produced significantly higher 18.52 gm and 18.62 gm per 250 ml of biomass production was recorded in *V. lecanii, M. anisopliae* respectively.

Organic Medium: Among the organic medium, yeast extract produced significantly higher 30.82 gm and 36.96 gm per 250 ml of biomass production was recorded in *V. lecanii, M. anisopliae* respectively. In all three categories other media also supported biomass production in all tested fungi (table-1, figure-1 A-H, and figure-2).

Table-1
Biomass production of entomopathogenic fungi

Media	**Microorganism (Temperature 25˚C, Duration 3 weeks)**			
Grain	***Verticillium lecanii***		***Metarhizium anisopliae***	
	Biomass (gm/ 250 ml)	**Frequency of biomass Growth (%)**	**Biomass (gm/ 250 ml)**	**Frequency of biomass Growth (%)**
Rice	10.31	55	16.22	65
Wheat	2.23	20	1.15	10
Pulses	26.50	80	20.70	75
Non Synthetic				
Saw dust	0.42	5	0.62	8
Rice Brawn	2.35	22	2.22	18
Carrot	18.52	70	18.62	72
Organic				
Yeast	30.82	85	36.96	90
Potato Dextrose	16.05	60	12.94	58
Jaggery	20.88	78	16.25	68



A. Microscopic view of *V. lecanii*. B. Mother culture of *V. lecanii.* C. Microscopic view of *M. anisopliae*. D. Mother culture of *M. anisopliae.* E. Slants of *V. lecanii.* F. 3 weeks in yeast medium *V. lecanii.* G. Slants of *M. anisopliae.* H. 3 weeks in yeast medium *M. anisopliae.*

Figure-1
(A-H) Biomass production of entomopathogenic fungi

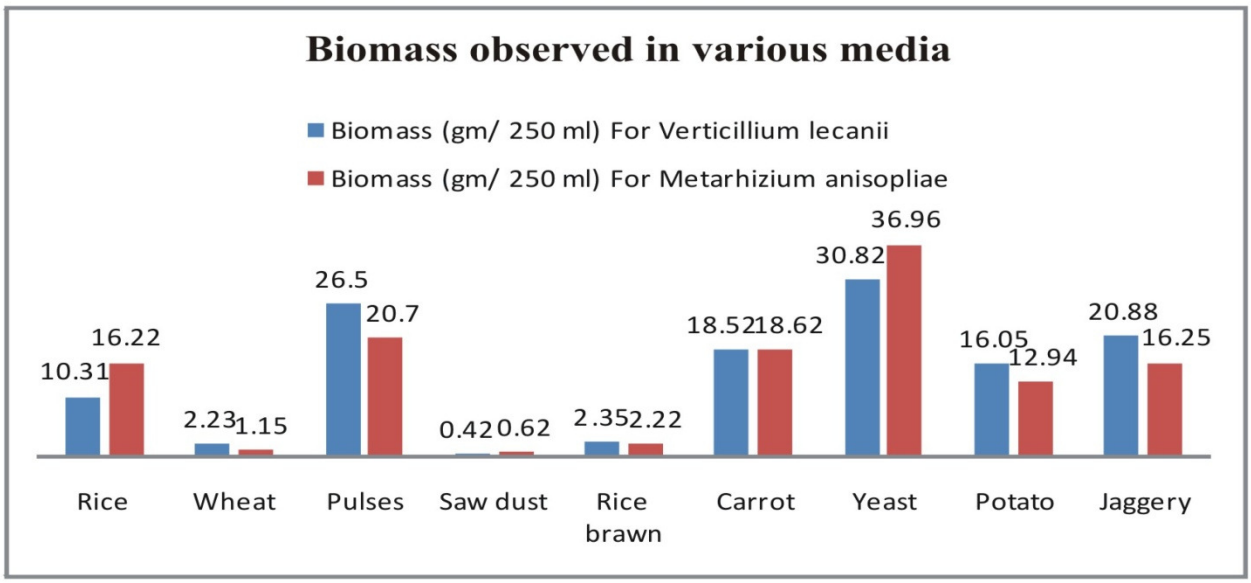


Figure-2
Biomass production of entomopathogenic fungi observed in various media

Conclusion

The excess use of chemical pesticides not only polluted the environment but also deteriorated the overall fertility of the soil. There can be benefits using pesticides at initial level but later use can counterproductively increase pest resistance and kill the natural enemies of pests and also adversely affect the fertility of soil. In turn, bio-pesticides not only increase the fertility of soil, but also are eco-friendly and do not affect the other beneficial microorganisms. The entomopathogenic fungi are considered natural mortality agents and environmentally safe bio-pesticides. Bio-pesticides are more effective than chemical pesticides in long term use, and also cost effective. Entomopathogenic fungi have been reported as an excellent bio-pesticide. From all above methods we were concluded that yeast medium showed a high growth rate for biomass production of entomopathogenic fungi (*Verticillium lecanii and Metarhizium anisopliae*) as compare to pulses and carrot medium.

Acknowledgement

We are grateful to Department of Microbiology and Pathology division, Vital Biotech Research Institute, Kota for providing laboratory facilities and also thankful to Mr. Jitendra Mehta of Vital Biotech for sincere efforts in writing this research paper. We are also grateful to Mrs Jayraj Kiran Dhaker, Miss Ambika Kavia, Miss Neha Kaushal, Miss Priya Sen**,** and Miss Sakshi Dutta for their continuous team work. We would also like to thank Mr. R. C. Mehta and staff members of Vital Biotech Research Institute for encouragement and we would like to thank the reviewers of this paper for their excellent comments.

Reference

1. Burges A.D. and Hussey N.W., Microbial Control of Insect Pests and Mite, Academic Press, London, 161-167 **(1998)**

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

3. Moore D., Higgins P.M. and Lomer C.J., The effects of simulated and nautral sunlight on the viability of conidia of *Metarhizium flavoviride* Gams and Rozsypal and interactions with termperature, *Biocontrol Science Technology*, **7,** 87-94 **(1996)**

4. Romback M.C., Production of *Beauveria bassinaa* Conidia in Submerged Culture, Entomophaga, **5,** 45-52 **(1989)**

5. Rousson S., Rainbautt M. and Lonsane B.K., Zymotics a Large Scale Fermenter Design and Evaluation, *Appl. Biochem. Biotechnol*., **42,** 161-167 **(1983)**

6. Burges A.D. and Hussey N.W., Microbial Control of Insect Pests and Mite, Academic Press, London, 161-167 **(1981)**

7. Samson, Entomopathogenic fungi, New Willey, New York, 123 **(1988)**

8. Dangar T.E., Geetha L. and Pillai G.B., Mass production of the entomopathogenic fungi *Metarhizium anisopliae* in coconut water, *Journal of Plantation Crop,* **19**, 54-69 **(1991)**

9. Dangar T.K., Geetha L., Jayapal S.D. and Pillai G.B., Mass Production of the Entomopathogens *Metarhizium anisopliae in* Coconut Water, *J. Plant. Crop,* **19,** 54-59 **(1999)**

10. Gopalakrishnan C. and Mohan S.C., A Simple and Cost Effective Invitro Method for the Mass Production of Conidia of *Nomuraea rileyi*, *Insect Environ*., **6,** 52-53 **(2000)**

11. Nakade Dhanraj B., Antibiotic sensitivity of common Bacterial Pathogens against selected Quinolones , *ISCA J. Biological Sci.,* **1(1),** 77-79 **(2012)**

12. Muthusamy P., Murugan S. and Manothi Smitha, Removal of Nickel ion from Industrial Waste Water using Maize Cob*, ISCA J. Biological Sci.,* **1(2),** 7-11 **(2012)**

13. Srivastava K.P. and Singh Vikash Kumar, Impact of Air-Pollution on pH of soil of Saran, Bihar, India, *Res. J. Recent Sci.*, **1(4),** 9 -13 **(2012)**

14. Mondal Debashri and Mondal Tamal, A Review on efficacy of Azadirachta indica A. Juss based biopesticides: An Indian perspective, *Res. J. Recent Sci.,* **1(3)**, 94-99 **(2012)**

15. Amanchi Nageswara Rao and Mohd. Masood Hussain, Ecophysiological and cytopathological impact of delfin insecticide (*B. thuringiensis*) to an unicellular ciliate protozoan, *Euplotes patella*, *Res. J. Recent Sci.,* **1(4)**, 64-67 **(2012)**

16. Chauhan R., Chaudhary R., Singh A. and Singh P.K., Salt Tolerance of *Sorghum bicolor* Cultivars during Germination and Seedling Growth, *Res J Recent Sci.,* **1(3)**, 1-10, **(2012)**

17. Patil S.G., Chonde S.G., Jadhav A.S. and Raut P.D., Impact of Physico-Chemical Characteristics of Shivaji University lakes on Phytoplankton Communities, Kolhapur, India, *Research Journal of Recent Sciences,* **1(2)**, 56-60, **(2012)**

18. Uttam Kumar, Sharma Pawan Kumar and Shrivastava Surabhi, Spectrum of Insect Pest Complex of Soybean (Glycine max (L) Merrill) at Lambapeepal Village in Kota Region, India , *ISCA J. Biological Sci.,* **1(1),** 80-82 **(2012)**

19. Kumar Praveen G.N. and Sumangala K. Bhat, Fungal Degradation of Azo dye- Red 3BN and Optimization of Physico-Chemical Parameters , *ISCA J. Biological Sci.,* **1(2),** 17-24 **(2012)**

20. Verma V., Bhardwaj A., Rathi S. and Raja R.B., A Potential Antimicrobial Agent from Cocos nucifera mesocarp extract; Development of a New Generation Antibiotic*, ISCA J. Biological Sci.,* **1(2),** 48-54 **(2012)**