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Potentialities entomopathogenic fungus *Beauveria bassiana* as a biocontrol agent: A Review

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Abstract
Entomopathogenic fungi are the predominant natural pathogens in most of the terrestrial ecosystems. The species richness, ubiquitous nature and observations of epizootics among insect population indicate the great potential of these microbes to regulate the pestiferous species. High virulence, eco-friendliness, bio-persistence, host specificity and the increased biodiversity in managed ecosystem, are some of the attributes of these insect pathogens that enable them to be incorporated as an eco-friendly component in integrated pest management programs. They have gained a great momentum worldwide as an effective biocontrol agent. The ecofriendly control of various insect pests involves the use of myco-insecticide based on *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Lecanicillium* spp., *Isaria fumosorosea* and *Paecilomyces fumosoroseus*. The entomopathogenic fungi belonging to Entomophthorales and Hypomycetes are responsible for epizootics in the insect population. Among the fungal pathogens *Beauveria bassiana* has assumed a prime role in the management of various arthropod species of forestry, veterinary, medical and agricultural importance.

Keywords: Entomopathogenic fungus, *Beauveria bassiana*, host range, mass multiplication, commercial avilability

1. Introduction
Entomopathogenic fungi being the prominent natural pathogens in the terrestrial ecosystems have gained a great momentum worldwide as effective biocontrol agent. The ecofriendly control of various insect pests involves the use of myco-insecticide based on *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Paecilomyces fumosoroseus*. Among the fungal pathogens *Beauveria bassiana* has assumed a prime role in the management of various arthropod species of forestry, veterinary, medical and agricultural importance [18]**.** Presently, six species of this genus i.e. *Beauveria bassiana*, *Beauveria clade*, *Beauveria brongniartii*, *Beauveria caledonica*, *Beauveria vermiconia* and *Beauveria amorpha* has been recognized worldwide [27, 10]. Amongst all the available species *B. bassiana* have been studied and exploited most for the control of insect and made commercially available as a biopesticide worldwide [20].
Beauveria bassiana has been integrated as the part of several biocontrol programmes. The funguswas included in the Gazette of India on March 26, 1999 for commercial production and distribution [14]. One essential component of biocontrol program is to produce adequate quantities of good quality inoculums [15]. *Beauveria bassiana* grows and sporulates on several natural substrates such as whole grains, vegetable wastes, hay and straw, chopped carrot, sugarcane bagasse and bran. The liquid media such as rice and wheat washed water, coconut water, sliced potato boiled water and rice cooked water can be used for the production of *Beauveria bassiana* [36]. The present paper discus about the mode of action, host range, mass production technique, avilable formulations, field doses, commercial availability and precautionary measures during application of *Beauveria bassiana*.

2. Mode of action
Beauveria bassiana is a facultative cosmopolitan soil inhabiting hyphomycetes fungus with a broad host range [34]**.** Its host range spans to the insects of all orders and extends to ticks and mites [28]. It causes white muscardine disease in the arthropod species when the respective host comes in contact with the infectious propagules. The infective propagules or conidia adheres to the host body, penetrates the cuticle where it proliferates, produces mycotoxin beauvericin that has been documented for its larvicidal properties [1]. The mycotoxin incites the progressive

Degeneration of the host tissues and obstructs the haemolymph circulation further leading to asphyxiation followed by death within a matter of few days. The fungus continues to grow saprophytically and under favorable conditions the hyphae emerges from the cadaver's intersegmental areas producing conidia. These conidia are dispersed to soil and other substrates where they lead to further infection in the host with which they come in contact. The fungal spores and mycelia are white and they have been isolated from diverse insects like *Helicoverpa armigera*, *Spodoptera litura* and *Anadevidia peponis.*

3. Method for mass production of *B. bassiana*

For the mass multiplication of this entomopathogenic fungus, 1 kg of disinfected, healthy and sound sorghum grains were taken and soaked with normal water for 48 hours. Then the soaked grains were allowed to shade dry and after proper drying, they were transferred into the small size heat resistant plastic bags at the rate of 100gm of the grains per bag. The plastic bags along with the grains were tightly packed with the help of cotton plug and plastic ring and then they were sterilized in autoclave at 121psi for 15 minutes. Grains were then inoculated with the fungal mycelium and kept under incubation. Shaking of the grains was done at an interval of 3-4 days. Full growth of whitish fungal mycelium on the grains was observed after 15-20 days of inoculation. The infected grains can be further processed for field application. The grains can be further crushed and mixed with talc. This can be used as a dust formulation for application in the field [36].

Table 1: Host Range

Order	Susceptible hosts
Coleoptera	*Lathrobium brunnipes* (rove beetle) *Otiorhynchu esulcatus*(vine weevil) *Sitona lineatus* (pea leaf weevil) *Anthonomus pomorum* (Apple blossom weevil) *Hylasterater* (bark beetle) *Leptinotarsa decemlineata* (Colorado potato beetle) *Pantorhytesplutus spp.* (cocao weevil) *Holotricha spp.* (whit grub)
Lepidoptera	*Hepialus spp.* (ghost moths) *Hypocrita jacobaea* (Cinnabar moth) *Cydia nigricans* (pea moth) *Spodoptera litura* (tobacco cutworm) *Helicoverpa aarmigera* (cotton bollworm)
Heteroptera	*Picromerus bidens* (spiny shield bug) *Anthocoris nemorum* (minute pirate bug)
Homoptera	*Eulecanium spp.* (scale insect) *Aphis craccivora* (cowpea aphid, groundnut aphid, black legume aphid) *A. gossypii*(melon aphid, cotton aphid) *Rhapalosiphum maidis* (corn leaf aphid) *Bemisia tabaci* (whitefly)
Diptera	*Leria serrata* (wool flies)
Orthoptera	*Schistocera gregaria*(desert locust) *Locusta migratoria*(migratory locust)

(Keswani *et al*., 2013) [14]

4. *Beauveria bassiana* as a bio-control agent against major agricultural pest

Agricultural pests continue to be one among the major concerns related to lower production of agricultural produce contributing major crop loss under field as well as storage condition. Synthetic insecticides used for controlling the pest menaces are already creating many problems and has raised serious concerns about their safety to environment as well as other natural enemies. Excessive application of chemicals to environment often have deleterious effects on beneficial insects like predator, parasitoids and pathogens. Apart from these, problems of pesticide residue, pest resurgence and secondary pest outbreak are the major drawbacks of synthetic pesticide being used in large scale. Health hazards to the workers during the spraying and handling of chemical insecticide cannot be avoided. Therefore, realizing the ill effects of these chemical insecticide, an alternate strategy should be developed. Promotion and largescale use of biological control can reduce excess dependence on chemical insecticide. Amongst the biological control agents, entomopathogenic fungi is growing popularity. The reasons for their popularity can be attributed as firstly, they are not considered as "introduced organism" because of their ubiquitous presence in soil trough out the world. Secondly although *Beauveria bassiana* is considered a broad - spectrum insect pathogen, strains can be developed that are more hosts specific. With research into pathogenicity and strain specificity, it is anticipated that fungal biological control agents can be selected to target specific insect pest [30]. Role of Beauveria as a bio control agent against major insect pests of agricultural importance are described below.

A) Lepidopteran Pest

Beauveria bassiana (Balsamo) primarily act as a chitin synthesis inhibitor, which attaches the fungal mycelium to the insect cuticular structures of insect and gains entery through the cuticle. Gradually the fungal hyphae germinate and proliferates in insect body. Decaying body tissues provide ample moisture for the growth of fungus. White coloured mycelium grows profusely over the insect body after 24-48 hours. After hyphal penetration, infected insects may live for three to five days. Fungus released chitinase enzymes and its action inhibits chitin formation. Hyphal penetration of the fungus into different parts of insect with respect to time was recorded by EISinary (2002) [7] and Quesada-Moraga (2006) [26]. Efficacy of the entomopathogenic fungi began clearly after 48 h after inoculation and advances inside tracheal cells and fat bodies after 72 hours and 100 hours of inoculation respectively.

Beauveria bassiana has been found effective against many lepidopteran insect pests. It inhibits the mitochondrial respiration by affecting NADH- C cytochrome reductase and complex 1 of insect mitochondria resulting in malformed pupal stage [16]. After 48 hours of inoculation of the fungus the hyphae penetrates the integument, the trachea and integument. After 72 hours damage in fat tissue was recorded with complete mortality after 96 hours [26]. Many researchers have proved the pathogenicity of *B. bassiana* against major lepidopteran pests under laboratory as well as field condition. In this paper we will throw light on some of those earlier works proving the pathogenicity of this fungus against major lepidopteran pests. A prolonged pupal duration of potato tuber moth (8.9 days) due to application of *B. bassiana* treatment (2.4×105 conidia per ml) [12]. Vanderberg *et al*. (1998) [35] reported that, the formulated products were found least effective against diamond back moth spores in comparision with the spores suspended in water and oil. Effectiveness of *B. bassiana* against diamond back moth has also been proved by Yoon, 1999, Selman *et al*. (1997) and Puzari *et al*. (2006) [37, 31, 25]. Increase in larval mortality of *Helicoverpa armigera* with increase in spore concentration was reported by Dhembare and Siddique (2004) [6]. Purwar and Sachan, (2006)

[24] reported higher toxicity of *B. baasiana* (4.9 times) against *Spilarctia oblique* in combination with endosulfan. Zibaee *et al.* (2013) [39] observed higher mortality of fall web worm upon application of *B. bassiana* along with two medicinal plant extract. El- khawas *et al.* (2002) [8] determined the LC_{50} value of Beauveria formulation for *Spodoptera litorallis* i.e. 30.65x 10^5 conidia/ cm3 at which elongation of the larval and pupal duration was observed with 59-92 percent mortality. EI-sufty *et.al* (1982) [9] and Boucias (1996) [5] found the fungi *Beauveria bassiana* (Balsamo) Vuillemin effective against the leaf worm and beet armyworm. Prasad and Syed (2010) [23] reported 86.7 per cent larval mortality of *Helicoverpa armigera* when treated with 0.25 x 10^8 conidia per ml) of fungus *Beauveria bassiana* (Balsamo) under laboratory condition. Ritu *et al.* (2012) [29] standardized different formulations of Beauveria against *Helicoverpa armigera* and concluded bentotite based formulation as most effective. Shakir *et al.* (2015) [32] recorded maximum mortality (61.91%) of *Cnaphalochrosis medinalis* with combined application of *B. bassiana*, Potassium silicate and imidacloprid.

B) Coleopteran pest

Apart from different lepidopteran insect pests, Beauveria has also been found effective against many stored grain coleopteran pest. Batta *et al.* (2018) [4] obtained mortality of *Sitophilus* granaries with in 10 days by mixing wheat grain flour with conidia of *B. bassiana* at the rate of 2.6 × 10^8 conidia/g of *B. bassiana* mixture. Presence of diatomaceous earth in the formulations reduced the median lethal concentration of *B. bassiana* by 16fold and increased its efficacy against *Tribolium castaneum* by increasing conidial attachment and making nutrients more available to conidia for their germination. Sheeba *et al.* (2001) [33] recorded a mortality of 75.8% among *Sitophilus oryzae* by mixing 7.6 log conidia/ml of *B. bassiana* with rice grain. Lord (2005) [17] recorded 95-97% reduction in adult progeny when adult female *Rhyzopertha dominica* were introduced onto wheat kernels with 200mg/kg of *Beauveria bassiana* and/or 100mg/kg of diatomaceous earth (DE).

C) Hemiptera

Sucking pests poses severe threats to several Agricultural, horticultural and plantation crops either by direct feeding or indirectly by transmitting plant viral diseases. Since sucking pests like plant and leaf hoppers, aphids, whiteflies, scale insects, thrips and mites have developed resistance to many insecticides, an alteranative should be developed for their efficient management. Amongst the alternate practices, the efficacy of entomopathogenic fungi *Beauveria bassiana* against different sucking pests are described in this paper. *B. bassiana* has also been found effective against major sucking pests infesting various agriculturally important crops. Zafar (2016) [38] found *B. bassiana* effective against different developmental stages of *B.tabaci* infesting *Gossypium hirsutum, Lycopersicum esculentum, Solanum melongena* and *Capsicum annum.* Effectiveness of *B. bassiana* in controlling different aphid species viz, *Schizaphis graminum, Rhopalosiphum padi, Brevicoryne brassicae* and *Lipaphis erysimi* were reported by Akmal *et al.* (2013) [2]. Spraying of 0.4 per cent *Beauveria bassiana* under field condition was found effective against Chilli thrips [11]. Efficacy of *Beauveria bassiana* against different thrips species has also been recorded by Ludwig and Oetting (2002), Naik and Shekharappa (2009), Shiberu *et al.* (2013) [18, 22, 33]. Ali *et al.* (2018) [3] proved the pathogenicity of *B. bassiana* strain 202 against multiple targeted sucking insect species that are serious pests of crops and ornamental plants. The insect species include *Myzus persicae* Sulzer (Hemiptera: Aphididae), *Jacobiasca formosana* Paoli (Hemiptera: Cicadellidae), *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), and *Stephanitis nashi* (Hemiptera: Tingidae).

D) Orthoptera

Beauveria has also been found effective against orthopteran pest grasshoppers and locusts. Mohammadbeigi and Port (2015) [21] recorded significant reduction in food consumption and faeces production of long horned grasshopper *Uvarovistia zebra* (Uvarov) (Orthoptera: Tettigoniidae) upon treatment with 5X10^6 spores/ml of *B. bassiana.* Incorporation of sublethal levels of Dimilin with conidia of *B. bassiana* increased efficacy of the fungus against grasshoppers in laboratory and field trials [13].

Table 2: Different commercial formulations and field doses of *Beauveria bassiana*

Crop name	Target pest	Dose	Dilution (in water)
Beauveria bassiana 1.15% W.P.			
Cotton	Bollworm	400gm/hac	750-1000 lit/hac
Beauveria bassiana 1.15% W.P. (1x10^8 /gm min) Strain BB-ICAR-RJP Accession No – MCC 1022			
Rice	Rice leaf folder (*Cnaphalocrosis medinalis*)	2.5 kg/ha	750-850 L/Ha
Beauveria bassiana 1.15% W.P. (Strain : BB – 5372, own R & D Isolate)			
Rice	Rice leaf folder (*Cnaphalocrosis medinalis*)	2.5 kg/ha	600-750 L/Ha
Beauveria bassiana 1.15% W.P. (1x10^8 /gm min) Strain ICAR, Research Complex, Umiam, Meghalaya, Accession No – NAIMCC-F-03045			
Rice	Rice leaf folder (*Cnaphalocrosis medinalis*)	2.5 kg/ha	750-850 L/Ha
Beauveria bassiana 1.15% W.P. (1x10^8 /gm min) Accession No – NAIMCC-F-03045 Strain No. NBAIM, MAU.			
Rice	Rice leaf folder (*Cnaphalocrosis medinalis*)	2.5 kg/ha	750 L/Ha
Beauveria bassiana 1.15% W.P. (1x10^8 /spores/ml) Strain BCRL, Accession No – BCRL Bbpx-6892			
Cabbage	Diamond back moth (*Plutella xylostella*)	1.1.5 litre/haformulation	500-750 litre/ha of water
Beauveria bassiana 1% WP Strain No: NBRI – 9947 (1x10^8 CFU/gm min)			
Chick pea	Pod borer (*Helicoverpa armigera*)	3 kg.	500 L/Ha
Beauveria bassiana 5% WP (1x10^8 CFU/gm min) Strain IARI, Accession No. ITCC-7353			
Cabbage	Diamond back moth (*Plutella xylostella*)	2.0 kg.	500litre/ha of water
Beauveria bassiana 5% SC Strain: NBAII, Bangalore, Accession No. ITCC-7102, (Strain Isolated by Project Directorate of Bio-logical control, Bangalore)			
Tomato	Fruit borer (*Helicoverpa armigera*)	500	500
Beauveria bassiana 1% WP (1x10^9 CFU/gm min) Strain No. IPL/BB/MI/01			

Okra	Fruit borer / spotted bollworm	3.75-5.0 kg	400-500 L/Ha
Beauveria bassiana 1% WP (1x10^8 CFU/gm min) Strain No. SVBPU/CSP/Bb-10, Accession No. ITCC-7520			
Chick pea	Pod borer (*Helicoverpa armigera*)	3.0 kg/ha	500 l/ha

Source: CIBRC-2018- Major Use of insecticides- biopesticides

Table 3: Commercial availability

***Beauveria bassiana* Products**	**Company**
RACERTM (Rs 200/ kg)	Agri Life, Hyderabad, Telangana
BB Power- *Beauveria bassiana* (Rs 250/ Pack(s))	KN Biosciences (India) Pvt. Ltd, Hyderabad
Beauveria Liquid (Rs 175/Litre)	Universal Industries, Kashipur, Uttarakhand
Beveron (*Beauveria bassiana*) (Rs 250/Bottle)	Florken Sciences, Nahsik, Maharastra
Kissan Beauveria (Rs 400/Litre)	South Indian Fertilizers, Kochi, Kerala
Beauveria Baseco (Rs 500/ Litre)	Ecosense Labs India Private Limited, Mumbai
Bio Bassiana Bio Pesticides	Universal Bio-con Private Limited, Pune, Maharastra
Beauveria Bassiana	Varsha Bioscience And Technology India Private Limited, Saidabad, Hyderabad
Myco-B2(Rs 490/Litre)	Farmers Bio-fertilizers And Organics Coimbatore, Tamil Nadu
Krishi Bio Prahar(Rs 250/Kilogram)	Krishi Bio Products & Research Private Limited, Indore, Madhya Pradesh
Cib Beauveria Bassiana Bio Pesticide (Rs 200/Kilogram)	Amit Biotech Private Limited, Kolkata, West Bengal
Kalpvrix (Liquid Beauveria Bassiana) (Rs 450/Bottle)	Kalpvrix, Surat, Gujarat
Beauv-Q (Rs 250/Litre)	OK Biosystems, Gudiyattam, Tamil Nadu
Beauveria(Rs 65/Kilogram)	The Peermade Marketing Cooperative Society Limited, Kerala
AGROLUTIONS Liquid Culture Pestilutions Beauveria Bassiana (Rs 360/Bottle)	Agrolutions, Bhopal, Madhya Pradesh
Biopesticides- Beauveria Bassiana Rs 450/Litre	RD Overseas, Pune, Maharastra
Ecoria	JU Agri Sciences Pvt. Ltd., Janakpuri, New Delhi

(https://dir.indiamart.com/impcat/beauveria.html)

5. Precautions

1. Workers must wear protective clothing and respiratory equipment while spraying, as mentioned in the product label.
2. Pesticide handlers and workers must enter into field after few days of application of pesticides.
3. Pesticides must not be applied nearer to bee hives or the areas where bees forage frequently.

6. Conclusion

Beauveria bassiana is a popular cosmopolitan entomopathogenic fungi infecting a wide range of insect pest. It also possess least detrimental effect on the nontarget organisms, soil microbes as well as on environment. Since the production technique is comparatively easy and cost effective, it is available in many different formulations now- a-days and being used as a registered product in India as well as in many other countries. So, it can be successfully incorporated in different IPM programmes as a potential bio control agent.

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