Effect of different Fungicides on Growth of *Beauveria Bassiana*

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Abstract— *Beauveria bassiana is an entomopathogenic fungus that grows naturally in soils throughout the world,which acts as a pathogen with significant host range and host specificity. Use of chemical products to protect plants and other environmental factors affects the conidial survival of beauveria bassiana fungus. This research paper mentions the compatibility of beauveria bassiana with fungicides such as Chloramphenicol, Tetracycline, Amoxicillin, Gentamicin, Mancozeb + Metalaxyl, Fluconazol. A positive result was obtained for Fluconazole, Mancozeb+Metalaxyl, Dithane (Amoxicillin + Streptomycin + Tetracycline + Chloramphenicol + Mancozeb).*

Keywords— Beauveria bassiana, Fungicides, soils, plants, environmental factors.

I. INTRODUCTION

The entomopathogenic fungi *Beauveria bassiana* functions as an effective control agent against many pests.*Beauveria bassiana* plays a key role in causing disease in arthropod populations.

Availability of adequate moisture and temperature helps *Beauveria bassiana* in acting as an important mortality factor of arthropod pests. Various commercial products are developed using this fungus due it's capability to control pests. *Beauveria bassiana* possesses all the attributes of an effective mycoinsecticide, i.e. high virulence, broad host range, flexibility of fungi to mass production and formulation and storage and product stability. Diversity and abundance of natural enemies in the soil such as *Beauveria bassiana* is affected due to man activities such as frequency and type of pesticide application .Although, abundant of bio-control agents has been introduced in the market need for chemical pesticides remains. Frequent use of chemical pesticides resulted in tremendous decline of population of entomopathogenic fungi in the soil. Furthermore, it affected their efficiency in pest regulation. Conidial survival of *Beauveria bassiana* gets affected due to environmental factors or due to use of chemicals such as fungicides and biopesticides used to control plant pests. When the fungus comes in contact with pesticides or herbicides, its efficacy gets either antagonized or synergized. Similarly, the insecticidal property of *Beauveria bassiana* also gets affected with a disruption of its natural epizootics. The effect of pesticides such as fungicides on *Beauveria bassiana* fungus was discovered after carrying out many experiments. Inhibition of *Beauveria bassiana* by many fungicides was indicated in various *in vitro* studies.

II. HISTORY OF FUNGUS BEAUVERIA BASSIANA

The entomologist BASSI Agostino of Lodi in 1985 discovered the cause of pebrine disease characterised by turning Italy's silkworms into white mummies. The symptoms of disease included appearance of cadavers covered with white powdery layer which gave rise to the name"white muscardine disease". Later, Beauverie jean described this pathogen as *Botrytis bassiana*. By twentieth century, Vuillemin (1912) claimed that *Botrytis bassiana* (Bals.-Criv) was a species that belongs to the genus Beauveria.

A new species, Cordyceps bassiana has been described from China on a carpenterworm larva, Prionoxystus robiniae (Lepidoptera: Cossidae), and is doubtless associated with *Beauveria bassiana*. The main characteristic of hyphomycetes (Deuteromycotina) is that they lack amphimixis, making the taxonomists consider only morpho-ontogenic characteristics to discriminate between species (De Kouassi 2001).

2.1 Morphology of *Beauveria Bassiana*

The fungus *Beauveria bassiana* (Bals.) Vuill acts as a pathogen for many insect orders such as: Lepidoptera, Hemiptera, Coleoptera, Hymenoptera, Homoptera, Hemiptera and Orthoptera. *Beauveria bassiana* consist of asexual spores called conidia of white to yellow colour with zig-zag transparent and septal filaments. Diameter of hyphae varies between 2.5 μm and 25 μm. Type of conidia produced by *Beauveria bassiana* depends on the environment. Spherical or oval conidia are produced in the presence of air while oval shaped blastospores are produced in anaerobic conditions.

2.2 *Beauveria Bassiana*: Mode of Action on Insects

Four steps included in the mode of action of *Beauveria bassiana* on pest insects include adhesion, germination, differentiation, penetration and dissemination. Once inside the insect, the fungus develops a hyphal body that uses haemocoel for dissemination. The insect dies after 3 days or two weeks due to infection in different muscle tissues, malpighian tubes, fatty bodies, haemocoel and mitochondria. The idea of using insect-infecting fungi for insect pest management belongs to Gilbert and Gill. Entomopathogenic fungi can be defined as a group of fungi that attack and infect the host of the insect in order to kill the insect. The major issues involved in mass production and utilization of myco-pathogens are selection of effective strains, development of cost effective methods of mass rearing, development of effective methods for storage and shipment and creation of effective formulation. So considering the importance of *Beauveria bassiana* in pest management, it felt worthwhile to research various aspects of this insect pathogen under South Gujarat conditions, where a damp atmosphere, which is favourable for multiplication of fungus, is available throughout the year. Entomopathogenic fungi play a significant role as a biocontrol agent. There are over 750 species of entomopathogenic fungi and their presence in the environment induces fungal infection in the insect population and this way they help in controlling insect pests. This fungi infect insect by a mechanism in which it initiates formation of germination tube by retaining spores on the integument surface and then by releasing enzymes such proteases, chitinases, quitobiases, Upases and lipoxygenases. Once the insect dies and lots of of the nutrients are exhausted, fungi start micelles growth and invade all the organs of the host. In the 1980s, the primary insect pathogenic studies were administered and their focus was to seek out the methods of disease management of the silkworm. These enzymes degrade the insect's cuticle and help within the process of penetration by mechanical pressure that's initiated by the appressorium, a specialized structure formed within the germinative tube. The cutworm, spodoptera litura (fabricus) (Lepidoptera:Noctuidae), may be a polyphagous sporadic pest with high mobility and reproducing capacity that has about 150 host species. In the end, the hyphae emerges outside the insect by penetrating through the cuticle and after reaching the surface, it begins spore formation when suitable environmental conditions are available .This will provide scientific information for development strategies for various insect pests. The rice weevil, Sitophilus oryzae (L.) (Coleoptera: Curculionidae) in rice grain, and the red flour beetle, Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) in wheat grain cause quantitative and qualitative damage to grain. Insects are categorised as important pest of seeds because they attack stored seeds and damages the seed embryo resulting in reduced rate of seed germination. Qualitative damage is thanks to product alterations like loss of nutritional and aesthetic value, increased levels of rejects within the grain mass, and loss of commercial (baking) characteristics.

The entomopathogenic fungus is the most effective agent in biocontrol. It can be found in all types of soil. Different isolates of *Beauveria bassiana* act as pathogens to a wide range of insects and mites. *Beauveria bassiana* can be considered as an eco-friendly method of pest control contrary to chemical pesticides. Culturing and maintenance of *Beauveria bassiana* is cheap compared to chemical pesticides. Due to longer lifespan, insecticidal activity of *Beauveria bassiana* is faster than other entomopathogenic agents. Conidia of *Beauveria bassiana* remains in the environment for a longer time by spreading epizootic or enzootic diseases. Their affect on beneficial insects and other non-targeted organisms are limited. Due to its ability to use various mode of action and adapt to host changes, insects cannot easily develop resistance against *Beauveria bassiana*.

III. MATERIALS AND METHODS

3.1 Materials

- *Beauveria bassiana* mother culture
- Water
- SDA agar plate

- L-shaped glass spreader
- Alcohol
- Bunsen burner
- Petri dish
- Filter paper
- Measuring scale
- Incubator
- Micropipette and tips
- Microscope
- Beaker
- Conical funnel
- Flask

Antibiotics:-Chloramphenicol, Tetracycline, Amoxicillin, Gentamicin, Mancozeb+Metalaxyl, Fluconazol

3.2 Observe growth of *Beauveria bassiana* on different concentrations of fungicides:

3.2.1 For 10 ml preparation:

1. Take 9 ml of distilled water in a glass beaker.
2. Add 1 capsule of fungicide.3.For Streptomycin add 350mg in 9 ml water and for Mancozeb + Metalaxyl 250mg, Dithane (Mancozeb) 350mg add in 9 ml water.
3. Dissolve properly with the help of glass rod.
4. Antibiotic solution is poured into a conical funnel fitted with a filter paper and collects antibiotic solution (filtrate) into a flask.
5. Make a fungal solution 10 ml.

3.2.2 10 ml combination of different fungicidal dose:

1. Take 1 ml of fungicide which is prepared by the above given method.
2. Take 1 ml of different fungicides and mix up in one test tube.

Different combinations of fungicides:

C_1 = Amoxicillin + Streptomycin + Tetracycline + Chloramphenicol

C_2 = Amoxicillin + Streptomycin + Tetracycline + Chloramphenicol + Mancozeb (Dithen)

$C_3 = C_2$ + Gentamicin

$C_4 = C_3$ + Fluconazole

$C_5 = C_1$ + (Mancozeb +Metalaxyl)

3.2.3 For 100 ml preparation:

1. Take 90 ml of distilled water in a glass beaker.
2. Add 1 capsule of fungicides.
3. For Streptomycin, add 350 mg in 100 ml water and for Mancozeb + Metalaxyl 250 mg, Dithen (Mancozeb) 350 mg add in 100 ml water.
4. Dissolve properly with the help of a glass rod.

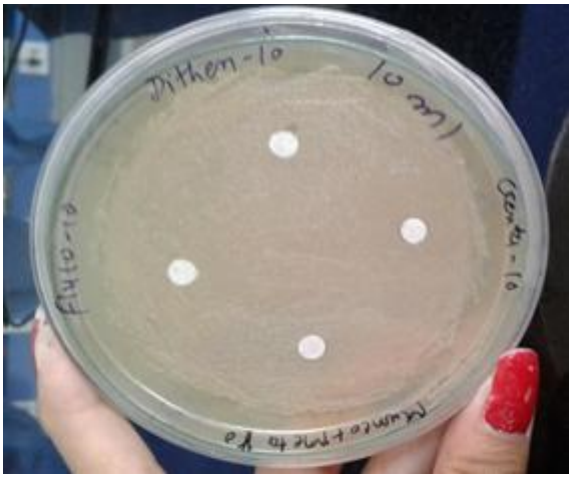
5. Fungal solution is poured into a conical funnel fitted with a filter paper and collect fungal solution (filtrate) into a flask.
6. Make up fungal solution 100 ml.

3.2.4 Disc Diffusion Method:

1. First of all, make a suspension using fungicides.
2. Take a loop of full mycelia growth of the fungus to the pure culture plate of Beauveria bassiana.
3. Dissolve it in 10 ml of sterile distilled water.
4. Pipette out 0.1 ml from the desired dilution series onto the center of the surface of an SDA agar plate.
5. Dip the L-shaped glass spreader into alcohol.
6. Flame the glass spreader over a Bunsen burner.
7. Spread the sample evenly over the spreader of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at the same time.
8. The disc diffusion assay was performed in a sterilized petri plate of 10 cm diameter.
9. Different dilutions of the fungicides were impregnated on the sterilized discs (5 mm in diameter) of whatman filter paper number 1.
10. The discs were placed on the surface of the SDA agar plate which was already inoculated with *Beauveria bassiana* culture suspension.
11. The plates were inoculated at 25℃ and examined after 48 hour for zones of inhibition, if any, around the disc.
12. The diameter of the zone of inhibition was measured with the help of a scale.

IV. RESULT AND DISCUSSION

Among the fungicides used, a maximum zone of inhibition was observed in the case of fluconazole covering an area of 0.9mm. On the other hand, the zone of inhibition recorded in case of gentamicin was the least with an area covering 0.2 mm. In case of mancozeb+metalaxyl, dithane and streptomycin the zone of inhibition recorded was 0.8, 0.7 and 0.6 respectively, which can be considered moderate. On the contrary to this, amoxicillin and streptomycin showed minimum inhibition to beauveria fungi in an area of 0.3 and 0.2 respectively. Fungicides such as fluconazole which showed maximum inhibition to beauveria when applied in the soil, restricts the growth of natural biocontrol agents like beauveria in the soil. Whereas, gentamicin application has minimal effect on the lives of *Beauveria bassiana* hence can be used in the soil without killing the beneficial organisms present in the soil.



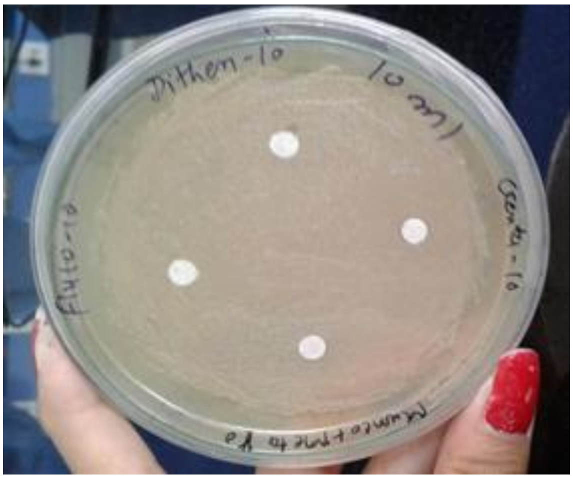


FIGURE 1: [10ml : Fluconazole, Dithane, Manco+Metalexyle, Amoxicillin, Chloramphenicol, Gentamicin, Tetracycline]

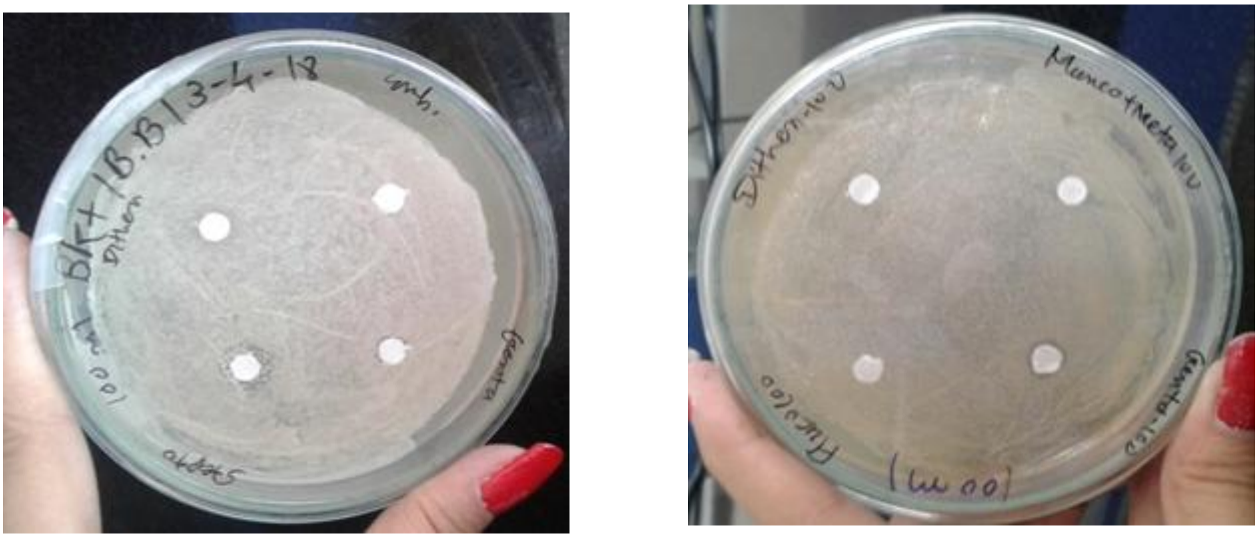


FIGURE 2: [100 ml : Dithen, Streptomycin, Fluconazole, Gentamicin, mancozeb + Metalaxyl, Amoxicillin]

V. CONCLUSION

The Use of biological control agents such as entomopathogenic fungi (EPS) can be used as a component of integrated pest management (IPM) 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. Hence it is very important to ensure availability of these bioagents in the soil by avoiding the use of fungicides and other pesticides that inhibit their growth. In this research paper we have discussed how affect of fungicides on *Beauveria bassiana* varies depending on the type of fungicide and the result helped in determining which fungicides should be used to ensure the survival and growth of beauveria bassiana in the soil. Instead of using fungicides such as fluconazole and mancozeb+metalaxyl, which shows high rate of inhibition of growth of *Beauveria bassiana*, fungicides such as gentamicin that shows minimum rate of inhibition should be used. Fungicides that inhibit growth of *Beauveria bassiana* such as fluconazole and mancozeb+metalaxyl, when applied in the soil, reduces the population of beneficial *Beauveria bassiana* that are naturally available in the soil and results in increased dependency on chemical pesticides for controlling pests. Use of fungicides like gentamicin helps in conserving the population of *Beauveria bassiana* in the soil, which acts as a natural biocontrol agent.

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