RESEARCH ARTICLE

White Muscardine Fungus, *Beauveria bassiana* (Hypocreales): Role in Plant Protection and Techniques for Mass Production and Cultivation – A Review

Praveen T[1*], Prakash K[2], Eniya A[2] and Nivedha M[3]

[1*]Kalasalingam School of Agriculture and Horticulture, KARE, Krishnankoil, Tamil Nadu
[2]Department of Entomology, Tamil Nadu Agricultural University, Coimbatore, India
[3]Department of Plant Pathology, Tamil Nadu Agricultural University, India

ABSTRACT

The white muscardine fungus, *Beauveria bassiana*, is a well-known entomopathogenic fungus with great potential in integrated pest management strategies. Its ability to infect and control a wide range of insect pests and its environmentally friendly profile have positioned it as a valuable tool for sustainable agriculture. This review provides a concise overview of the techniques and considerations for the mass production and cultivation of *Beauveria bassiana*. The successful mass production of *Beauveria bassiana* relies on various factors, including strain selection, culture media, environmental conditions, and bioreactor design. This review also outlines vital methodologies and strategies to optimize the growth and sporulation of the fungus, ensuring a high-quality product for pest control applications. Furthermore, the cultivation of *Beauveria bassiana* in liquid fermentation, solid-state fermentation, and submerged fermentation systems is discussed, along with their respective advantages and limitations. Different substrates, nutritional requirements, and aeration parameters are highlighted in these cultivation methods, allowing for a better understanding of the most suitable approach for specific applications.

INTRODUCTION

Beauveria bassiana is a fungus that can effectively control pest populations without harmful chemicals, making it an ideal choice for integrated pest management. The fungus is known for being able to infect a wide range of insects and is named after the French mycologist, Xavier Beauverie, who discovered it in the early 1900s. *B. bassiana* is widely used against pests because it produces insecticidal compounds, including Beauvericin and other secondary metabolites, which cause the host insect to die. Additionally, the fungus can form resting structures called conidia, which allow it to survive in different environmental conditions. Many studies have been conducted on *B. bassiana* to understand its ecological interactions,

*Corresponding author mail: tpraveen1993@gmail.com

molecular mechanisms, and genetic diversity. Its genome has also been sequenced, which has helped researchers better understand its biocontrol abilities. This fungus is a promising alternative to chemical pesticides, and its versatility and effectiveness make it an essential tool for sustainable agriculture. (Bischoff *et al.,* 2009; Ortiz-Urquiza and Keyhani, 2013).

Beyond its role in agriculture, *B. bassiana* has garnered interest in various biotechnological applications. Researchers have explored its potential in industries such as medicine and bioenergy. The fungus produces enzymes with industrial significance, including chitinases and proteases, which have applications in the degradation of organic materials and bioconversion processes. Understanding the molecular mechanisms underlying the pathogenicity of *B. bassiana* is crucial for optimizing its efficacy as a biopesticide. Recent studies have delved into the expression profiles of essential genes involved in host recognition, penetration, and immune evasion. This knowledge is instrumental in enhancing the fungus's virulence and improving its biocontrol capabilities (Feng*,* 2002). As the demand for sustainable pest management practices grows, assessing the environmental impact and safety of *B. bassiana* becomes paramount. Research has focused on evaluating its ecological interactions, persistence in the environment, and potential non-target effects. This information is crucial for developing guidelines to ensure this biopesticide's safe and responsible use (Goettel *et al.,* 2008). Commercial formulations have been developed for practical use. Studies have investigated the formulation strategies to improve the stability and shelf life of the fungus. Field trials and case studies have provided insights into the practical efficacy and challenges of applying *B. bassiana* in agroecosystems (Lacey *et al.,* 2015).
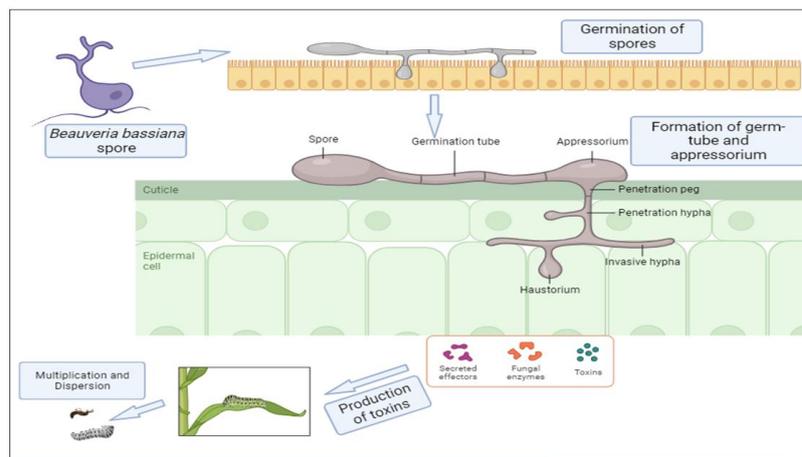
MODE OF ACTION

While the primary route of infection was through the integument, evidence indicated that *B. bassiana* could also infect insects via oral secretion, especially in those with chewing mouthparts. Notable examples include the Colorado potato beetle, *Lepinotarsa decemlineata* (Say) (Allee *et al.,* 1990), and the red imported fire ant, *Solenopsis invicta* Buren (Siebeneicher *et al.,* 1992), and others mentioned in various studies (Cai & Liu, 1988; McDowell *et al.,* 1990; Ignoffo *et al.,* 1982; Huang, 1988). Upon invading the hemocoel, the fungus underwent proliferation. Septate mycelia

from elongated germ tubes released blastospores, leading to the death of host insects due to hemolymph nutrient depletion and toxemia caused by fungal toxic metabolites (Khachatourians, 1998; Roberts, 1981). In moist conditions, the fungus emerges and produces aerial conidia on the host cadaver surface.

Beyond its lethal effects, *B. bassiana* infection can lead to sub-lethal or secondary outcomes. For example, doses close to the LD50 were shown to decrease the reproductive potential of adult *Sitona lineatus* (L.), as well as the fertility (N'Doye, 1976) and fecundity (Faizi, 1978) of adult *Chilo suppressalis* (Walker) that survived infection. The fungus also affected the fecundity and egg fertility of adult Colorado potato beetles that survived larval infection and the egg development of rice plant hoppers and leaf hoppers (Homoptera) (Zhang & Huang, 1988).

The adherence of conidia from *B. bassiana* to the insect's integument, along with the penetration of the cuticle by the germ tube emerging from the appressorium through a penetration peg, facilitates access to the host insect's hemolymph (Figure 1). Subsequently, the mature haustorium liberates blastospores and releases toxic metabolites, initiating the germination process and causing the destruction of internal tissues within the host. This sequence ultimately culminates in the mortality of the host organism.

Formation of germ tube and mode of entry: B. bassiana has no known sexual cycle. Insects become infected when conidia attach to their cuticle, germinating in humid environments. The germ tubes penetrate the host cuticle and invade the hemocoel. This infection relies on various enzymatic activities to degrade proteins, chitin, and lipids in the insect integument, as noted in studies by Ferron (1981) and Khachatourians (1986). Infection begins with the attachment of conidia to the host cuticle through physical forces, followed by germination and penetration of the cuticular layers, aided by hydrolytic enzymes (such as proteases, lipases, and chitinases), mechanical pressure, and other factors (Ortiz-Urquiza & Keyhani, 2013). Once the hyphae reach the nutrient-rich hemolymph, they can transform into single-celled, yeast-like blastospores (or hyphal bodies), which are specialized for rapid proliferation, nutrient exploitation, internal tissue colonization, and evasion of the host immune response (Humber, 2008).

Fig. 1: Mode of action of B. bassiana

Colonization: Colonization is accompanied by the production of various toxic metabolites (antimicrobial peptides) that help suppress the host's immune response, damage internal tissues, deplete nutrients, and ultimately lead to host mortality (Ortiz-Urquiza *et al.,* 2010; Gibson *et al.,* 2014). Studies have demonstrated that the pathogenesis and virulence of *Beauveria* isolates are linked to the production of *in vivo* toxic metabolites. These enzymes degrade the cuticle and possess antioxidant properties, as well as active vegetative growth within the host, resulting in physiological starvation of the host (Quesada-Moraga & Vey, 2003; Zimmermann, 2007; Ortiz-Urquiza *et al.,* 2010).

Production of toxic substances: However, it is important to note that insect-toxic secreted peptides from *Beauveria* are not always essential for its virulence (Quesada-Moraga and Vey, 2003). *Beauveria* is widely recognized as an environmentally safe biocontrol agent that poses minimal risk to human health and is generally harmless to non-target organisms (Zimmermann, 2007). The dimorphic cycle of *Beauveria*, which involves the transition from yeast-like blastospores to aerial conidia, is regarded as a virulence trait, as these yeast-like blastospores have adapted to evade host immune defenses and effectively utilize host nutrients (Pendland *et al.,* 1993; Holder *et al.,* 2007). Unlike aerial conidia, blastospores lack hydrophobin components or hydrophobic rodlet layers, indicating that the electrostatic charges on the blastospore surface may significantly influence the host-pathogen interaction (Holder *et al.,* 2007). Research has shown that *Beauveria* blastospores can exhibit similar or even greater virulence compared to aerial or submerged conidia against various arthropod pests (Hegedus *et al.,* 1992; Holder *et al.,* 2007; Mascarin *et al.,* 2015).

Dispersion: After the host dies, the fungus emerges from the cadaver and produces aerial conidia on the surface when environmental conditions, especially humidity, are suitable. Conidia can be dispersed by wind, rain splash, and various abiotic and biotic factors. The genetic and biochemical mechanisms that govern the infection cycle and virulence of *Beauveria* have been extensively reviewed by Ortiz-Urquiza and Keyhani (2013), Ortiz-Urquiza *et al.* (2015), and Valero-Jiménez *et al.* (2014).

BIOTIC AND ABIOTIC LIMITATION

Beauveria's efficiency in laboratory and field settings are regarded as highly reliant on humidity. Beauveria grows best at temperatures ranging from 34 to 36 °C. However, there are considerable intraspecific differences. According to Noma and Strickler (1999) and Ugine (2011), elevated temperatures considerably reduce efficacy. According to Jaronski (2010), fungus effectiveness may surpass forecasts based merely on the temperature and humidity of its surroundings. The phylloplane microclimate of insects, tiny insects in close contact with leaf surfaces, such as thrips or *Bemisia tabaci* nymphs, can differ significantly from their surrounding environment. Additionally, the chemical and physical properties of the surfaces where fungi are applied present another challenge that greatly influences host-pathogen interactions and, ultimately, the effectiveness of biocontrol. For instance, western flower thrips were six times more

sensitive to Beauveria when exposed to leaf disks of Phaseolus vulgaris than *Impatiens walleriana* (garden impatiens) (Ugine *et al.,* 2011).

In vitro experiments examined the impact of temperature, relative humidity, and water activity on conidia germination from an isolate of *B. bassiana* (Bals.) Vuill., which harms *Rhodnius prolixus* Stal. The triatomine vector that causes Chagas' disease. In a saturated environment, germination occurs between 15 and 35 °C, with an optimal temperature range of 25 to 40 °C. The germination process was longer at the highest measured temperatures of 15 °C and 35 °C, yet germination rates still exceeded 95%. Conditions related to moisture have a significant impact on conidia germination. Changes in germination rates and timeframes were related to water availability in both liquid and air conditions. For instance, germination occurred in 20 hours at 95.5% RH at 25 °C + 0.5 °C, while it required 72 hours of incubation at 90% RH. As the water activity decreased from 0.96 water activity (w_a) to 0.92 w_a, germination times increased. After 72 hours of incubation, germination was seen below 0.92 aw (Luz and Fargues, 1997). *B. bassiana* conidia were maintained in sterile and nonsterile soil under various conditions of temperature, relative humidity, soil water content, and pH. The main factors affecting conidia survival were water content and temperature. Half-lives of the conidia ranged from 14 days at 25 °C with 75 percent water saturation to 276 days at 10 °C with 25 percent water saturation. At 15 °C, conidia showed minimal to no loss in viability, regardless of pH, water content, or relative humidity. However, conidia could not be revived after 10 days in soils maintained at 55 °C. While conidia typically lost viability in sterile soil within 22 days, their survival was significantly lower in nonsterile soil enriched with carbon or nitrogen sources, or a combination of both (Lingg & Donaldson, 1981). Additionally, one strain of *Metarhizium anisopliae* and three isolates of the entomopathogen *B. bassiana* were cultured in seven different media with varying carbon/nitrogen (C/N) ratios. The effect of nutrition on the pathogenicity of these isolates was evaluated by measuring colony growth, spore production, germination speed, conidial C/N ratio, and the activity of Protease 1 (a serine protease). The "osmotic stress" medium resulted in the slowest colony formation across all isolates due to its low conidial yield. However, the pathogenicity and germination rate of these conidia were high. Nonetheless, some isolates exhibit low conidial Pr1 activity. Conidia from low (10:1) C/N

medium, 2% peptone, and 1% yeast extract exhibited more excellent Pr1 activity than conidia from other media most of the time, but not always. Conidia from artificial cultures had a better C/N ratio than conidia transferred by insects under different circumstances. As a consequence, they should be more harmful than in vitro conidia. Given that germination rate, conidial Pr1 activity, and C/N ratio are not dependent on the host, the virulence of fungal isolates towards host insects may be regulated by host-related par 99ameters such as insect physiology, cuticle, and environmental conditions (Safavi *et al*., 2007).

ISOLATION OF *B. bassiana*

The isolation of *B. bassiana* typically involves specific techniques to obtain pure cultures from environmental samples. The use of selective media is a common practice for isolating *B. bassiana*. Media formulations such as Sabouraud dextrose agar or other specialized formulations can be supplemented with antibiotics or other additives to inhibit the growth of contaminants and promote the growth of Beauveria. Insect baiting involves placing insect cadavers or other suitable substrates in the field to attract and isolate Beauveria from infected insects. This method is beneficial for isolating strains naturally associated with arthropod hosts. The soil dilution plating technique involves diluting soil samples and plating them onto selective media. *B. bassiana* has been identified both on the surfaces and within plants. Using selective media, *B. bassiana* was isolated from the bark of elm trees and the surrounding soil (Doberski and Tribe, 1980), as well as from the bark of *Carpinus caroliniana* (ironwood, hop hornbeam) (Bills and Polishook, 1991). Recently, this species has been found naturally on the phylloplanes of various hedgerow plants (Meyling and Eilenberg, 2006). Further details about the natural occurrence of *B. bassiana*, including its role as an endophyte in different plant species, can be found in the sections discussing host range and effects on plants. Introducing the 'Galleria-bait' technique (Zimmermann, 2007) and various selective media for isolating *Beauveria* spp. from soil has significantly increased the global discovery of *B. bassiana*. Although *Beauveria* spp. are not typically found as airborne fungi, they have been isolated from the air. In a study on fungal biodiversity in the air of Turin, Italy, *B. bassiana* was detected at an average of 0.2 CFU/m³ for air samples collected over 10 months, while *B.*

brongniartii was found at a mean of 0.1 CFU/m^3 for just one month (Airaudi and Marchisio, 1996). The natural density of B. bassiana in the air of a forest in Japan ranged from 0 to 3.1103 CFU/m^3 (Shimazu *et al.,* 2002). In laboratory conditions, this fungus was isolated alongside 98 other fungal species (Rainer *et al.,* 2000).

SCREENING OF *B. bassiana*

Molecular techniques such as Polymerase chain reaction (PCR) and DNA sequencing can be employed to confirm the identity of isolated Beauveria strains. Specific primers targeting conserved regions of the *Beauveria* genome can be used for PCR, and sequencing the amplified DNA can provide definitive identification. It was discovered that *B. bassiana* could grow successfully at high pHs of more than 10 after its growth at various pHs was examined. Additionally,, the fungus was resistant to reduced sucrose content and CuCl2. CuCl$_2$, minimal sugar, and brilliant green or crystal violet were added to the medium to create new selective media to isolate *B. bassiana.* The produced medium was utilized to extract *B. bassiana* from soil samples. It was discovered that the isolation efficacy of developed media was greater than that of VEEN and FERRON, which are often used to isolate *B. bassiana* selectively (Shimazu and Sato, 1996). *Diaphorina citri,* an Asian citrus psyllid, is a global citrus pest. It assists in the spread of a set of phloem-limited bacteria (*Candidatus Liberibacter* spp.) that cause Huanglongbing (HLB) disease. In this investigation, a novel Chinese native fungal strain (F-HY006) was isolated from *D. citri* cadavers collected in Ganzhou, Jiangxi, China (114°94′ E, 25°87′ N). The isolate proved virulent to adult female *D. citri*, with a corrected mortality of 76% and an LT50 of 4.09 Days after following treatment of a suspension containing 10^7 conidia/ml (400 microlitre/citrus seedling) (Awan *et al.,* 2021). Numerous investigations examining ITS gene analysis have shown a correlation between global climate zones and genetic groupings of *B. bassiana* (Ghikas *et al.,* 2010). Furthermore, phylogenetic analyses based on nuclear ITS sequences have shown the monophyly of Beauveria and the presence of at least two lineages within *B. bassiana (*Rehner and Buckley, 2003). According to Valero-Jiménez *et al.* (2016), the genome sequences of five *B. bassiana* isolates provide a significant resource for further studies on this crucial biological control agent and a better knowledge of the natural diversity in virulence.

According to Coates *et al.* (2002) and Bhattacharya *et al.* (2005), molecular genetic markers were generally helpful in identifying the genetic types of *B. bassiana* and allied species. Ten *B. bassiana* isolates were sequenced in the ITS1-5.8S-ITS2 region as a whole, which yielded further information on polymorphism and allowed the isolates to be grouped into three major groups based on relative similarity (Costa *et al.,* 2011). In Taif, 94 soil samples were collected from different locations. Only 11 samples had the *B. bassiana* fungus, suggesting an 11.7% ratio. According to incomplete COI (437 bp) and ITS (593 bp) sequences, four isolates exhibited diverse genetic make-ups. The pairwise genetic distances (D) between the four isolates under examination range from 0.002 to 0.008, showing a close genetic link (Sayed *et al.,* 2018). Researchers looked at the possibility of employing an isolate of *B. bassiana* obtained from aphids to biologically manage the hop aphids in both the lab and the field. Using conidial suspensions at doses ranging from 10^4 to 10^8 conidia/ml in early bioassays, the isolate showed strong virulence against *Phorodon humuli* (Schrank), with an LC50 of 1.37×10^5 conidia/ml. As conidial dosage increased, LT 50 values dropped and were 11.1 ± 4.2, 8.4 ± 4.0, 5.0 ± 1.5, 3.6 ± 1.5, and 3.1 ± 1.1 days at 10^4, 10^5, 10^6, 10^7, and 10^8 conidia/ml, in that order (Dorschner et al., 1991).

MASS PRODUCTION OF *B. bassiana*

Culturing of B. bassiana: B. bassiana grew well on liquid and solid media, including broth and potato dextrose agar or Sabouraud. Aerial conidia formed on solid surfaces were similar in morphology and infectivity to those produced on the surface of insect cadavers. In submerged cultures, six developmental stages were observed (Bidochka *et al.,* 2001). Bacterospores, resembling an insect's hemocoel, were often produced by schizolytic dissociation at septa or mechanical hyphae fragmentation caused by shearing pressures in the liquid media. Furthermore, blastospores could be produced via yeast-like budding from parent single cells. Numerous studies on blastospore growth in submerged culture were undertaken (Samsináková, 1966; Rombach, 1989; Trinci *et al.,* 1990). Because its growth method was regarded as functionally comparable to short hyphal cells, *B. bassiana* blastospores were more correctly referred to as "hyphal bodies" (Rombach, 1989). *B. bassiana* blastospores had thin walls and were unstable when applied in the

field and after fermentation (Yin, 1983). Conidia were significantly more resilient, and attempts to produce them in large numbers had piqued the scientific community's curiosity. The process of producing large quantities of aerial conidia via diphasic fermentation, which involved producing vegetative mycelia via liquid batch culture and then allowing the mycelia to surface conidia on a nutrient or inert carrier (Soper and Ward, 1981), was thought to be labor-intensive and incompatible with the traditional fermentation method of processing fungal material (Rombach, 1989). This motivated scientists to investigate the possibility of developing submerged conidia that matched airborne conidia in terms of morphology, environmental stability, and virulence.

MASS PRODUCTION METHODS OF *B. bassiana*

Diphasic fermentation method: Soper and Ward (1981) pointed out that diphasic fermentation combined the advantages of both solid and liquid substrates. The fungus was cultured in fermenters until the end of the log phase to maximize mycelial biomass output. It was then transferred to nutrient- or inert-rich substrates to develop aerial conidia in the form of naturally occurring inocula. Despite its simplicity, the diphasic technique was considered the most expensive and labor-intensive (Soper & Ward, 1981; Rombach, 1989).

Alves and Pereira (1989) obtained a yield of 2 × 10^8 *B. bassiana* conidia of powdered preparation using rice as the fundamental growth substrate. Five hundred grams of rice were soaked for one hour in 200 mL of deionized water in autoclavable polypropylene bags measuring 25 × 45 cm. The bags and their contents were removed from the autoclave after 45 minutes at 121°C, and their tops were folded and stapled. The conidial powder was put in one corner of each bag of rice after it had cooled to inoculate it. The bag was then resealed, evenly dispersing the conidia throughout the grains. The bags were incubated at 25°C for five days under 16-24 hours of light, allowing for proliferative mycelial development on the rice kernels. The contents of the bags were then dumped onto plastic trays measuring 30 × 46 or 40 × 40 cm, with a rice layer no more than 6 cm thick. To multiply the conidia, the cultures were wrapped with plastic or tray covers to conserve moisture and stored for 12 to 15 days. The trays were then put in a cold chamber set at 3°C to allow the cultures to dry. The wrap was then taken off. Finally, the conidia were extracted using a vibrating sieve mechanism enclosed in a container. 3 kg of conidial powder was generated for every 100 kg of rice media-grown culture. It should be noted that when the rice was ready to be infected, it was as firm as hard rubber, which guaranteed that the moisture content of the rice was uniform. The amount of rice and water in each plastic bag varied somewhat. For example, rice could be cooked for 4-5 minutes in a metal barrel with water instantly heated by steam from a boiler, or it could be soaked for 1-2 hours before autoclaving.

Rombach et al. (1988b) produced *B. bassiana* conidia on wheat bran using a diphasic fermentation process in a small mass production facility. After almost three years of storage at 4°C, more than 95% of the conidia germinated in viability tests. In field tests three weeks after administering an aqueous solution of *B. bassiana* (4—5 X 10^{12} conidia ha"1), *Nilaparvata lugens* (Stál) mortality varied from 65 to 95% in rice fields. Other simple materials and procedures were also used to mass manufacture *B. bassiana* and after 10 days of surface culture at 28°C, a mixture of 70% wheat bran, 25% maize flour, 5% bean flour, and water (1:1.25) yielded a dry powder with 2.9 × 10^{10} conidia g/l. Surface culture provided twenty kilograms of conidial powder from twenty kilograms of wheat flour, three hundred kilograms of wheat bran, and a little maize flour (approximately 2%). Lin et al. (1988) collected 2.9 × 10^9 conidia/g of dry powder from a medium containing 25% wheat bran, 5% maize flour, and 70% cotton-seed-shell powder (with water) using the diphasic approach on *B. brongniartii*. Aregger (1992) discovered a maximum production of 1 × 10^8-2 × 10^9 conidia g/1 with the same fungus grown in polyamide bags at 23°C after 24 or 42 days of growth on shelled barley mixed with water and sunflower oil after 24 or 42 days of growth, respectively, on shelled barley mixed with water and sunflower oil.

Solid phase fermentation: Beauveria and other hypocrealean entomopathogenic fungi disseminate infection by aerial conidia. Mass producing this hydrophobic asexual spore is not too difficult or costly. Solid-substrate fermentation (SSF) using sterile, moist cereal grains often produces aerial conidia (Jaronski, 2023). Although solid-substrate fermentation may be time-consuming and labor-intensive, it is optimal for low-tech handcraft manufacturing. A one- or two-stage mass manufacturing method may produce

aerial conidia. In the first phase of the manufacturing procedure, conidia from solid culture are promptly injected into the substrate. In the second production stage, submerged liquid fermentation was employed to produce an inoculum, usually blastospores, which are subsequently used in the final solid-phase mass production.

Ferron (1981) reported that a pilot facility in the former Soviet Union could manufacture 22 tons of *B. bassiana* as Boverin (6 × 10^9 conidia g/1) yearly. In Brazil, the primary method of producing aerial conidia is still solid-substrate fermentation (Li *et al.,* 2010). Some businesses used the mushroom spawn bag or tray technique (Alves and Pereira 1989) in large-scale production facilities to produce Beauveria conidia, yields varied substantially according to the substrate, oxygen content, initial moisture content, and isolation. Steamed rice, which is still the primary starch-rich substrate employed in SSF for conidial production of *Metarhizium* spp. and Beauveria spp. in Brazil, produced yields of up to 6.2 x 10^9 conidia/g in earlier tray technique experiments conducted in the 1980s (Alves and Pereira 1989). Nonetheless, there is a tremendous possibility of automating and scaling up the solid substrate fermentation process. An automated solid-substrate fermentation system is routinely used to produce the commercial strain GHA (ARSEF 6444) in the United States. This system yields approximately 2.2 × 10^{13} conidia/kg for 114 kg of high-purity conidial powder (1.4 × 10^{17} conidia) from a batch size of 6.35 metric tons of sterilized cereal grain in a 10-day cycle (Jaronski, unpublished observations). Filamentous fungi, including *Metarhizium spp.* and *Beauveria spp.,* have also been grown on fabric filaments, or non-woven fiber material, implanted in an appropriate artificial culture medium. The fungus develops quickly on this nutrient-rich matrix and eventually generates a substantial number of conidia per square meter of textile surface (Dubois *et al.,* 2004).

Liquid fermentation in submerged condition: Earlier attempts to generate large quantities of Beauveria blastospores through liquid culture techniques resulted in yields of less than 1 x 10^9 blastospores mL^{-1}, prolonged fermentation periods exceeding 5 days, inadequate resistance to desiccation, and inadequate stability during storage (Samsinakova 1966; Humphreys *et al.,* 1989; Rombach 1989; Vega *et al.,* 2008; Pham et al. 2009; Chong-Rodriguez *et al.,* 2011; Lohse *et al.,* 2014).

The steps involved in liquid fermentation begin with inoculating a liquid medium using harvested spores or mycelium from a fresh culture. First, a suitable fermentation medium is prepared and autoclaved for sterilization before being inoculated with the prepared culture. The culture is then incubated under optimal conditions for *B. bassiana* growth, maintaining aseptic conditions to prevent contamination. Adequate aeration and agitation are provided, particularly in bioreactors, to enhance fungal growth. Regularly monitoring parameters such as biomass concentration, pH, and temperature is essential. Once the desired growth stage is reached, typically when spore production is at its peak, the biomass is harvested. The harvested biomass is then formulated into products suitable for field application, such as wettable powders or emulsifiable concentrates. Finally, the formulated product should be stored in a cool, dry place until it is ready.

Recent research has discovered crucial environmental and nutritional factors required to rapidly generate large quantities of Beauveria and similar species blastospores under optimal nutritional settings (Mascarin *et al.,* 2015). Blastospore yields dramatically rose when aeration rates were increased to better oxygenate the mixture by altering the volume-to-surface ratio and agitation speed after two or three days of fermentation. As a result, there was a 2×10^9 mL^{-1} blastospore concentration, and more than 60% of these cells were still alive after spray drying or air drying (Mascarin *et al.,* 2015a; Jackson and Mascarin, 2016). Blastospore production was increased further by employing ionic or nonionic osmolytes, such as salts and carbohydrates, to raise the osmotic pressure of liquid cultures. This resulted in up to 3 ×10^9 blastospores mL^{-1} concentrations without impairing the cell's capacity to tolerate desiccation when exposed to air or spray drying (Mascarin *et al.,* 2015b; Jackson and Mascarin, 2016).

Blastospores seem to tolerate the hyperosmotic environment of insect hemolymph (0.7-1.2 MPa). In contrast to the low yields of aerial conidia produced under osmotic stress conditions on solid culture media (Rangel *et al.,* 2015), increased osmotic pressure in the liquid medium seems to be the source of enhanced blastospore formation. The ability of Beauveria blastospores to accumulate endogenous osmoprotectant compounds, such as glycerol, to

maintain an osmotic balance has been linked to the high osmolarity glycerol (HOG) pathway, in which osmosensing proteins are responsible for mediating adaptation to the insect hemocoel (Xiao *et al.,* 2012). Compared to cells grown in lower osmotic stress liquid media supplemented with 4% glucose, blastospores grown in liquid media supplemented with 10% glucose demonstrated superior resistance to desiccation and increased infectivity towards whitefly nymphs (Mascarin *et al.,* 2015).

MASS PRODUCTION OF SUGARCANE BYPRODUCTS

B. bassiana radial growth, biomass production, and spore formation were best suited for concentrations of three to six percent sugarcane molasses out of all tested concentrations. When the development and spore formation of *B. bassiana* were investigated, it was shown that different concentrations of discarded wash, a waste product from sugar plants, provided no support for fungal growth. Rice had the most excellent mycelial dry weight of the fungus (0.67×10^{10} g/100 ml) of the grains and tubers examined, followed by sorghum (0.64×10^{10} g/100 ml). When compared to the PDA medium (1.45×10^{10} spores/100 ml), chopped carrots (2.08×10^{10} spores/100 g), tapioca (1.74×10^{10} spores/100 g), and potatoes (1.67×10^{10} spores/100 g) produced *B. bassiana* spores. Sugarcane press mud had the most remarkable spore growth of the agricultural outputs tested (1.85×10^{10} spores/100 g). The gingelly cake produced *B. bassiana* spores per 100 g of the oil cakes tested, totaling 5.35×10^{10} spores/100 g. Cotton seed cake came in second with 4.31×10^{10} spores/100 g, while neem cake came in third with 3.80×10^{10} spores/100 g (Abraham *et al.,* 2003).

FORMULATION AND QUALITY CONTROL

Commercial products are more complex and available in dry and liquid form. The former comprises fillers, adjuvants that work well together, and other ingredients required to develop stable preparation. Environmental considerations and the ultimate cost of the product are challenged by the need for natural (biodegradable) and affordable formulation components.

Pure conidial powder could be stored unformulated for up to 21 months in sealed containers at 4°C with a 71% viability rate, as long as the water content was less than 10% (Chen *et al.,* 1990). The water content of conidia was considered to impact their shelf life substantially. According to Yin (1983), the conidial viability of an 8% water preparation was 81% after 12 months at 4°C but fell to 4.5% after 46 months. The identical preparation could only be stored for 6 months if the water content was increased to 15%. There was no noticeable decline in virulence after a year of storage at 26°C and the inclusion of attaclay X-250 as a filler (Chen *et al.,* 1990). Shi (1988) performed a test in which quick lime was utilized as a desiccant in a storage setting. The findings showed that unformulated conidial powder could be stored under natural conditions for extended periods. The viability of the conidial powder was maintained at approximately 83% after six months of storage.

Oil formulations have been shown to improve disease resistance at low humidity (Prior *et al.,* 1988; Bateman *et al.,* 1993), heat stress tolerance (Hedgecock *et al.,* 1995; Hong *et al.,* 1997), sun survival (stability of fungal formulation to solar radiation and increased leaf temperature) (Moore *et al.,* 1993; Alves *et al.,* 1998), and application efficiency to large areas with ultralow volume (ULV) application (Burges, 1998). Hydrophobic dry aerial conidia may be readily combined with pure oil (for ULV applications) or emulsifiable oil formulations for spraying (Burges, 1998). Although some researchers have emphasized the advantages of using oil-based formulations of *Beauveria* and other fungal entomopathogens, other research has shown that oil and aqueous preparations of conidia are equally effective and have comparable persistence on phylloplanes after conventional or ultra-low-volume (ULV) applications (Inglis *et al.,* 1993; Behle *et al.,* 2009). The apparent short durability of oil-containing formulations might be explained by the oil absorbed by plant tissues, resulting in the loss of any photoprotective qualities. Burges (1998) and Brar *et al.* (2006) mentioned several carriers and additives used in formulation procedures for microbial biocontrol agents.

The objective of producing liquid-produced submerged conidiospores for Beauveria seed coatings or foliar spray applications was to establish endophytic colonization while reducing target insects successfully (Lohse *et al.,* 2015). The incorporation of *Beauveria* into fungal bands was a formulation that targets adult beetles or gypsy moth larvae, which were known to climb trees and were likely to cross or wander under the fungal bands and get contaminated. The bands might

be treated with conidia in an oil-based formulation or impregnated with medium, infected with fungus, and cultured until the fungus sporulates. The fungal bands may then be attached to tree trunks or branches using allelochemical lures or not. This is a simple and low-cost way of spreading fungus throughout the target insect population. The "fungal band" application method was developed in Japan to combat native cerambycids damaging orchards (Higuchi *et al.,* 1997). Since then, it has been investigated for the integrated control of invasive, destructive wood-boring beetles in North America (Ugine et al., 2013). Storm et al. (2013) recently created a novel formulation to control grain storage arthropods based on electrostatically charged micro-powder composed of kaolin, carnauba wax, and Beauveria conidia.

COMMERCIALIZATION

Koppert in Brazil employs solid-state fermentation on wet rice to create Beauveria (Boveril® WP, strain ESALQ-PL63) and produces up to 10 tons of pure dry conidia for spray application over about 50,000 hectares, primarily made up of coffee and eucalyptus plantations. This product has been recommended for the control of eucalyptus snout beetles (*Gonipterus scutellatus*), whiteflies (*B. tabaci),* coffee berry borer (*Hypothenemus hampei*), and spider mites (*Tetranychus urticae*). Because of whiteflies on soybean crops in Brazil, the worldwide market for Beauveria is expected to approach 30 million hectares.

Beauveria strain GHA is widely used to control thrips, Hemiptera (which includes whiteflies, aphids, psyllids, mealybugs, plantbugs, and leafhoppers), a variety of Coleoptera Curculionidae and Chrysomelidae, and Orthoptera (which includes grasshoppers) (Faria and Wraight (2001), Faria and Wraight (2007), Lacey *et al.,* (2015)).

In Colombia, MicosPlag®, a mixture of M. *anisopliae, Purpureocillium lilacinum, and B. bassiana,* is used to treat insect pests such as the coffee berry borer as well as plant parasitic nematodes. In addition, a variety of *B. bassiana* mycoinsecticides seem to be accessible in the Indian biopesticide market; they may be accessed online at sites such as http://dir.indiamart.com/search.mp?ss=beauveria.

The most prevalent Beauveria-based product is wettable powder (WP), followed by concentrated suspension (CS) and emulsifiable suspension (ES) formulations. A few Beauveria products, such as beetles (BiolisaMadar®, Japan), flies (Bb Moscas®, Argentina), and Tephritidae, are intended to be spread automatically to specific targets. Beauveria and other botanical insecticides produced from natural sources may be used in conjunction, which has been helpful in various conditions (Islam and Omar 2012). Certain firms, such as LAM International (Butte, MT, USA), are adding certain botanical compounds, such as azadirachtin (neem), into their Beauveria-based treatments to build synergy and eventually boost the effectiveness of pest control in field settings.

APPLICATION OF *B. bassiana*

Bandani (2006) developed an emulsifier for ultra-low volume sprays of a *B. bassiana* conidial suspension via airplane application against pine caterpillars. This emulsifier comprises 38.9% oxidized resin, 22.2% diesel, 5.6% Na_2CO_3, and 33.3% water. It has been determined that this emulsifier does not have any detrimental effects on conidial viability and, in fact, significantly enhances virulence. Additionally, it was found that when the conidial suspension is mixed with 12.5% of the emulsifier as mentioned above (v/v), it provides optimal forest canopy coverage. An airplane carrying a whole load of 800 liters of the emulsion can spray approximately 533 hectares, with a dry powder content of 125 grams (containing more than 1.2 × 10 conidia per gram) per hectare.

CONCLUSION

B. bassiana is an entomopathogenic fungus recognized for its versatility in biological pest control. Named after Xavier Beauverie, the fungus is known for its wide range of insect hosts and its production of insecticidal compounds, making it a sustainable alternative to chemical pesticides. The introduction covers critical aspects such as life cycle and applications in integrated pest management. It also touches upon biotechnological applications, molecular mechanisms of pathogenicity, environmental impact, safety considerations, and the development of commercial formulations. These diverse research areas contribute to our comprehensive understanding of *Beauveria bassiana* and its potential to shape sustainable pest control practices in agriculture and beyond.

them to write a review on the Mass production techniques of *Beauveria bassiana*. Also, thank the co-authors for their support and for sharing information.

Consent for Publication*:*

All authors must agree to the content of the article and its publication in the journal.

Competing Interests*:*

The authors declare that no conflict of interest

Data Availability*:*

It is essential to mention where the data supporting the manuscript's conclusions can be found. If the data is included within the manuscript, this should be stated. If additional data is required, contact information for the corresponding author should be provided.

REFERENCES

Abraham, T., Easwaramoorthy, S., and Santhalakshmi, G. (2003). Mass production of *Beauveria bassiana* isolated from sugarcane root borer, *Emmalocera depresella* Swinhoe. *Sugar Tech,* **5**: 225-229. https://doi.org/10.1007/BF02942477

Airaudi, D., & Marchisio, V. F. (1996). Fungal biodiversity in the air of Turin. *Mycopathologia,* **136**(2): 95-102. https://doi.org/10.1007/BF00437502

Allee, L. L., Goettel, M. S., Gol'berg, A., Whitney, H. S., & Roberts, D. W. (1990). Infection by *Beauveria bassiana* of *Leptinotarsa decemlineata* larvae as a consequence of fecal contamination of the integument following per os inoculation. *Mycopathologia,* 111: 17-24. https://doi.org/10.1007/BF02277296

Alves, R. T., Bateman, R. P., Prior, C., & Leather, S. R. (1998). Effects of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. *Crop protection*, 17(8): 675-679. https://doi.org/10.1016/S0261-2194(98)00074-X

Aregger, E. 1992. Conidia production of the fungus *Beauveria brongniartii* on barley and quality evaluation during storage at 2°C. *Journal of Invertebrate Pathology* 59, 2-10. https://doi.org/10.1016/0022-2011(92)90104-C

Awan, U. A., Xia, S., Meng, L., Raza, M. F., Zhang, Z., & Zhang, H. (2021). Isolation, characterization, culturing, and formulation of a new *Beauveria bassiana* fungus against *Diaphorina citri*. *Biological Control*, 158: 104586. https://doi.org/10.1016/j.biocontrol.2021.104586

Bateman, R. P., Carey, M., Moore, D. E., and Prior, C. (1993). The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Annals of Applied Biology*, 122(1): 145-152. https://doi.org/10.5897/AJMR2013.6593

Bandani, A. R., Esmailpour, N. (2006). Oil formulation of entomopathogenic fungus, *Beauveria bassian,* against Sunn pest, Eurygaster integriceps puton (Heteroptera: Scutelleridae). *Commun Agric Appl Biol Sci*. 71(2):443-8. PMID: 17385512

Behle, R. W., Compton, D. L., Laszlo, J. A., and Shapiro-Ilan, D. I. (2009). Evaluation of soyscreen in an oil-based formulation for UV protection of *Beauveria bassiana conidia. Journal of economic entomology*, 102(5): 1759-1766. https://doi.org/10.1603/029.102.0505

Bhattacharya, D., Reeb, V., Simon, D. M., and Lutzoni, F. (2005). Phylogenetic analyses suggest reverse splicing spread of group I introns in fungal ribosomal DNA. BMC Evolutionary Biology, 5: 1-11. https://doi.org/10.1186/1471-2148-5-68

Bidochka, M. J. (2001). Monitoring the fate of biocontrol fungi. Fungal biocontrol agents: progress, problems and potential. *CAB International,* Wallingford, United Kingdom, 193-218. https://doi.org/10.1079/9780851993560.0193

Bills, G. F., and Polishook, J. D. (1991). Microfungi from *Carpinus caroliniana. Canadian Journal of Botany*, 69(7): 1477-1482. http://dx.doi.org/10.1139/b91-191

Bischoff, J. F., Rehner, S. A., & Humber, R. A. (2009). A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia*, 101(4): 512-530. https://doi.org/10.3852/07-202

Brar, S. K., Verma, M., Tyagi, R. D., & Valéro, J. R. (2006). Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. *Process biochemistry*, 41(2): 323-342. https://doi.org/10.1016/j.procbio.2005.07.015

Burges, H.D., (1998). Formulation of microbial biopesticides: beneficial organisms, nematodes and seed treatments. Kluwer Academic, Dordrecht, p 412. https://doi.org/10.1007/978-94-011-4926-6

Cai, B. L., & Liu, A. X. (1988). Pathogenicity of *Beauveria bassiana* and *Metarhizium*

anisopliae to cockroaches. *Study and Application of Entomogenous Fungi in China*, 1: 101-103. https://scholar.google.com/scholar?cluster=15528626649577171736&hl=en&as_sdt=2005&sciodt=0,5

Chen, C. J., Wu, J. W., Li, Z. Z., Wang, Z. X., Li, Y. W., Chang, S. H., ... and Tan, Y. C. (1990). Application of microbial pesticides in IPM. Integrated Management of Pine Caterpillars in China. *China Forestry Publishing House, Beijing*: 214-308. http://dx.doi.org/10.1016/B978-0-12-824451-7.00004-8

Chong-Rodríguez, M. J., Maldonado-Blanco, M. G., Hernández-Escareño, J. J., Galán-Wong, L. J., and Sandoval-Coronado, C. F. (2011). Study of *Beauveria bassiana* growth, blastospore yield, desiccation-tolerance, viability and toxic activity using different liquid media. *African Journal of Biotechnology*, 10(30): 5736-5742. http://dx.doi.org/10.5897/AJB10.2264

Coates, B. S., Hellmich, R. L., and Lewis, L. C. (2002). Allelic variation of a *Beauveria bassiana* (Ascomycota: Hypocreales) minisatellite is independent of host range and geographic origin. Genome, 45(1): 125-132. https://doi.org/10.1139/g01-132

Costa, E. M. D., Pimenta, F. C., Luz, C., Oliveira, V. D., Oliveira, M., Bueno, E., Petrofeza, S. (2011). *Beauveria bassiana*: quercetinase production and genetic diversity. *Brazilian Journal of Microbiology*, 42: 12-21. https://doi.org/10.1590/S1517-83822011000100002

De Faria, M. R., Wraight, S. P. (2007). Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological control*, 43(3): 237-256. https://doi.org/10.1016/j.biocontrol.2007.08.001

Doberski, J. W., and Tribe, H. T. (1980). Isolation of entomogenous fungi from elm bark and soil with reference to ecology of *Beauveria bassiana* and *Metarhizium anisopliae*. *Transactions of the British Mycological Society*, 74(1): 95-100. https://doi.org/10.1016/S0007-1536(80)80013-1

Dorschner, K. W., Feng, M. G., Baird, C. R. (1991). Virulence of an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) to the hop aphid, *Phorodon humuli* (Homoptera: Aphididae). *Environmental Entomology*, 20(2): 690-693. https://doi.org/10.1093/ee/20.2.690

Dubois, T., Li, Z., Jiafu, H., Hajek, A. E. (2004). Efficacy of fiber bands impregnated with *Beauveria brongniartii* cultures against the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Biological Control*, 31(3): 320-328. https://doi.org/10.1016/j.biocontrol.2004.03.011

Fang, W., Azimzadeh, P., Leger, R. J. S. (2012). Strain improvement of fungal insecticides for controlling insect pests and vector-borne diseases. *Current opinion in microbiology*, 15(3): 232-238. https://doi.org/10.1016/j.mib.2011.12.012

Faria, M., Wraight, S. P. (2001). Biological control of *Bemisia tabaci* with fungi. *Crop protection,* 20(9): 767-778. https://doi.org/10.1016/S0261-2194(01)00110-7 .

Feng, MG. (2002). Microbial Control of Insect Pests with Entomopathogenic Fungi in China : A Decade's Progress in Research and Utilization. In: Upadhyay, R.K. (eds) Advances in Microbial Control of Insect Pests. Springer, Boston, MA. https://doi.org/10.1007/978-1-4757-4437-8_12

Ferron, P. (1981). Pest control by the fungi Beauveria and Metarhizium. *Microbial control of pests and plant diseases*: 1970-1980. https://doi.org/10.1146/ANNUREV.EN.23.010178.002205

Ghikas, D. V., Kouvelis, V. N., Typas, M. A. (2010). Research article Phylogenetic and biogeographic implications inferred by mitochondrial intergenic region analyses and ITS1-5.8 S-ITS2 of the entomopathogenic fungi *Beauveria bassiana* and *B. brongniartii. BMC Microbiol* **10**, 174 (2010). https://doi.org/10.1186/1471-2180-10-174

Gibson, D. M., Donzelli, B. G., Krasnoff, S. B., Keyhani, N. O. (2014). Discovering the secondary metabolite potential encoded within entomopathogenic fungi. *Natural Product Reports*, 31(10): 1287-1305. https://doi.org/10.1039/c4np00054d

Goettel, M. S., Koike, M., Kim, J. J., Aiuchi, D., Shinya, R., Brodeur, J. (2008). Potential of Lecanicillium spp. for management of insects, nematodes and plant diseases. *Journal of invertebrate pathology*, 98(3): 256-261. https://doi.org/10.1016/j.jip.2008.01.009

Hedgecock, S., Moore, D., Higgins, P. M., Prior, C. (1995). Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviride* conidia in an oil formulation. *Biocontrol Science*

and Technology*, 5(3): 371-378. https://doi.org/10.1080/09583159550039828

Hegedus, D. D., Bidochka, M. J., Miranpuri, G. S., Khachatourians, G. G. (1992). A comparison of the virulence, stability and cell-wall-surface characteristics of three spore types produced by the entomopathogenic fungus *Beauveria bassiana. Applied microbiology and biotechnology*, 36: 785-789. https://doi.org/10.1007/BF00172195

Higuchi, T., Saika, T., Senda, S., Mizobata, T., Kawata, Y., Nagai, J. (1997). Development of biorational pest control formulation against longicorn beetles using a fungus, *Beauveria brongniartii (Sacc.) Petch. Journal of Fermentation and Bioengineering*, 84(3): 236-243. https://doi.org/10.1016/S0922-338X(97)82061-2

Holder, D. J., Kirkland, B. H., Lewis, M. W., Keyhani, N. O. (2007). Surface characteristics of the entomopathogenic fungus *Beauveria (Cordyceps) bassiana. Microbiology*, 153(10): 3448-3457. https://doi.org/10.1099/mic.0.2007/008524-0

Hong, T. D., Ellis, R. H., Moore, D. (1997). Development of a model to predict the effect of temperature and moisture on fungal spore longevity. *Annals of Botany*, 79(2): 121-128. https://doi.org/10.1006/anbo.1996.0316

Humber, R. A. (2008). Evolution of entomopathogenicity in fungi. *Journal of invertebrate pathology*, 98(3): 262-266. https://doi.org/10.1016/j.jip.2008.02.017

Humphreys, A. M., Matewele, P., Trinci, A. P., Gillespie, A. T. (1989). Effects of water activity on morphology, growth and blastospore production of *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces farinosus* in batch and fed-batch culture. *Mycological research*, 92(3): 257-264. https://doi.org/10.1016/S0953-7562(89)80063-2

Inglis, G. D., Goettel, M. S., Johnson, D. L. (1993). Persistence of the entomopathogenic fungus, *Beauveria bassiana,* on phylloplanes of crested wheatgrass and alfalfa. *Biological control*, 3(4): 258-270. https://doi.org/10.1006/bcon.1993.1035

Islam, M. T., Omar, D. B. (2012). Combined effect of *Beauveria bassiana* with neem on virulence of insect in case of two application approaches.

J Anim Plant Sci, 22(1): 77-82. https://www.thejaps.org.pk/Volume/2012/22-1/abstracts/29.php

Jackson MA, Mascarin. GM (2016). Stable fungal blastospores and methods for their production, stabilization and use. Patent US20160075992. https://patents.google.com/patent/US20160075992A1/en

Jaronski, S. T. (2010). Ecological factors in the inundative use of fungal entomopathogens. BioControl, 55: 159-185. https://doi.org/10.1007/s10526-009-9248-3

Jaronski, S. T. (2023). Mass production of entomopathogenic fungi—state of the art. *Mass production of beneficial organisms:* 317-357. https://doi.org/10.1016/B978-0-12-822106-8.00017-8

Khachatourians, G. G. (1986). Production and use of biological pest control agents. *Trends in Biotechnology*, 4(5): 120-124. https://doi.org/10.1016/0167-7799(86)90144-7

Khachatourians, G. G. (1996). *Biochemistry and molecular biology of entomopathogenic fungi. In Human and animal relationships* (pp. 331-363). Berlin, Heidelberg: Springer Berlin Heidelberg. http://dx.doi.org/10.1007/978-3-540-79307-6_3

Lacey, L. A., Grzywacz, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M., Goettel, M. S. (2015). Insect pathogens as biological control agents: Back to the future. *Journal of invertebrate pathology*, 132: 1-41. https://doi.org/10.1016/j.jip.2015.07.009

Li, Z., Alves, S. B., Roberts, D. W., Fan, M., Delalibera Jr, I., Tang, J., ... Rangel, D. E. (2010). Biological control of insects in Brazil and China: history, current programs and reasons for their successes using entomopathogenic fungi. *Biocontrol Science and Technology*, 20(2): 117-136. http://dx.doi.org/10.1080/09583150903431665

Lin, L. X., Yu, Y. C., Hu, J. W. (1988). Study on white grub control by *Beauveria brongiartii* in soybean field. *Study and application of entomogenous fungi in China*, 1: 135-139.

Lingg, A. J., Donaldson, M. D. (1981). Biotic and abiotic factors affecting stability of *Beauveria bassiana* conidia in soil. *Journal of Invertebrate Pathology*, 38(2): 191-200. https://doi.org/10.1016/0022-2011(81)90122-1

Lohse, R., Jakobs-Schönwandt, D., Patel, A. V. (2014). Screening of liquid media and fermentation of an endophytic *Beauveria bassiana* strain in a bioreactor. *AMB Express*, 4: 1-11. https://doi.org/10.1186/s13568-014-0047-6

Lohse, R., Jakobs-Schönwandt, D., Vidal, S., Patel, A. V. (2015). Evaluation of new fermentation and formulation strategies for a high endophytic establishment of *Beauveria bassiana* in oilseed rape plants. *Biological Control*, 88: 26-36. http://dx.doi.org/10.1016/j.biocontrol.2015.05.002

Luz, C., Fargues, J. (1997). Temperature and moisture requirements for conidial germination of an isolate of *Beauveria bassiana*, pathogenic to *Rhodnius prolixus*. *Mycopathologia*, 138: 117-125. https://doi.org/10.1023/A:1006803812504

Mascarin, G. M., Jackson, M. A., Kobori, N. N., Behle, R. W., Júnior, Í. D. (2015a). Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *Beauveria bassiana* and *Isaria fumosorosea* strains. *Journal of invertebrate pathology*, 127: 11-20. https://doi.org/10.1016/j.jip.2014.12.001

Mascarin, G. M., Jackson, M. A., Kobori, N. N., Behle, R. W., Dunlap, C. A., Delalibera Júnior, Í. (2015b). Glucose concentration alters dissolved oxygen levels in liquid cultures of *Beauveria bassiana* and affects formation and bioefficacy of blastospores. *Applied microbiology and biotechnology,* 99: 6653-6665. https://doi.org/10.1007/s00253-015-6620-3

McDowell, J. M., Funderburk, J. E., Boucias, D. G., Gilreath, M. E., Lynch, R. E. (1990). Biological activity of *Beauveria bassiana* against *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae) on leaf substrates and soil. *Environmental Entomology*, 19(1): 137-141. https://doi.org/10.1093/ee/19.1.137

Meyling, N. V., and Eilenberg, J. (2006). Isolation and characterisation of *Beauveria bassiana* isolates from phylloplanes of hedgerow vegetation. *Mycological research*, 110(2), 188-195. https://doi.org/10.1016/j.mycres.2005.09.008

Moore, D., Bridge, P. D., Higgins, P. M., Bateman, R. P., & Prior, C. (1993). Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. *Annals of Applied Biology*, 122(3): 605-616. https://doi.org/10.1111/j.1744-7348.1993.tb04061.x

Noma, T., Strickler, K. (1999). Factors affecting *Beauveria bassiana* for control of Lygus bug (Hemiptera: Miridae) in alfalfa seed fields. *Journal of Agricultural and Urban Entomology*, 16(4): 215–233

Ortiz-Urquiza, A., and Keyhani, N. O. (2013). Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects*, 4(3): 357-374. https://doi.org/10.3390/insects4030357

Ortiz-Urquiza, A., Riveiro-Miranda, L., Santiago-Álvarez, C., Quesada-Moraga, E. (2010). Insect-toxic secreted proteins and virulence of the entomopathogenic fungus *Beauveria bassiana. Journal of invertebrate pathology*, 105(3): 270-278. https://doi.org/10.1016/j.jip.2010.07.003

Pendland, J. C., Hung, S. Y., Boucias, D. G. (1993). Evasion of host defense by in vivo-produced protoplast-like cells of the insect mycopathogen *Beauveria bassiana*. *Journal of bacteriology*, 175(18): 5962-5969. https://doi.org/10.1128/jb.175.18.5962-5969.1993

Pham, T. A., Kim, J. J., Mm, S. G., Kim, K. (2009). Production of blastospore of entomopathogenic *Beauveria bassiana* in a submerged batch culture. *Mycobiology*, 37(3): 218-224. https://doi.org/10.4489/myco.2009.37.3.218

Prior, C., Jollands, P., Le Patourel, G. (1988). Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). *Journal of Invertebrate Pathology*, 52(1): 66-72. https://doi.org/10.1016/0022-2011(88)90103-6

Quesada-Moraga, E., Vey, A. (2003). Intra-specific variation in virulence and in vitro production of macromolecular toxins ctive against locust among *Beauveria bassiana* strains and effects of in vivo and in vitro passage on these factors. *Biocontrol Science and Technology*, 13(3): 323-340. https://doi.org/10.1080/0958315031000110346

Rainer, J., Peintner, U., Pöder, R. (2001). Biodiversity and concentration of airborne fungi in a hospital environment. *Mycopathologia*, 149: 87-97. https://doi.org/10.1023/a:1007273131130

Rangel, D. E., Braga, G. U., Fernandes, É. K., Keyser, C. A., Hallsworth, J. E., Roberts, D. W. (2015). Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions

during conidial formation. *Current genetics*, 61: 383-404. https://doi.org/10.1007/s00294-015-0477-y

Rehner, S. A., Buckley, E. P. (2003). Isolation and characterization of microsatellite loci from the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Hypocreales). *Molecular Ecology Notes*, 3(3): 409-411. https://doi.org/10.1046/j.1471-8286.2003.00464.x

Roberts, D. W. (1981). Toxins of entomopathogenic fungi. *Microbial control of pests and plant diseases*, 441. https://link.springer.com/chapter/10.1007/978-1-4684-7112-0_10

Rombach, M. C. (1989). Production of *Beauveria bassiana* [Deuteromycotina. Hyphomycetes] sympoduloconidia in submerged culture. *Entomophaga,* 34: 45-52. https://link.springer.com/article/10.1007/BF02372586

Rombach, M. C., Humber, R. A., Evans, H. C. (1987). *Metarhizium album*, a fungal pathogen of leaf- and planthoppers of rice. *Transactions of the British Mycological Society*, 88(4): 451-459. https://doi.org/10.1016/S0007-1536(87)80028-1

Safavi, S. A., Shah, F. A., Pakdel, A. K., Reza Rasoulian, G., Bandani, A. R., Butt, T. M. (2007). Effect of nutrition on growth and virulence of the entomopathogenic fungus Beauveria bassiana. *FEMS Microbiology Letters*, 270(1): 116-123. https://doi.org/10.1111/j.1574-6968.2007.00666.x

Samšináková, A. (1966). Growth and sporulation of submersed cultures of the fungus *Beauveria bassiana* in various media. *Journal of Invertebrate Pathology*, 8(3): 395-400. https://doi.org/10.1016/0022-2011(66)90056-5

Sayed, S. M., Ali, E. F., El-Arnaouty, S. A., Mahmoud, S. F., Amer, S. A. (2018). Isolation, identification, and molecular diversity of indigenous isolates of *Beauveria bassiana* from Taif region, Saudi Arabia. *Egyptian Journal of Biological Pest Control*, 28: 1-6. https://doi.org/10.1186/s41938-018-0054-z

Shi, Z. M. (1988). Technology for conidial preparation of *Beauveria bassiana*. *Study and Application of Entomogenous Fungi in China*, 1: 114-115.

Shimazu, M., SATO, H. (1996). Media for selective isolation of an entomogenous fungus, *Beauveria bassiana* (Deuteromycotina: Hyphomycetes).

Applied Entomology and Zoology, 31(2): 291-298. https://doi.org/10.1303/aez.31.291

Shimazu, M., Sato, H., Maehara, N. (2002). Density of the entomopathogenic fungus, *Beauveria bassiana* Vuillemin (Deuteromycotina: Hyphomycetes) in forest air and soil. *Applied Entomology and Zoology,* 37(1): 19-26. http://dx.doi.org/10.1303/aez.2002.263

Soper, R. S., and Ward, M. G. (1981). Production, formulation and application of fungi for insect control. *Biological control in crop production*: 161-180.

Storm, C. G., Wakefield, M. E., Chambers, J. (2013). Control of arthropod infestation using particles comprising an entomopathogen and wax U.S. Patent Application No. 13/704,291. https://patents.google.com/patent/WO2011157983A1/en

Trinci, A. P. J., Lane, B. S., Humphreys, A. M. (1990). Optimization of cultural conditions for the production and longevity of entomopathogenic fungi. *In Proceedings and abstracts, Vth International Colloquium on Invertebrate Pathology and Microbial Control*, Adelaide, Australia, 20-24 August 1990. (pp. 116-120). Department of Entomology, University of Adelaide.

Ugine, T. A. (2011). The effect of temperature and exposure to *Beauveria bassiana* on tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae) population dynamics, and the broader implications of treating insects with entomopathogenic fungi over a range of temperatures. *Biological Control*, 59(3): 373-383. http://dx.doi.org/10.1016/j.biocontrol.2011.08.004

Ugine, T. A., Jenkins, N. E., Gardescu, S., Hajek, A. E. (2013). Comparing fungal band formulations for Asian longhorned beetle biological control. *Journal of invertebrate pathology*, 113(3): 240-246. https://doi.org/10.1016/j.jip.2013.04.007

Valero-Jiménez, C. A., Debets, A. J., van Kan, J. A., Schoustra, S. E., Takken, W., Zwaan, B. J., Koenraadt, C. J. (2014). Natural variation in virulence of the entomopathogenic fungus *Beauveria bassiana* against malaria mosquitoes. *Malaria journal*, 13(1): 1-8. https://doi.org/10.1186/1475-2875-13-479

Valero-Jimenez, C. A., Faino, L., Spring in't Veld, D., Smit, S., Zwaan, B. J., van Kan, J. A. (2016). Comparative genomics of *Beauveria bassiana*: uncovering signatures of virulence against mosquitoes. BMC genomics, 17: 1-11. https://doi.org/10.1186/s12864-016-3339-1

Vega, F. E., Posada, F., Aime, M. C., Pava-Ripoll, M., Infante, F., Rehner, S. A. (2008). Entomopathogenic fungal endophytes. *Biological control*, 46(1): 72-82. https://doi.org/10.1016/j.biocontrol.2008.01.008

Vidal, C., Fargues, J., Lacey, L. A., Jackson, M. A. (1998). Effect of various liquid culture media on morphology, growth, propagule production, and pathogenic activity to Bemisia argentifolii of the entomopathogenic Hyphomycete, Paecilomyces fumosoroseus. *Mycopathologia*, 143: 33-46. http://dx.doi.org/10.1023/A:1006962808712

Xiao, G., Ying, S. H., Zheng, P., Wang, Z. L., Zhang, S., Xie, X. Q., ... Feng, M. G. (2012). Genomic perspectives on the evolution of fungal entomopathogenicity *in Beauveria bassiana. Scientific reports*, 2(1): 483. https://doi.org/10.1038/srep00483

Yin, F. M. (1983). Storage of conidral powder of *Beauverra bassiuna. Guangdong Forestry Newsletter*, 3(4): 13-16.

Zhang, Z. G., and Huang, X. Y. (1988). Study of the influence of *Beauveria bassiana* on oviposition of *Nephotettix cincticeps* and *Nilaparata lugens. Study and Application of Entomogenous Fungi in China*, 1: 85-89.

Zimmermann, G. (2007). Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii. Biocontrol Science and Technology*, 17(6): 553-596. https://doi.org/10.1080/09583150701309006