



***BEAUVERIA BASSIANA* (Bals.-Criv.) Vulliemini – USE AS A MAGICAL BIOCONTROL AGENT**

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ABSTRACT

The sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times. Of the several microbial pathogens *viz.*, bacteria, fungi, viruses, protozoans and entomopathogenic nematodes reported, only a few have been studied systematically for their usefulness. A careful evaluation of these beneficial pathogens can lead to gainful exploitation in microbial control programmes. India is bestowed with a rich biodiversity of entomopathogens and exploitation of these natural and renewable resources are essential in a successful biocontrol strategy. Entomogenous fungi are potentially the most versatile biological control agents, due to their wide host range that often results in natural epizootics. An attractive feature of these fungi is that infectivity is by contact and the action is through penetration these fungi comprise a heterogeneous group of over 100 genera with approximately 750 species, reported from different insects. Many of these offer a great potential in pest management. The most important fungal pathogens are *Metarhizium* spp., *Beauveria* spp., *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella* spp. of the over 750 species of fungi known to be pathogenic to insects, six have been commercialized and the cosmopolitan pathogens, such as *B. bassiana*, *M. anisopliae* are the best known far spectacular epizootics with large number of pathogenic insects showing visible fungal outgrowth. Conservation and periodic enhancement of efficacy of biological control agents will help in crop protection and in producing agricultural commodities free from pesticide residues. There is a general feeling that, the development and spread of biological control will empower the resource of poor farmers to manage their pest problems in an eco-friendly way. This assumes importance since the growth rate of the use of biopesticides alone over the next 10 years has been forecast at 10-14 per cent per annum, in contrast to two per cent for chemical pesticides. Environmental factors like temperature, humidity and sunlight play a profound role on the field persistence of entomopathogenic fungi. One of the critical factors in the effective use of microbial agents as insecticides is their relatively short persistence on leaf surfaces. The realization of the economic potential of mycoinsecticides would benefit from advances in biotechnology.

KEYWORDS - *Beauveria bassiana*, Entomopathogenic fungus, White muscardine disease, Soil saprophyte, White bloom appearance.

INTRODUCTION

Beauveria bassiana is a fungal pathogen that was discovered by Agostino Bassi de Lodi in 1835. Bassi was researching the heavy decline in larval silkworms, which are used to produce silk. He determined that the “muscardine” was caused by a fungus that multiplied in and on the host (Mahr). This was actually the first reported microorganism to be recognized as a contagious agent of animal disease. Its use in the control of malaria-transmitting mosquitoes is under investigation. This pathogen was later named after Agostino Bassi himself, who was also deemed the “Father of Insect Pathology.” His discovery not only laid the foundation for microbial pest control, but also significantly influenced the work Louis Pasteur, Robert Koch and other pioneers of microbiology (Ainsworth, 1956; Porter, 1973; Van Driesche & Bellows, 1996). Bassi himself recognized the potential to use organisms such as *Beauveria bassiana* to

control insect pests (Bassi, 1836; cit. in Van Driesche & Bellows, 1996). Today, over 100 years later, *Beauveria bassiana* is used as a magical biocontrol agent and there are no significant adverse effect has been reported that can be attributed to the use of these organisms in biocontrol.

Morphology of *Beauveria bassiana*

In culture, *B. bassiana* grows as a white mold. On most common cultural media, it produces many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells. The conidiogenous cells of *B. bassiana* are short and ovoid, and terminate in a narrow apical extension called a rachis. The rachis elongates after each conidium is produced, resulting in a long zig-zag extension. The conidia are single-celled, haploid, and hydrophobic. *B. bassiana* has a variety of characteristics that make it unique to other pathogens. It produces sexually and asexually. It is usually filamentous and has definite cell walls. The growth rate is moderately rapid. The colonies can

reach a diameter of 3cm following an incubation time of seven days at 27°C on potato glucose agar. It possesses many strains that exhibit considerable variation in virulence, pathogenicity and host range.

How it works upon pathogens

B. bassiana is an aggressive parasite of many different insect host species. Insects are attacked at larval or adult stages. Insects can also spread the fungus through mating (Long *et al.* 2000). The hyphae and spores are non pigmented (hyaline) and so colonies appear white in cultures or tufts of white mycelium bearing masses of powdery spores burst out through the body parts of infected insects.

B. bassiana produces spores that are resistant to environmental extremes and are the infective stage of the fungal life cycle. The spores infect directly through the outside of the insect's skin. Under favorable temperature and moisture conditions, a conidium (singular of "conidia") adhering to the host cuticle will germinate. The fungal hypha growing from the spore secretes enzymes which attack and dissolve the cuticle, allowing it to penetrate the skin and grow into the insect body. Fungal spores are readily killed by solar radiation and infect best in cool to moderate temperatures (Goettel *et al.*, 2000, Wraight and Ramos 2002). Once inside the insect it produces a toxin called Beauvericin that weakens the host's immune system. After the insect dies, an antibiotic (oosporein) is produced that enables the fungus to outcompete intestinal bacteria. Eventually the entire body cavity is filled with fungal mass. When conditions are favorable the fungus will grow through the softer parts of the insect's body, producing the characteristic "white bloom" appearance. Relative humidity must be 92% or more for *B. bassiana* to grow outside the insect. These external hyphae produce conidia that ripen and are released into the environment, completing the cycle.

Types of pests it controls

Beauveria bassiana is a common soilborne fungus that occurs worldwide. It attacks a wide range of both immature and adult insects. Besides silkworm, the extensive list of hosts includes such important pests as whiteflies, aphids, grasshoppers, termites, Colorado potato beetle, Mexican bean beetle, Japanese beetle, boll weevil, cereal leaf beetle, bark beetles, lygus bugs, chinch bug, fire ants, European corn borer, codling moth, and Douglas fir tussock moth. Natural enemies, such as lady beetles, are susceptible too, and it has even been found infecting the lungs of wild rodents, and the nasal passages of humans. There are many different strains of the fungus that exhibit considerable variation in virulence, pathogenicity and host range. It occurs in the soil as a saprophyte.

Pathogenicity and Infectivity on humans and the environment

An extensive literature search was conducted to evaluate risks related to human exposure to *B. bassiana*. Like any micro-organism, *B. bassiana* has the potential to act as an opportunistic pathogen, but as the literature study

demonstrates, *Beauveria* infections are extremely rare events. A detailed analysis of case reports allegedly involving *B. bassiana* reveals that extraordinary circumstances, such as a severely compromised immune system or a history of surgery/injury, are required for a *B. bassiana* infection to occur.

The most severe human cases of *Beauveria* infections are two recent reports of disseminated mycoses (Henke *et al.*, 2002; Tucker *et al.*, 2004). Both of these infections occurred in severely immuno-compromised patients with acute leukemia. Prior the development of mycoses, one patient underwent 4 full cycles of chemotherapy, the other was in her first cycle of chemotherapy and had been diagnosed with *Streptococcus viridans* in her bloodstream. Despite their poor health, both patients responded well the antimycotic treatments and fully recovered from their mycoses.

While there are some reports of *Beauveria* spp. isolated from patients with corneal keratitis, the *B. bassiana* can certainly not be considered a significant eye pathogen. Of four reports linked to *Beauveria*, only two (Sachs *et al.*, 1985; Kisla *et al.*, 2000) can be conclusively attributed to *Beauveria bassiana*. In these cases the affected eye had undergone surgery following traumatic injury to the eye, and in all reported cases, the therapy of the injured eye involved corticosteroids and antibiotics, which, according to Sachs *et al.* (1985) can predispose the eye to fungal infections by otherwise non-pathogenic fungi.

None of the studies conducted for the registration of *B. bassiana* strain GHA in the US (acute oral toxicity/pathogenicity; acute dermal toxicity; acute pulmonary toxicity/ pathogenicity; acute intraperitoneal toxicity/ pathogenicity) showed any pathogenicity against eye irritation (US EPA, 2006) and (Ishibashi *et al.*, 1987; Begley & Waggoner, 1992) show no unacceptable effects related *B. bassiana* exposure to the healthy eye. There are no expected health risks to humans who apply this insecticide or to people who eat the crops that have been treated with the fungus. No negative effects of Botanigard were found in ecotoxicology studies with mammals, birds or fish (US EPA, 2006). Literature reports of *B. bassiana* infections in captive reptiles (Georg *et al.*, 1962; Fromtling *et al.*, 1979; Gonzalez *et al.*, 1995) can be attributed to inappropriate captivity conditions, and no reports of any vertebrates infected by *B. bassiana* in the wild were found.

Efficacy of *Beauveria bassiana*

The variability of control of biological organisms are generally more dependent on environmental conditions, such as climatic factors, timing of sprays, and the stage of the insect, as well as the insect's inherent susceptibility to the fungus than those achieved with conventional pesticides. *Beauveria bassiana* has been tested in a wide range of pest control scenarios and has been successfully used in many countries. While, under suitable conditions, efficacy rates of Botanigard can exceed 90 %, in many instances, a considerably lower level, but longer-term suppression can be sufficient to prevent crop damage. It is important to recognize that biological control agents such as *B. bassiana* significantly differ from chemical pesticides in

their properties and this should be taken into account when designing and reviewing efficacy studies.

Compatibility with other pesticides

Beauveria products should not be tank-mixed with other products such as sticking agents, insecticidal soaps, emulsifiable oils, fungicides or used with beneficial insects. Chlorpyrifos is a common insecticide used against the sum of same targets as *B. bassiana*. This substance acts as neurotoxin (acetylcholine esterase inhibitor) and is therefore highly toxic to a wide range of non-target organisms, including mammals and birds, aquatic organisms and bees. *Beauveria* products are compatible with a number of adjuvants and chemical and biological insecticides. Imidacloprid appears to synergize the activity of *Beauveria* for several pest insects, including potato beetles.

Commercially available as a microbial insecticide

B. bassiana is available commercially as a microbial insecticide since *Beauveria* can now be mass produced by a fermentation process and formulated to enable the fungus to withstand ultraviolet light, and temperature and humidity extremes commonly encountered in the field. There are several products that contain *B. bassiana*, including Naturalis and Mycotrol; it is likely additional products will be registered with the EPA. Because it takes 3-7 days to kill an insect with *B. bassiana*, it will take some time to suppress the pest population when using these products. Thorough spray coverage is essential because fungal spores must contact the insect for infection to occur.

CONCLUSIONS

A careful evaluation of these beneficial pathogens can lead to gainful exploitation in microbial control programmes (Burgess, 1998). Entomogenous fungi are potentially the most versatile biocontrol agents (Boucias D. & R, Pendland JC. 1998), due to their wide host range that often results in natural epizootics (Kpindou, *et al.*, 1997; M. Faria, *et al.*, 2001; Feng, *et al.*, 2004; S.P. Wraight, *et al.*, 1998). An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Nadeau *et al.*, 1996). Additionally, commercial considerations such as identification of existing or novel isolates, quality control of product and patent protection (Leathers *et al.*, 1993) would benefit the development of efficient process for large scale multiplication. Furthermore, it has been used in biocontrol for over 100 years with no reports of illness related to exposure to *B. bassiana* strains used in biocontrol.

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