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REVIEW

Biological Control of Planococcus citri by Entomopathogenic Fungi: A Review of Mechanisms and Applications

Control Biológico de Planococcus citri mediante Hongos Entomopatógenos: Una Revisión de Mecanismos y Aplicaciones

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ABSTRACT

Introduction: entomopathogenic fungi (EPFs) represent a sustainable and effective alternative for the biological control of agricultural pests, such as the citrus scale (Planococcus citri), which significantly affects lemon crops in Ecuador. These fungi, including Beauveria bassiana, Metarhizium anisopliae, and Lecanicillium lecanii, act as natural insect pathogens, reducing dependence on chemical pesticides.
Objective: to review the potential of EPFs as biological control agents against Planococcus citri, analyzing their mechanisms of action, efficacy, and applications in Ecuadorian agriculture.
Development: EPFs infect scale insects by adhesion, germination, penetration, and colonization, producing enzymes and toxins that degrade the insect's cuticle. Studies show that strains such as Metarhizium anisopliae and Lecanicillium lecanii are especially effective against P. citri nymphs and adults. However, their effectiveness can be limited by environmental factors such as humidity, temperature, and the presence of waxy coatings on the insect.
Conclusions: HEPs are a promising tool for integrated pest management in citrus, although their success depends on optimal environmental conditions and stable formulations. Further local research evaluating native strains and application strategies is recommended to maximize their effectiveness in controlling P. citri.

Keywords: Entomopathogenic Fungus; Planoccocus Citri; Biological Control; Invertebrates; Bioinsecticide.

RESUMEN

Introducción: los hongos entomopatógenos (HEPs) representan una alternativa sostenible y eficaz para el control biológico de plagas agrícolas, como la cochinilla de los cítricos (*Planococcus citri*), que afecta significativamente cultivos de limón en Ecuador. Estos hongos, entre los que destacan Beauveria bassiana, *Metarhizium anisopliae y Lecanicillium lecanii*, actúan como patógenos naturales de insectos, reduciendo la dependencia de pesticidas químicos.
Objetivo: revisar el potencial de los HEPs como agentes de control biológico contra *Planococcus citri*, analizando sus mecanismos de acción, eficacia y aplicaciones en el contexto de la agricultura ecuatoriana.
Desarrollo: los HEPs infectan a las cochinillas mediante adhesión, germinación, penetración y colonización, produciendo enzimas y toxinas que degradan la cutícula del insecto. Estudios demuestran que cepas como *Metarhizium anisopliae* y *Lecanicillium lecanii* son especialmente efectivas contra ninfas y adultos de P. citri. Sin embargo, su eficacia puede verse limitada por factores ambientales como la humedad, temperatura y la presencia de capas cerosas en el insecto.

Conclusiones: los HEPs son una herramienta prometedora para el manejo integrado de plagas en cítricos, aunque su éxito depende de condiciones ambientales óptimas y formulaciones estables. Se recomienda profundizar en investigaciones locales que evalúen cepas nativas y estrategias de aplicación para maximizar su efectividad en el control de *P. citri*.

Palabras clave: Hongo Entomopatógeno; *Planoccocus Citri*; Control Biológico; Invertebrados; Bioinsecticida.

INTRODUCTION

Entomopathogenic fungi (EPFs) parasitize and kill arthropods at various stages of their life cycle, whether as larvae or adults.[1] In recent years, 90 genera and 700 species of EPHs have been identified in various conditions, of which *Beauveria bassiana* and *Metarhizium anisopliae* are species that host a wide range of hosts.[2]

On the international market, some HEP-based products have *Beauveria bassiana* as their main component.[3] Aphids and whiteflies are repelled by mycoinsecticide products that have been created to combat a variety of pests, including desert locusts and Colorado potato beetles in Russia. Like those mentioned above, other products are environmentally beneficial substitutes for synthetic pesticides.[4]

Due to their entomopathogenic capacity, they have been used as a biological control method, resulting in commercial products. The formulations usually consist of a suspension of spores of the fungus or entomopathogenic consortium, which can be applied to certain parts of the plant within the crop.[5]

In Chile, according to the Agricultural and Livestock Service, 34 HEP-based products are registered. The country's agricultural research institute has developed more stable formulations using encapsulation, as one of the main drawbacks of using HEPs is exposure to relative humidity and ultraviolet radiation. Coated beads have proven to be more viable and promote fungal growth by absorbing water vapor from the environment.[5]

In Colombia, 47 companies market fungi and entomopathogenic consortia, the latter including associations between *Beauveria* bassiana, Metarhizium anisopliae, and Purpureocillium lilacinum; Hirsutella thompsonii Fisher and *Akanthomyces* sp.; *and Metarhizium anisopliae (*Metchnikoff) *Sorokin* and *Paenibacillus popilliae (*Dutky), among others.[6]

HEP serves as a biological control for pests such as cochineal. In the study by Góngora and Gil-Palacio[7], strains of *Metarhizium anisopliae and Metarhizium robertsii* were analyzed against this insect in plants under controlled conditions. It was concluded that *Metarhizium robertsii* should be used in further studies as it shows a higher mortality rate compared to the methods evaluated in the study.

The study by Cloyd and Herrick [8] suggests that treatments based on HEPs and those applied in conjunction with insect growth regulators were not effective due to the waxy coating that covers mealybugs. Other drawbacks related to the use of HEPs were unfavorable relative humidity or temperature conditions, in addition to the fact that the bioinsecticides left whitish residues on the leaves that could affect their appearance and, consequently, their sale price.

Another study in Mexico determined the pathogenicity of three species of HEPs: *Beauveria bassiana*, *Metarhizium anisopliae*, and *Lecanicillium lecanii* in nymphs of *Dactylopius opuntiae*. The last two strains showed the most effective results. *Metarhizium anisopliae* was the most effective strain in the second instar of the pest.[9]

DEVELOPMENT

Table 1. Taxonomy Planococcus citri (Risso)

Kingdom	Animalia
Order	Hemiptera
Suborder	Sternorrhyncha
Superfamily	Coccoidea
Family	Pseudococcidae
Subfamily	Pseudococcinae
Genus	Planococcus Ferris, 1950
Species	Planococcus citri (Risso, 1813)

Source: Global Biodiversity Information Facility[13]

The citrus mealybug *Planococcus citri* (Risso, 1813) is a sucking insect of the order Hemiptera and the family Pseudococcidae that feeds on plant sap.[10,11,12] It has an extensive geographical distribution according to the

Global Biodiversity Information Facility database [13], with 703 georeferenced records, including Ecuador, which was registered as introduced in 2019 by Pagad.[14] The optimal temperatures for maximum population yield are between 28° and 30°C.[15] It is a pest that has been affecting forest and urban areas of Guayaquil, Ecuador, from November 2020 to the present.

Morphology

The eggs are small, oval-shaped, and light yellow when freshly laid. As the embryo develops, the egg turns dark yellow. The eggs are deposited in a white ovisac formed by filamentous secretions from the female. This structure is almost straight and measures between 0,38 and 0,75 mm in length. At this stage, males and females are identical.[16]

Nymph I is the first stage of development of the mealybugs. They are small, light yellow, and elongated oval in shape. When it hatches, it remains in the ootheca for 2 to 3 days and then moves to the leaf veins. It has small antennae, and its three pairs of legs do not yet have filaments around them. The body is soft, and both males and females have the same characteristics at this stage.[16]

The second instar nymph of Planococcus citri is bright yellow and oval in shape. It is covered with a white powdery substance, and its movement slows down. At this stage, female and male nymphs begin to differentiate. Females maintain the same characteristics as nymph I, while males grow to an average length of 1,31 mm and a width of 0,58 mm. After two or three days, before the end of this stage, the male's color turns almost pink and thin, and it becomes covered with a cottony substance, forming a cottony cocoon.[16]

Nymph III is very similar to the adult female but smaller, and here the caudal filaments are much more formed around the body. It already has a whitish waxy covering, and the body segments can also be observed. Its movement is slower. The nymph has an average length of 1,65 mm and a width of 0,80 mm.[16]

The pre-pupa of the male mealybug is an intermediate stage in its development. It finishes forming inside a white cotton cocoon and has almost no movement. Its wings are not yet developed and are pale yellow in color. It then becomes a pupa, and its appearance changes as it now has an elongated shape, is dark brown in color, moves slowly, and has no wings at this stage.[16]

Adult females of this species, Planococcus citri, are yellow, oval-shaped, and measure approximately 3 to 4 mm long and 1,5 to 2 mm wide.[17,18,19,20] These scale insects are covered with a white, waxy coating that gives them a powdery appearance. They have 18 pairs of waxy filaments, the last of which are slightly longer and located at the rear. Their color varies from yellow to reddish brown.

Figure 1. Life cycle of Planococcus citri

The males, on the other hand, have a trunk divided into distinct segments, known as tagmata. They are more elongated than the females and can be winged or wingless. Their legs are elongated, and the dimensions of the organism are 0,95 to 1 mm in length and 0,2 to 0,3 mm in width. The abdomen is an elongated cylinder that is completely attached to the body.[21] They do not have a mouthpart, or it is atrophied; therefore, they do not produce honeydew, nor do they have symbiotic relationships with other species. They also have a short lifespan.[16]

Scale insects and ants

Ants and some species of scale insects have a symbiotic relationship based on the exchange of resources, according to Bravo & Cordoba[22]. Ants transport and protect the pest from its natural enemies, and in turn, the scale insects produce honeydew, a sugary liquid that serves as food for the ants.[23]

Entomopathogenic fungi (EPFs)

EPFs are microorganisms [24] that are natural enemies of insects and capable of wiping out entire populations of insect pests.[25,26] They are a sustainable alternative to chemical pesticides, as they are safe for human health and the environment.[27]

Most EPPs belong to the Entomophthoromycota and Hypocreales divisions. Both phyla produce conidia and other asexual spores, and when they develop, they can live as obligate or facultative arthropod pathogens.[28]

HEP infects insects through a process that is generally divided into four stages.[29] The first is known as fungal adhesion to the surface. It involves the attachment of conidia, where the reproductive structures adhere to the insect's cuticle through nonspecific hydrophobic and electrostatic interactions.[30,31] The adhesion phase is a determining factor and the most critical phase, since everything depends on the number of spores that adhere to the cuticle to kill the insect.[30,32]

The second stage is germination, which occurs once the conidia have adhered, germinated, and formed the appressorium, a structure that penetrates the insect's cuticle. During this stage, penetration continues, and in addition to hydrolytic enzymes, molecules that turn off the host's immune responses also participate.[30,32] so that the fungus can finally enter and secrete phospholipase C to hydrolyze the phosphodiester bonds found in the host's cell membrane.[32,33]

In the third stage, the fungus reaches the hemocoel, changing from a mycelial form to a yeast-like form, which allows it to multiply more easily, giving it an advantage over the insect.[32,34] The multiplication of hyphae produces toxins that kill the insect.[29]

Due to the death of the insect, nutrients such as nitrogen sources are depleted, causing it to acquire its mycelial form.[32,35] Finally, spores are dispersed and adhere to a new host.[32,36]

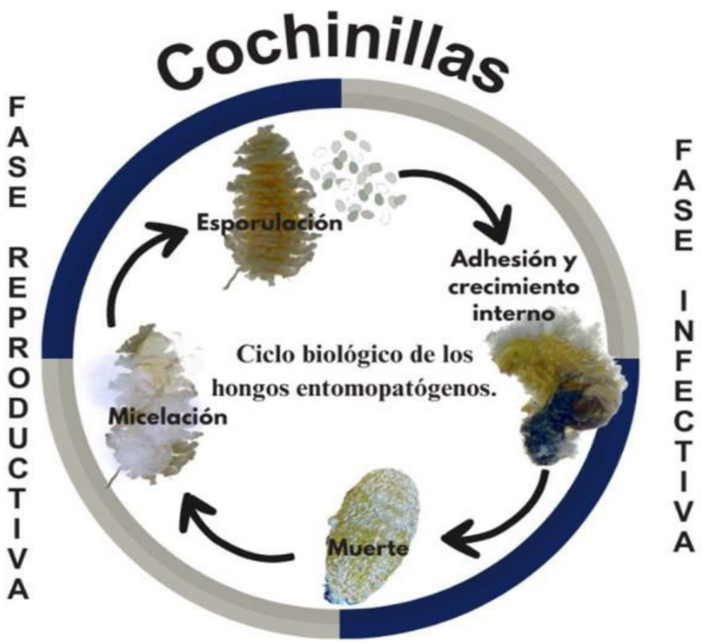

Figure 2. Biological cycle of entomopathogenic fungi

Pathogenic mechanisms

EPFs use a variety of mechanisms to cause the death of insects. The most common mechanisms include toxicity, where they produce toxins that kill insects. Secondly, infection occurs when the pathogen's hyphae invade the insect's tissues, causing death by malnutrition or mechanical damage. Another common mechanism is the induction of immune responses: fungi induce immune responses in insects, which can result in the death of the insect.[37] EPPs have a complex life cycle that includes a parasitic phase and a saprobic phase. In the parasitic phase, they infect and kill an insect; in the saprobic phase, the organism grows on dead organic material.[38]

The most relevant entomopathogenic fungi in the tropics include Beauveria bassiana, one of the most studied in the world because it is a common pathogen for insects, belonging to the order Hypocreales and the family Cordycipitaceae. This pathogen has a high diversity of hosts, with approximately 700 species [39,40], causing white mycelial growth in nymphs and adults. The asexual spores are shaped like flattened hemispherical threads and are generated after 20 days at 25°C.[41]

Table 2. Taxonomy Beauveria bassiana

Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Cordycipitaceae
Genus	Beauveria Vuill., 1912
Species	*Beauveria bassiana* (Bals.-Criv.) Vuill.

Source: Global Biodiversity Information Facility[13]

Metarhizium anisopliae is one of the most studied pathogens in the tropics. It belongs to the order Hypocreales, family Clavicipitaceae,[42] and generates septate mycelial growth that starts out white- , but changes over time to a dark green color. It grows on both nymphs and adults. Its asexual spores are cylindrical or ovoid in shape and are generated after 15 days at 25°C.[41]

Table 3. Taxonomy of Metarhizium anisopliae

Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Clavicipitaceae
Genus	Metarhizium Sorokin
Species	Metarhizium anisopliae (Metschn) Sorokin

Source: Global Biodiversity Information Facility[13]

The fungus *Lecanicillium lecanii* has a slow colonization rate, can affect both nymphs and adults,[43] has a wide range of hosts, including arthropods such as mealybugs and aphids, among others, and is characterized by its long shelf life, effective mechanism of action, and high host specificity.[44]

Table 4. Taxonomy of Lecanicillium lecanii

Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Cordycipitaceae
Genus	Akanthomyces Lebert
Species	Lecanicillium lecanii (Zimm)

Source: Global Biodiversity Information Facility(13)

The fungus *Purpureocillium lilacinum* belongs to the Ophiocordycipitaceae family and was classified based on its medical importance.[45] This pathogen can survive in a wide range of temperatures, from 8 to 38 °C, with optimal temperatures of 26 to 30 °C.[46]

Beauveria bassiana, Metarhizium anisopliae, and *Lecanicillium lecanii* are fungi that are effective against different insect pests and at different stages of insect development. These pathogens, when applied to mealybugs, have proven to be effective, with their mechanisms of action tending to have different durations in each phase of their biological cycle.[43,47]

The organisms *Lecanicillium lecanii* and *Beauveria bassiana* have been shown to penetrate the cuticle of *Planococcus citri* nymphs, as they produce lipases, proteases, and chitinases that combine to degrade the cuticle.

Table 5. Taxonomy of Purpureocillium lilacinum	
Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Ophiocordycipitaceae
Genus	Purpureocillium Luangsa-ard et al.
Species	Purpuerocillium llacium (Thom)
Source: Global Biodiversity Information Facility[13]	

Beauveria bassiana and Metarhizium anisopliae specimens have different mechanisms of action, as they eliminate the insect and also form beneficial relationships with plants (Litwin et al., 2020).

Various studies show that the optimal average temperature for HEPs of the Hypocreales phylum is 30-° C and a relative humidity of 95 %.[48]

Applications of entomopathogenic fungi

EPF are used for pest control in a wide range of crops, including cereals, fruits, vegetables, and ornamentals, and are also used for forest and urban pest control.

These microorganisms used as bioinsecticides have notable characteristics such as: safety, as they have little or no impact on the environment or human health; resistance, since, although they can generate resistance, this is not absolute;[50] selectivity, since some species infect certain species or a group of insects; they do not produce residues; they can be mass-produced with accessible substrates such as rice.[28,51]

Although they are a sustainable alternative, HEPs are susceptible to temperature, humidity, ultraviolet radiation, and the use of other pesticides. For these reasons, it is recommended to store HEP-based bioinsecticides correctly and apply them as recommended by the manufacturer. It is also recommended to be aware of phytosanitary management in nearby crops in case they may cause damage, for example, through the use of pesticides.[28,51]

Lemon (Citrus × aurantiifolia (Christm.) Swingle)

Lemon *Citrus* × *aurantiifolia (Christm.) Swingle* is one of the most widely consumed fruits worldwide and one of the most cultivated in Ecuador.[52] Commonly known as lime, it has a slightly round shape with a conical end. Its flavor is very acidic, juicy, and has a strong aroma.[53] The pests that usually attack it are whiteflies, scales, or mealybugs on the trunk and foliage. Mealybugs are usually found on the calyx, and their bites can cause the foliage to fall. Once the fruits are large, mealybugs prefer to locate themselves in the area of contact between the fruit and the leaf, attracting sooty mold activity, which damages the plant.[54]

Table 6. Taxonomy Citrus × aurantiifolia (Christm.) Swingle	
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Sapindales
Family	Rutaceae
Genus	Citrus L.
Species	Citrus × aurantiifolia
Source: Global Biodiversity Information Facility[13]	

CONCLUSIONS

Entomopathogenic fungi are a viable and environmentally friendly alternative for controlling Planococcus citri in lemon crops. Their mechanism of action, based on infecting and colonizing the insect, demonstrates high specificity and low environmental impact. However, factors such as the wax coating of the scale insect, climatic conditions, and interaction with other pesticides can affect their efficacy. Future research should focus on developing improved formulations and application protocols adapted to the specific conditions in Ecuador.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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