PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.10274

eISSN 2348-1900

REVIEW ARTICLE

Agro-industrial residues as functional materials for production of entomopathogenic fungi: Materials science applied to bark beetle control

Ricardo Gallardo Espinoza[1], Rosa Idalia Narro Céspedes[1]*, Raúl Rodríguez Herrera[1], Gabriela Hernández Ramírez[2], Francisco José González González[1], María Guadalupe Neira Velázquez[3]

[1]Faculty of Chemical Sciences, Autonomous University of Coahuila, Blvd. V. Carranza and Ing. José Cárdenas s/n, Col. República, C.P. 25280, Saltillo, Coahuila, Mexico

[2]National Technological Institute of Mexico, Tierra Blanca Campus, Veracruz Avenue S/N, corner of Heroes of Puebla Street, Pemex, Tierra Blanca 95180, Veracruz, Mexico

[3]Macromolecular Chemistry and Nanomaterials, Research Center for Applied Chemistry (CIQA), Enrique Reyna H. 140, San José de los Cerritos, Saltillo 25294, Coahuila, Mexico

*Correspondence email - rinarro@uadec.edu.mx

Received: 26 June 2025; Accepted: 14 October 2025; Available online: Version 1.0: 17 November 2025

Cite this article: Ricardo GE, Rosa INC, Raúl RH, Gabriela HR, Francisco JGG, María GNV. Agro-industrial residues as functional materials for production of entomopathogenic fungi: Materials science applied to bark beetle control. Plant Science Today (Early Access). https://doi.org/10.14719/pst.10274

Abstract

The bark beetle has been considered one of the main forest pests that causing serious damage to agricultural crops and trees by feeding on plant bark, affecting the health of ecosystems. In recent decades, the use of chemical pesticides has been the traditional method of control, generating negative impacts on the environment, pest resistance and threats to human health. For this reason, research is being conducted on the production and application of entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae*, as a biological and sustainable alternative that acts as a biological control agent. These fungi can be produced using traditional methods in solid, liquid and biphasic states, involving the use of agro-industrial waste as a low-cost and easily accessible substrate for their growth. The use of waste reduces pollution and promotes ecological balance. Scientific publications and texts show that entomopathogenic fungi inoculated into agro-industrial substrates are suitable for controlling pests, mainly bark beetles. This promotes environmental and agricultural sustainability, mitigating environmental damage and favoring residence in production systems.

Keywords: agronomy; bark beetle; entomopathogenic; residues; sustainable agriculture

Introduction

In recent years, modern agriculture has undergone various changes due to the significant pollution it generates, caused by the indiscriminate use of synthetic pesticides and fertilizers. Pest and disease control treatments based on synthetic compounds have, for many years, been and continue to be the main source of damage to ecosystems, through air and water pollution and soil degradation, the latter mainly due to the death of beneficial microorganisms present in the soil (1). On the other hand, human health is also affected due to the indiscriminate use of synthetic chemical pesticides. Several investigations have shown that residues of these products in food can generate serious negative effects on both human and animal health, due to bioaccumulation processes. The persistent use of synthetic agrochemicals over time has significantly contributed to the emergence of resistant strains of plant pathogens. As a result, many of these organisms have become increasingly aggressive and difficult to control. This situation is further exacerbated by the progressive degradation of soil quality, as chemical inputs promote processes such as erosion and leaching, ultimately reducing agricultural productivity (2). Considering these challenges, recent advances in biotechnology have paved the way for more sustainable and environmentally responsible approaches, including the use of entomopathogenic microorganisms for biological pest control (3).

Since their introduction more than fifty years ago, synthetic pesticides have served as a primary tool in combating agricultural pests. While initially effective, their intensive and prolonged use has led to unintended environmental consequences (4). In response, alternative methods have emerged to mitigate these impacts. Among the most promising are biological strategies that not only reduce environmental harm but also provide economically viable and effective pest control solutions (5).

One notable example is the application of entomopathogenic fungi. Countries such as Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Venezuela and Cuba have made notable advances in the cultivation and use of these fungi. Cuba, in particular, has become a regional leader, developing biopesticides that not only target pest populations but also promote plant health through formulations categorized as biostimulants and biofertilizers (6). Among the entomopathogenic fungi, *Metarhizium anisopliae* stands out for its efficacy in controlling pests like the bark beetle. Its pathogenic activity is primarily mediated by hyphae and conidia key infectious structures capable of initiating widespread infections in insect hosts. In addition to causing physiological and biological changes, it leads to death through mechanical or enzymatic action (7).

The use of agro-industrial residues has been a viable option for the mass production of fungi, as it positively impacts the environmental balance. These organic materials reduce production costs and are easily obtained, making them suitable as fermentable substrates (8). As mentioned earlier, the production of entomopathogenic fungi has developed thanks to the use of by-products from the agro-industry, as they complement or combine to achieve a high concentration of conidia, replacing food-grade substrates, which are more expensive and are not considered waste, such as cereals (rice, wheat, corn, sorghum, or oats). It is important to emphasize that the effectiveness of biological control of a bioinsecticide depends on the inoculum quantities for its evaluation, as well as the concentration, formulation, application methods and the effect of abiotic factors such as UV radiation, temperature and humidity (9).

Materials and Methods

This work focused on a comprehensive literature search and review, prioritizing the use of entomopathogenic microorganisms such as *Beauveria bassiana* and *Metarhizium anisopliae* in the monitoring and biological control of bark beetle pests (*Dendroctonus* spp.). The advantages of developing these microorganisms using agro-industrial waste materials were highlighted, which are not only obtained at low cost but also promote environmental sustainability. Similarly, the most recent advances in the production and development of these microorganisms were investigated, based on academic sources such as Elsevier and Google Scholar, highlighting the most relevant results for their implementation in agricultural and forestry systems.

Insect pests in agriculture

A pest is defined as a group of animal or organisms that cause damage to plants and crops. One of the main challenges faced by the agri-food industry is the presence of pests, which influence physical deficiencies and economic losses. This is due to their behavior as vectors, as they can transmit diseases to commercially important crops, settling as hosts in large land areas where they create favorable environments for reproduction (10). For over fifty years, humans have tried to protect plants from insect attacks using traditional methods such as the application of chemical products. Plants are susceptible to attack by different types of microorganisms, such as viruses, fungi, bacteria and insects, with the latter being the most damaging to various crops in agriculture (11). There is evidence of various practices for pest control and management; however, these have been limited due to the resistance of insect populations to synthetic chemical substances. Furthermore, many of these substances are toxic to humans or have harmful health effects (12). The use of entomopathogenic agents, such as fungi, bacteria and viruses, for microbial control has increased in recent years, offering the advantage of being environmentally friendly. It is specific to the whitefly population and no cases of poisoning caused by these entomopathogenic formulations have been reported in mammals or humans (13).

One of the main concerns for farmers is the presence of pests, as they limit the proper development of crops, thus decreasing both yield and the quality of the final product, leading to significant economic losses. Pests can be classified depending on the level of damage they cause, as well as their behavior. Occasional pests are those that, while they may cause significant losses, only appear occasionally. Secondary pests do not cause significant losses even though they are always present. Key pests are the most important for the farmer, as they are always present and cause considerable losses, both in production and in management costs (14).

Performance of entomopathogenic fungi in agricultural residues as functional materials

Within the country's economy, the agro-industrial sector is a key pillar, providing raw materials for agricultural and livestock purposes. By-products are utilized and transformed into new products with higher added value and more efficient commercial advantages. An agro-industrial product is one that results from the transformation of raw materials, whether of animal or plant origin, which can be processed and yield better utility for daily consumption (15).

Most solid organic materials must undergo a composting process to be suitable as substrates (such as rice husk, cereal straw, coconut fiber, grape pomace, tree bark, sawdust, wood chips, urban solid waste, wastewater treatment sludge, etc.) (16). Agro-industrial waste and by-products play an important role in the scientific sector due to the various alternatives they offer, becoming a topic of growing research, within the main objectives and scopes when using waste is the formulation of bioproducts based on fungi that function as control agents against pests such as the bark beetle and in this way contribute to forest protection with less polluting and economically viable methods(17).

The use of agro-industrial by-products offers several advantages: waste reduction, income generation and resource conservation. The reuse of agro-industrial waste to produce entomopathogenic fungi has become a sustainable and profitable practice, helping to reduce the amount of waste generated during the production and processing of agricultural products. Agricultural waste acts as a catalyst for the mass production of conidia and blastospores; however, it can cause limitations; variability in composition, risk of contamination and the need for a standardization process (18).

Entomopathogenic fungi

There are various microorganisms used in agriculture for disease control, pest management and as biostimulants for plant growth and development. Among them, entomopathogenic fungi are the most important group in biological control. These fungi are naturally present in the environment and their main function is to regulate natural arthropod populations by infecting and causing death through epizootics. They are primarily found in insect cadavers, obtaining nutrients from other organisms as well as from organic matter to support their fungal development, which prolongs their survival within the agroecosystem (19).

The bark beetle (*Dendroctonus spp.*) is one of the most damaging forest pests in forests and conifers. Its reproduction is characterized by the massive invasion of weak trees, where adults begin their development by breaking the bark, promoting oviposition and the development of larvae that interfere with nutrient flow, causing the death of the host. Significant economic, ecological and social losses have increased due to the spread of this beetle, which causes alterations in forest ecosystems

Economic impact of bark beetles: Current control methods and the use of entomopathogenic fungi

The most commonly used entomopathogenic fungi for pest insect control include *Beauveria bassiana*, *Lecanicillium lecanii*, *Isaria fumosorosea*, *Hirsutella thompsonii* and *Metarhizium anisopliae*. These fungi cause a range of diseases that result in insect death. Entomopathogens are associated with insects from different orders (*Coleoptera* - beetles, *Lepidoptera* - butterflies, *Diptera* - mosquitoes and *Hemiptera* - true bugs), which are known to affect economically important crops (20). Generally, entomopathogenic fungi exhibit the following developmental stages on their hosts: germination, formation of appressoria and penetration structures, colonization and reproduction inside the host, followed by sporulation and dissemination under suitable humidity and temperature conditions (≤70 % and up to 28 °C, depending on the fungal species). These fungi typically attack the immature stages (nymph or larva) of insects. Their host specificity varies, as most fungi have a broad host range, while others are specific to a single insect species (21).

Unlike other pathogens, one of the features that makes these fungi attractive for biological control is their mode of action. Typically, the fungus infects insects through active penetration by its hyphae, breaking through the insect's cuticle. Several studies indicate that infection can also occur through ingestion or entry via spiracles or other external openings. In the case of *Metarhizium anisopliae* and *Beauveria bassiana*, penetration is achieved through enzymatic and physical actions, as they produce proteases and chitinases. The route of action depends on the insect's cuticle: for soft teguments, the hypha or germ tube penetrates directly without forming appressoria, whereas for thick cuticles, the germ tube forms appressoria and often penetration plates and hyphal bodies. These fungi also can synthesize toxins that play a role in the pathogen-host interaction cycle (22).

Entomopathogenic fungi in pest management: Advances, challenges and prospects for mass production

Metarhizium anisopliae is an entomopathogenic microorganism commonly used in biological control systems due to its ability to invade many insect pests. It grows mainly in the soil, where it is found naturally as a fungus that feeds on decaying organic matter. Its mechanism of action begins when the conidia invade the insect's cuticle, germinate and penetrate until they reach the hemolymph, causing the death of the host. This entomopathogen is characterized by its wide selection of insects of the order *Coleoptera, Hemiptera, Lepidoptera* and other groups within the agricultural and forestry sectors, not excluding the bark beetle (*Dendroctonus spp.*). Its response as a biological control agent is due to its ability to produce secondary metabolites with insecticidal activity, which makes it an effective microorganism (23-25).

Another key player in biological control is *Beauveria bassiana*, an anamorphic fungus that causes white muscardine disease in silkworms (*Bombyx mori*). This fungus occurs naturally in the environment and is notable for its stability outside the host, making it an ideal tool for insect bioremediation (26). Like *Metarhizium*, it is one of the most widely used fungi for biological control due to its ability to survive in different environments such as soil, insects and plants. *B. bassiana* is one of the most widely used fungi for pest control due to its wide range of hosts, colonizing more than 200 species of insect pests, such as the coffee borer (*Hypothenemus hampei*), the cabbage moth (*Plutella xylostella*) and the banana weevil (*Cosmopolites sordidus*) (27).

Beauveria bassiana is characterized by colonies with a cottony, shiny and powdery appearance. After 14 days on potato dextrose agar (PDA) culture medium, the fungus appears white and gradually turns to a creamy yellow color; it has well-defined edges and the surface may be flat or elevated. When cultured in Petri dishes, the color of this entomopathogen may vary depending on factors such as the culture medium, temperature and humidity. The most common color of *Beauveria bassiana* is white; the mycelium can develop as a white, fluffy growth on the surface of the culture (Fig. 1a). As the entomopathogen matures, it may acquire a light green pigment (28).

Under the microscope, the microorganism exhibits septate hyphae ranging from 2.5 to 25 µm in diameter (Fig. 1c). The conidiophores are simple, approximately 1-2 µm in diameter and are either verticillate or branched (Fig. 1b). Conidia develop at a temperature of 25 °C, with an optimal pH for growth between 5.7 and 5.9 and they typically form within 7-9 days (29).

The infection cycle of both entomopathogens begins with the invasion of the conidium into the host's cuticle. The process occurs in two phases: in the first parasitic phase, the entomopathogen grows, develops a germ tube and penetrates the cuticle with the help of enzymes that break down sclerotized and membranous barriers. Once inside, blastopores are produced that colonize the hemocoel, absorb nutrients from the hemolymph and release toxins, causing the death of the insect. Subsequently, in the saprophytic phase, the mycelium covers the corpse with conidiophores that release new conidia into the environment, restarting the infectious process. (Fig. 2) illustrates the process of host death caused by entomopathogenic colonization (30, 31).

Agricultural waste as a substrates for the production of entomopathogenic fungi

A substrate is any surface used as support or a growth medium on which an animal, plant, or microorganism can develop and begin reproducing. There are different types of substrates, such as chemically inert substrates like granite or silica sand, gravel, volcanic rock, perlite, clay, etc. There are also chemically active substrates, which include cucumber bark, vermiculite and lignocellulosic materials. Both types of substrates serve the same purpose: to provide essential nutrients for the plant system. However, each has its own characteristics. Inert substrates mainly provide support for the plant, without participating in the adsorption or fixation of nutrients, meaning nutrients must be added through fertilization. Chemically active substrates serve a dual function: they support the plant and also act as a reservoir for nutrients supplied through fertilization (32).

In addition, there are substrates of natural origin (such as rice husk, coconut fiber, animal manure, among others), which are characterized by their biological decomposition. There are also synthetic substrates, such as non-biodegradable organic polymers, which are obtained through chemical synthesis; examples include polyurethane foam, polyethylene and others (33).

Substrates have been used as a support where fungi grow and develop their morphological structures, such as conidia. The most used substrates for entomopathogenic fungi production methods are solid organic natural substrates, composed of cereal

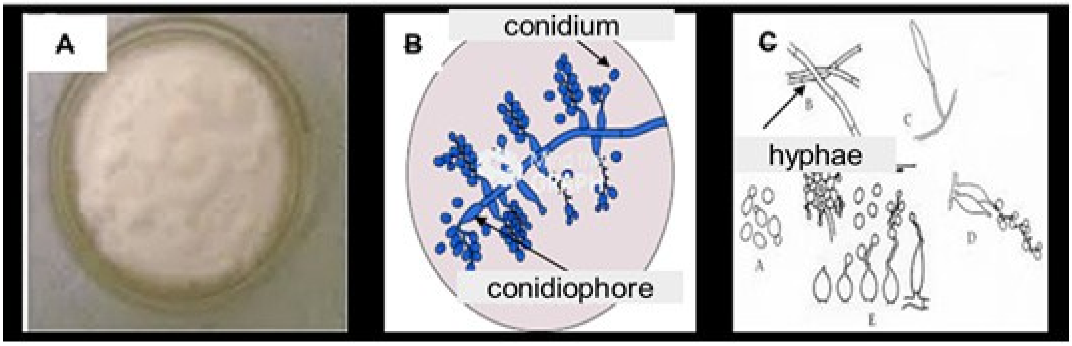

Fig. 1. Macroscopic and microscopic morphology of the growth and development of *Beauveria bassiana* in its different phases: a) Germination phase, b) Conidia formation phase, c) Dispersal phase.

and legume grains, which serve as sources of starch, cellulose and fiber. The main reason because these materials are used is that, once hydrated and sterilized, they easily absorb nutrients from the liquid culture, providing the necessary elements for biomass reproduction under healthy conditions (34).

For entomopathogenic fungi like *Beauveria bassiana* and *Metarhizium anisopliae*, substrates as: rice (*Oryza sativa* L.) and barley (*Hordeum vulgare*) are used. These fungi easily penetrate the hydrated grains and efficiently absorb their nutrients. Since the grains are small and separate, they offer more surface area and moisture, which promotes rapid growth of the entomopathogen on the support (35).

The agroindustry is one of the main sources of solid waste, which often contributes to the formation of pollutants that harm the environment. In response, the scientific community has encouraged the development of new methodologies that incorporate emerging technologies to better utilize agro-industrial residues (36). The residues used as supports must meet certain criteria such as availability, low cost, the microorganism to be cultivated and chemical composition, among others (37). The reutilization of solid waste offers a valuable avenue for economic development in many emerging nations, particularly through the transformation of waste into high-value-added products. Frequently used substrates include residues from agriculture, forestry and the food processing industry. These materials are typically rich in polysaccharides components like hemicellulose, cellulose and lignin, that form the structural framework of plant cell walls (38).

Materials science perspective on substrate functionality

Agro-industrial waste is not just a biological support; materials science recognizes that it is a functional material whose structure, composition and physicochemical properties determine the efficiency of entomopathogenic fungal cultivation. It has a porous structure and particle size that favor aeration and hyphal penetration; it can retain moisture, which is essential for maintaining metabolic activity in solid-state fermentation; and it exhibits surface properties such as roughness, hydrophobicity and electrostatic charge, which influence adhesion and sporulation. These types of materials can be pretreated with hydrolysis, heat plasma and grinding, improving their enzymatic accessibility and reducing contaminants (39).

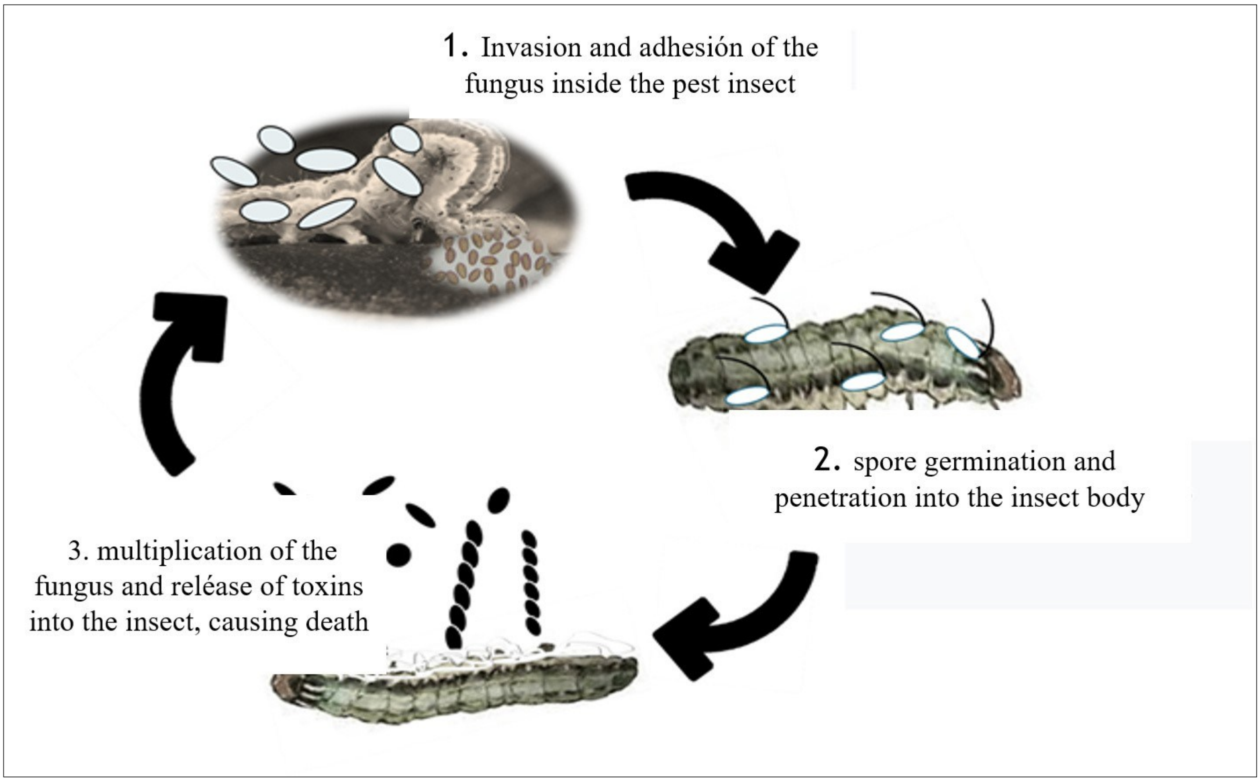

Fig. 2. Diagram of the infection process of an entomopathogenic fungus on the bark beetle.

Results and Discussion

The bark beetle belongs to the Scolytinae family within the Curculionidae family. It is characterized by its great capacity to affect coniferous forests in Mexico, becoming known as one of the most important forest pests. Despite its small size (less than 1 cm), its cylindrical morphology and the development of legs and antennae allow it to develop adequately between the bark and the wood, causing damage to the vascular system of trees. Thirteen species of the genus *Dentroctonus* have been discovered in Mexico, the main ones being *D. adjunctus, D. brevicomis, D. frontalis, D. mexicanus and D. rhizophagus*. These species are capable of invading 25 of the 42 species of pine recognized in the country, with *P. engelmannii, P. durangensis, P. leiophylla, P. hartwegii and P. oocarpa* being the most affected. Detailed information on the life cycle and reproduction of *Dentroctonus* species is essential for designing pest management and control strategies for pine forests. Due to this problem, there has been increased interest in developing bioinsecticides capable of improving the conditions of plants and trees in agriculture. Below are the most notable projects addressing the use of agro-industrial waste using entomopathogenic fungi with applications in agriculture. The information presented is documentary and based on an exhaustive review of the literature, based on publications in indexed journals, articles and reliable sources related to the study (40).

Previous researchers point out that the bark beetle problem in Mexico is significant, primarily affecting temperate forests dominated by the *Pinus* genus. Between 1993 and 2016, bark beetle outbreaks impacted 296165 hectares, making them the main biotic threat to these ecosystems. However, the damage level is lower than that observed in the United States and Canada (41).

The efficacy of different strains of *Beauveria bassiana* against *Dendroctonus valens* was reported earlier. The results showed that strain Bb1801 was the most virulent, achieving 100 % mortality in treated insects, with a mean lethal time of 4.60 days at a concentration of 1×10^7 conidia/Ml. These findings reinforce the importance of using *B. bassiana* as a biological control agent, highlighting the need to select specific strains to optimize their effectiveness in integrated forest pest management programs (42).

Previous researchers evaluated the effect of agro-industrial waste: *Saccharum officinalis* bagasse, *Zea mayz* crown and *Oryza sativa husk*, as substrates in the production of *Trichoderma harzianum* spores through solid substrate fermentation. Four substrates were used: the control, consisting of whole *O. sativa* grains and the three aforementioned agro-industrial wastes; the latter were pretreated with 2.4 % NaOH and enriched with a saline solution to the four substrates, then sterilized and inoculated with *T. harzianum* at a concentration of 2×10^7 spores mL^{-1} and incubated at 22 ± 2 °C for 7 days. Each day, the spores were counted in the four substrates until the end of the incubation period. Rice favored spore production by *T. harzianum*, with a mean value of 8.99×10^8 spores g^{-1} and, among the three substrates evaluated, the highest mean concentration was obtained in *surgance bagasse* (2.28×10^7 spores g^{-1}), followed by corn cobs (2.09×10^7 spores g^{-1}) and rice husks (1.35×10^7 spores g^{-1}). Spore production was achieved in *S. officinalis bagasse*, *Z. mayz corn cobs and O. sativa husks;* however, there was only a statistically significant difference between the control and the three substrates evaluated ($P < 0.05$), but not between the substrates ($P > 0.05$) (43).

In previous studies, the mass production of *Metarhizium robertsii* and *Metarhizium pinghaense* using solid-state fermentation with three substrates: rice, barley and rolled oats. Their results showed that rice was the most efficient substrate, producing an average of 8.2 g ± 4.38 g of dry conidia for *M. pinghaense* and 6 g ± 2 g for *M. robertsii*. Barley favored the production of *M. robertsii* with 1.83 g ± 1.47 g, while oats did not support dry conidia production for either fungus (44).

In previous studies, *Beauveria bassiana* strain INRS-242 in an assisted self-dissemination device, achieving 98 % mortality with a median lethal dose (LD_{50}) of 2.1×10^9 conidia using agro-industrial waste as substrates: sugarcane husk (*Saccharum officinalis*), corn cob (*Zea mays*) and husk (possibly rice or similar) as support materials. The waste was treated by sterilization and humidity and temperature conditions were adjusted for rapid spore and mycelium production (45).

Previous researchers studied the production of *Beauveria* and *Trichoderma* in solid-state fermentation using rice husk, apple pomace, whiskey mash and other substrates. This results showed that rice husk and potato peel were the most efficient substrates, yielding up to 1×10^9 conidia/g (48).

The mass production of *Metarhizium robertsii* and *Metarhizium pinghaense* using solid-state fermentation with three substrates: rice, barley and rolled oats. These results showed that rice was the most efficient substrate, producing an average of 8.2 g ± 4.38 g of dry conidia for *M. pinghaense* and 6 g ± 2 g for *M. robertsii*. Barley favored the production of *M. robertsii* with 1.83 g ± 1.47 g, while oats did not support dry conidia production for either fungus (49).

Former researchers evaluated the mass production of *Metarhizium anisopliae* using solid-state fermentation with rice, sorghum and mung bean. Rice was the most efficient, yielding 4.8×10^9 conidia/g when soaked in a 2 % sucrose solution. *Sorghum* yielded 3.2×10^9 conidia/g and mung bean 2.7×10^9 conidia/g, making it the least efficient (50). The mass production of *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana Z1* using a biphasic fermentation method with solid substrates (rice, wheat bran, wheat, barley and sorghum) followed by a liquid phase (whey) was reported earlier. Rice bran and wheat bran were the most efficient for *M. anisopliae,* producing $1.08 \times 10^7 \pm 2.42 \times 10^5$ and $1.06 \times 10^7 \pm 1.86 \times 10^5$ conidia/mL, respectively. For *B. bassiana*, wheat bran produced 5.14×10^6 conidia/mL. One gram of conidial powder obtained by sieving rice for *M. anisopliae* and wheat for *B. bassiana* contained approximately 2.85×10^{10} and 3.09×10^{10} conidia, respectively (51).

Lethal concentrations (LC50) at 24 hr were evaluated earlier. Topical application of bifenthrin showed it was the most toxic, with an LC50 of 0.94 mg/L. Cypermethrin applied to bark had an LC50 of 5.04 mg/L. Regarding bioaccumulation, deltamethrin showed the highest body concentration in insects (622.41 µg g^{-1} dry weight), while cypermethrin showed the least (183.09 µg g^{-1} dry weight). Despite this, cypermethrin proved effective at lower doses in killing *D. mexicanus* adults. However, bifenthrin's persistence in forest ecosystems (soil, water, sediments) poses significant environmental risks due to bioaccumulation and trophic chain disruption. This highlights the importance of assessing environmental impacts when using synthetic insecticides for forest pest management (52). Agro-industrial residues (rice hulls, apple pomace, soybean fiber, rice fiber, wheat straw, brewer's marc and potato peels) as substrates for the production of conidia of *Beauveria bassiana* and *Trichoderma harzianum* by solid-state fermentation (SSF). The results showed that for *B. bassiana* the best production results (≥ 1×10^9 conidia per gram of dry matter) were achieved with rice hull and potato peel residues. For *T. harzianum*, the highest yields (more than 5×10^9 conidia per gram of dry matter) were achieved with brewer's marc and potato peels (53). This work, outlines the strategies and technical aspects for the mass production of entomopathogenic fungi, essential for their application as biocontrol agents against insect pests. These authors argue that for excellent production of entomopathogens, it is essential to correctly select strains capable of forming stable propagules, producing large quantities of conidia and maintaining good viability during storage and application. Furthermore, the mass production method is another important point, since solid-state and submerged fermentation is the ideal option for the propagation of entomopathogenic agents. According to these authors, all of the above leads to improved product stability, reduced costs, improved field formulations and the development of strains that are more stable against abiotic factors (54). Finally, developed a liquid fermentation process for the production of desiccation-tolerant blastospores with high storage stability and biocontrol efficacy, used *B. bassiana* and *I. fumorosa* strains in liquid media with different nitrogen sources (cottonseed meal), achieving rapid production in just 3 days, reaching concentrations of up to 1×10^9 blastospores/mL. Desiccation tolerance after drying between 61-86 % of blastospores remained stable, regarding stability, blastospores stored at 4 °C remained viable for more than 9-14 months (55).

Formulation and application for bark beetle control

In the large-scale production of *Beauveria bassiana* and other entomopathogenic fungi, solid-state fermentation (SSF) remains the preferred technique. This is largely due to its capacity to yield high concentrations of conidia, which are the core component in the formulation of effective bioinsecticides, widely used by both small and industrial-scale producers of biological control agents As mentioned in previous paragraphs, entomopathogenic fungi are sustainable alternatives due to their effective response to agricultural problems such as pests. The formulation and application of bioproducts is important to achieve a good insect mortality rate: wettable powders (these allow for adequate foliar or log application), granules and pellets (these are used for the release of propagules in specific areas of the forest), emulsifiable oils (these increase inoculum adhesion to the insect cuticle), nano formulations and encapsulation (these are emerging technologies that seek to protect spores from environmental degradation and prolong their viability). In terms of their application, they can be applied in various ways, the main ones being direct inoculation, dispersal traps, aerial or ground spraying and finally, integration with pheromones. All these application techniques seek to maintain the inoculum, achieving their effectiveness on the insect (56).

Table 1 summarizes the research conducted in recent years on the mass production of entomopathogenic fungi, as well as the main results obtained according to the production process. The research demonstrates that bioinsecticides are an effective tool for curbing agricultural and environmental problems. The information contained in Table 1 presents a broad methodology for the different processes used to achieve the mass production of entomopathogenic agents in the agricultural sector. The research focuses on the reuse of designer materials inoculated with entomopathogens under different fermentation processes. It is important to mention that the research documented in this work is highly relevant as a scientific contribution to the development of sustainable new agricultural processes. This research is also part of the improvement of field production for greater pest-free production and high yields without affecting ecosystems.

Table 1. Summary of bibliographic contributions from control agents

YEARS	AGENTE CONTROL	SUBSTRATES	RESULTS	REFERENCE
2010	*Beauveria bassiana*	Half crop	100 % mortality Concentration $1x10^7$ conidia/Ml	(42)
2024	*T. harzianum*	Surgance bagasse, rice and corn	8,99 x10 ^{8}	(43)
2022	*Metarhizium robertsi y Metarhizium pinghaense*	rice, barley and rolled oats	M. *robertsi* producing an average of 8.2 g ± 4.38 g *M. robertsii* with 1.83 g ± 1.47 g,	(44)
2016	*Beauveria bassiana*	sugarcane husk corn cob	98 % mortality with a median lethal dose (LD_{50}) of 2.1×10^9 conidia	(45)
2016	*Metrahizium anisopliae*,		92 % mortality in larvae after 7 days and 100 % mortality in adults	(46)
2023	*Beauveria bassiana*	Sorghum, rice and corn	biomass yield of 0.62 g, 10.92×10^7 conidia/mL and a germination rate of 86.94 %.	(47)
2021	*Beauveria* and *Trichoderma*	rice husk, apple pomace, whiskey mash	Yielding 1×10^9 conidia/g	(48)
2022	*Metarhizium robertsii* and *Metarhizium pinghaense*	rice, barley and rolled oats -	8.2 g ± 4.38 g of dry conidia for *M. pinghaense* and 6 g ± 2 g for *M. robertsii*	(49)
2025	*Metarhizium anisopliae*	with rice, sorghum and mung bean	*M. anisopliae*, producing 1.08×10^7 ± 2.42×10^5	(50)
2020	*Metarhizium anisopliae*	rice, wheat bran, wheat, barley and sorghum -	yielding 4.8×10^9 conidia/g when soaked in a 2 % sucrose solution. Sorghum yielded 3.2×10^9 conidia/g and mung bean 2.7×10^9 conidia/g,	(51)
2022	Synthetic insecticides		Topical application of bifenthrin showed it was the most toxic, with an LC50 of 0.94 mg/L	(52)
2021	*Beauveria bassiana*	rice hulls, apple pomace, soybean fiber, rice fiber, wheat straw, brewer's marc and potato peels -	1×10^9 conidia per gram of dry matter	(53)

Currently implemented control methods include silvicultural practices (elimination of infected trees), chemical insecticides and pheromone traps. However, these control practices present environmental and sustainability limitations. The use of entomopathogenic microorganisms appears to be the best integrated management alternative, acting as natural agents of bark beetle mortality. Significant knowledge gaps remain, limiting large-scale production (57). These include:

Variability in strain efficiency

Prior research is needed on the adaptability and persistence of different entomopathogen strains under field conditions (58).

Formulation and stability

It is essential to develop nano formulations, encapsulation and organic carriers that prolong the shelf life and effectiveness of inoculants (59).

Optimization of agro-industrial substrates

Waste presents a great opportunity for mass production; further research is needed into physical/chemical pretreatments that standardize their composition and minimize microbial contamination (60).

Integration into management programs

Evaluations of the compatibility of entomopathogenic fungi with other techniques, such as pheromones and traps, are required to design integrated management programs. Prospects should consider focusing primarily on selecting native strains with high efficacy against bark beetles, accompanied by formulations resistant to extreme environmental conditions and finally, field studies that validate the efficacy of bioproducts.

Recommendations

The mass production of entomopathogenic fungi is an alternative for reducing environmental pollution and increasing agricultural productivity, so further research is needed to optimize the development of microorganisms and improve crop quality. It is recommended to evaluate different agro-industrial waste to increase the source of substrates, with the goal of diversifying available inputs and promoting a circular economy. It is also important to explore and test different techniques that enhance the process and efficiency for the successful development of the agricultural sector. However, it is essential to inform farmers about the economic benefits of using bioinsecticides and biological products to generate changes that modify and benefit society. To promote their use, it is recommended to implement outreach programs, technical advice through demonstration plots, educational materials and field visits where the effectiveness of the products can be demonstrated, especially in areas affected by pests such as the bark worm, thus creating an initiative for transformation in the agricultural sector.

Conclusion

The production of entomopathogenic fungi from agro-industrial waste is proving to be a sustainable and effective alternative for biological pest control. The developed methodologies allow for high levels of sporulation and fungal viability, surpassing conventional methods based on commercial substrates. Studies indicate that the use of these control agents can significantly reduce the need for synthetic chemical pesticides, promoting safer and more environmentally friendly agriculture. However, further research is needed to optimize fermentation and formulation processes and ensure their effectiveness on a large scale. The use of biological products is an effective tool for improving agricultural land. Their production method does not alter environmental effects; on the contrary, it favors crop production, diminishing the traditional approach to mitigating agricultural problems based on hazardous products that make pests more resistant. In short, biological products not only improve rural life but also human quality of life by generating a more balanced and resilient environment that allows people to enjoy healthier food and achieve a healthy lifestyle.

Acknowledgements

The authors thank the Mexican Council of Humanities, Sciences and Technologies (CONAHCYT) for the grant awarded to Ricardo Gallardo Espinoza (CVU 1183277) in support of his doctoral studies.

Authors' contributions

RGE conducted the research and drafted the original manuscript. RINC provided supervision and contributed to writing, review and editing. RRH contributed to writing, review and editing. GHR, MGNV and FJGG contributed to writing and review. All authors made substantial contributions to the development of the work and actively participated in writing and revising the manuscript. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no conflicts of interest to declare.

Ethical issues: None

References

1. LOS40. Demostrado: los plaguicidas afectan gravemente a la biodiversidad. Madrid: LOS40; 2025.
2. Intriago IZ, Moreira Salazar M, Romero Yupanqui J, Vera Menéndez M, Ganchozo Z. Impacto de agroquímicos en la agricultura. Rev FDM. 2021.
3. Cruz-Cárdenas CI, Zelaya Molina LX, Sandoval Cancino G, Santos Villalobos SDL, Rojas Anaya E, Chávez Díaz IF, et al. Using microorganisms for a sustainable agriculture in Mexico: considerations and challenges. Rev Mex Cienc Agríc. 2021;12(5):899-913. https://doi.org/10.29312/remexca.v12i5.2905
4. Villota HJA. Importancia del desarrollo sustentable en la producción agrícola [bachelor's thesis]. Babahoyo: Universidad Técnica de Babahoyo; 2024. http://dspace.utb.edu.ec/handle/49000/15900
5. Mamani A. Biofertilizers based on beneficial microorganisms and organic matter: a systematic review. Rev Med Actions. 2023;2(4):43-55.
6. Zelaya-Molina LX, Chávez-Díaz IF, de los Santos-Villalobos S, Cruz-Cárdenas CI, Ruíz-Ramírez S, Rojas-Anaya E. Control biológico de plagas en la agricultura mexicana. Rev Mex Cienc Agríc. 2022;13(SPE27):69-79. https://doi.org/10.29312/remexca.v13i27.3251
7. Abro NA, Wang G, Ullah H, Long GL, Hao K, Nong X, et al. Influence of

Metarhizium anisopliae (IMI330189) and Mad1 protein on enzymatic activities and Toll-related genes of migratory locust. Environ Sci Pollut Res Int. 2019;26(17):17797-808. https://doi.org/10.1007/s11356-019-05158-2

8. Romero-Sáez M. Los residuos agroindustriales, una oportunidad para la economía circular. TecnoLógicas. 2022;25(54). https://doi.org/10.22430/22565337.2505
9. Cadena Cuamacás AM, Gianella SBK. Análisis de ciclo de vida de la producción de los hongos *Beauveria bassiana* A21 y *Metarhizium anisopliae* A13 en sustratos sólidos [engineering thesis]. Chimborazo: Escuela Superior Politécnica de Chimborazo; 2022. http://dspace.espoch.edu.ec/handle/123456789/21377
10. Jiménez Martínez E, Jarquín EJ. Plagas de cultivos. Managua: Universidad Nacional Agraria; 2021. https://repositorio.una.edu.ni/4459/
11. Salgado EQ. Manejo integrado de plagas en el cultivo de papa: control de la mosca blanca. Rev Latinoam Cienc Agrar RLCA. 2024;2(2):31-43.
12. Zepeda-Jazo I. Manejo sustentable de plagas agrícolas en México. Agric Soc Desarro. 2018;15(1):99-108. https://doi.org/10.22231/asyd.v15i1.752
13. Ruiz MLH, Navarro NGV, Alvarado JH, Arellano DA, García LFG, Castro MCDR. Estudio de agentes de control biológico virales y bacterianos hacia plagas agrícolas. Jóvenes en la Ciencia. 2021;10.
14. Tangarife García NS. Control biológico, la nueva era de la agricultura [undergraduate monograph]. Bogotá: Universidad de Ciencias Aplicadas y Ambientales (UDCA); 2021:76.
15. Aguiar S, Estrella ME, Cabadiana HU. Residuos agroindustriales: su impacto, manejo y aprovechamiento. AXIOMA. 2022;27:Artículo 27. https://doi.org/10.26621/ra.v1i27.803
16. Hidalgo Mata DA, Tello Torres CM. Manual para la producción de hongos entomopatógenos y análisis de calidad de bioformulados. Santo Domingo: INIAP-Estación Experimental Santo Domingo, Protección Vegetal; 2022.
17. Jaramillo HY, Vasco-Echeverri O, Camperos JAG. Characterization of the coffee husk: a potential alternative for sustainable construction. Civil Eng Archit. 2023;11(4):1902-8. https://doi.org/10.13189/cea.2023.110418
18. Ángeles-Vega LS, Ramos-Jaimes HG, Espitia-López J, Garza-López PM, Angel-Cuapio A. Fermentación sólida de *Metarhizium robertsii*: sustrato y condiciones de cultivo en la producción de conidios y la eficacia biológica. Rev Mex Cienc Agríc. 2025;16(3). https://doi.org/10.29312/remexca.v16i3.3596
19. Palomino-Martínez JA, Martínez-Sánchez DR, Torres-Cruz N, Sandoval-Gasca PB, Avalos AMC. Potencial de hongos entomopatógenos en el control biológico de insectos plagas agrícolas. Jóvenes en la Ciencia. 2024;28. https://doi.org/10.15174/jc.2024.4278
20. Delgado PAM, Murcia-Ordoñez B. Hongos entomopatógenos como alternativa para el control biológico de plagas. Ambient Agua. 2011;6(2):77-90. https://doi.org/10.4136/ambi-agua.187
21. Pucheta Díaz M, Flores Macías A, Rodríguez Navarro S, de la Torre M. Mecanismo de acción de los hongos entomopatógenos. Interciencia. 2006;31(12):856-60.
22. Bórquez Cerda DI. Estado del arte de las interacciones entre nemátodos entomopatógenos y hongos entomopatógenos para el control de insectos plaga [thesis]. Santiago de Chile: Universidad de Chile; 2021.
23. Gutierrez AC, Hipperdinger ML, Lopez Lastra CC. Preservación de entomopatógenos y normativas y funciones de las colecciones de cultivos microbianos. In: Patología de insectos: metodologías y técnicas de laboratorio. La Plata: Universidad Nacional de La Plata; 2021.
24. Musso A. Alternativa agroecológica mediante el uso de hongos entomopatógenos para el control de insectos plaga en granos almacenados [thesis]. La Plata: Universidad Nacional de La Plata; 2023.
25. Liu Y, Yang Y, Wang B. Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* play roles of maize (*Zea mays*) growth promoter. Sci Rep. 2022;12(1):15706. https://doi.org/10.1038/s41598-022-19899-7
26. Pólit Solórzano FX, Echeverria Vergara ZL. Evaluación patogénica de *Beauveria bassiana* y *Metarhizium anisopliae* para el biocontrol del *Cosmopolites sordidus* en cultivos de banano [bachelor's thesis]. Cuenca: Universidad Politécnica Salesiana; 2023.
27. Swathy K, Parmar MK, Vivekanandhan P. Biocontrol efficacy of entomopathogenic fungi *Beauveria bassiana* conidia against agricultural insect pests. Environ Qual Manag. 2024;34(1):e22174. https://doi.org/10.1002/tqem.22174
28. Veloz Badillo GM. Efecto de la actividad endófita del hongo entomopatógeno *Beauveria bassiana* debido a diferentes métodos de inoculación [thesis]. [Place unknown]: [Institution unknown]; 2025.
29. Espinosa Velázquez I. Efectividad biológica de hongos entomopatógenos para el control de *Rhipicephalus microplus* [bachelor's thesis]. Chiapas: Universidad de Ciencias y Artes de Chiapas; 2024.
30. Osorio AC, Calvo JGM, Ordoñez MAQ, Mainicta FBA, Quispe MC, Lovaton RL, et al. Formulación de medios de cultivo líquidos para la producción de biomasa micelial de hongos entomopatógenos nativos de la región Cusco, Perú. Q'EUÑA. 2024;15(1):Artículo 1. https://doi.org/10.51343/rq.v15i1.1441
31. Rodríguez Ríos PE. Manual de protocolos de aislamiento, purificación, conservación y multiplicación de hongos entomopatógenos [Internet]. Paraguay: CONACYT; 2019. http://repositorio.conacyt.gov.py/handle/20.500.14066/3614
32. García Cruz I, del Pozo Núñez EM, Hernández Pérez Y. Producción y conservación de conidios del aislado Ma-005 de *Metarhizium anisopliae* (Metsch.) Sorokin. Cent Agríc. 2019;46(1):5-12.
33. Moreno Tume RDA. Efecto de desechos agroindustriales utilizados como sustratos en la producción de esporas de *Trichoderma harzianum* por fermentación en sustrato sólido. 2024. https://hdl.handle.net/20.500.14414/22161
34. Calle-Cheje YH, Aguilar-Anccota R, Rafael-Rutte R, Morales-Pizarro A. Formulación y conservación del hongo antagonista *Trichoderma asperellum* como polvo mojable y emulsionable. Idesia (Arica). 2023;41(4):43-53. https://doi.org/10.4067/S0718-34292023000400043
35. Leal Rocha P, Rodríguez Moreno LC. Desarrollo y evaluación de las propiedades mecánicas de un biomaterial para el desarrollo de biobloques obtenido a partir de biomasa fúngica y residuos agroindustriales. 2022. https://hdl.handle.net/20.500.11839/9038
36. Schulze B, Gómez DG, Posadas JB. Evaluación de sustratos para la producción de *Escovopsis weberi* por fermentación en estado sólido. 2021. https://agris.fao.org/search/en/providers/124845/records/670505f1b1dfe472e1449e83
37. Vargas PS, Hoyos JL, Mosquera SA. Uso de hojarasca de roble y bagazo de caña en la producción de *Pleurotus ostreatus*. Biotecnol Sect Agropecu Agroind. 2012;10:136-45.
38. Gutiérrez-Román M, López JA, Hernández P. Review of the potential of *Beauveria bassiana* as a biological controller of pathogens in agricultural crops. BioTecnología. 2025;29(1):9-20.
39. Manzano A, Pérez R, López J. Sustratos para la producción de conidios de *Beauveria bassiana*. Instituto Nacional de Investigaciones Microbiológicas; 2024. Informe técnico.
40. Ramírez J. Evaluación de la incidencia del género *Ips* en bosques de Tlaxcala [bachelor's thesis]. Tlaxcala: Universidad Autónoma de Tlaxcala; 2024.
41. Pacheco G, Ramírez L, Torres A. Impacto de los descortezadores en

los bosques templados de México. INIFAP; 2021.

42. Zhang LW. Virulence of *Beauveria bassiana* strains against *Dendroctonus valens*. J Insect Pathol. 2010;105(3):200-6.
43. Verma ML, Kumar A, Chintagunta AD, Samudrala PJK, Bardin M, Lichtfouse E. Microbial production of biopesticides for sustainable agriculture. Sustainability. 2024;16(17):7496. https://doi.org/10.3390/su16177496
44. Mathulwe G, Malan AP, Stokwe NF. Solid-state production of *Metarhizium* species on agricultural substrates. J Invertebr Pathol. 2022;189:107694.
45. Moreno Tume RDA. Efecto de desechos agroindustriales utilizados como sustratos en la producción de esporas de *Trichoderma harzianum* por fermentación en sustrato sólido [master's thesis]. Perú: Universidad Nacional de Trujillo; 2024.
46. Loera-Corral O, Porcayo-Loza J, Montesinos-Matias R, Favela-Torres E. Production of conidia by the fungus *Metarhizium anisopliae* using solid-state fermentation. Methods Mol Biol. 2016;1477:61-9. https://doi.org/10.1007/978-1-4939-6367-6_6
47. Rashki M. Two-phase fermentation for entomopathogen mass production using agro-industrial residues. Appl Microbiol Biotechnol. 2023;107(5):1925-38.
48. Rivera OL. Bioaccumulation and toxicity of insecticides in bark beetles. Environ Toxicol Chem. 2022;41(10):2512-20.
49. Sun Y, Guan Q, Wang Q, Yang L, Pan N, Ma Y, et al. Quantitative assessment of the impact of climatic factors on phenological changes in the Qilian Mountains, China. For Ecol Manag. 2021;499:119594. https://doi.org/10.1016/j.foreco.2021.119594
50. Muñoz EJ, Díaz JD, Quijada XG, Pérez PAL, Peón AN, Vera CJ. The complexity of the genus *Arthrospira*. Pädi Bol Cient Cienc Bás Ing ICBI. 2025;13(25):6-15.
51. Pham TA, Kim JJ, Kim K. Optimization of solid-state fermentation for improved conidia production of *Beauveria bassiana* as a mycoinsecticide. Mycobiology. 2010;38(2):137-43. https://doi.org/10.4489/MYCO.2010.38.2.137
52. Sala A, Echegaray T, Palomas G, Boggione MJ, Tubio G, Barrena R, et al. Insights on fungal solid-state fermentation for waste valorization: conidia and chitinase production in different reactor configurations. Sustain Chem Pharm. 2022;27:100702. https://doi.org/10.1016/j.scp.2022.100624
53. Murietta M, Sánchez A, Artola A, Barrena R. Scanning agro-industrial wastes as substrates for fungal biopesticide production: use of *Beauveria bassiana* and *Trichoderma harzianum* in solid-state fermentation. Bioresour Technol. 2021;342:125989.
54. Jackson MA, Jaronski ST, Mascarin GM. Mass production of entomopathogenic fungi for biological control of insects. In: Wraight SP, Ramos ME, Kaya HK, editors. Microbial Control of Insect and Mite Pests: From Theory to Practice. Cambridge, MA: Academic Press; 2010:209-30.
55. Mascarin GM, Jackson MA. Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *Beauveria bassiana* and *Isaria fumosorosea* strains. Biol Control. 2019;132:109-17.
56. Wen TC, Yang YY, Lin CC. Production of cordycepin in different fermentation modes of *Cordyceps militaris* and its effects on immune cells. J Food Drug Anal. 2019;27(4):859-68.
57. Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS, et al. Insect pathogens as biological control agents: back to the future. J Invertebr Pathol. 2015;132:1-41. https://doi.org/10.1016/j.jip.2015.07.009
58. Inglis GD, Goettel MS, Butt TM, Strasser H. Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N, editors. Fungi as biocontrol agents: progress, problems and potential. Wallingford: CABI Publishing; 2001:23-69. https://doi.org/10.1079/9780851993560.0023
59. Meyling NV, Eilenberg J. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol Control. 2007;43(2):145-55. https://doi.org/10.1016/j.biocontrol.2007.07.007
60. Ortiz-Urquiza A, Keyhani NO. Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects. 2013;4(3):357-74. https://doi.org/10.3390/insects4030357

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etcSee https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.