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REVIEW ARTICLE

Status and scope of entomopathogenic fungus, *Beauveria bassiana* in sustainable pest management : A review

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ABSTRACT: Biopesticides are biological alternatives to synthetic pesticides and environmentally sustainable pest management tools. The demand for biopesticides in agriculture is rising due to increased awareness among farmers about the safety of biopesticides to human health and environment. As a result, the global consumption of biopesticides has steadily increased. The anamorphic hyphomycete, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), is a well-recognized cosmopolitan microbial agent known to infect broader insect groups. This paper provides an overview of current knowledge on isolation, culturing, identification, mode of action, the genes contributing to virulence and formulation of *B. bassiana*. The fungus can develop three distinct infective units *viz*., aerial conidia, blastospores, and submerged conidia. *Beauveria bassiana* grows as a white mold in culture media and generates many dry, powdery conidia in unique white spore balls on most standard culture media. The *B. bassiana* can be isolated through the galleria bait technique and use of specific media. The molecular characterization of *B. bassiana* can be confirmed using the gene sequences of the nuclear intergenic region (bloc), beta-tubulin (bt), and ITS region. Understanding the potential factors of genetic variation on the virulence of *B. bassiana* and its insect-fungus interactions will improve usage of this fungus as a cost-effective and sustainable mycoinsecticide.

Keywords: *Beauveria bassiana*, biopesticide, entomopathogenic fungi, mycoinsecticide, endophyte, safety, ecofriendly, pathogenicity.

INTRODUCTION

Entomopathogenic fungi (EPF) are natural biocontrol agents with global distribution. Selective nature of infection, and their safety to environment combined with simple mass production techniques, have made EPF an effective and viable alternative to synthetic insecticides (Rani *et al*., 2021). The empirical and unilateral use of chemicals to control pests failed to provide a long lasting solution (Archana *et al*., 2022). Pest resurgence and upsets in the natural balance due to the poisons used against them clearly show that a rapid and drastic change is necessary to achieve control of pests in an ecologically and economically satisfactory manner. Implementing fundamental ecological principles in managing pest problems is the most effective approach to significantly reduce the use of insecticides, with some agroecosystems even being able to eliminate their usage (Deguine *et al*., 2021). In the present review, we attempted to provide an overview of current knowledge on isolation, culturing, identification, mode of action, the genes contributing to virulence in entomopathogenic fungi and formulation of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), a widely used biopesticide in agriculture.

History, natural occurrence, geographical distribution and host range of *Beauveria bassiana*

Microbial biopesticides have had considerable success in controlling crop pests. Among EPF's *Beauveria* sp. is the most commonly reported natural enemy of insects causing regular epizootics (Roberts and St. Leger, 2004). Currently, 16 species are included in the genus, *Beauveria*. Rehner *et al*. (2011) recognized 12 species of *Beauveria*, *i.e*., *B. bassiana*, *B. brongniartii*, *B. caledonica*, *B. amorpha*, *B. asiatica, B. australis*, *B. kipukae*, *B. pseudobassiana*, *B. varroae*, *B. sungii*, *B. malawiensis* and *B. vermiconia*. Later 4 more species were described, *i.e., B. lii* (Zhang *et al*., 2012), *B. sinensis* (Chen *et al*., 2013), *B. rudraprayagi* (Agrawal *et al*., 2014) and *B. hoplocheli* (Robène *et al*., 2015). However, only two species, *B. bassiana* and *B. brongniartii*, were most studied and commercially exploited for pest management.

The anamorphic hyphomycete, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), is a well-recognized cosmopolitan microbial agent known to infect broader insect groups. *Beauveria bassiana* was one of the most intensively studied fungal entomopathogens from which thousands of isolates have been collected from different parts of the world (Rehner *et al*., 2011). The history of research on *B. bassiana* started in the early nineteenth century. In 1835, the Agostino Bassi, an entomologist discovered the causal agent of pebrine disease that turned legions of Italy's silkworms into white mummies (Lord, 2005). The fungus was subsequently named after Bassi by Vuillemin. The characteristic appearance of a white powdery layer on the cadavers gave rise to the descriptor white muscardine disease. One of the first and most prominent early attempts to extensively use *Beauveria* was made in the mid-1800s in the US Midwest to control chinch bugs, *Blissus leucopterus* (Lord, 2005).

Beauveria bassiana is a generalist entomopathogen with a broad ecological host range of over 700 arthropod species, covering most orders of the class Insecta (Feng *et al*., 1994). However, *B*. *brongniartii* (Saccardo) Petch has a more restricted host range, mainly infecting coleoptera and the other seven orders in the field. For several species, such as *B*. *vermiconia* or *B*. *caledonica*, the number of strains available in collections needs to be larger to conclude their host range (Rehner *et al*., 2011). To date, the species *B*. *hoplocheli* has only been isolated in natural conditions from the white grub, *Hoplochelus marginalis* (Fairmaire) (Coleoptera: Scarabaeidae) (Robène *et al*., 2015). Many studies have compared the virulence of several strains of *Beauveria* spp. on a given insect host, especially strains of *B*. *bassiana* (Quesada-Moraga *et al*., 2003). Few works have studied the physiological host range of *Beauveria* spp. strains by comparing their pathogenicity and virulence on several insect species. For example, 43 *B*. *bassiana* strains collected worldwide exhibited a substantial variation in virulence against eight lepidopteran species (Wraight *et al*., 2010). Twenty-nine genetically diverse *B*. *bassiana* strains were pathogenic to nine insect species from five orders, with significantly different levels of virulence (Uma Devi *et al*., 2008). Despite a few preliminary studies, the physiological host range of many species of *Beauveria*, excluding *B*. *bassiana* and *B*. *brongniartii*, has not been investigated extensively.

Cultural, morphological and molecular identification of *B. bassiana*

The taxonomic hierarchy of *Beauveria bassiana* is as follows kingdom- Fungi, division- Ascomycota, class- Sordariomycetes, order- Hypocreales, and Family- Cordycipitaceae. Under varying nutritional and climatic conditions, *B. bassiana* can develop three distinct infective units: aerial conidia, blastospores, and submerged conidia. *B. bassiana* is the anamorphic stage (asexually reproducing form) of *Cordyceps bassiana*. Potato Dextrose Agar (PDA) and sporulation media (SM) can be utilized for the growth and multiplication of *B. bassiana* at two different temperatures (25 ^{0}C and 28 ^{0}C) with a relative humidity (RH) of 65-70 % for 10 days. *B. bassiana* grows as a white mold in culture media. It generates many dry, powdery conidia in unique white spore balls on most standard culture media. Each spore ball is made up of a group of conidiogenous cells. *B. bassiana* conidiogenous cells are short and oval, with a slender apical projection known as a rachis. The rachis elongates after each conidium is produced, resulting in a long zig-zag extension. Conidia are single-celled, haploid, and hydrophobic organisms.

The microscopic observation (100× magnification) of morphological characteristics is the most widely used criterion for characterizing EPF during their asexual stages. It requires adequate observation of both conidia and conidiogenous cells. Commonly two methods are employed for microscopic observation, *i.e.,* the whole mount method, a straightforward and rapid method used for observing fungi under a light microscope. The disruption of conidiogenous cells and dehiscence of conidia are also widespread while preparing the slide. However, it can be avoided with the slide culture preparation method, but the culture must be incubated long enough to develop conidiogenesis for examination **(**Senthilkumar *et al*., 2021**)**. Based on microscopic observation, hyphae branched and formed conidiogenous cells and single cell *B. bassiana* conidium is round and tends to be oval with hyaline color.

Molecular characterization has become essential to confirm the identity of the EPF, *Beauveria spp*. using molecular detection tools. The molecular characterization can be confirmed using the gene sequences of the nuclear intergenic region (bloc), beta-tubulin (bt), and ITS region. Molecular identification can be achieved by isolating fungal DNA from the pure cultures and re-isolated on PDA media, as reported by Liu *et al*. (2013). Later the species of *Beauveria* was confirmed at the molecular level through the amplification, sequencing, and phylogenetic analysis of the internal transcribed spacer (ITS) sequence of 5.8S rDNA of the fungus (Sharma *et al*., 2015). Universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) are employed for amplifying a partial sequence of ITS1-5.8S rDNA-ITS4 (Kimaru *et al*., 2018). For *Beauveria* isolates, approximately 1500-bp segments of bloc gene region amplified by the primer pairs of B5.1F (5′-CGACCCGGCCAACT ACTTTGA-3′) and B3.1R (5′-GTCTTCCAGTACCA CTACGCC-3′) as described by Rehner *et al*. (2006).

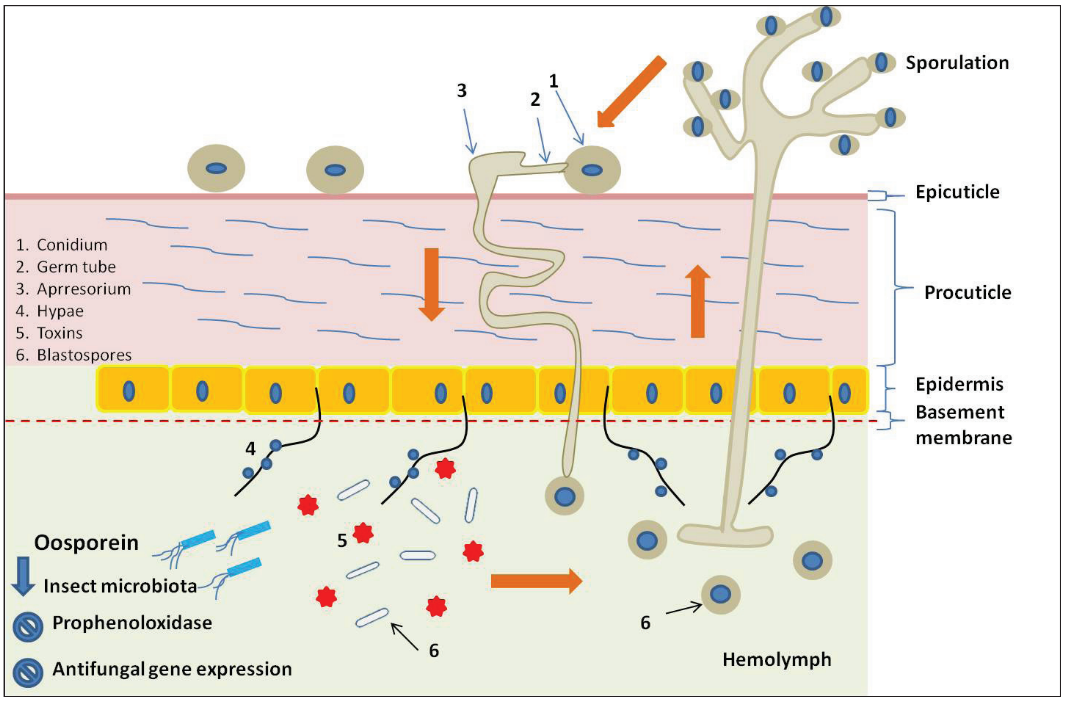

Fig 1. A schematic view of *Beauveria bassiana* pathogenesis

Isolation of EPF from soil and infected insects

Two methods commonly employed for EPF isolation from the soils are (1) baiting the environment with a susceptible insect host, *i.e.,* the Galleria Bait technique, or (2) the use of selective media (Sharma *et al*., 2021).

Isolation from insects

EPF was isolated from dead insects using direct isolation techniques and incubated on the prepared PDA plate at 28 ^{0}C for one week (Parker *et al*., 2003). All dead insects were placed in 9-cm plastic Petri dishes on a sterilized paper towel moistened with a solution of 0.001 g/L of penicillin G and 0.005 g/L of streptomycin sulfate. The Petri dishes were sealed with Parafilm and held at 26^{0}C for 4 weeks. Unopened Petri dishes were examined daily for the presence of fungal outgrowth. The isolate was subcultured several times to obtain a pure culture (Awan *et al.,* (2021).

Isolation of *Beauveria* spp. from soil

Galleria Bait Technique

Isolation of EPF using selective media manipulates the saprotrophic ability of EPF. To manipulate the fungi's ability to infect the host, *Galleria* Bait Technique was commonly used (Zimmermann, 1986). The EPF was isolated from soil using *Galleria mellonella* L. (greater wax moth) larvae. Place four *G. mellonella* larvae in a plastic container containing a soil sample; seal the containers with perforated lids and hold them at room temperature. Place the three to five *G. mellonella* larvae in containers with sterile soil (negative control), no soil (negative control), or sterilized soil to which fungi obtained from one plate of each of the three known EPF cultures were added (positive controls). Examine the containers every other day, and collect dead larvae. Surface-sterilize the cadaver for 3 min in a 1% sodium hypochlorite solution and rinse in sterile distilled water, plate, and incubate at 27°C in a humidity chamber at 100% RH to permit the growth of fungi (Brownbridge *et al*., 1993).

Isolation of *Beauveria* spp. from soil using selective media

Soil is the primary source of the EPF (Sanchez-Pena *et al*., 2011). Insect bait is a susceptible detection method, and EPF can be selectively isolated. However, some insect species may select for specific fungal pathogens, and challenging to quantify inoculum levels. Although the isolated fungi must be evaluated for their pathogenicity to target insects, by contrast, selective media have some advantages for the mass collection of positive EPF and quantitative data. Therefore, various selective media have been developed for the mass collection of EPF from soil (Meyling, 2007). A selective medium is available for the recovery of *B. bassiana,* having been developed by Veen and Ferron (1966) to isolate *B. tenella (B. bassiana,* fide de Hoog, 1972) from natural sources. Using a selective medium, *B. bassiana* was isolated from elm trees' bark and soil (Doberski and Tribe, 1980). For the success of

Table 1. Efficacy of *Beauveria bassiana* against different insect pests

Target pests	Percent efficacy (strain)	Study location	Authors
Sitophilus granarius L. (Coleoptera: Curculionidae)	93.66%	Turkey (Laboratory)	Ak (2019)
Callosobruchus maculatus F. (Coleoptera: Chrysomelidae)	100 % mortality (TR-217)	Turkey (Laboratory)	Ozdemir *et al* (2020)
Frankliniella occidentalis (Thysanoptera: Thripidae)	69%–96% (RSB)	China (Laboratory)	Gao *et al*. (2012)
Thrips tabaci (Thysanoptera: Thripidae)	83%–100% (SZ-26)	China (Laboratory)	Wu *et al.* (2013)
Rhynchophorus ferrugineus (Oilv.) (Coleoptera: Curculionidae)	Up to 90 %	Egypt (Field)	Sewify *et al*. (2009)
Cosmopolites sordidus (Germar, 1824) (Coleoptera: Curculionidae)	54% to 66% (IBCB 74, IBCB 87 and IBCB 146)	Brazil (Laboratory)	Almeida *et al*. (2009)
Polyphylla fullo (L.) (Coleoptera: Scarabaeidae)	71.6 to 79.8% (PPRI5339)	Turkey (Laboratory)	Erler and Ates, (2015)
Thaumastocoris peregrinus Carpintero & Dellapé (Hemiptera: Thaumastocoridae)	37 to 80.1%	Brazil (Laboratory)	Lorencetti *et al*. (2018)
Helicoverpa armigera, *Spodoptera litura* *Earias vittella* (Lepidoptera: Noctuidae)	*H. armigera* (86.67%), *S. litura* (86.67%) and *E. vittella* (73.33%)	India (Laboratory)	Karthikeyan and Elvanarayanan (2011)
Plutella xylostella Linn.	72.64 %	India (Field)	Kamal *et al*. (2018)

EPF-based commercial bio-pesticides, conidia production is crucial. The biphasic growth culture method involving liquid- and solid-state culture is mainly used to produce EPF.

During the isolation of the fungus, one gram of a given soil sample and 10 ml of the sterilized distilled water were mixed in 15 ml test tubes, which were vortexed for 10 min to obtain a homogenous solution. Then, a serial dilution from 10^{-1} to 10^{-7} for each soil sample was prepared to isolate a single colony of fungi. The 1 ml of the soil extracts spread on a selective medium SDA (Sabouraud Dextrose Agar) containing 0.2 µg/ml dodine (N-dodecylguanidine monoacetate), 100 µg/ml chloramphenicol, and 50 µg/ml streptomycin sulfate) and incubated at 28 °C for 2 weeks (Goettel and Inglis, 1997). At the end of the incubation period, growing single colonies were transferred to other SDA plates to get pure cultures. Store all purified fungal isolates in 20% glycerol at − 20 °C. Veen's medium is designed to maximize recovery of naturally occurring *Beauveria* sp.

Pathogenesis and mechanism of *B. bassiana* against plant diseases

The EPF encounters several host obstacles in each generation to produce enough viable infectious spores to perpetuate healthy populations. They would first come close to a susceptible host, then stick to and puncture the host's cuticle. They must subsequently overpower and avoid host immunological systems to receive nutrients and grow. The EPF causes infection at low conidia concentrations, which can be as low as one or two conidia per host (Oduor *et al*., 1997). The adhesion of spores to the host's epicuticle, followed by germinating and pre-penetration proliferation, is a crucial stage of pathogenicity (Ortiz-Urquiza and Keyhani, 2013). Most

EPF has hydrophobic conidia, which allow quick adhesion to the waxy epicuticle. Hydrophobin proteins that form enclose and protect layers on the conidial surface increase adhesion of conidia in *B. bassiana* (Holder *et al*., 2007). Immediately after the initial contact, secrets sticky lime (Boucias and Pendland, 1991).

During the pre-germination stage, moist conidia release proteases, probably for nutrient absorption and invasion (Qazi and Khachatourians, 2007). *B. bassiana* has at least 16 fungal enzymes involved in the oxidative breakdown and assimilation of epicuticle lipids (Pedrini, 2022). The process of infection of arthropods and fungal invasion, attachment to hosts and penetration of the cuticle, virulence enzymes associated with EPF and interaction with the host immune response are well described by Sharma and Sharma (2021) and Chandler (2017). As shown in Figure 1, most pathogenic fungi infect insects through the epidermis and then multiply in the Hemolymph system. The figure 1 shows that the fungal infection cycle not only depends on the successful penetration of the epidermis but also requires a dimorphic transition in vivo, *i.e.,* the transformation of conidia into hyphae. Chitinase, lipases, and proteases are the most important enzymes produced by *B. bassiana*. However, different studies have determined that it can produce other enzymes, such as amylase, asparaginase, cellulase and galactosidase (Petlamul and Boukaew, 2019). Various studies have reported the presence of beauvericin, bassianolide, bassiacridin, and oosporein toxins in *B. bassiana* culture supernatants (Ortiz-Urquiza *et al*., 2010).

Genes involved in virulence and production of toxins-Molecular approaches to improve their virulence

Most microbial biopesticides are found in the microbiome of the agricultural fields, where they are in combination with both pathogenic and beneficial organisms. These fungal biopesticides bio-actively deter harmful insect pests (Archana *et al*., 2022). Their action is often parasitic or may secrete bioactive metabolites like enzymes, *i.e.,* contingent on both the pesticidal fungus applied and the targeted pest. e.g., *B. bassiana* germinates, grows and spreads its spores in the targeted insect body, colonization by degradation, draining nutrients and releasing toxins causing its death (Raya-Díaz *et al*., 2017). Manifold reports have shown the importance of virulence genes to understand better the infection mechanisms deployed by EPF. The implication of various virulence genes directly involved in biocontrol mechanisms are presented in Table 2.

Over the past decade, immense advances in molecular biology and genetic techniques have helped in the understanding of the life history as well as the genetic mechanisms of fungal virulence of *B. bassiana* for a robust and sustainable solution to arthropod pests. Rapid progress in understanding the genetics that constitutes virulence in insects can be made due to the recent availability of the whole genome sequence of *B. bassiana* (Xiao *et al*., 2012). In general, the host–fungus biological interactions are more prominent in the host insect and can be further magnified for research purposes (Joop and Vilcinskas, 2016). Many of the genes that were functionally analyzed thus far involve general biological processes (e.g., conidiation, stress response) that pleiotropically affect virulence.

Studies on comprehensive information of genetic variation and identification of virulence variants and their evolutionary dynamics help to understand their mechanism of inhibition. Knock-out mutant approaches are crucial and will play an essential role in verifying the action of the candidate genes. Valero-Jiménez *et al*. (2016) sequenced the genomes of five isolates of *B. bassiana* with low/high virulence against mosquitoes. Understanding the potential factors of genetic variation on the virulence of *B. bassiana* and its insect-fungus interactions will improve our methods to use this fungus as a cost-effective and sustainable mycoinsecticide. However, do these genetic mutations play a role in virulence, or how does it regulate virulence in biological processes, which is exciting and will need further study (Zhang *et al*., 2020).

***B. bassiana* as an endophyte**

The fungal entomopathogens are found naturally as an endophyte (Vega, 2018) and also colonize plants *via* seed dressings, seed soaking, foliar sprays, and soil drenching (Tefera and Vidal, 2009). They protect their host plant against disease pathogens by enhancing plant growth through plant disease antagonism and rhizosphere colonization. Colonization by fungal endophytes may be systemic, localized or partitioned within plant parts. The artificial introduction of *B. bassiana* as an endophyte has been successful in maize, coffee, banana, broad beans, cotton, the common bean and tomato (Behie *et al*., 2015).

Interaction of EPF with environmental factors

In general, fungi inhabiting higher latitudes experience a wider range of temperatures due to seasonality (Wielgolaski and Inouye, 2003). Thus, abiotic stressors (mainly temperature) at higher latitudes may predominantly drive population genetics and adaptability of EPF. In temperate regions, EPF must adapt to a broad range and greater climatic intensities (Maggi *et al*., 2013; Wang *et al*., 2017), whereby abiotic factors primarily influence generalist pathogen's survival

Table 2. Different virulence genes of *B. bassiana* involved in biological processes of inhibition

***B. bassiana* Genes**	**Gene encoded proteins**	**Genes involved in biological functions**	**References**
hyd1 and *hyd2*	Hydrophobin gene	**Host adherence:** Involved in surface hydrophobicity, adhesion, virulence and composition of the rodlet layer	Zhang *et al*., (2011b)
Bbhog1 & *Bbmpk1,*	MAP kinase gene	**Germination and penetration peg development:** Required for conidial adhesion, appressorium formation and penetration	Zhang *et al*., (2009)
Bbcyp52x1	cytochrome P450 gene	**Enzymatic degradation of the waxy layer:** Act as fatty acid hydroxylase activity	Zhang *et al*., (2012)
Pr1, Pr2	Subtilisin/ trypsin-like protease	**Cuticle degradation**: Cuticle-degrading proteases are important for cuticle breakdown	Pedrini (2022)
Putative gene clusters (BbbeaS BbbslS; BbtenS	Non-ribosomal peptide synthase (NRPS) protein; beauvericin synthetase gene (*BbbeaS*); bassianolide synthetase gene (*BbbslS*); tenellin synthetase gene	**Role in biosynthesis and production of metabolites** / **toxins** *viz*., tenellin, beauvericin, Oosporein, and bassianolide role in virulence	Zhang *et al*., (2020);
Bbpks11, Bbpks15, BbopS1	Polyketides synthetases (PKS); Oosporein polyketide synthase (*BbopS1*)	Important functions in fungal asexual development and cell wall integrity and **virulence function**. *BbopS1* directly participate in the evasion of insect immunity	Pedrini (2022)
Bbchit1, Chi1, Chi2, ChsA2	Endo chitosanase and Chitinase D	**Chitin-degrading enzymes for lysis** of insect chitin	Dionisio *et al*., (2016).
Bbmtd, Bbmpd	Mannitol dehydrogenase	Involved in mannitol biosynthesis for growth & colonization. Also involved in **multi-stress tolerance**	Wang *et al*., (2012)
Mdr1, Mrp1, Pdr1, Pdr2, Pdr5	ABC transporters	**Interactions with the insect immune system** for multi drug resistance	Song *et al*., (2013)
Bbac	cAMP signaling/ adenylate cyclase gene	**Regulatory functions**: Regulating multi-stress responses (osmolarity, oxidation, cell wall damage and different chemicals) and helps in conidiation	Wang *et al*., (2014a)
BbGPCR3, Bbrgs1	G-protein coupled receptor (GPCR)	**Role in Hyphal extrusion and conidiation:** Involved in conidiation (transition to blastospore), conidial viability, nutrient sensing and thermo/ stress tolerance	Fang *et al*. (2008)

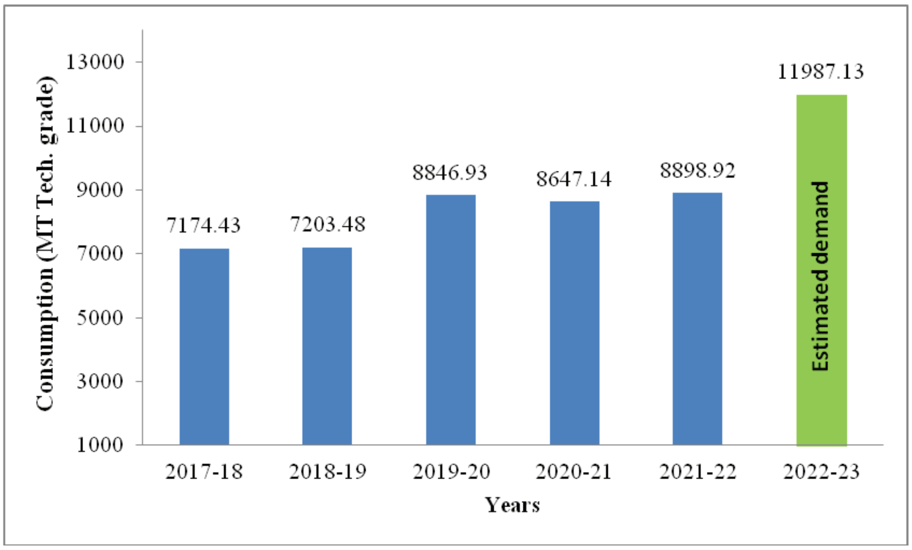

Fig 2. Consumption of biopesticides in India during last 5 years and estimated demand during 2022- 23 (Source: DPPQS, Ministry of Agriculture & Farmers Welfare, Government of India).

(Lennon *et al*., 2012). Phylogenetic *B. bassiana* cluster by habitat type more at seasonally variable high latitudes (Ormond *et al*., 2010). However, one study found no seasonal effect in regions of sub-tropical climates (Garrido-Jurado *et al*., 2015). Phylogenetically structured investigations suggest *B. bassiana* adapts gene regulation to environmental conditions, with habitat adaptation driving population dynamics (Bidochka *et al*., 2002; Xiao *et al*., 2012). The optimal temperature for growth and virulence against insect hosts of *Beauveria* species is generally between 25 and 30 °C (Luz and Fargues, 1997; Devi *et al*., 2005). However, significant variation exists in a fungal pathogen species' thermal preference and their effects on potential hosts due to the environment in which the pathogens evolved (Alali *et al*., 2019), and individual strains can differ in their thermal optima (Alali *et al*., 2019).

The sub-tropical *B. bassiana* strains collected from hotter areas of Syria demonstrated more remarkable thermo-tolerant ability than the outlier collected from a site experiencing lower temperatures (Alali *et al*., 2019). Regarding virulence against insects, temperate isolates of *B. bassiana* were significantly more effective against the elm bark beetle (*Scolytus scolytus* F.) at low temperatures (2 to 6 °C) (Doberski, 1981). The strains of *B. bassiana* are sensitive to ultraviolet radiation, prompting UV protectant use in oil-based field sprays (Kumar *et al*., 2018). UV tolerance often varies among isolates from different latitudes (Fernandes *et al*., 2008) and habitat types (Bidochka *et al*., 2001).

Development of the entamopathogenic fungus in liquid and solid cultures

Microorganism-based bio-pesticide forms the most substantial portion of bio-pesticide products. Worldwide attention has been focused on the mass manufacturing of promising EPFs like *Metarhizium anisopliae*, *Beauveria bassiana, Verticillium* sp., *Trichoderma* sp., *Chaetomium* sp., *Aspergillus* sp., and *Hirsutella sp* for crop protection against various pests. *B. bassiana* has also been found to be one of the potential biocontrol agents effectively used in IPM because of its wide natural distribution and ability to control aphids, lepidopteron larvae and other pests (Abidin *et al*., 2017). The fungus, *Beauveria bassiana* was cultured with excellent results on a medium consisting of yeast extract, *i.e.,* Sabouraud-dextrose-yeast extracts (Ramle *et al*., 2005). Several approaches have been made to increase the effectiveness of *B. bassiana* with suitable mass-production techniques for commercial formulation. Among them, the most common and inexpensive techniques used for cultivation are either a surface culture with a solid substrate or a submerged culture with a liquid medium (Fang *et al*., 2000).

Solid-type microbial culture has a relatively long preservation time due to the hydrophobic nature of conidia. It is suitable for making oil formulation but requires an extended activation time. On the other hand, the liquid-type microbial culture is disadvantaged in making oil formulations due to less viability during storage. It can also be grown by following a biphasic system, in which the fungus is first grown under submerged conditions to produce metabolic active blastospores (hydrophilic) and then allowed to conidiate (hydrophobic) in solid-state conditions (Lopez-Perez *et al*., 2015). To ensure the effective implementation of potential micro-organisms, solid substrate fermentation is one of the proper methods for mass production of *B. bassiana*. Gola *et al*. (2019) made innovative attempts to produce three stable formulations of *Beauveria bassiana* targeted against multimetal (Cu, Cr, Cd, Ni, Zn, and Pb) containing synthetic wastewater. The micro-granules, myco-tablets, and myco-capsules formulations can potentially remediate multimetal-containing wastewater. It will also help extend the formulation's shelf life at ambient temperature and solve the problem of storability and transportation.

Fate and behaviour in the environment and effect on non-target organisms including humans

A widespread application entomopathogenic fungus in various crop protection systems raises the concern of potential adverse effects on non-target organisms like human health, earthworms, pollinator and other beneficial arthropods. When *B. bassiana* was tested for pathogenicity against the adults of *Folsomia fimetaria*, *Hypogastrura assimilis*, and *Proisotoma minuta*, no strains of *B. bassiana* were found to be toxic (Zimmermann, 2007).

B. bassiana has been extensively used in agricultural practices in various Asian countries since the past century. The critical issue microbial ecologists raise is that host specificity is a strain-specific trait. A difference was observed between the physiological and ecological host range of *B. bassiana* strains isolated from different parts of the world. The ecological host range shows the susceptibility of insects under natural or field conditions, while the physiological host range demonstrates which insects can be infected in the laboratory. *B. bassiana* has a diverse range of hosts, yet data suggests that using it can have little effect on beneficial organisms (Zimmermann, 2007).

The safety of *B. bassiana* to humans was cautiously evaluated before its registration as a biocontrol agent. In minor cases, some workers involved in the mass production of *B. bassiana* exposed to high spore concentrations likely had allergies. Besides allergy, there are some cases where Mycotic keratitis has been linked to *Beauveria bassiana* in humans and other mammals. The genus *Beauveria* is not mentioned in the medical charts of rare but crucial fungal infections (Zimmermann, 2007).

Formulations of *B. bassiana* and their compatibility with insecticides

Different formulations of *B. bassiana* have been tested against house flies (bait, encapsulation, and emulsion), whiteflies (oil, talc, and crude), and other agricultural pests (Prithiva *et al*., 2017; Saeed *et al*., 2017). The results found that oil formulation (45.86 %), followed by talc (29.62 %) and crude formulations (21.63 %) were most effective against whitefly on tomato. Similarly, oil and water-based formulations of *B. bassiana* were suitable for application to control coffee berry borer, *Hypothenemus hampei*. Ritu *et al*. (2012) studied the different formulations (Bentonite oil-based liquid formulation (BOBLF), oil-based liquid formulation (OBLF), and Carrier-based powder formulation (CBPF) of *Beauveria bassiana* tested against larvae of *Helicoverpa armigera*. It was found that the bentonite-based liquid formulation exhibited the highest efficacy at the optimum concentration (60%).

Pesticides can be substituted by biopesticides (Rani *et al*. 2021; Archana *et al*. 2021). Various biologically derived compounds had pesticide action against insect pests and diseases (Shivakumara *et al*., 2022, Darshan *et al*., 2020). The successful formulation depends on its compatibility with other insecticides used in pest management programs. Many research groups have checked the compatibility of *B. bassiana* with several pesticides at different concentrations. Various parameters were studied, like conidial germination, vegetative growth, and fungus sporulation. Alizadeh *et al*. (2007) reported that *B. bassiana* (isolate DEBI008) was compatible with imidacloprid and showed synergistic interaction. However, flufenoxuron was highly incompatible and inhibited conidial germination significantly.

The combination of compatible insecticides and synergistic bioagents at lower doses can help manage the pest sustainably with a low risk of resurgence. Abidin *et al*. (2017) reported the compatibility of *B. bassiana* with various insecticides. Imidacloprid (77.72%) and deltamethrin (76.02%) were compatible and showed the highest vegetative growth and conidial germination. The combined applications (Beta cypermethrin (10%) with *B. bassiana PfBb* (1×10^7), imidacloprid (0.5 x DF) with *B. bassiana*) showed effective pesticidal action on insects than applications of insecticides alone (Chen *et al*., 2021). *B. bassiana* was also shown good compatibility with acaricides formulation like Avermectin and pyrethroids (De Olivera and Neves, 2004). In summary, a detailed compatibility evaluation of insecticides with biocontrol agents is required for simultaneous usage in integrated pest management programs. Knowledge of this will facilitate the choice of entomopathogenic fungi and pesticides used in a cocktail for crop protection.

Demand and production needs of *B. bassiana*

According to the DPPQS (Directorate of Plant Protection, Quarantine and Storage, Ministry of Agriculture, Gov. of India), 361 biocontrol laboratories and units are working in India. However, only a few of them are involved in the production. They can meet the demand of less than 1% of the cropped area. A wide gap can only be bridged by setting up more units for Biopesticides production. However, data suggests that in India, the consumption of biopesticides has increased in the last few decades. Data obtained from DPPQS suggested that the all-India consumption of biopesticides gradually increased for five years, and the estimated demand for 2022-23 was 11987.13 MT Tech. Grade (Fig. 2).

Currently, there are 970 biopesticides products registered with the Central Insecticides Board and Registration Committee (CIBRC) for all types of usage of biopesticides in India. Among which 107 products are *B. bassiana.* Currently, CIBRC recommends the use of *B. bassiana* against different insect pests like cotton bollworm complex, rice leaf folder, *Cnaphalocrosis medinalis*; Diamondback moth, *Plutella xylostella* on cabbage; chickpea pod borer, *Helicoverpa armigera*; Fruit borer and spotted bollworm on Okra; *Helicoverpa armigera* on Tomato. Further, the *B. bassiana* recommended (ad-hoc) for the management strategies for invasive thrips (*Thrips parvispinus*) in Chilli and Fall Armyworm, *Spodoptera frugiperda* in Maize (DPPQS, 2022).

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