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Review article

Organismos entomopatógenos como control biológico en los sectores agropecuario y forestal de México: una revisión

Entomopathogenic organisms for pest control in the mexican agriculture, livestock and forest sectors: a review

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Resumen

El control biológico es uno de los métodos de manejo de plagas compatibles con el ambiente, ofrece beneficios a la economía de los agricultores, protección al ambiente y a la salud de los consumidores. En esta compilación, se presenta una revisión actualizada sobre las investigaciones que se han realizado hasta el momento en el tema de organismos entomopatógenos para combate de plagas en los sectores agrícola, pecuario y forestal de México. Existen varios tipos de organismos entomopatógenos, tales como hongos, bacterias, nematodos y virus, de los cuales se mencionan las principales aplicaciones, así como las empresas que comercializan en el país productos elaborados con estos. Se resume el progreso y las investigaciones realizadas en los últimos años como componentes de las estrategias de manejo integrado de plagas en cultivos, bosques, hábitats urbanos y de importancia médica y veterinaria. Cabe resaltar el amplio interés en el estudio de hongos ─principalmente *Beauveria bassiana* y *Metarhizium anisopliae*─ en el sector agrícola; seguido de las aplicaciones en el sector pecuario y por último en el forestal. En cuanto a nematodos, caben resaltar los trabajos y usos, sobre todo, contra la mosca de la fruta. Las bacterias*,* así como los virus utilizados en el combate de plagas han sido explorados muy escasamente.

Palabras clave: Bacterias entomopatógenas, control biológico, hongos entomopatógenos, nematodos entomopatógenos, plagas, virus entomopatógenos.

Abstract

Biological control, one of the methods of pest management compatible with the environment, offers benefits to the farmers' economy, environmental protection and consumer health. This is an updated review about the research that has been carried out on entomopathogenic organisms for pest control in the Mexican agriculture, livestock and forest sectors. There are several types of entomopathogenic organisms such as fungi, bacteria, nematodes and viruses used for this purpose. It is summarized the progress and research carried out in recent years as components of the integrated pest management strategies in crops, forests, urban habitats, medical and veterinary importance. It is worth highlighting the wide interest in research of entomopathogenic fungi -mainly *Beauveria bassiana* and *Metarhizium anisopliae*- in the agricultural sector, followed by their use in the livestock and lastly in the forestry sector. More research and applications should be done regarding entomopathogenic nematodes. Bacteria, as well as viruses, applied to combat pests have been explored very rarely. The main companies that commercialize products with these organisms in the country are also listed.

Key words: Entomopathogenic bacteria, biological control, entomopathogenic fungi, entomopathogenic nematodes, plagues, entomopathogenic virus.

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Introduction

The indiscriminate use of synthetic pesticides is the direct cause of the resistance of different organisms, and therefore of the loss of their effectiveness. Given this situation, it is common to increase the doses and prepare mixtures of several pesticides, often more toxic, so that the problem of resistance, far from being solved, becomes worse. Chemical control of pests also produces other effects such as secondary pest outbreaks, pest resurgence and decreased populations of natural enemies. From this situation, it is necessary to develop methods of pest management compatible with the environment, one of which is biological control.

Biological control offers, at the same time, benefits to the farmers' economy, environmental protection and consumer health. In addition, it has contributed to the development of agriculture in Mexico and many countries (Arredondo, 2008). Technicians and producers, intuitively, have realized that the use of biological control allows them to fight pests against which entomophageal or entomopathogenic species are available, at a lower cost than the expenditures generated by the use of chemical pesticides. Therefore, it is currently assumed that this alternative constitutes (from its economic, environmental and ecological virtues) the most desirable strategy for the management of populations of agricultural, livestock and forest pests.

Contrary to what one might think, this is not a new strategy for crop protection in Mexico. Its history covers almost 100 years, during which some important successes have been obtained. However, since 1990, the role of biological control has been formalized at the federal and state levels of government and within the academic community. In 1991, the *Centro Nacional de Referencia para el Control Biológico (CNRCB)* National Reference Center for Biological Control (CNRCB, for its acronym in Spanish) was inaugurated in *Tecomán*, *Colima*, Mexico, and was recognized by the International Organization for Biological Control as an international reference center. The CNRCB has the mission of developing and establishing biological control strategies for regulated pests, for this purpose it generates and provides alternative technology to the use of chemical pesticides. Likewise, it contributes with phytosanitary programs or campaigns in which the use of beneficial organisms as agents of biological control is promoted, in order to strengthen the health of plant crops in Mexico (CNRCB, 2018).

The *Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria* (Senasica) (National Agrifood Service of Health, Safety and Quality (Senasica, for its acronym in Spanish in Mexico), a decentralized institution of the *Secretaría de Agricultura y Desarrollo Rural* (Sader) (Department of Agriculture and Rural Development (Sader, for its acronym in Spanish in Mexico), has the responsibility of monitoring and controlling plant and animal health, food safety and inspection of agricultural and animal products at national borders and inspection points. Fortunately, the authorities of these institutions have considered and supported biological control when planning governmental responses to emerging pest problems, so, in emergency situations, Senasica asks the CNRCB reference center to evaluate possible pest options that represent important threats to the country (Trevor *et al.,* 2013).

There are more and more scientists and researchers involved in the disciplines related to this task, so in 1989 the *Sociedad Mexicana de Control Biológico* (Mexican Society of Biological Control) was established; this group holds an annual conference, as well as courses and workshops that attract several hundred people each year. However, the development of biological control as a scientific discipline and as a technology for crop protection is still under development (SMCB, 2018).

In the near future it is envisioned that the use of biological control will increase as a result of the globalization of the economy, and openness to international trade, so the use of bioinsecticides and biopesticides will be the norm, since the latter with organisms Entomopathogens have become a good tool for the biological control of many insect pests and are receiving more attention due to their environmentally friendly nature, their effectiveness against pests and their easy mass production protocols. The term entomopathogens refers to microorganisms capable of causing a disease to the pest insect, leading to its death after a short incubation period. There are several types of entomopathogenic organisms, such as fungi, bacteria, nematodes and viruses (García and González, 2013).

Information on entomopathogenic organisms as pest control agents is described below, which may be of interest to generations of producers, students, scientists and scholars in Mexico and thereby stimulate research and practice of biological control, as a responsible option for improving the environment.

Entomopathogenic fungi

Entomopathogenic fungi are the most important group in the biological control of pest insects. Virtually all insects are susceptible to diseases caused by these fungi. When their spores come into contact with the cuticle of susceptible insects, they germinate and grow directly through it towards the inside of their host's body. Therefore, the fungus proliferates throughout the body of the insect producing toxins and consuming nutrients from the insect, and eventually destroys it. At the beginning of the infection, symptoms may or may not be observed, but the insect begins to lose mobility and appetite. After seven or ten days, it dies due to nutritional deficiency (Pérez, 2004).

The diseases they cause are known as *muscardinas*, a term that was first applied to *Beauveria bassiana* (Bals.-Criv.) Vuill. The color of the conidia is very variable, hence, there are different names such as green *muscardina*, for *Metarhizium anisopliae* (Metchnikoff) Sorokin and Nomuraea rileyi (Farlow) Samson, and red *muscardina* for *Paecilomyces fumosoroseus* (Wize) Brown & Smith. The use of these organisms is one of the best alternatives used in biological control because it is economical, simple and ecologically sustainable. However, it is essential to provide adequate temperature and humidity conditions to achieve its purpose. In addition, when it is intended to be used as bioinsecticides, it is necessary to carry out an exhaustive characterization of isolates in order to select those with high virulence and good conditions for field application. This characterization includes studies referring to the mode of infection (Godwin and Shawgi, 2000; Arredondo *et al.,* 2008; Caballero, 2014). Nowadays it is important to carry out studies on the molecular and biochemical determinants related to the specificity of the fungus to the host.

It is well known that *B. bassiana* infects more than 200 species of insects of different orders, which include pests of economic importance such as the *cogollero* worm (*Spodoptera frugiperda* (J.E. Smith)), borer worm (*Diatrea magnifactella* Dyar, 1911), coffee drill (*Hypothenemus hampei* Ferrari, 1867), among others. While *M. anisopliae*, with a broader spectrum of toxicity, has been found infecting between 300 and 400 species of lepidoptera, beetles, dipterans and homoptera (Table 1).

Table 1. Main entomopathogenic fungi used for pest control in the agricultural sector.

Fungi	Plague	Crop	Reference
Beauveria bassiana (Bals.-Criv.) Vuill.	Coffee drill: *Hypothenemus hampei* Ferrari, 1867 (Coleoptera: Scolytinae)	Coffee	Gerónimo, 2016
	Coffee drill: *H. hampei* Ferrari, 1867	Coffee	Díaz and Roblero, 2007
	Predators of *Diaphorina citri* Kuwayama*: Ceraeochrysa valida* Banks and *Eremochrysa punctinervis* McLachlan	Citrus	Gandarilla *et al.,* 2013
	Ligus bug: nymphs of *Lygus lineolaris* Palisot de Beauvois	Strawberry	González *et al*., 2010
	Mexican bean beetle: *Epilachna varivestis* Mulsant	Beans	Castrejón, 2017
	Grasshoppers: *Brachystola magna* Girard and *B. mexicana* Bruner	Beans	Lozano and España, 2006
	White fly: *Bemisia tabaco* Gennadius	Green vegetables	Ruiz, 2009
	Plague insect of J*atropha curcas* L. fruit*: Pachycoris torridus* Scopoli	*Jatropha curcas*	Chávez, 2016
	Codling moth: *Cydia pomonella* L.	Apple	Solís, 2006
	Opuntia weevil: *Metamasius spinolae* Gyllenhal	Edible opuntia	Sánchez *et al*., 2016
	Opuntia weevil: *M. spinolae* Gyllenhal	Edible opuntia	Tafoya, 2004
B. bassiana (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokin	White fly *B. tabaci* Gennadius	More than 500 ornamental species	García *et al*., 2013
	Agave weevil: *Scyphophorus interstitiales* Gyllenhal	Agave hard liquors	Aquino, 2006
	Borer worm: *Diatrea magnifactella* Dyar, 1911	Sugar cane	Castro *et al*., 2013
	Bacteria *Candidatus Liberibacter* spp.	Citrics	Mellín *et al*., 2016
	Blind hen larvae: *Phyllophaga crinita* (Burm.) (Coleoptera: Melolonthidae)	Maize, green areas, golf greens	Nájera, 2005
	Blind hen: *Phyllophaga vetula* Horn	Maize	Hernández *et al*., 2011
	Scale insects or scales (Sap sucking insects): *Aulacapsis tubercularis* Newstead	Mango	Pérez-Salgado, 2013
	Fruit flies: *Anastrepha obliqua* (Macquart)	Mango	Díaz-Ordaz *et al*., 2010
	Potatoe psyllid*: Bactericera cockerelli* Šulc.	Potatoe	Villegas, 2017
M. anisopliae (Metchnikoff) Sorokin	Borer worm: *D. magnifactella* Dyar, 1911	Sugar cane and maize	Buenosaires, 2013
	Grasshoppers: *Sphenarium purpurascens* (Charpentier) and *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae)	Maize and beans	Tamayo, 2009.
	Central Anerican locust: *Schistocerca piceifrons piceifrons* Walker	Maize, sorghum, beans, sugar cane, soy, cotton, sesame, bananas and peanuts.	Barrientos, 2005

M. anisopliae (Metchnikoff) Sorokin and entomapathogenic nematodes	Blind hen larvae *: Phyllophaga vetula* Horn	Maize	Ruiz, 2012
B. bassiana (Bals.-Criv.) Vuill., *M. anisopliae* (Metchnikoff) Sorokin and *P. fumosoroseus* (Wize) Brown & Smith	Weevil: *Anthonomus fulvipes* Boheman	Barbados Cherry (Acerola)	Lezama *et al*., 1997
B. bassiana (Bals.-Criv.) Vuill., *M. anisopliae* (Metchnikoff) Sorokin and *P. fumosoroseus* (Wize) Brown & Smith	Cabbage White butterfly: *Pieris rapae* Linnaeus; diamond-back worm: *Pluxtella xylostella* Linnaeus, cabagge looper: *Trichoplusia ni* Hübner; cabbage aphid: *Brevycorine brassicae* Linnaeus	Green vegetables	García and González, 2010
B. bassiana (Bals.-Criv.) Vuill., *Lecanicillium lecanii* (Zimm.) Zare & W. Gams. and *P. fumosoroseus* (Wize) Brown & Smith	Citrus sadness virus Brown citrus aphid : *Toxoptera citricida* Kirkaldy	Citrus	Hernández *et al*., 2007
B. bassiana (Bals.-Criv.) Vuill., *M. anisopliae* (Metchnikoff) Sorokin, *N. rileyi* (Farlow) Samson, *P. fumosoroseus* (Wize) Brown & Smith and *P. javanicus* (Friederichs & Bally) Brown & Smith	Maize fall armyworm: *Spodoptera frugiperda* (J. E. Smith)	Maize	Lezama *et al*., 1996a o b
N. rileyi (Farlow) Samson and *P. fumosoroseus*(Wize) Brown & Smith	Maize fall armyworm larvae: *S. frugiperda* (J. E. Smith)	Maize	Lezama *et al*., 1994
H. citriformis Speare	Huanglongbing (HLB). Asian citrus psyllid: *Diaphorina citri* Kuwayama	Citrus	Pérez-González, 2013
Trichoderma spp.	Onion root rot: *Fusarium oxysporum* Schltdl.	Onion	Pulido-Herrera *et al.,* 2012
Verticillium and *P. fumosoroseus* (Wize) Brown & Smith	White fly: *Bemisia argentifolli* Bellows y Perring	Cotton	Ceceña *et al.,* 2017

Entomopathogenic fungi against agricultural pests

Table 1 summarizes the main research studies carried out in the most important crops in the country where entomopathogenic fungi were used. The extensive study of *Beauveria bassiana* strains is outstanding, especially in the fight against pests such as the coffee drill, in which Gerónimo *et al.* (2016) observed a pathogenic effectiveness of 100 % at 144 h in this crop; Díaz and Roblero (2007) recorded that the optimal time for the application of the fungus is in July, before the drill enters the coffee fruit.

From Mexico's interest in citrus production, Pérez-González *et al.* (2013) conducted studies against the Asian citrus psyllid (*Diaphorina citri* Kuwayama) in which they used the fungus *Hirsutella citriformis* Speare; Mellín *et al.* (2016) with *Paecilomyces fumosoroseus* and Hernández *et al.* (2007) with *Lecanicillium lecanii* (Zimm.) Zare &

W. Gams.; in addition to studies conducted with *B. bassiana* and *M. anisopliae* by Gandarilla *et al.* (2013). In all these studies, the strains analyzed were considered as promising for the development of biological control technology of *D. citri*, as in the case of *M. anisopliae*, which caused 93-100 % mortalities in nymphs, while in adults the Mortality range ranged from 40 to 95 % (Mellín *et al*., 2016).

Phyllophaga vetula Horn, 1887, known in Mexico as blind hen and the sprout worm (*Spodoptera frugiperda*) are two of the main corn pests that have been studied to combat them with *M. anisopliae* with which the highest level of virulence was recorded and where the treatments recorded a mortality of 80 % at 30 days (Nájera, 2005). Buenosaires (2013) reported an LC_{50} of 2.0518×10^8 conidia ml^{-1}. In a similar way, strains of the *N. rileyi* fungus have been explored, which parasitized 100 % of the neonatal larvae of these pests, with a TL_{50} between 4.1 and 6.3 d, as well as strains of *P. fumosoroseus* that parasitized between 92.5 and 98.8 %, with a TL_{50} between 2.5 and 4.3 d (Lezama *et al*., 2004); these same authors in 2006, published a more extensive study where they evaluated other strains of entomopathogenic fungi concluding that the strains of *M. anisopliae*, *P. fumosoroseus* and *P. javanicus* (Friederichs & Bally) Brown & Smith, were highly virulent in eggs and larvae with a mortality of 94 and 100 %, and a TL_{50} of 1.3 to 3.3 d. The strains of *B. bassiana* had a very variable virulence, with a parasitism between 3-90 % in eggs and 54-100 % in larvae (Lezama *et al.,* 2006b). On the other hand, Ruiz *et al.* (2012) concluded in their studies that *S. carpocapsae* (1.500 JI $plant^{-1}$) in combination with *M. anisopliae* (2 × 108 spores $plant^{-1}$) are recommended for the control of larvae of *P. vetula.*

Entomologic fungi against livestock plagues

In studies with entomopathogenic fungi for the control of pests in the livestock sector (Table 2), it is observed that *M. anisopliae* and *B. bassiana*, mainly, have been effective for the control of pests of cattle ticks, fleas of dog, bugs bream, grasshopper and *dengue* transmitting mosquitoes. There are few records in which other strains of entomopathogenic fungi such as *Trichoderma, Cordyceps* or *Isaria* have been explored, to name a few.

Table 2. Main entomopathogenic fungi used for pest control in the livestock sector.

Fungi	Plague	Animal	Reference
Beauveria bassiana (Bals.-Criv.) Vuill.	Kissing bug: *Meccus pallidipennis* Stål, 1872	Kissing bug, causative of Chagas disease	Zumaquero, 2014
	Ctenocephalides canis Shaftesbury, 1934	Dog flea	Pacheco, 2015
	Grasshopper (Orthoptera: Acrididae)	Grasshopper	García and González, 2009
B. bassiana (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokin	Garrapata *Rhipicephalus microplus* Canestrini, 1888	Cattle ticks	Rivera-Oliver *et al.*, 2013
	Tick: *Rhicephalus* (Boophilus) *microplus*	Cattle ticks	Bautista, 2017
	Flea: *C. canis* Shaftesbury, 1934	Dog flea	Rivera-Ramírez, 2013
M. anisopliae (Metchnikoff) Sorokine and *Isaria fumosorosea* Wize	Kissing bug: *M. pallidipennis* Stål, 1872	Kissing bug, causative of Chagas disease.	Flores, 2016
Trichoderma, *M. anisopliae* (Metchnikoff) Sorokine, *A. aculeatus* Lizuka, *G. virens* Corda	*Dengue's* vector: *Aedes aegypti* Linnaeus,1762	*Dengue's* vector: *Aedes aegypti* Linnaeus,1762 mosquitoes	Molina *et al.*, 2013

Among the most economically important pests in this sector is the cattle tick (*Rhipicephalus microplus* Canestrini, 1888); according to Rivera (2013), the use of entomopathogenic fungi such as *M. anisopliae* and *B. bassiana* are found to be pathogenic for the three, eight, and 17-day-old eggs of the insect, and it is accentuated the younger the tick egg is. For Bautista (2017), *M. anisopliae* and *B. bassiana* are an alternative for the control of adult ticks in the XIII *Maya* region of *Chiapas* and the *Ríos* region of the state of *Tabasco*, Mexico.

Entomopathogenic fungi have been tested to combat mosquitoes that transmit serious diseases. Thus, Flores *et al.* (2016) investigated the effects of *M. anisopliae* and *I. fumosorosea* Wize on nymphs of *Meccus pallidipennis* Stål, 1872, which is the main triatomine vector of Chagas disease in Mexico, in terms of insect survival and immune response. Zumaquero *et al.* (2014) studied an isolation of *B. bassiana* from *San Antonio Rayón, Puebla,* Mexico and its entomopathogenic effects in *Meccus pallidipennis*, from which they concluded that this strain was 100 % virulent. A strain of *T. longibrachiatum* showed high entomopathogenic activity on larvae and adult females of *Aedes aegypti* Linnaeus, 1762 mosquitoes, which are *Dengue* vectors; therefore, this fungus can be a good candidate to be developed as a bioinsecticide

Rivera-Ramírez *et al*. (2013) docunented that *B. bassiana* and *M. anisopliae* are pathogens for dog fleas (*Ctenocephalides canis* Shaftesbury, 1934) under laboratory conditions; and Pacheco *et al*. (2015) assessed the pathogenicity of two strains of *B. bassiana* at concentrations of 10, 15 and 20 % mineral oil, sterile water and Tween 80, inoculated by immersion on *C. canis*; it turned out that the treatment formulated at 10 % recorded mycosis of 86.3 % on fleas, confirming that it was the most pathogenic.

Entomologic fungi against forest plagues

There are very few reports in Mexico regarding the use of entomopathogenic fungi to combat forest pests (Table 3). Due to the economic importance of red cedar wood, *B. bassiana* has been one of the most studied fungi against the combat of the meliaceous borer (*Hypsipyla grandell* Zeller, 1898).

Table 3. Main entomopathogenic fungi used for pest control in the forestry sector.

Fungi	Plague	Affected species	Reference
Beauveria bassiana (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokin	*Hypsipyla grandella* Zeller, 1898	*Cedrela odorata* L.	Díaz *et al.*, 2009
B. bassiana (Bals.-Criv.) Vuill.	*H. grandella* Zeller, 1898	*Cedrela odorata* L.	Caballero, 2014
	Meliaceae borer: *H. grandella* Zeller, 1898	Precious woods	Barrios *et al.*, 2017
Trichoderma sp	Bark beetles: *Dendroctonus* spp.	*Pinus* spp.	Gijón *et al.*, 2015
	Bark beetles: *Dendroctonus* spp.	*Pinus greggii* Engelm. ex Parl.	Arriola *et al.*, 2016

Díaz *et al.* (2009) evaluated chemical insecticides such as Novaluron, Pyrethroids, Amitraz (Ovicides) and Carbofuradan, as well as *Beauveria bassiana* and *Metarhizium anisopliae*, an organic insecticide based on Neem (*Azadirachta indica* A. Juss.) and a control against this forest pest. His conclusion was that the treatments with *B. bassiana* and *M. anisopliae* had the same degree of control as the chemicals, with the advantage of not being contaminants and of not representing a risk of toxicity for the personnel that applies.

For Caballero (2014), the inoculation of *B. bassiana* in washed, disinfected and conserved larvae of *H. grandella* in an area with a controlled climate at 22 °C, was achieved as they colonized them , which means that in the laboratory stage they *B. bassiana* was found effective for the control of *H. grandella*. On the other hand, Barrios *et al.* (2017) selected two native strains of B*. bassiana* for use in the control of this pest, and the evaluation of pathogenicity of the two isolates demonstrated mortality on third instar larvae at a dose of 1×10^8. According to the authors, these strains could have a high potential to be used in the nursery or in the field for the integrated management of the borer, since they recorded 92.84 % dead of after five days, on average.

Regarding the applications of *Trichoderma* for the biological control of the pest of debarkers in laboratory conditions, the contribution of Gijón *et al.* (2015) showed, by statistical analysis, that a strain of this fungus caused 100 % mortality of these insects. The following year, Arriola *et al*. (2016) announced their results on the same fungus to combat the same pest in the *Sierra Gorda* Biosphere Reserve, Qro.; there, five treatments were applied to *Pinus greggii* Engelm. ex Parl. trees, which consisted of three concentrations of conidia / milliliter: high 3.6×10^8, average 9×10^7 and low 5×10^7, a control based on a commercial product with *M. anisopliae* (5×10^9) and an absolute water control. The highest concentration was the one that recorded the most severe mortality.

Entomopatogenic nematodes

Entomopathogenic nematodes (NEP) in the *Steinernema* and *Heterorhabditis* genera are potent agents for biological control. Nematodes parasitize their hosts (in this case plague insects) by direct penetration through the cuticle to the hemocele or by penetration through natural openings (spiracles, mouth and anus). The infection can be passive or active, and the way in which the infection process continues will depend on the species of nematode that attacks the insect. In the case of *Steinernema* and *Heterorhabditis*, once the infective juvenile manages to penetrate the hemocele, it releases the associated bacteria, which reproduces in the hemolymph of the host and causes death (Pérez, 2004).

In the last decade, substantial advances have been made in its research and application, since the number of target pests that are susceptible to NEPs has continued to increase (Table 4). The progress is also due to the advances in the technology of its production, which use *in vivo* and *in vitro* systems, and the new methods of application (injections, sprays, etc.), as well as advances in genomics, nematode symbiont -bacteria interactions and ecological relations.

Table 4. Main entomopathogenic nematodes used for pest control in Mexico.

Nematode	Plague	Affected species	Reference
Six nematode species of entomopathogens of *Steinernema* and *Heterorhabditis*	Seven-day larvae, prepupa and pupa of the core worm: *Spodoptera frugiperda* (J.E. Smith)	Maize, golf greens	Molina, 1996
Steinernema carpocapsae Weiser, 1955 (All and Tecomán strains), *Steinernema feltiae* Filipjev, 1934, *S. glaseri* Steiner, 1929 (cepa NC), *S. riobravis* Cabanillas, Poinar & Raulston, 1994 and *Heterorhabditis bacteriophora* Tecomán	Third stage larvae of the Mexican fruit fly: *Anastrepha ludens* Loew, 1873	Plague of several fruit species, in citrus and mango in particular	Lezama *et al.*, 1996b
S. feltiae Filipjev, 1934	Mexican fruit fly: *A. ludens* Loew, 1873	Fruit plagues	Toledo *et al.*, 2001
H. bacteriophora Tecomán	Fruit fly: *Anastrepha obliqua* Macquart, 1835	Mango, plum and guaba	Toledo *et al.*, 2005

In Mexico, *Steinernema carpocapsae* Weiser, 1955 has been declared efficient in the combat of larvae of the third stage of Mexican fruit fly (*Anastrepha ludens* Loew, 1873), which is a pest that attacks especially citrus and mango. Lezama *et al.* (1996b) demonstrated that this pest is susceptible to varying degrees to various tested nematodes. *S. riobravis* Cabanillas, Poinar & Raulston, 1994 and *S. carpocapsae* All strain killed 90 % of the larvae and pupae; *H. bacteriophora* Poinar 1975 NC strain killed 82.5 %; *Steinernema feltiae* Filipjev, 1934, 81.25 %; the S*. carpocapsae* Tecomán strain caused 76 % mortality, while *H. bacteriophora* Tecomán and *S. glaseri* Steiner, 1929, 52.5 %. These results suggest that the *S. riobravis* and *S. carpocapsae* strain species have potential as biological control agents against the fruit fly.

On the other hand, Toledo *et al.* (2001) assessed the parasitic capacity of *S. feltiae* in laboratory conditions on larvae of *A. ludens* of third stage, and pupae of five and 12 days of age, in three soils of different texture and three temperature regimes Their results showed that there was no difference when the nematodes were applied to the soil before or after the larvae. The pupae were not susceptible to attack. In adults who emerged and left through the treated soil, parasitism was only 10 %. Later, in 2005, the same authors studied this plague, but this time using another nematode: *H. bacteriophora* (Toledo *et al.,* 2005). These studies allowed them to describe the effect of temperature, soil texture and depth of the host on the ability of nematode infection in third stage larvae of *Anastrepha obliqua* Macquart, 1835 and, although the potential of the nematode was demonstrated to infect and kill *A. obliqua* larvae, it was evident that there was an important difference in the susceptibility of the six-day-old larvae, compared to those of eight days.

In a study on the control of the maize core worm with nematodes, Molina *et al.* (1996) identified that *S. carpocapsae* strain All, *S. riobravis* and *H. megidis* Poinar, Jackson & Klein, 1988 have potential as biocontrol agents against *S. frugiperda*. The LC_{50} varied from 1.5 to 20.6 and 3.4 to 37.2 mL^{-1} nematodes, for larvae and prepupes, respectively, and in their studies the cumulative mortality in pupae was 5-43 % with the concentration of 100 mL^{-1} nematodes.

Entomopatogenic bacteria

The greatest success in the microbial control of insects in the world has been achieved by *Bacillus thuringiensis* Berliner, 1915 (Bt). The crystals (Cry) that it produces under stress conditions are aggregates of a large protein (130-140 kDa) that is not really active in itself (it is a protoxin) as it is insoluble. When protoxin is subjected to very basic conditions (pH > 9.5) such as those existing in the intestines of some insects, it is solubilized and transformed by means of the insect proteases into an active toxin of about 60 kDa. This is the toxin known as "δ-Bt endotoxin", which acts by binding to receptors of epithelial cells of the intestine of the insect, which leads to the formation of pores and osmotic lysis of the cells that finally they cause their death (Galitsky *et al.,* 2001).

At present, the gene responsible for the production of this endotoxin has been introduced into tobacco, corn, tomato, cotton, potato, beet and cabbage plants, among other crops, giving rise to genetically modified (GMO) crops. The wide acceptance of GM crops, especially in the United States of America, has increased the yield per hectare and the income of farmers in these countries. The first success achieved in this field was obtained in 1987 when it was possible to produce transgenic tobacco plants capable of producing by themselves a toxin of the bacteria *B. thuringiensis*, which had lethal effects for certain phytophagous insects (Rubio and Fereres, 2005).

Some entomopathogenic bacteria have been developed for the control of commercial scale insect pests, among which the subspecies of *Bacillus thuringiensis, Lysinibacillus sphaericus* Neide, 1904, *Paenibacillus* spp. and *Serratia entomophila* Grimont. The subspecies *B. thuringiensis kurstaki* is the most commonly used for the control of insect pests of crops and forests, and the subspecies *israelensis* and *L. sphaericus* of *B. thuringiensis* are the main pathogens used for the control of pests of medical importance (Ponce *et al.*, 2003).These pathogens combine the advantages of chemical pesticides and biological control agents: they are fast acting, easy to produce at a relatively low cost, easy to formulate, have a long shelf life and allow delivery using conventional application equipment and systems systemic *(i.e.* in transgenic plants) (De la Rosa *et al.*, 2005; Camacho *et al.*, 2017; García *et al.*, 2018).

There is very little experience on the use of bacteria for pest control In Mexico, and these are limited to the use of *Bacillus thuringiensis* for tobacco worm control, the sugarcane borer worm and the coffee drill in the agricultural sector (Table 5), and for control of the mosquito vector of the *dengue* virus.

Table 5. Main entomopathogenic bacteria used for pest control in Mexico.

Bacteria	Plaguea	Species/ crop	Reference
Bacillus thuringiensis Berliner, 1915	Coffee drill: *Hypothenemus hampei* Ferrari	Coffee	De la Rosa *et al.*, 2005
	Boring worm: *Diatraea considerata* Heinrich, 1931	Sugar cane	Camacho *et al.*, 2017
	Tobacco worm: *Manduca sexta* Linnaeus, 1763	Tobacco	García *et al*., 2018
Bacillus thuringiensis Berliner var. *kenyae*	Lepidóptera species, one Coleóptera and one Díiptera	Tobacco and cabagge worm	Barboza *et al.*, 1998
B. thuringiensis Berliner var. *israelensis* Barjac	*Dengue's* vector: *Aedes aegypti* mosquitoes Linnaeus,1762	*Dengue's* vector mosquitoes	Ponce *et al*., 2003

In the case of the coffee drill (*Hypothenemus hampei* Ferrari, 1867), its most susceptible stage was its first larval instar, with an average lethal time average of 6.4 ± 1.8 days (De la Rosa *et al*., 2005). For the sugarcane borer worm (*Diatraea consideta* Heinrich, 1931), Camacho *et al*. (2017) managed to isolate eight strains of dead insects in agricultural fields, which were from *B. thuringiensis* and observed a high mortality with the strains of interest.

From different tobacco plants obtained from southeastern Mexico, García *et al* (2018) made isolates, from which they selected bacterial colonies of *Bacillus thuringiensis*, which caused 100 % mortality of *Manduca sexta* Linnaeus larvae, 1763 at 96 h of exposition. In other studies against tobacco pests the toxicity of *Bacillus thuringiensis* ssp Kenyae was demonstrated against eight species of Lepidoptera, one of Coleoptera and one of Diptera (Barboza *et al.*, 1998).

On the other hand, against the control of the mosquito vector of the *dengue* virus (*Aedes aegypti*), in 2003 the bioinsecticide Vectobac 12 AS was formulated based on *B. thuringiensis* var *israelensis* Barjac, 1978 (Bti). The product was applied in pipe trucks that deliver water to several communities in the metropolitan area of *Monterrey*, NL; in this way, the people received it with the means to interrupt the biological cycle of the virus. Bti proved effective as a larvicide against *A. aegypti* even in the presence of chlorine in the water. However, the results showed that the efficiency of Bti applied in pipes was reduced mainly due to water temperature, larval density, sunlight and the effect of association with filter organisms (Ponce *et al.,* 2003).

Entomopathogenic virus

Insect pathogenic viruses are an important source of microbial control agents, particularly for the control of lepidopteran pests. Baculoviruses are accepted as safe, easily mass-produced, highly pathogenic and easily formulated and applied control agents. New baculovirus products are appearing in many countries and gaining greater market share. However, the absence of a practical *in vitro* mass production system, higher production costs, limited persistence after application, slow death rate and high host specificity contribute to its restricted use in control Of pests. Overcoming these limitations are key research areas for which progress could open the use of insect viruses to much larger markets. The Baculoviridae family is the most numerous and studied of entomopathogenic viruses. The use of the *Anticarsia gemmatalis* Hübner 1818 NPV nucleopoliedrovirus, (AgMNPV) to control *A. gemmatalis* in soybeans in Brazil was a successful program and was considered the most important in the world (Nava, 2012).

Biopesticides based on entomopathogenic organisms

Currently, there are several companies mainly in Holland, France, Italy, Great Britain and Russia, which sell products for biological control with entomopathogenic organisms (Rubio and Fereres, 2005). The fact of marketing products with living organisms has its limitations, among which stand out:

- Problems with patents (since living organisms are not patented, a specific use of them can be patented, but the difficulty lies in the fact that commercializing a living organism makes it easy for anyone to use it as a starter culture to multiply or replicate the product and this cannot be controlled).
- High production costs, since specialized labor is required with the consequent increase in the final product. This means that biocontrol agents generally have a higher price than chemical pesticides.

- Short shelf life of the product, this is because ultimately they are living organisms that require a certain temperature and humidity to survive.
- Too specific in some cases, which requires the use of several different biocontrol agents to control pests that usually appear in the same crop. Instead, chemical pesticides usually control several pests at the same time.
- Complex application as it requires qualified personnel.

Until almost 10 years ago, the commercial production of bioinsecticides and other biological control agents in Mexico was carried out in at least 68 companies and 25 states, although these numbers have now increased; they reproduce entomopathogenic fungi (mainly *Beauveria bassiana* and *Metarhizium anisopliae*; entomopathogenic bacteria (*Bacillus thuringiensis*) and nematodes (*Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. These microorganisms are the basic active ingredients in the formulation of bioinsecticides (Table 6); carrier, an inert material as a support, and adjuvants, as well as compounds that promote and maintain the viability of the active ingredient and protect it from UV radiation, rain, moisture and dehydration, which facilitates its handling, application and effectiveness (García and Mier, 2010).

Table 6. Main Mexican companies that sell bioinsecticides based on entomopathogenic organisms.

Company	Products based on:	Location of the company by state
Agrobiológicos del Noroeste, S. A de C.V.	*Beauveria bassiana* (Bals.-Criv.) Vuill, *Metarhizium anisopliae* Metchnikoff Sorokin var. *anisopliae, Isaria fumosorosea* Wize, *Lecanicillium lecanii* (Zimm.) Zare & W. Gams., *Paecilomyces lilacinus* (Thom) Samson and *Trichoderma harzianum* Rifai	*Sinaloa*
Bioagris	Biological fungicides with spores of *Trichoderma viride* Pers, or *Beauveria bassiana* (Bals.-Criv.) Vuill, or *Metarhizium anisopliae anisopliae* (Metchnikoff) Sorokin or spores of *Paecilomyces lilacinus* (Thom) Samson	*Ciudad de México*
Bioamin	Biological insecticides based on spores of *Beauveria bassiana* (Bals.-Criv.) Vuill, *T. harzanium* Rifai and *T. viride* Pers, *Bacillus thuringiensi* Berliner and entomopathogenic fungi of the *Paecilomyces* genus	*Saltillo, Coahuila*
Biotecnologia Agroindustrial	*Bacillus thuringiensis, B. thuringiensis* Berliner and three entomopathogenic fungi, *Subtilis*, *Trichoderma* and *Bacillus subtilis* (Ehrenberg)	*Morelia, Michoacán*
Bio- Zentla	*B. bassiana* (Bals.-Criv.) Vuill, *M. anisopleae* (Metchnikoff) Sorokin, micorrhyzae, *Paecilomyces fumosoroseus* (Wize) Brown & Smith	*Zentla, Veracruz*
Desarrollo Lácteo, S.P.R de R.L	*Spalangia endius* Walker, *Trichogramma pretiosum* Riley, *Chrysoperla carnea* Stephens, *B. bassiana* (Bals.-Criv.) Vuill, *I. fumosorosea* Wize, *M. anisopliae* Metchnikoff Sorokin *var. anisopliae* and *T. harzianum* Rifai	*Gómez Palacio, Durango*
EcoAgro	Biological plagues control, bioinsecticides and biofertilizant production, bioecológicos procedures applied to agriculture	*Sinaloa*
FMC Agroquímica de México S. de R. L. de C.V.	Agrochemical products in México and Latin America. Biofungicide based on a very singular bacterial strain that belongs to *B. subtilis* (Ehrenberg)	*Zapopan, Jalisco*
Grupo Solena	*Azospirillum brasilense* Tarrand, Krieg & Döbereiner, *B. subtilis* (Ehrenberg), *B. thuringiensis* Berliner, *B. bassiana* (Bals.-Criv.) Vuill, *I. fumosorosea* Wize, *L. lecanii* (Zimm.) Zare & W. Gams., *M. anisopliae* (Metchnikoff) Sorokin, *Paecilomyces lilacinus* (Thom) Samson, *Rhizobium* sp., *Streptomyces* spp., *T. harzianum* Rifai	*León, Guanajuato*
Microvida Innovación Agrícola S.A de C.V.	*A. brasilense* Tarrand, Krieg & Döbereiner, *Azotobacter* sp., *B. subtilis* (Ehrenberg), *B. thuringiensis* Berliner var. *kurstaki, B. thuringiensis* Berliner var. *aizawai, B. thuringiensis* Berliner var. *israeliensis, B. bassiana* (Bals.-Criv.) Vuill, *Glomus intraradices* Błaszk, Wubet, Renker & Buscot, *M. anisopliae* (Metchnikoff) Sorokin, *Pseudomonas fluorescens* Flügge, *T. harzianum* Rifai	*Morelia, Michoacán*
Minerales y nutrientes Plantifor	*Bacillus megatherium* de Bary, *B. subtilis* (Ehrenberg), *Beauveria bassiana* (Bals.-Criv.) Vuill, *Metarhizium, Trichoderma* spp.	*San Luis Potosí*
Organismos beneficos.com	Entomopathogenic fungi	*Jalisco*
Profertinnova	Natural bioinsecticide for biological control 100 % organic made from entomopathogenic fungi (*Metharizium* sp. and *Verticillium* sp.).	*Estado de México*
Profungi	Entomopathogenic fungi, biological insecticides	*Sinaloa*
SummitAgro México	Biological insecticides based on *Bacillus thuringiensis* Berliner var. kurstaki serotipo 3a, 3b and *Bacillus subtillis* (Ehrenberg)	*Ciudad de México*
Tierra de Monte	*Beauveria bassiana* (Bals.-Criv.) Vuill,*Metharhizium, Paecilomyces, Thricodherma,* entomopatogenic microorganism concentration and stimulators of the vegetal immune system	*Querétaro*
Ultraquimia Agrícola, S.A de C.V.	*B. bassiana* (Bals.-Criv.) Vuill, *I. fumosorosea* Wize, *L. lecanii* (Zimm.) Zare & W. Gams., *Metarhizium anisopliae* Metchnikoff Sorokin var. *acridium* and *M. anisopliae* Metchnikoff Sorokin var. *anisopliae, P. lilacinus* (Thom) Samson,*Trichoderma* spp., *Bacillus subtilis* (Ehrenberg) and *B. thuringiensis* Berliner	*Morelos*
Valent de México	Bioinsectcides based on *Bacillus thuringiensis* Berliner ssp. kurstaki	*Zapopan, Jalisco*

The regulations in Mexico (NOM-032-FITO-1995) (Sagarpa, 2015) indicate that for the registration and commercialization of bioinsecticides, official provisions must be complied with and the opinion of different institutions that regulate crop health, the risk of the substances that are used as pesticides must be met, as well as care for the environment and human health (García and González, 2013). Likewise, it is necessary to carry out biosecurity studies and risk of the environmental impact of the agents used in biological control (qualitative and quantitative evaluation), environmental monitoring of these organisms and assessment of their effect on non-target organisms.

Conclusions

Based on the impact of climate change, the intense exploitation in agricultural, livestock and forestry production systems, the use of extensive monocultures without management in forests and worldwide commercial opening, it is necessary to promote biological control of pests (bioinsecticides and biological agents with entomopathogenic organisms) as well as assessing the environmental impact of these products with more tests under field conditions to identify the effects of biotic and abiotic factors on efficacy and persistence, since these are also useful to agriculture, they favor human health and the environment. On the other hand, it is also essential for these new biological products to design more efficient production, formulation and mass delivery systems to supply a growing market.

From the review carried out in this work, it can be concluded that as in the rest of the world, studies of entomopathogenic fungi for combating agricultural, livestock and forest pests abound in Mexico. Special attention has been given to *Beauveria bassiana* and *Metarhizium anisopliae* in recent years, since its toxicity has been very effective against a broad spectrum of pests, in addition to the fact that stable commercial products with mixtures of entomopathogenic organisms have been developed to enhance their insecticidal action. However, further studies are required to overcome the current difficulties related to the production and development of these bioinsecticides.

To a much lesser extent, nematodes, bacteria have been addressed and very little interest has been given to viruses against pests, so it is suggested to promote these lines of research and their use in integrated pest management programs; in particular, strategies that incorporate entomopathogenic organisms in combination with predators and parasitoids should be defined to ensure compatibility and maximize their effectiveness.

Conflict of interests

The authors declare no conflict of interests.

Contribution by author

Ma. de Lourdes Pacheco Hernández: literature review and writing of the manuscript; J. Francisco Reséndiz Martínez and Víctor J. Arriola Padilla: review and correction of the manuscript.

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