LA GRANJA:
REVISTA DE
CIENCIAS DE LA VIDA
pISSN:1390-3799; eISSN:1390-8596
http://doi.org/10.17163/lgr.n40.2024.08

Review / Reseña bibliográfica

BIOTECHNOLOGY

ABYA YALA UNIVERSIDAD POLITÉCNICA SALESIANA

PRODUCTION OF *BEAUVERIA BASSIANA* FOR THE FORMULATION OF BIOPESTICIDES

PRODUCCIÓN DE *BEAUVERIA BASSIANA* PARA LA FORMULACIÓN DE BIOPLAGUICIDAS

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Received on January 10th, 2022. Accepted, after revision, on May 19th, 2022.

Abstract

The harmful effects of chemicals in conventional agriculture and the growing demand for food free of toxic residues has developed environmentally sustainable strategies. An effective alternative for integrated pest management in agricultural crops are biopesticides formulated with microorganic structures or from the production of active compounds. This paper describes the production processes of *Beauveria bassiana* for formulating biopesticides for agricultural use. The information was collected through a systematic search in Research Gate, Google Scholar, Science Direct and PubMed, using keywords such as production, *Beauveria bassiana*, solid fermentation, liquid fermentation and metabolites. The results affirm that *B. bassiana* is one of the microorganisms with great potential to produce biopesticides, due to the entomopathogenic mechanism of action and secondary metabolites, which can be used for the biological control of phytophagous insects. Likewise, for the formulation of *B. bassiana* it should be considered a profitable culture medium for large-scale production, also the control of environmental variables such as temperature at 25 °C, relative humidity 65-70%, pH of 5.4, propagation time between 4 to 8 days, and for the liquid fermentation process, a constant agitation between 200 to 400 rpm must be maintained. Biological products represent an alternative to minimize the use of synthetic pesticides, reduce environmental pollution and ensure food safety and security.

Keywords: Biopesticides, *Beauveria bassiana*, solid fermentation, liquid fermentation, metabolites.

Resumen

Los efectos nocivos de los productos químicos en la agricultura convencional y la creciente demanda de alimentos libres de residuos tóxicos, ha dado lugar al desarrollo de estrategias sostenibles con el medio ambiente. Una alternativa eficaz para el manejo integrado de plagas en los cultivos agrícolas son los bioplaguicidas formulados con estructuras de microorganismos o a partir de la producción de los compuestos activos. En este contexto, el presente trabajo describe los procesos de producción de *Beauveria bassiana* para la formulación de bioplaguicidas de uso agrícola. La recolección de la información se realizó mediante una búsqueda sistemática en ResearchGate, Google Académico, ScienceDirect y PubMed, empleando palabras claves como producción, *Beauveria bassiana*, fermentación sólida, fermentación líquida y metabolitos. A partir de los resultados de la investigación se afirma que *B. bassiana* es uno de los microorganismos con gran potencial para la producción de bioplaguicidas, por el mecanismo de acción entomopatógeno y los metabolitos secundarios, que pueden ser utilizados para el control biológico de insectos fitófagos. Así mismo, en la producción de *B. bassiana* se debe considerar un medio de cultivo rentable a gran escala, además de controlar las variables ambientales como temperatura a 25 °C, humedad relativa 65-70 %, pH de 5.4, tiempo de propagación entre 4 a 8 días, y para el proceso de fermentación líquida agitación constante entre 200 a 400 rpm. Los productos biológicos representan una alternativa para minimizar el uso de plaguicidas sintéticos, reducir la contaminación ambiental y garantizar la seguridad e inocuidad de los alimentos.

Palabras clave: Bioplaguicidas, *Beauveria bassiana*, fermentación sólida, fermentación líquida, metabolitos.

Suggested citation: Lucero, J., Manzano, J., Loaiza, I. and Orellana, Y. (2024). Production of Beauveria bassiana for the formulation of biopesticides. *La Granja: Revista de Ciencias de la Vida.* [Accepted version] http://doi.org/10.17163/lgr.n40.2024.08.

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1 Introduction

Agricultural crops are affected by bacteria, fungi, weeds, insects and nematodes, causing yield reductions (Thakur et al., 2020). Since 1960, agricultural pest control methods have been carried out by applying synthetic pesticides, such as dichlorodiphenyl-trichloroethane (DDT), and other organophosphate pesticides and carbamates (Kumar, 2012).

Green revolution technologies contributed to increase food production through intensive agriculture by using chemical fertilizers and pesticides (Kumar and Singh, 2015). However, adverse effects such as soil degradation, water pollution, insect resistance, and toxic residues in food (Lengai and Muthomi, 2018), have demanded the production of healthy food, decreasing the use of natural resources, and strengthening sustainable agriculture (Kumar, 2012).

The production of microbial biopesticides has increased due to the demand for chemical-free food, being essential in organic agriculture (Mascarin and Jaronski, 2016). Biopesticides are generally composed of beneficial bacteria, viruses, fungi, and nematodes with chitinolytic, entomopathogenic and antagonistic activities, used as biological controllers of phytopathogens, insects and phytophagous nematodes (Lengai and Muthomi, 2018).

Biochemical and genomic analyses have shown that the metabolites produced by the microorganisms have great potential in biological pest control (Luo et al., 2017). The identified metabolites of *Beauveria bassiana* are beauvericin and bassiacridin with insecticidal action (Al Khoury et al., 2019; Quesada-Moraga and Vey, 2004), are used for controlling *Tetranychus urticae, Bemisia tabaci* and *Locusta migratoria*; oosporein with antiviral and antibacterial effects on *Enterococcus faecalis* and *Stenotrophomonas* spp. (Jeffs and Khachatourians, 1997), and bassianin as an ATP inhibitor (Patočka, 2016).

Formulation technologies of biological control agents for commercial scale-up must consider certain criteria for the production process (Ávila-Hernández et al., 2020) such as stabilization of the microorganism in the production; hence, distribution and storage stage conditions conducive to field application should also be provided (Dannon et al., 2020). Evaluations of persistence after application and adaptation to environmental conditions without altering the physicochemical properties of the microorganism should be carried out (Ávila-Hernández et al., 2020).

Pesticides made from entomopathogenic fungi are frequently used in phytosanitary programs to control populations of phytophagous insects (Luo et al., 2014). One of the most relevant entomopathogenic fungi in the agricultural field is *Beauveria bassiana*, used to control pests such as the coffee berry borer (*Hypothenemus hampei*), black banana weevil (*Cosmopolites sordidus*), aphids and spider mites, among others (Gerónimo et al., 2016; Al Khoury et al., 2019; Ávila-Hernández et al., 2020). This fungus is considered a natural enemy of insects in ecosystems, crop residues and colonized hosts (Marín et al., 2018).

Commercial formulations of *Beauveria bassiana* include artisanal methods such as fermentation on solid substrates, in which the fungus is inoculated on a substrate and the application is done by filtering the conidia of the microorganism. On the other hand, the most innovative methods consist of developing dry and liquid formulations, boosting the fungal propagule (conidia or blastospores). The drying process is performed by spray drying, air drying, rotary vacuum drying and fluidized bed drying; these techniques are employed to stabilize *Beauveria bassiana* propagules on a large scale and have a satisfactory shelf life (Mascarin and Jaronski, 2016). Regarding the analysis on the use of chemical pesticides and the need to produce environmentally friendly inputs, a systematic review has been carried out with the aim of describing the production processes of *Beauveria bassiana* for the formulation of biopesticides for agricultural use.

2 Methodology

A systematic review of 60 manuscripts was carried out in specialized search engines and databases of ResearchGate (18), Semanticscholar (10), Google Scholar (14), Springer (2), SciELO (2), ScienceDirect (6) and PubMed (8), dedicating approximately 1920 hours for the bibliographic search, review and scientific writing. The research focused on the entomopathogen *Beauveria bassiana* by its biocidal potential for controlling phytophagous insects, and the *in vitro* and commercial scale production processes for formulating biopesticides for agricultural use. The search was carried out in journals such as the Journal of Applied Entomology, Plant Protection Science, World Journal of Microbiology and Biotechnology, Biology, Journal of Invertebrate Pathology and others as primary sources; and in institutions such as the National Agricultural Health Service and companies as secondary sources that formulate insecticides containing *Beauveria bassiana*. The information selected corresponds to articles published during the last 10 years, with some exceptions considered by their relevance to the bibliographic review.

3 *Beauveria bassiana* as biological controllers

The entomopathogen *B. bassiana* was first observed in silkworms. The larvae had a white outer covering with multiple inflorescences that could infect healthy larvae in a short period of time (Bassi, 1835). In 1954, the first outbreaks of infection in acridids were reported, but it was in 1987 when the pathogenicity of the microorganism in grasshoppers was proven under laboratory conditions (Inglis et al., 1993).

In recent years, about 700 species of insect hosts of *B. bassiana* have been discovered (Xiao et al., 2012). This fungus can infect major insect taxa when it finds the right conditions to inoculate the host. However, studies related to the pathogenicity of *B. bassiana* have focused on insects considered pests (Meyling and Eilenberg, 2007). Genome sequencing of infected hosts showed that *B. bassiana* evolved from insects; it is also assumed that the expression of certain protease and chitinase genes are associated with functions necessary for insect pathogenesis and convergent evolution (Xiao et al., 2012).

4 Biopesticidal potential of *Beauveria bassiana*.

Beauveria bassiana is an entomopathogenic fungus used as a biological controller of agricultural crop pests (Barcenilla, 2021). The pathogen enters insects through conidia that attach to the host cuticle. Formation of the germ tube and appressorium allows attachment to the insect integument by pressure. The action of hydrolytic enzymes such as lipases, proteases and chitinases enter the insect body through the soft parts (Lara-Juache et al., 2021). The hyphae have contact with the hemolymph containing high nutrient initiating a stage of unicellular blastospore budding (Mascarin and Jaronski, 2016). The fungus colonizes the internal tissues of the insect and during this process they release metabolites such as beauvericin and bassiacridin that help inhibit the immune system, facilitating entry into the internal organs of the host, causing its death (Figure 1) (Harith-Fadzilah et al., 2021). Infected insects show a cottony, powdery, creamy-yellow, cottony cover that coats the external part of the host (Barcenilla, 2021).

In the colonization stage, *B. bassiana* secretes proteins and enzymes that can be used as cell factories for producing commercial inputs. The evaluation of the metabolic activity by complete genome sequencing expresses that B. bassiana has an open pangenome with the capacity to colonize different hosts (insects, nematodes and plants). In addition, 10366 genes coding for proteins such as proteases and 145 carbohydrate-active enzymes of the chitinases, cellulases and hemicellulases type were identified (Vidal and Jaber, 2015).

Gene expression of *B. bassiana* in the insect inoculation process demonstrates the presence of peroxidase, trehalase, lipase, peptidase, phosphatase and lyase enzymes responsible for host cuticle degradation. The chitinase enzymes of *B. bassiana* hydrolyze the b-bonds of chitin polymers into N-acetyl b-D-glucosamine monomers present in the arthropod exoskeleton (Amobonye et al., 2020). The cuticle of insects consists of surface lipids that act as a protective barrier to pathogens. However, lipases produced by B. bassiana contribute to the degradation of the insect cuticle (Salazar et al., 2020); these are water-soluble enzymes that act on insoluble substrates that can hydrolyze triglycerides and transform them to fatty acids and glycerol. Entomopathogenic proteases act on the host cuticle and attack weakened tissue by chitinolytic actions (Amobonye et al., 2020).

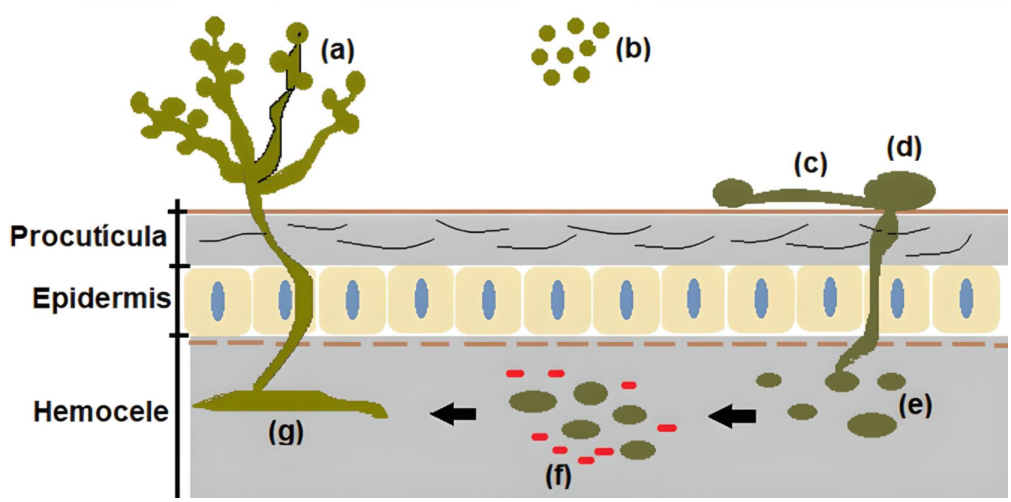

Figure 1. Infection process of *Beauveria bassiana* in the cuticle of insects. **(a)** Structures of *B. bassiana* **(b)** Dissemination of *B. bassiana conidia* **(c)** Formation of the germ tube on the surface of the insect body **(d)** Formation of the appressorium and entry of the hyphae into the procuticle and epidermis. **(e)** Production of blastospores and invasion into hemocele. **(f)** Release of secondary metabolites. **(g)** Formation of the fungal structures (hyphae, mycelium and conidia) and release into the outer space.

The diversity of toxins produced by *B. bassiana* can range from simple compounds such as biological macromolecules, e.g. oxalic acid, 2,6- pyridindicarboxylic acid (dipicolinic acid), and compounds with more complex structures of cyclic and linear peptide nature such as beauvericin, bassiacridin, beauverolides and bassianolides (Borges et al., 2010). Toxins alter the natural and artificial permeability of membranes, induce cell fluid loss, produce changes in the nucleus during molting and metamorphosis processes, deform the external structures of insects and interfere with fertilization processes (Patočka, 2016). Finally, lipase, chitinase, protease and amylase enzymes degrade the exoskeleton of the host and allow the fungus to enter its internal tissues (Cortés and Mosqueda, 2013).

5 *In vitro* production of *Beauveria bassiana*

The processes for the conidia production of *B. bassiana* can range from very simple methods to processes that involve more technification (Vela et al., 2019). The first step for *B. bassiana* production consists of isolating the strains of the microorganism, for which it is important to determine the composition of the culture medium and the optimal parameters for growth. Greenfield et al. (2016) state that *B. bassiana* strains can be grown on glucose agar, peptone of soy, potato-dextrose agar (PDA), dextrose-Sabouraud agar (SDA) or oatmeal agar. Optimal temperatures for growth range from 22-26°C, alternating light and dark for eight days. In contrast, Bugti et al. (2018) state that a spore suspension of the microorganism should be made and inoculated on dextrose-Sabouraud agar culture medium (20 g agar, 10 g peptone, 40 g dextrose, 0.5 mg potassium, in 1000 mL of distilled water) and incubated at 24 $\pm$ 1 °C for twelve days.

The second step is to test the pathogenicity of *B. bassiana* by inoculating healthy insects by preparing conidial suspensions in 0.05% Tween 80 solution, with constant agitation for five minutes, adjusting the spore concentration and spraying the insects (Bugti et al., 2018). Table 1 details studies conducted to test the pathogenesis of B. bassiana on different hosts.

6 Fermentations in solid substrates

The production of *Beauveria bassiana* on solid substrates by aerial conidial reproduction is the most used method at commercial level for small, medium and large companies dedicated to the production of biopesticides (Feng et al., 2008). Culture media for the production of *B. bassiana* must have a nutrient composition and controlled conditions of pH, temperature, light, water availability and atmospheric gas mixture, crucial factors for the growth of the microorganism and sporulation (Patočka, 2016). The production process consists of inoculating *B. bassiana* conidia on nutrient solid substrates. Fermentation on solid substrates, under controlled conditions can achieve yields from 4×10^{12} to 4×10^{13} conidia·kg^{-1}.

The most commonly used materials for solid-state fermentation are rice, barley, wheat, rye, sorghum and corn, (Jaronski, 2014). Likewise, agro-industrial wastes allow reducing production costs and energy consumption. An example of this is potato skin and rice husk, which allow reaching a production of 1.3×10^9 spores·g^{-1} of *B. bassiana* under optimal conditions of humidity 65-70 %, temperature 25°C and time between 7 to 8 days (Sala et al., 2021).

Another important factor is the fermentation containers, which can be high temperature resistant plastic bags or large chambers, considering aeration since propagules require a balanced gas exchange for mycelial growth. The production of aerial conidia can be done in one or two stages. The first stage consists of inoculating the fungus directly from the solid substrate and the second stage involves the production of the inoculum by liquid fermentation and subsequent inoculation into the solid substrate (Mascarin and Jaronski, 2016).

Table 1. Studies conducted to test the pathogenesis of *Beauveria bassiana* on different hosts and their mortality percentage.

Conidia concentration/ml	Host	Morphological phases	Mortality percentage	Reference
1×10^8	*Lygus lineolaris* *Anthonomus signatus* *Otiorhynchus ovatus*	Adultos	77.47 60.35 54.50	Sabbahi et al. (2008)
1×10^7	*Bemisia tabaci*	Adults	67	Ruiz et al. (2009)
1.7×10^8	*Ceratitis capitata*	Adults	91.90	Muñoz et al. (2009)
1×10^7	*Bemisia tabaci*	Eggs	27	Ruiz et al. (2009)
1×10^8	*Premnotrypes vorax*	Larvae	96	García et al. (2013)
2×10^7	*Panonychus citri*	Nymphs Adults	94	Alayo and Krugg (2014)
1×10^7	*Hypothenemus hampei*	Adults	100	Gerónimo et al. (2016)
1×10^8	*Helicoverpa zea*	Larvae	100	Everton et al. (2016)
1×10^8	*Tenebrio molito*	Larvae	100	López-Sosa et al. (2018)
1×10^6	*Rhynchophorus palmarum L.*	Adults	43.33	León et al. (2019)
4×10^{10}	*Monalonion velezangeli*	Nymphs	84	Góngora et al. (2020)

The substrate must be previously sterilized at 121°C, 15 lb pressure for 30 min, the substrate is inoculated with the suspension of *B. bassiana* conidia or a proportion of mycelium. The inoculated substrates are incubated at 25 °C for 7 days and contaminated substrates are removed (López-Sosa et al., 2018). Consequently, bags are shaken to oxygenate the inoculum and achieve a homogeneous mixture, incubated for 21 days at 24 °C (Monzón, 2001) preserving a relative humidity of 53 %. Once the fungus is sporulated throughout the substrate, the drying process is carried out at temperatures between 16-20 °C for a period of 5 to 6 days to reduce the relative humidity to 15 % (Gómez et al., 2014).

The main problem of solid fermentation is commercial scale-up, whose focus is the efficient and economic production of a large number of conidia to reduce production costs and be able to compete with traditional pesticides (Rodríguez-Gámez et al., 2017). Therefore, it is important to use good quality substrates and containers, but at the same time cheap; and to constantly monitor each of the production processes to avoid contamination, performing a correct sterilization process and handling of materials. The substrates and environmental conditions for the solid fermentation of *Beauveria bassiana* are shown in Table 2.

Table 2. Substrates used for the solid fermentation of *Beauveria bassiana*.

Inoculum	Substrate	Incubation	Production in the substrate	Reference
1×10^7 conidia·mL^{-1}	Barley	25 °C/14 days	s/n	Sabbahi et al. (2008)
1×10^8 conidia·mL^{-1}	Rice husk	28 °C/14 days	4.3×10^8 esporas·g^{-1}	Mishra et al. (2016)
1×10^8 spores·mL^{-1}	Wheat bran	28 °C/14 days	2.1×10^8 spores·g^{-1}	Mishra et al. (2016)
3.5×10^9 conidia·mL^{-1}	Torn rice	25 °C/7 days	10^9 conidia·g^{-1}	López-Sosa et al. (2018)
1×10^6 blastospores·mL^{-1}	Oatmeal	25 °C/14 days	10^8 conidia·g^{-1}	Rodríguez-Gámez et al. (2017)
6×10^6 spores·mL^{-1}	Rice flour	30 °C/5 days	$6{,}2 \times 10^{10}$ spores·g^{-1}	Deepak et al. (2019)
6.6×10^6 spores·mL^{-1}	Rice husk	25 °C/7 days	1.3×10^9 spores·g^{-1}	Sala et al. (2021)

7 Fermentations on liquid substrates

The liquid fermentation process facilitates massive scale-up for the formulation of biopesticides. This method allows better control of environmental variables and reduces production times (Jaronski, 2014). However, implementation on a commercial scale requires considerable investment in equipment for mass production of mycoinsecticides, and is one of the most widely applied methods on a commercial scale (Mascarin and Jaronski, 2016).

Liquid fermentation can be performed stationary or by submerged fermentation. The former produces mycelia and aerial conidia; on the contrary, the latter produces blastospores, microspore conidia, or microsclerotia in an agitated and aerated liquid medium (Jaronski, 2014). Culture media should be nutrient-rich with high concentrations of carbon and nitrogen to induce the production of blastospores or conidia. An optimal C:N ratio induces fungal growth under controlled conditions (Pham et al., 2009).

According to García et al. (2013), SDA (Sabouraud dextrose agar) culture medium provides adequate nutrients for the development of *B. bassiana* in an incubation period of 15 days at 30 °C. Likewise, Pham et al. (2009) point out that conidial inoculum for liquid culture media should be obtained from two-week-old sporulated cultures on Papa Dextrose Agar (PDA) at a temperature of 25 ± 1 °C. Conidia are harvested by scraping the inoculum with 0.02% tween 80 solution (Lee et al., 2016). Flasks containing liquid culture medium with molasses as a carbon source (García et al., 2013), yeast broth or glucose are inoculated for 6 days at a temperature of 24-26 °C (Lee et al., 2016) on a rotary shaker at 200 rpm until a stable suspension is obtained (Pham et al., 2009). The suspension obtained is used to inoculate the fermenter, at a concentration of 10% relative to the fermenter volume, with a propagation phase of 4 days until the fungus reaches 80% of the logarithmic growth phase (García et al., 2013).

The substrates used for elaborating liquid culture media should be low cost, and at the same time should provide suitable conditions to produce blastospores or conidia (Mascarin et al., 2015).

In this context, Pham et al. (2009) report that a culture medium with 3% corn flour, 2% corn maceration powder and 2% rice bran yields 8.54×10^8 blastospores·mL^{-1}. In contrast, García et al. (2013) report that the optimal medium for blastospore production should have molasses 14.5mL·L^{-1}, $(NH_4)^2 SO_4$ 6 g·L^{-1}, KH_2PO_4 3.5 g·L^{-1}, $MgSO_4$ 0.5 g·L^{-1}, NaCl 0.1g·L^{-1}, $CaCl_2$ 0.1g·L^{-1}, and a production of 8.40×10^8 blastospores·mL^{-1} can be obtained. On the other hand, Elías-Santos et al. (2021) report that a culture medium with 20 g of peanut pericarp flour and 200 g of corn glucose allows obtaining 5.10×10^8 blastospores·mL^{-1}.

Similarly, environmental conditions are important for Beauveria bassiana production in submerged culture media. The temperature range is between 25-30 °C, pH of the culture medium 5.4, constant agitation from 200 to 400 rpm, until the fungus can reach 80% of the logarithmic growth phase (Pham et al., 2009; García et al., 2013; Elías-Santos et al., 2021). Table 3 shows the culture media and environmental conditions to produce *B. bassiana* by fermentation of liquid substrates.

Table 3. Fermentation in liquid substrates of *Beauveria bassiana*.

Strain	Inoculum	Cultivate substrate	Incubation	Production	Reference
GHA	1×10^7 conidium·mL^{-1}	50 g glucose, 50 g sucrose and 20 g corn macerate liquor	26 °C/3 days 300 rpm	6.38×10^9 blastospores·mL^{-1}	(Chong-Rodríguez et al., 2011)
Iran 441c	1×10^4 conidium·mL^{-1}	Sugarcane molasse	25 °C/3 days	2.4×10^8 spores·mL^{-1}	(Latifian et al., 2013)
ATP-02	5×10^4 spores·mL^{-1}	5% sugar beet	25 °C/8 days 600 rpm	5.32×10^{10} spores·mL^{-1}	(Lohse et al., 2014)
GHA	5×10^6 conidium·mL^{-1}	100 g glucose 25 g cotton seed flour	28 °C/3 days 350 rpm	$11.6 \pm 0.6 \times 10^8$ blastospores·mL^{-1}	(Mascarin et al., 2015)
ESALQ1 432	5×10^6 conidium·mL^{-1}	100 g glucose and 25 g cotton seed flour	28 °C/3 days 350 rpm	12.4×10^8 blastospores·mL^{-1}	(Mascarin et al., 2015)
KK5	5×10^7 conidium·mL^{-1}	3% corn flour 2% powder corn 2% rice bran	25 °C/2 days 200 rpm	8.54×10^8 blastospores·mL^{-1}	(Atef and Behle, 2017)

8 Production of secondary metabolites of emphBeauveria bassiana

Beauveria bassiana secretes a wide variety of biologically active enzymes and metabolites (Amobonye et al., 2020), with potential applications in the industrial, agricultural and pharmaceutical sectors, among others (Mancillas-Paredes et al., 2019). The most important enzymes produced by *B. bassiana* are chitinases, lipases and proteases; although they also produce amylase, asparaginase, cellulase, galactosidase, etc. (Amobonye et al., 2020); and metabolites with insecticidal activity (Table 4) such as beauvericin (Al Khoury et al., 2019), bassiacridin (Quesada-Moraga and Vey, 2004), bassianolide (Patočka, 2016), antiviral and antibacterial as oosporein (Jeffs and Khachatourians, 1997), and bassianin as an ATP inhibitor (Patočka, 2016). The production of secondary metabolites is influenced by environmental conditions and nutrients available in the culture medium (Ávila-Hernández et al., 2020) (Table 5).

Table 4. Metabolitos secretados por *Beauveria bassiana* para el control de artrópodos.

Metabolites	Action mode	Dose	Objective	Mortality percentage	Reference
Bassiacridin	Insecticide	2.8 μg·g^{-1}	*Locusta migratoria*	50 %	(Quesada-Moraga and Vey, 2004)
Oosporein	Antibacterial	100 μg·mL^{-1}	*Enterococcus faecalis*	81 %	(Fan et al., 2017)
Oosporein	Antibacterial	10 μg·mL^{-1}	*Stenotrophomonas* sp.	34 %	(Fan et al., 2017)
Beauvericina	Insecticide	100 μg·g^{-1}	*Tetranychus urticae*	100 %	(Al Khoury et al., 2019)

9 Use of biopesticides in the agriculture

Population growth, environmental degradation (Thakur et al., 2020), demand for chemical-free crops and stringent pesticide regulations in European countries and North America (Mascarin and Jaronski, 2016), pose a challenge to food production worldwide. Conventional agriculture relies on pesticides for integrated pest and disease management in agricultural crops.

An alternative that contributes to reducing the incidence of phytophagous or phytopathogens are biopesticides formulated through the reproduction of beneficial microorganisms or the production of metabolites with insecticidal, fungicidal or bactericidal activities (Table 6). The advantages of these formulations are that they degrade naturally in the environment, do not store residues in plant tissues, do not create resistance to the active ingredients they produce, and reduce the presence of natural enemies in crops (Thakur et al., 2020).

The growing acceptance of biological products has allowed the development of formulations from entomopathogenic fungi by solid, liquid, biphasic fermentations and secondary metabolites. They are now frequently used in agricultural crop protection programs to control phytophagous insect populations (Luo et al., 2014). The insecticidal activity of *B. bassiana* is faster compared to other entomopathogenic microorganisms, and conidia can persist longer in the environment.

In addition, Sabbahi et al. (2008) affirm that the chances of insects acquiring resistance to *B. bassiana* are null, due to the different modes of action that the fungus uses to invade the host body and it can adapt to host changes by being a living organism.

Table 5. Culture media formulated to produce secondary metabolites of *Beauveria bassiana*.

Fermentation	Metabolites	Cultive media	Dose	Reference
Submerged	Tenellin/ Bassianin	Glucose (20 g·L^{-1}), Ammonium tartrate (4.6 g·L^{-1}), KH_2PO_4 (1 g·L^{-1}), $MgSO_4·7H_2O$ (0.5 g·L^{-1}), NaCl (0.1 g·L^{-1}), $CaCl_2$ (0.1 g·L^{-1}), $CuSO_4$+$5H_2O$ (3.93 × 10^4 g·L^{-1}),	60 mg·L^{-1}	(Basyouni et al., 1968)

Table 5 – Table continuation

		H_3BO_3 (5.7×10^{-5} g·L^{-1}), $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (3.68×10^{-5} g·L^{-1}), $MnSO_4\cdot H_2O$ (6.1×10^{-5} g·L^{-1}), $ZnSO_4\cdot 7H_2O$ (8.79×10^{-3} g·L^{-1}), $FeSO_4\cdot 7H_2O$ (9.96×10^{-4} g·L^{-1}).		
Submerged	Oosporein	Glucose (20 g·L^{-1}), Difco neopeptone (20 g·L^{-1}), Glycine (5 g·L^{-1}), KH_2PO_4 (2 g·L^{-1}), $MgSO_4\cdot 7H_2O$ (1 g·L^{-1}).	100 mg·L^{-1}	(Basyouni et al., 1968)
Submerged	Bassiacridin	40 g Glucose·L^{-1}, 40 g Yeast extract·L^{-1}, 30 g macerated corn liquor·L^{-1}	2.8 μg·g^{-1}	(Quesada-Moraga and Vey, 2004)
Submerged	Tenellin	Mannitol (50 g·L^{-1}), KNO_3 (5 g·L^{-1}), KH_2PO_4 (1 g·L^{-1}), $MgSO_4\cdot 7H_2O$ (0.5 g·L^{-1}), NaCl (0.1 g·L^{-1}), $CaCl_2$ (0.2 g·L^{-1}), $FeSO_4\cdot 7H_2O$ (20 mg·L^{-1}), Mineral ion msolution-2 (10 mL), $ZnSO_4\cdot 7H_2O$ (880 mg·L^{-1}), $CuSO_4\cdot 5H_2O$ (40 mg·L^{-1}), $MnSO_4\cdot 4H_2O$ (7.5 mg·L^{-1}), Boric acid (6 mg·L^{-1}), $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (4 mg·L^{-1}).	No information	(Eley et al., 2007)
Submerged	Red coloring	Glucose (40 g·L^{-1}) Yeast extract (5.0 g·L^{-1}), $NaNO_3$ (1,0 g·L^{-1}), KH_2PO_4 (2,0 g·L^{-1}), KCl (0,5 g·L^{-1}), $MgSO_4\cdot 7H_2O$ (0,5 g·L^{-1}), $FeSO_4\cdot 7H_2O$ (0,02 g·L^{-1}).	480 mg·L^{-1}	(Amin et al., 2010)

Source: Ávila-Hernández et al. (2020).

The efficacy of the biopesticide *Beauveria bassiana* depends on the integration into integrated pest management programs to enhance efficacy in insect control (Mascarin and Jaronski, 2016). Although *B. bassiana* is an effective microorganism for the biological control of insects, environmental conditions play an important role in the mechanism of action of the entomopathogen, so it is essential to develop new technologies to produce active compounds that enhance biopesticide formulations.

Table 6. Commercial formulations of *Beauveria bassiana*.

Commercial Name	Active Ingredients	Concentration	Crop	Pest	Dose	Area of application	Reference
EFICAX	*Beauveria bassiana*	1×10^9 conidia·mL^{-1}	Cocoa Plantain Corn	Crickets Bedbugs Leafhoppers Bugs	5cc/L	Foliage Soil	(Agroinsumos del Sur, 2021)
Bovetrópico WP	*Beauveria bassiana*	1×10^9 viable spores·g^{-1}	Cotton Orange Coffee Avocado Rose	White fly Coffee berry borer Fruit pin Spider mite	500 g/ha	Foliage	(Invesa, 2021)
BOTANIGARD CS	*Beauveria bassiana* cepa GHA	$2{,}11 \times 10^{10}$ conidia·mL^{-1}	Cotton Horticultural	White fly	1-1.50 L/ha	Foliage	(Certiseurope, 2021)
Biotech BMI	*Beauveria bassiana*	1×10^6 spores·mL^{-1}	Solanáceas	White fly Fruit maggot	1-2 L/ha	Foliage	(FAGRO, 2022)
			Corn Sorghum	Budworm Worm Fruit maggot Blind hen	1-3 L/ha	Foliage Soil	
			Brocoli Cabbage Cauliflower Brussels sprouts	Fruit maggot Soldier worm	1-4 L/ha	Foliage	
			Avocado tree	Branch borer	1-4 L/ha	Foliage	

Commercial Name	Active Ingredients	Concentration	Crop	Pest	Dose	Area of application	Reference
Biotech BMI	*Beauveria bassiana*	1×10^6 spores·mL^{-1}	Cucumber Watermelon, Melon, Pumpkin	White fly	1-2 L/ha	Foliage	(FAGRO, 2022)
MICOSIS	*Beauveria bassiana*	1×10^{10} spores·g^{-1}	Coffee	Coffee berry borer	1 a 1.5 kg/ha	Leaf	(Agroactivo, 2020)
			Citrics	Citrus weevil Mites Whitefly	1 kg/ha	Leaf	
			Avocado	Whitefly Mites and thrips	1 kg/ha	Leaf	
			Banana Plantain	Striped weevil Black Weevil	20 g a 1 kg/ha	Tramps soil	
			Soursop	Lace bug Scales	1 a 1.5 kg/ha	Leaf	
			Flowers Foliage	Thrips Whitefly	1.5 kg/beds	Leaf	
			Potato	Loggers White grub Flea beetle	1 to 1.5 kg/ha	Foliage Soil	
			Rice	Defoliators Mites	1 kg/ha	Leaf	
			Oil palm	Fruit scrapers Weevil	1 kg/ha	Leaf	
			Forestry	Defoliators	1 kg/ha	Leaf	
			Pasifloras	Thrips Whitefly Scales	1 kg/ha	Leaf	

10 Conclusions

The production of biopesticides based on *Beauveria bassiana* can be carried out through solid and liquid fermentations. The first protocol has been widely used because it requires a low investment in equipment and does not involve complex protocols in production and formulation. However, it is necessary to use a low-cost substrate and to exhaustively control the phases of each process to avoid contamination events.

On the other hand, liquid fermentation allows more control of environmental variables and reduces production times, but implementation on a commercial scale requires considerable investment in equipment for mass production of mycoinsecticides.

Regarding fermentation processes, it is necessary to control environmental variables since these factors influence the production of spores and blastospores. Optimal conditions consist of maintaining a temperature of 25°C, relative humidity of 65 to 70%, pH of 5.4, propagation time between 4 to 8 days, and the inoculum requires constant agitation between 200 to 400 rpm for the liquid fermentation process.

Author contribution

JRLM; Conceptualization, Data curation, Formal analysis, Research, Methodology, Software, Original draft writing, review and editing. JWMT; Acquisition of financing and supervision. ICLM; Project validation and administration. YAOG; Resources and visualization.

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