Evaluation of Various Parameters in Mass Multiplication of *Beauveria bassiana* in Modified Method.

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Received:- 01 August 2021/ Revised:- 09 August 2021/ Accepted:- 15 August 2021/ Published: 31-08-2021

Abstract— *In the recent years, the environmental contamination caused by excessive use of chemical pesticides increased the interest in integrated pest management, where bio-pesticides are used to control plant pests and plant diseases. Present study deals with use of different media like SDA, rice bran, wheat bran, sorghum and to find their ability as substrates for mass multiplication of beauveria bassiana and creates effective production methodology which can be easily adopted. Biomasses of fungal grain media, organic media and non-synthetic media have been used for the production. Mass multiplication of Beauveria bassiana on different grain media, different temperature like with incubator and without incubator method and calculate biomass of fungus, microscopic examination. Development of SDA was the result which was considered as a best media for quickly growth of Beauveria bassiana and rice bran produced spore production which are most suitable for Beauveria bassiana.*

Keywords— Beauveria bassiana, chemical pesticides, SDA, rice bran, wheat bran, sorghum, fungal grain media, organic media and non-synthetic media.

I. INTRODUCTION

Biopesticide as defined by the United States Environmental Protection Agency, biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria and certain minerals.

In commercial terms, biopesticides include microorganisms that control pests, naturally-occurring substances that control pests, and pesticidal substances produced by plants containing added genetic material. The EPA separates biopesticides into three major classes based on the type of active ingredient used, namely, biochemical, plant incorporated protectants and microbial pesticides. Biochemical pesticides, which are naturally occurring substance that control pest by non toxic mechanisms. Though biopesticides cover about 1% of the total plant protection products globally, their number and the growth rate have been showing an increasing trend in the past two decades about 175 biopesticides active ingredients and 700 products have been registered worldwide. Regulatory system favorable to chemical pesticide and the gradual disappearance of multiple or mixed cropping, which is known to keep away the magic bullet chemical pesticide.

The main advantages of these biocontrol agents are their specificity to target pests, safety to the non-target organism, they do not cause ill effects on environment and human health and can be used against pests which develop resistance to the conventional insecticides, and they fit as ideal components in integrated pest management.

II. BIOPESTICIDE

B.bassiana, the white muscardine fungus belonging to class Deuteromycetes, is one of the important disease-causing pathogens in insects. B. bassiana formerly known as Botrytis bassiana is a widely distributed soil inhabiting fungus. Two fungi, B. bassiana and M. anisopliae are known to be pathogenic to the larval stage of the silkworm. A member of the hyphomycetes class of fungi, B. bassiana is categorized as a white muscardine fungus due to the white color of sporulating colonies. In culture, B. bassiana grows as a white mold. On most common culture media, it produces many dry, powdery conidia in distinctive white spore balls. The conidiogenous cells of B. bassiana are short, ovoid and terminate in a narrow apical extension called a rachis.

III. REVIEW LITERATURE

3.1 Historical development related to B. bassiana

The origins of microbial pest control date back to the early 19th century, when the Italian scientist Agrostino Bassi spent more than 30 years studying white muscardine disease in silkworms. He identified B.bassiana as the cause of the disease. Bassi himself recognized the potential to use organisms such as B.bassiana to control the insect pest (Bassi, 1836; Van Driesche & Bellows, 1996) and by the early 20th century ,field trials had been conducted with B.bassiana , B.brongniartii.v.

3.2 Mode of Action of B.Bassiana on Pests

The major issues involved in mass production and utilization of my pathogens are selection of effective strain, development of cost effective methods of mass rearing, development of effective methods for storage and shipment and creation of effective formulation. Environmental factors like temperature, humidity and sunlight play an important role in the field persistence of entomopathogenic fungi. One of the critical factors in the effective use of microbial agents as insecticides in their relatively short persistence on leaf surface. The commercial consideration such as identification of existing or novel isolation. Quality control of product and patent protection would benefit development of efficient strains.

The cutworm, *spodoptera litura* (fabricus) (Lepidoptera:Noctuidae), is a polyphagous sporadic pest with high mobility and reproducing capacity (Hollyways, 1989) that has about 150 host species (Rao et al. 1993). It is one of the most economically important insect pests in many countries including india. The efficacy of *B. bassiana* against *S. litura* was successfully studied by many scientists (Rangaswami et al., 1969: Robert and Marchal 1980 and Dayakar and Kanajujia 2001).

The mycoinsecticides based on *B. bassiana* have been reported to be useful to control *S.litura, Achaea janata* (Linn.) and *Euproctis fraternal* (Misra). As far as research is concerned, negligible work has been done in Gujarat regarding *B. bassiana*. There was a report of *B.bassiana* on *Helicoverpa armigera* infesting cotton at Junagadh by Baraiya(2003).

So considering the significance of *B. bassiana* in pest management, it is felt worthwhile to investigate various aspects of this insect pathogen under South Gujarat condition, where a humid atmosphere prevails throughout the year, providing a congenial environment for multiplication of the fungus. This will provide scientific information, for development strategies for various insect pests.

In the 1980s, the first insect pathogenic studies were carried out and their focus was to find the methods of disease management of the silkworm. Bassi in 1835, first time formulated the germ theory by the use of white muscardine fungus on the silkworm that was then named in his honor as *Beauveria bassiana*. Gilbert and Gill described that this silkworm disease gave the idea of using insect infecting fungi for the control of insect pest management. A group of fungi that kill an insect by attacking and infecting its insect host is called entomopathogenic fungi. The main route of entrance of the entomopathogen is through integument and it may also infect the insect by ingestion method or through the wounds or trache. Entomopathogenic fungi have a great potential as control agents, as they constitute a group with over 750 species and when dispersed in the environment, provoke fungal infections in insect populations. These fungi begin their infective process when spores are retained on the integument surface, where the formation of the germinative tube initiates, the fungi starting to excrete enzymes such as proteases, chitinases, quitobiases, Upases and lipoxygenases. These enzymes degrade the insect's cuticle and help in the process of penetration by mechanical pressure that is initiated by the appressorium, a specialized structure formed in the germinative tube. Once inside the insect, the fungi develop a hyphal bodies that disseminate through the haemocoel and invade diverse muscle tissues, fatty bodies, Malpighian tubes, mitochondria and haemocytes, leading to death of the insect 3 to 14 days after infection. Once the insect dies and many of the nutrients are exhausted, fungi start micelles growth and invade all the organs of the host. Finally, hyphae penetrate the cuticle from the interior of the insect and emerge at the surface, where they initiate spore formation under appropriate environmental conditions.

Fungi	**Target**
Beauveria bassiana (White muscardine fungus)	Colorado potato beetle, Corn rootworm, Citrus root weevil, Cotton bollworms, Coffee berry borer, codling moth, Japanese beetle, Pod borer, Mango mealy bug, Boll weevil, Cotton leaf hopper, Chinch bug, Yellow stem borer, Rice leaf folder, Brown plant hopper, etc

(Source: Pawar and Singh 1993 and Zimmermann, 1993)

Classification of B.bassiana : Steinhaus gave a brief idea of classification of insect pathogenic fungus including B.bassiana, according to him, insect pathogen fungi divided into four large classes.

1. Phycomycetes
2. Ascomycetes
3. Basidiomycetes
4. Deuteromycetes

Class Deutromycetes have an order Moniliales which include most of the insect pathogen fungus including B.bassiana.

Order: moniliales

Genus: Beauveria

Species: bassiana.

By the time so many modification came in classification finally at present B.bassiana is classification as:

Kingdome: Fungi

Phylum: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Cordycipitaceae

Genus: Beauveria

Species: bassiana

Biochemical name: Beuvaria bassiana.

3.3 Mass Multiplication of Beuvaria Bassiana:

3.3.1 Media for growth and sporulation of *B. bassiana:*

- **Solid medium:**

Pandey and Kanaujia recorded the highest number of conidia produced in Sabouraud dextrose agar medium. Santa et al. recorded that solid substrate (mixture of potato and sugarcane bagasse) gave the highest spore production due to better aeration, less compaction problems and greater surface for spore production. Subouraud dextrose medium with yeast extract was superior over all other media supported the maximum biomass , conidial count and viability of conidia , Rodriguez et al. studied that medium which contain glucose in the pre culture and sucrose and corn steep liquor in the culture medium produced highest spore.

- **Different grain substrates for their effect on sporulation and growth of B. bassiana:**

Among the liquid media, rice powder recorded higher spore production of b.bassiana. Rice and wheat wash water also supported the growth and sporulation of all the tested fungi. Alves and Percira tested different grain substrates for their effect on growth and sporulation of B.bassiana, clearly indicating that inoculation of rice media in plastic bags was highly successful in spore yield. Nirmala et al.notice that rice and its substrate was most suitable for productive growth of B.bassiana. Patel and Kanaujia found that sorghum grain medium was the best substrate for growth and sporulation of B.bassiana. Pandey and Kanaujia suggested that sorghum medium gave higher biomass and conidial count of B.bassiana. Sorghum grain yielded highest conidia/g of substrate due to the presence of rich source of carbon and nitrogen, essential for higher growth and sporulation

IV. METHODOLOGY

4.1 Mass Multiplication Method

1. Multiplication of *B. bassiana* on sorghum seed with incubator.
2. Multiplication of *B. bassiana* on sorghum seed without incubator.
3. Multiplication of *B.bassiana* on wheat bran with incubator.

4. Multiplication of *B. bassiana* on wheat bran without incubator.
5. Multiplication of *B. bassiana* on rice bran with incubator.
6. Multiplication of *B. bassiana* on rice bran without incubator.
7. Mass multiplication on A media given by vise innovative enterprise Pvt. Ltd to observe *B. bassiana* growth on different content of moisture.

4.2 Parameters:

4.2.1 CFU count by Hemocytometer

- Counting cells in a hemocytometer:

Total cells/ml = Total cells counted ×dilution factor/ no. of square × 10,000 cells/ml

So, for example , if you diluted your sample 1:1 with Trypan blue, and you counted 325 cells in 4 corner square plus the central big square.

Total cells per ml = 325 cells ×2(dilution factor)/5 square ×10,000 cells/ml = 130×10^4 cells/ml

If you want to know how many cells you have in your original sample, just multiply the cell concentration by total sample volume. For example, if your original sample volume is 5 ml , than your sample has a total = 130×10^4 cells/ml × 5ml = 650×10^4 cells.

4.2.2 BIOMASS calculation of Beauveria bassiana:

- ***Beauveria bassiana* growth with incubator:**

The dry weight of the fungus was calculated by using the following formula:

Dry weight = (weight of petri plate with mycelia) - (weight of petri plate)

- ***Beauveria bassiana* growth without incubator:**

The dry weight of the fungus was calculated by using the following formula:

Dry weight = (weight of petri plate with mycelia) - (weight of petri plate)

V. RESULT AND DISCUSSION

5.1 Zone of inhibition on different antibiotics dose on Beauveria bassiana:

100 ml dose of Different Antibiotic	Zone of inhibition on petri plate
Streptomycin	0.6 mm
Gentamicin	0.2 mm
Amoxicillin	0.3 mm
Chloramphenicol	0.5 mm
Fluconazol	0.9 mm
Dithen	0.7 mm
Mancozeb + Metalaxyl	0.8 mm

5.2 Biomass calculation of *Beauveria bassiana* with incubator and without incubator:

After 5-6 days with the incubator, it gives good results of fungal biomass calculate the dry weight of fungus give by below formula:

Dry weight = (weight of petri plate with mycelium) - (weight of petri plate)

- Biomass of B. bassiana with incubator = (7.017) – (6.4443) = 0.5727
- Biomass of B. bassiana without incubator = (7.2777) – (6.7057) = 0.5725

5.3 Effect of different grain substrate medium on sporulation of *Beauveria bassiana* with incubator:

B. bassiana maximum spore production on rice 5.8×10^8 CFU/gm. wheat bran and sorghum also produce good sore production.

Grain (20g)	Spore count ($\times 10^4$) (Series 1)	Spore count ($\times 10^6$) (Series 2)	Spore count ($\times 10^8$) (Series 3)
Rice	**7.9**	**6.5**	**5.8**
Wheat bran	**6.8**	**6.02**	**5.5**
Sorghum	**6.6**	**5.8**	**4.5**

5.4 Effect of different grain substrate medium on sporulation of Beauveria bassiana without incubator:

B. bassiana maximum spore production on rice 7.24×10^8 CFU/gm which is more than with incubator method. On wheat and sorghum spore production is less than with the incubator method.

Grain (20g)	Spore count ($\times 10^4$)	Spore count ($\times 10^6$)	Spore count ($\times 10^8$)
Rice	**8.87**	**7.89**	**7.24**
Wheat	**6.7**	**5.76**	**5.45**
Sorghum	**6.58**	**5.77**	**4.45**

5.5 Mass multiplication on A media given by vise innovative enterprise Pvt. Ltd to observe *B. bassiana* growth on different content of moisture:

Volume of Suspension	Growth of B.bassiana
4 ml	64%
6 ml	75%
8 ml	88%
9 ml	95%

VI. CONCLUSION

Using biological control agents such as entomopathogenic fungi can be used as a component of integrated pest management of many pests. Several fungal species such as Beauveria bassiana are being used as biocontrol agents for a number of crops, livestock and human nuisance pests. Various agriculture products and by-products such as grain, vegetable waste, seeds, rice husk, and sawdust were evaluated for mass multiplication. Here we take wheat bran, rice bran and sorghum for mass multiplication. Beauveria bassiana effect on rice weevil, Sitophilus oryzae.and rice leaf folder in rice grain.

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