

Secondary Metabolites of Entomopathogenic Fungi, Biological Alternative for the Control of Agricultural Pests and Disease: Present and Perspectives

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Abstract

The use of entomopathogenic fungi (EF) in recent years has been highly effective against the different orders of insects considered pests of agricultural importance and their conidia have been commonly applied, but it has been reported that these are sensitive to the environmental conditions. For this reason, biopesticides products have been formulated based on secondary metabolites, recently. These biomolecules participate as biological control agent, such as: cyclic depsipeptides, amino acids, polyketides, polyphenols and terpenoids, affecting their morphology, life cycle and insect behavior. The use of secondary metabolites of entomopathogenic fungi opens the possibility of application in a more efficient way for the control of agricultural pests in a compatible with the environment and human health; therefore, it is important to know, analyzing the type of molecules, their effects, and their different methods of application.

Keywords

Secondary Metabolites, Entomopathogenic Fungi, Biological Control Agent

1. Introduction

Entomopathogenic fungi (EF) have shown that they are used efficiently as bio-

logical control agents, since they are responsible for causing diseases in insects [1]. Until now, more than 90 genders and 700 species of EF have been described and the isolation of new strains continues, among the most used are: *Metarhizium anisopliae* with 33.9%, *Beauveria bassiana* with 33.9%, *Cordyceps (=Isaria) fumosorosea* with 5.8% and *Beauveria brongniartii* with 4.1% [2] [3]. EF present a life cycle marked by a stage at which conidia are formed as a form of asexual reproduction, in which fungi remain until adhere to the insect's cuticle [4]. According to [5], its pathogenicity mechanism for EF consists of germination, and penetration by mechanical and enzymatic action, mainly by proteases, lipases and chitinases, affecting the host cuticle. Later, [6] mentioned that a germ tube is formed and quickly reaches the hemocoel, and the mycelium of the fungus is transformed in blastospores, which evade the insect's immune system, producing toxins, such as secondary metabolites (SM), such as depsipeptides, flavonoids, terpenes, polyketides. SM does not participate directly in the growth of the insect, otherwise, with their survival. SM cause alterations in the epithelial cells of the trachea, disable the mechanisms of the immune system and interrupt the physiological processes of the host, reduce feeding rates and the weight of some insect larvae, damage the nervous system, and they reduce their capacity to defend from the insect [7] [8] [9]. Conidia from EF are generally used in pest control, but EF has a high sensitivity to the variation of climatic conditions such as extreme temperatures and ultraviolet light, and also to their infection time, which is long compared to chemical products [10]. Therefore, there is a potential for formulating new bioinsecticides for the control of agricultural pests from these metabolites [11]. SM can be used together with conidia and thus reduce the time of infection in insects. Therefore, the objective of this review is to analyze the advances in research on the SM produced by EF and the potential they have as biological control agents.

2. Secondary Metabolites of Entomopathogenic Fungi

2.1. Secondary Metabolites

EF produce a wide variety of organic molecules that have no direct function in primary metabolic processes, such as nutrient assimilation, protein, carbohydrate or lipid synthesis, and which are referred to as SM, which are important for the survival and interaction of the organism with its environment [6]. SM serve as: organisms used against other bacteria, fungi, plants, and insects, metal transporters, and symbiosis agents [12]. [13] report that cordycepin (a), ergosterol (b) and polysaccharides (c) (**Figure 1**) are identified as the main metabolites of entomopathogens. On the other hand, other types of compounds have been reported, such as: polyphenolics with functional properties found in the plant and fungal kingdom, such as terpenes, flavonoids and alkaloids [14]. For EF, protein synthesis is essential and ribosomes participate in an important way in this process. However, there are SM called non-ribosomal peptides, which unlike ribosomes, are independent of messenger RNA and among these are polyketides,

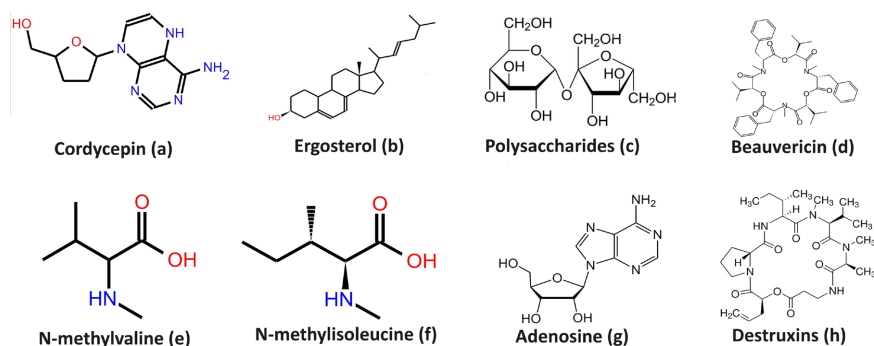


Figure 1. Cordycepin, ergosterol, polysaccharides and depsipeptides produced by entomopathogenic fungi.

non-ribosomal depsipeptides [15] [16] [17]. Several EF species such as *Beauveria spp.*, *Lecanicillium lecanii*, *C. fumosorosea* and *M. anisopliae* are capable of producing organic acids and some non-peptidic pigments, where these have participated in the infective process against insects considered pests; These compounds have been described as an infectivity factor and it has also been suggested that they may be an element that contributes to the solubilization of the cuticular protein [18].

2.2. Classification of Secondary Metabolites

The template is used to According to their chemical structure, they can be divided into the following groups: cyclic depsipeptides (cyclic tetradepsipeptides and cyclic hexadepsipeptides), peptides (octadepsipeptides, dipeptides and depsipeptides), amino acid derivatives, polyketides, polyphenols, terpenoids as shown in **Figure 1** [19].

a) Cyclic depsipeptides: [20] define cyclic depsipeptides, as a family of cyclic peptide-related compounds, whose ring is mainly composed of amino acid and hydroxy acid residues linked by amide and ester bonds (at least one), resulting in a wide diversity of chemical structures. Beauvericin (d) (**Figure 1**) present in *B. bassiana* and *Fusarium spp.*, is a recognized mycotoxin that according to [21] forms a cyclic hexadepsipeptide composed of three molecules of N-methyl phenyl alanine and three molecules of 2-hydroxy isovaleric acid and its synthesis is carried out by a non-ribosomal mechanism specific to fungi using the beauvericin synthetase enzyme complex, which has the ability to increase the permeability of cell membranes to ions causing cell death [22] [23]. These have antibacterial, antifungal, cytotoxic and insecticidal activities, possess low toxicity in humans and do not need special requirements for their use [24]. [25] [26] described that beauvericin belongs to a new group of emerging mycotoxins mainly composed of enniatins (EN) and these are divided into different classes: ENA, ENA1, ENB, ENB1 and BEA, the latter being of most recent interest; the difference between enniatins is due to the amino acid composition and presence of functional groups in different positions, similarly in type A and B EN they are composed of: N-methylvaline (e) or N-methylisoleucine (f) (**Figure 1**) or mix-

tures of these two amino acids, with a wide range on biological activities. Destruxins (h) (**Figure 1**) are cyclic hexadepsipeptides, isolated mainly from *Metarhizium*, more than 40 types have been reported, of which A, B and E are the most significant in the pathogenesis process and are well known for their insecticidal, fungicidal, and bactericidal activity [27]. Two bioactive compounds have been detected from *C. militaris*, cordycepin (3'-deoxyadenosine), which is an important bioactive compound and has a broad spectrum of biological activity, on the other hand, it has also been reported to produce adenosine (g) (**Figure 1**), which is a nitrogenous base and acts as a cellular nucleoside, which is necessary for the various molecular processes in cells, such as DNA and/or RNA synthesis [28] [29] [30].

b) Alkaloids: Fumosorinone (a) (**Figure 2**) is a 2-pyridone alkaloid isolated from *Cordyceps* (= *Isaria*) *fumosorosea*, it is a potent non-competitive inhibitor of the protein tyrosine phosphatase which is essential in the regulation of cell function in eukaryotes [31] [32]. [33] mentions that in *Cordyceps farinosa*, new yellow pigments, farinosones A (f) and B (g) together with farinosone C (h) (**Figure 2**), a metabolite derived from an early step in the biosynthesis of pyridine alkaloid (b) (**Figure 2**), were isolated. The alkaloid (+) N-deoxy militarinone A was reported by [34] which was extracted from this same fungus, besides three new pyridone alkaloids were isolated from *Paecilomyces militaris* which were: militarinone A, B and D (c, d, e) (**Figure 2**), having a pharmaceutical activity, as well as a biological control agent presenting changes in the host [35] [36].

c) Flavonoids: Flavonoids are MS with broad health-promoting insecticidal use and as biocatalysts, in nature they are mainly found in the form of glycosides [37]. EF are effective in the biotransformation of flavonoids, as demonstrated by [38] obtaining 2'-hydroxy-5'-methyl chalcone 3-O- β -D-(4''-O-methyl)-gluco pyranoside from *B. bassiana* and *C. fumosorosea*, also in the latter, flavonones were detected (a) (**Figure 3**) such as 6-methyl 4'-O- β -D-(4''-O-methyl)-gluco pyranoside and 2-phenyl-6-methyl chroman 4-O- β -D-(4''-O-methyl)-gluco pyranoside.

d) Terpenoids and steroids: Stigmasterol (b) (**Figure 3**), a phytosterol with a side chain containing ten carbon atoms attached to the steroid backbone, was

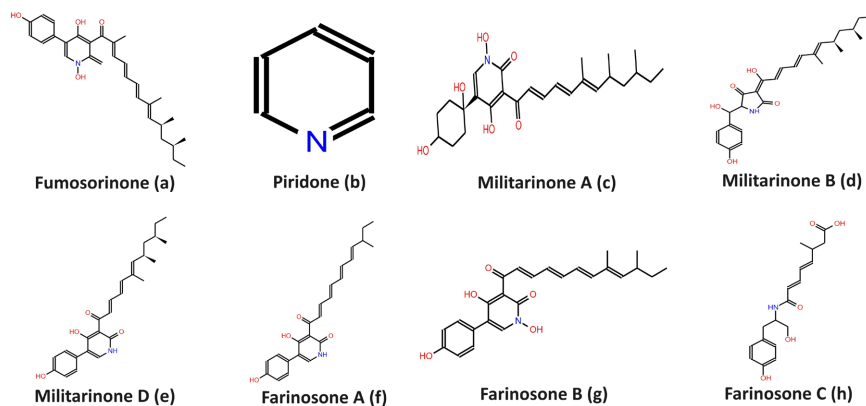


Figure 2. Alkaloids produced by entomopathogenic fungi.

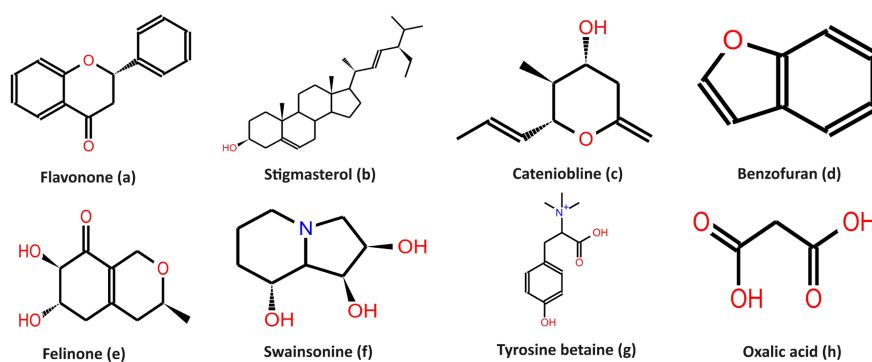


Figure 3. Flavonoids, terpenoids and organic acids produced by entomopathogenic fungi.

isolated from *Paecilomyces* sp. from marine sponge [39]. On the other hand, [40], report the terpenoid, cateniobline C (c) (**Figure 3**) causing an inhibitory effect on the growth in larvae of *Helicoverpa armigera* [41]. In addition, [32] carried out an analysis of different terpenoids reported in the literature, in which they mention that the benzofuran meroterpenoid was isolated from *Cordyceps annulata*; Benzofurans (d) (**Figure 3**), felinone (e) (**Figure 3**) and chromene meroterpenoids were isolated from *Cordyceps felina*, and they also mention that in *B. bassiana* the following alkaloids were identified: swainsonine (f) and tyrosine betaine (g) (**Figure 3**). Similarly, [42] reported the terpenes: β -elemene, α -chamigrene and β -bisabolene found in *C. fumosorosea*.

e) Organic acids: In *B. bassiana* contains an oxaloacetate hydrolase enzyme in which, through the cytoplasmic way and the mitochondrial pathway, produces oxalic acid (h) (**Figure 3**), which has been reported to have effects involved in the acidification of insect tissues; it also participates in the sequestration of metallic ions such as calcium, manganese, magnesium and iron; participating in the inhibition or reduction of the host defensive system [43] [44] [45]. Another important compound produced by *Cordyceps* spp. and *M. anisopliae* is dipicolinic acid (a) (**Figure 4**) also known as: 2,6-pyridinedicarboxylic acid, where it modifies its pH by acidifying it to allow a greater robustness in the growth of HE conidia as well as, it reduces the sensitivity to ultraviolet rays and finally it has been reported to possess insecticidal properties against the Diptera family. Its mode of action involves cation coordination through the formation of a ligand with three anions that can act as an enzyme inhibitor by subtracting essential ions from metalloenzymes [46] [18] [47] [48].

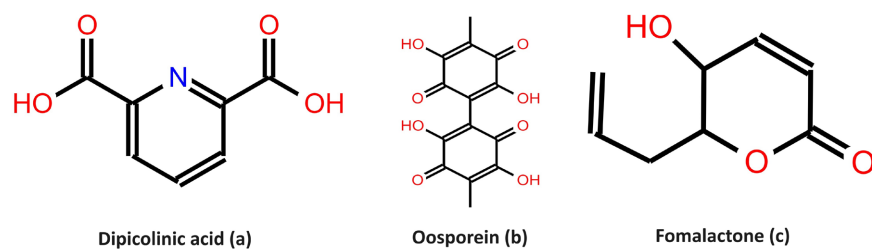


Figure 4. Metabolites produced by entomopathogenic fungi.

f) Non-peptide and polyketide pigments: In the same way, *B. bassiana* is well known to produce a diverse variety of biologically active DMs including non-peptidic and polyketide pigments such as: oosporein (b) (Figure 4), bassianin and tenellin [49], the former is a red benzoquinone, which can oxidize proteins and amino acids showing a broad insecticidal, antifeedant and immunosuppressive effect, tested on adults of *Hylobius abietis* (Linnaeus, 1758) and larvae of *Galleria mellonella* (Linnaeus, 1756), on the other hand, bassianin and tenellin affect cuticular membrane ATPases, performing important pathogenesis functions [50] [51].

3. Secondary Metabolites and Its Use as a Biological Control Agent

Secondary metabolites have a broad toxic effect on hosts as it causes severe damage to fatty tissues and organs [52], another reported symptom is the outflow of ions due to the loss of fluids through the peritrophic membrane, causing cellular dehydration through pores, in the same way it has been reported convulsions lack of coordination, altered behavior and paralysis in the host [53], the latter is caused as a consequence of the contraction of insect muscles through muscle depolarization due to the opening of Ca²⁺ channels in the membrane that slows growth [54], also these molecules have effects on nutrient intake in the host presenting a loss of appetite [54] [55]. On the other hand, extracts of phytopathogenic fungi such as: *Fusarium oxysporum* and *Fusarium solani* have insecticidal effect on different orders of insects among them: Hemiptera, Lepidoptera, Coleoptera, Hymenoptera, Blattodea, Diptera, Thysanoptera and Orthoptera, thus finding the greatest effect (96.6%) in the orders: Coleoptera and Diptera [56] [57], Therefore, they have insecticidal effects and can be a form of biological control of insects with a broad spectrum.

3.1. Diptera

Different concentrations of SM belonging *M. anisopliae* have been applied to control larvae of *Anopheles stephensi* (Liston, 1901), *Aedes aegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (Say, 1823), observing a mortality of 85 to 97% from 24 hours after application, having an effect of these SM against these insects [58]. It has also been showed that extracts of *Lecanicillum attenuatum* isolated from soil with high infective capacity, can be applied against larvae of *Aedes albopictus* (Skuse 1895), in the same way it presents negative physiological effects on the same stage of *Plutella xylostella* (Linnaeus, 1758) interfering in its development: such as the inability to withdraw from the head capsule, causing it to not completely remove from its old cuticles [59] called this behavior as insecticidal effect, which is manifested through lack of appetite, inhibition in growth, development and oviposition of the insect. On the other hand, SM of phytopathogenic fungus *F. oxysporum* have also been tested for the control of larvae and pupae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* under laboratory conditions; where damage has been detected in the intestinal tissue epithelial

cells, adipose tissue and muscles [60]. And phytopathogenic fungus *Ganoderma applanatum* has also been used under laboratory conditions for the control of larvae of these same insects, reporting a mortality of 50% after 24 hours of application, observing damage in the epithelial cells of the midgut and damage to muscle tissues in insects [9]. Another method commonly applied for the control of some biopesticides is the use of organic biopreparations such as whey and grain liquor, complemented with *B. bassiana* extract for adults of adults *Drosophila melanogaster* (Fallén, 1823) intensifying its effectiveness, being able to observe 100% mortality two days after application [61]. It can be observed that the synergistic use of these organic biopreparations with the extract of this fungus has a faster effect for the control of this pest.

3.2. Lepidoptera

Cordyceps (= *Isaria*) SM exhibit nematicidal, bactericidal, antiparasitic and insecticidal effects, as is the case of Oosporein (b) (Figure 4), which has been used for the control of hemiptera and lepidopteran insects, such as aphids and whiteflies [62]. Furthermore catenobline (s) (Figure 3) and fomalactone (c) (Figure 4) in particular, have effects on the growth of *H. armigera* (Hübner, 1805), as demonstrated by [63]. The effect of cordycepin (a) (Figure 1), SM produced by *Cordyceps militaris* tested on *Galleria mellonella* (Linnaeus, 1756), has been analyzed and found to cause a significant decrease in the mean survival time of the insect [40]. Similarly, crude extracts of *B. bassiana* have been tested on this same insect, producing a 55% larval death, after 96 hours of application [64]. The use of conidia has been the most common for biological control, but the effectiveness of applying extracts of EF has been analyzed, as is the case of the comparison between them by applying them on larvae of two species of *Spodoptera* (Guenée, 1852) under laboratory conditions, where 90% mortality can be obtained with the extract and 30% mortality with conidia for both species after 72 hours of application [15]. And when testing only the SM of this fungus on larvae of *Spodoptera exigua* (Hübner, 1808) and *S. frugiperda* (Walker), a mortality of 26% and 44% respectively was obtained after 7 days of application [65]. The use of fungal extracts is a mixture of several toxins that can produce a potential insecticidal effect together with traditional application of conidia [66].

3.3. Hemiptera

It has been reported that the application of *B. bassiana* extracts has a wide effectiveness for the control of *Bemisia tabaci* (Gennadius, 1889), showing a significant reduction in the population of eggs and nymphs stages [11]. Similarly, extracts of *Lecanicillum lecanii* and *B. bassiana* have also been tested on of *Planococcus citri* (Risso, 1813) nymphs *in vitro*, where it was observed that their extracts produce an insectistatic effect in *P. citri* such as: loss of appetite and poor mobility [6]. On the other hand, MS of *B. bassiana* have been tested of *Aphis gossypii* (Glover, 1877), showing 100% mortality on the third day of application [67]. Similarly the effect that of SM produced by *B. bassiana* on the cellular im-

immune defenses of *Eurygaster integriceps* (Puton, 1881), has been shown to inhibit the phagocytic activity of *E. integriceps* hemocytes and hinder nodule formation and it was also observed that these toxins deactivate several immune mechanisms allowing the fungus to overcome and then kill its host [63]. Dipicolinic acid produced by *C. fumosorosea* from a 6-day supernatant has also been reported to have a toxic effect, an example of which is the mortality observed against third instar nymphs of *B. tabaci* in the first 24 to 48 hours [46] [68].

3.4. Trombidiformes

The combination of plant SM and EF have a wide potential for control of some mites, as demonstrated with the use of 1-chloro octadecane and *Metarhizium anisopliae* extract on *Oligonychus afrasiaticus* (Berlese, 1886), obtaining 100% mortality after 15 days of application, demonstrating their synergy, reducing the mortality time and increasing the insecticidal capacity with this combination [41].

3.5. Prostigmata

The effect of beauvericin against *Tetranychus urticae* (CL Koch, 1836) has been tested with 100% mortality using two different concentrations against motile stages, and it was also found that this toxin was able to inhibit egg hatching up to 69.3 % under laboratory conditions [23].

3.6. Coleoptera

Oxalic acid produced by *B. bassiana* has been found to have insecticidal effects against adults of *Alphitobius diaperinus* (Panzer, 1797), obtaining a mortality of 50.39% [48]. On the other hand, it has been seen that the application of beauvericin (d) in adults of *Hypothenemus hampei* (Ferrari, 1867) negatively affects the insect and blocks its response mechanisms, in the same way it progressively degenerates the tissues, structural changes of the membranes are observed causing changes in the electrical activity of the nerves in the host and this is caused by the increase of oxygen consumption in an attempt of the insect to restore itself [46].

3.7. Nematodes and Others

Beauvericin was shown to have effective insecticidal activity against *Bursaphelenchus xylophilus* (Steiner and Buhner, 1934), with 46% mortality [25]. Similarly extracts of *B. bassiana* are reported to have a broad effect against *Caenorhabditis* sp. (Dougherty, 1955) obtaining a mortality of 100% at 48 hours of application, it was also tested on *Glycines heterodera* (Ichinohe, 1952), obtaining the same percentage of mortality at the same time of application, the same was applied in *Aphelenchoides besseyi* (Christie, 1942), where a mortality of 64.8% was observed in the first 48 hours and finally at the same hours a mortality of 99.7% was found in *Meloidogyne incognita* [62]. Oosporein and beauvericin, besides having an insecticidal effect, also limit the growth of pathogens in plants *in vitro*, so it is inferred that they have fungicidal effects [69]. In the same way, it has been reported

that the use of *B. bassiana* extract works as a plant growth factor, for example, increasing the SM production of *Allium schoenoprasum* (Linneo) [70].

4. Conclusions and Perspectives

As previously observed, the highest mortality is obtained when HE extract is applied, and this is due to the fact that this extract contains several toxic molecules in which both enzymes and MS participate, thus increasing toxicity and accelerating mortality. The metabolites of entomopathogenic cannot be synthesized *in vitro* because they are the result of a synthesis by metabolic pathways or by non-ribosomal synthesis peptides [71]. It is important to know how SM are formed and what is their classification, therefore, this review helps us to study the toxins produced by EF, analyzing the different strategies for their application, obtaining highly significant results, having a wide insecticidal capacity both in the larval stage and in insect pupae, as having an insectistatic effect. This effect causes the insects to have changes in their behavior and/or morphology, in which they do not develop normally as if they were 100% healthy, therefore, they become clumsy and thus, susceptible to being prey of other insects, on the other hand, the effect on their behavior is noticeable in their feeding when they stop eating and die. In various studies, it has been shown that the use of conidia and metabolites improves biological control of pests but it depends on the type of pest and its stage [58] [72]. At this time, the production of EF extracts in solid state fermentation maybe it was to be scaled using tray bioreactor and production costs are low because used waste agricultural and marine. Greenhouse evaluation has been carried out using metabolites to control the larvae of *Bradysia impatiens* in *Pinus montezumae* showing that there was no damage to the seedlings [73]. To combat pests in different crops, producers have resorted to the use of organic biopreparations, but by using the extract of an EF in a synergistic way, it increases the infectivity for insects of the order of diptera, so the panorama and a wide possibility of use, is friendly to the environment, as well as to its applicators.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Gandarilla-Pacheco, F.L., Elías-Santos, M., Del Socorro Flores-González, M., De Jesús De Luna-Santillana, E. and Quintero-Zapata., I. (2018) Virulence of Blastospores of *Isaria fumosorosea* Native to Northeastern Mexico on *Anastrepha ludens*

- (Diptera: Tephritidae). *Colombian Journal of Entomology*, **44**, 187-192.
<https://doi.org/10.25100/socolen.v44i2.7316>
- [2] González Baca, G., Venegas Barrera, C.S., González Gaona, O.J., Vargas Madriz, H., Jiménez Gómez, M.A., Pérez Arriaga, E. and Domínguez Azuara, A. (2019) Abundance and Distribution of Entomopathogenic Fungi in Different Locations and Environments in Southern Tamaulipas. *Mexican Journal of Agricultural Sciences*, **10**, 669-681. <https://doi.org/10.29312/remexca.v10i3.1550>
- [3] Alcantara-Vargas, E., Espitia-López, J., Garza-López, P.M. and Angel-Cuapio, A. (2020) Production and Quality of Conidia of Strains of Entomopathogens of the Genus *Metarhizium anisopliae*, Isolated in Agricultural Areas of the State of Mexico. *Mexican Journal of Biodiversity*, **91**, e912912.
<https://doi.org/10.22201/ib.20078706e.2020.91.2912>
- [4] Vara, S., Karnena, M.K., Dash, S. and Sanjana, R. (2020) Entomogenous Fungi and the Conservation of the Cultural Heritage. In: Yadav, A., Rastegari, A., Gupta, V. and Yadav, N., Eds., *Microbial Biotechnology Approaches to Monuments of Cultural Heritage*, Springer, Singapore, 41-69.
https://doi.org/10.1007/978-981-15-3401-0_4
- [5] Cepeda-Siller, M., Garrido Cruz, F., Castro Narro, E., Sánchez Peña, S.R. and Dávila Medina, M.D. (2018) *In vitro* Infection of Strains of *Beauveria* spp. on *Globodera rostochiensis* Wollenweber (1923). *Acta Universitaria*, **28**, 25-30.
<http://doi.org/10.15174/au.2018.1714>
- [6] Avalos Vela, K.L. and Wilson-Kugg, J. (2015) Effect of *Lecanicillium lecanii* and *Beauveria bassiana* on *Planococcus citri* under Laboratory Conditions. *Rebiolest*, **1**, 63-70.
- [7] Ali, S., Ren, S. and Huang, Z. (2014) Extracellular Lipase of an Entomopathogenic Fungus Effecting Larvae of a Scale Insect. *Journal of Basic Microbiology*, **54**, 1148-1159.
<https://doi.org/10.1002/jobm.201300813>
- [8] Mi Woo, R., Gu Park, M., Young Choi, J., Hwan Park, D., Young Kim, J., Wang, Minghui., Ji Kim, H., Dong Woo, S., Su Kim, J. and Ho Je, Y. (2020) Insecticidal and Insect Growth Regulatory Activities of Secondary Metabolites from Entomopathogenic Fungi, *Lecanicillium attenuatum*. *Journal of Applied Entomology*, **144**, 655-663. <https://doi.org/10.1111/jen.12788>
- [9] Logeswaran, C., Vivekanandhan, P. and Shivakumar, M.S. (2019) Chemical Constituents of Thermal Stress Induced *Ganoderma applanatum* (Per.) Secondary Metabolites on Larvae of *Anopheles stephensi*, *Aedes Aegypti* and *Culex quinquefasciatus* and Histopathological Effects in Mosquito Larvae. *Biocatalysis and Agricultural Biotechnology*, **20**, Article 101253. <https://doi.org/10.1016/j.bcab.2019.101253>
- [10] Cañedo, V. and Ames, T. (2004) Laboratory Manual for the Management of Entomopathogenic Fungi. 62 Editions, International Potato Center, Lima.
- [11] Sani, I., Izera Ismail, S., Abdulah, S., Jalinas, J., Jamalán, S. and Saad, N. (2020) A Review of the Biology and Control of Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), with Special Reference to Biological Control Using Entomopathogenic Fungi. *Insects*, **11**, Article 619. <https://doi.org/10.3390/insects11090619>
- [12] Keswani, C., Singh, H.B., Hermosa, R., García-Estrada, C., Caradus, J., He, Y.-W., Mezaache-Aichour, S., Glare, T.R., Borriss, R., Vinale, F. and Sansinenea, E. (2019) Antimicrobial Secondary Metabolites from Agriculturally Important Fungi as Next Biocontrol Agents. *Applied Microbiology and Biotechnology*, **103**, 9287-9303.
<https://doi.org/10.1007/s00253-019-10209-2>
- [13] López Rodríguez, L. and Burrola-Aguilar, C. (2019) Hongos parásitos de insectos y

- otros hongos: Una alternativa de alimento funcional. *Agro Productividad*, **12**, 57-62. <https://doi.org/10.32854/agrop.v0i0.1398>
- [14] Sharif Swallah, M., Sun, H., Affoh, R., Fu, H. and Yu, H. (2020) Antioxidant Potential Overviews of Secondary Metabolites. *International Journal of Food Science*, **2020**, Article ID: 9081686. <https://doi.org/10.11155/2020/9081686>
- [15] Villegas-Mendoza, J.M., Mireles-Martínez, M., Rodríguez-Castillejos, G.C., Santiago-Adame, R., Morales-San Claudio, P.D.C. and Rosas-García, N.M. (2019) Antimicrobial Evaluation of a Methanolic Extract of *Beauveria bassiana* against Pathogenic Bacteria of Nosocomial Importance. *Ars Pharmaceutica*, **60**, 169-176. <https://dx.doi.org/10.30827/ars.v60i3.9219>
- [16] Elbanhawy, A.A., Elsherbiny, E.A., Abd El-Mageed, A.E. and Abdel-Fattah, G.M. (2019) Potential of Fungal Metabolites as a Biocontrol Agent Against Cotton Aphid, *Aphis gossypii* Glover and the Possible Mechanisms of Action. *Pesticide Biochemistry and Physiology*, **159**, 34-40. <http://doi.org/10.1016/j.pestbp.2019.05.013>
- [17] Oide, S. and Turgeon, B.G. (2020) Natural Roles of Nonribosomal Peptide Metabolites in Fungi. *Mycoscience*, **61**, 101-110. <https://doi.org/10.1016/j.myc.2020.03.001>
- [18] Pucheta Díaz, M., Flores Macías, A., Rodríguez Navarro, S. and De La Torre, M. (2006) Mechanism of action of entomopathogenic fungi. Mecanismo de acción de los hongos entomopatógenos. *Interciencia*, **156**, 856-860.
- [19] Wang, X., Gong, X., Li, P., Lai, D. and Zhou, L. (2017) Structural Diversity and Biological Activities of the Cyclic Dipeptides of Fungi. *Molecules*, **22**, Article 2026. <https://doi.org/10.3390/molecules22122026>
- [20] Taevernier, L., Wynendaele, E., Gevaert, B. and De Spiegeleer, B. (2017) Chemical Classification of Cyclic Dipeptides. *Current Protein and Peptide Science*, **18**, 425-452. <https://doi.org/10.2174/1389203717666161128141438>
- [21] Rachmawati, R., Kinoshita, H. and Nihira, T. (2018) Production of Insect Toxin Beauvericin from Entomopathogenic Fungi *Cordyceps militaris* by Heterologous Expression of Global Regulator. *AGRIVITA Journal of Agricultural Science*, **40**, 177-184. <https://doi.org/10.17503/agrivita.v40i1.1727>
- [22] Vásquez-Bonilla, J.N., Cabrera-Vega, E.J., Vázquez-Olvera, J.I., Gutiérrez-Nava, M.A., Hurtado-Y De La Peña, M. and Barranco-Florido, J.E. (2017) Biological Activity of Mycotoxin Beauvericin in Cancer Cells. Actividad biológica de la micotoxina beauvericina en células cancerosas. *Revista Mexicana de Ciencias Farmacéuticas*, **48**, 17-27.
- [23] Al Khoury, C., Guillot, J. and Nemer, N. (2019) Lethal Activity of Beauvericin, a *Beauveria bassiana* Mycotoxin, against the Two-Spotted Spider Mites, *Tetranychus urticae* Koch. *Journal of Applied Entomology*, **143**, 974-983. <https://doi.org/10.1111/jen.12684>
- [24] Chávez Ibañez, E., Rodríguez Navarro, S., Sánchez Pérez, L.D.C., Hamdam Partida, A. and Barranco Florido, J.E. (2014) *In vitro* Insecticidal Activity of Crude Extract of *Beauveria bassiana* (Balsam) Vuillemin on Larvae of *Phyllophaga spp.* (Harris). *Revista Protección Vegetal*, **29**, 226-230.
- [25] Stanciu, O., Juan, C., Miere, D., Loghin, F. and Mañes, J. (2017) Analysis of Enniatins and Beauvericin by LC-MS/MS in Wheat-Based Products. *CyTA-Journal of Food*, **15**, 433-440. <https://doi.org/10.1080/19476337.2017.1288661>
- [26] Krížová, L., Dadáková, K., Dvoráková, M. and Kašparovský, T. (2021) Feedborne Mycotoxins Beauvericin and Enniatins and Livestock Animals. *Toxins*, **13**, Article 32. <http://doi.org/10.3390/toxins13010032>
- [27] Litwin, A., Nowak, M. and Rózalska, S. (2020) Entomopathogenic Fungi: Uncon-

- ventional Applications. *Reviews in Environmental Science Biotechnology*, **19**, 23-42. <https://doi.org/10.1007/S11157-020-09524-1>
- [28] Tuli, H.S., Sandhu, S.S. and Sharma, A.K. (2014) Pharmacological and Therapeutic Potential of *Cordyceps* with Special Reference to Cordycepin. *Biotech*, **4**, 1-12. <http://doi.org/10.1007/s13205-013-0121-9>
- [29] Wen, T.C., Long, F.Y., Kang, C., Wang, F. and Zeng, W. (2017) Effects of Additives and Bioreactors on Cordycepin Production from *Cordyceps militaris* in Liquid Static Culture. *Mycosphere*, **8**, 886-898. <http://doi.org/10.5943/mycosphere/8/7/5>
- [30] Raethong, N., Wang, H., Nielsen, J. and Vongsangnak, W. (2020) Optimizing Cultivation of *Cordyceps militaris* for Fast Growth and Cordycepin Overproduction Using Rational Design of Synthetic Media. *Computational and Structural Biotechnology Journal*, **18**, 1-8. <http://doi.org/10.1016/j.csbj.2019.11.003>
- [31] Nascimiento, M., Zhang, W.-W., Ghosh, A., Houston, D.R., Berghuis, A.M., Oliver, M. and Matlashewski, G. (2006) Identification and Characterization of a Protein-Tyrosine Phosphatase in *Leishmania*. *The Journal of Biological Chemistry*, **281**, 36257-36268. <https://doi.org/10.1074/jbc.M606256200>
- [32] Zhang, L., Yue, Q., Wang, C., Xu, Y. and Molnár, I. (2020) Secondary Metabolites from Hypocrealean Entomopathogenic Fungi: Genomics as a Tool to Elucidate the Encoded Parvome. *Natural Product Reports*, **37**, 1164-1180. <https://doi.org/10.1039/D0NP00007H>
- [33] Zimmermann, G. (2008) The Entomopathogenic Fungi *Isaria farinosa* (Formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* Species Complex (Formerly *Paecilomyces fumosoroseus*): Biology, Ecology and Use in Biological Control. *Biocontrol Science and Technology*, **18**, 865-901. <https://doi.org/10.1080/09583150802471812>
- [34] Cheng, Y., Schneider, B., Riese, U., Schubert, B., Li, Z. and Hamburger M. (2006) (+)-N-Deoxymilitarinone A, a Neuritogenic Pyridone Alkaloid from the Insect Pathogenic Fungus *Paecilomyces farinosus*. *Journal of Natural Products*, **69**, 436-438. <https://doi.org/10.1021/np050418g>
- [35] Schmidt, K., Günther, W., Stoyanova, S., Schubert, B.L. and Hamburger M. (2002) Militarinone A, a Neurotrophic Pyridone Alkaloid from *Paecilomyces militaris*. *Organic Letters*, **4**, 197-199. <https://doi.org/10.1021/ol016920j>
- [36] Schmidt, K., Riese, U., Li, Z. and Hamburger M. (2003) Novel Tetramic Acids and Pyridone Alkaloids, Militarinones B, C, and D, from the Insect Pathogenic Fungus *Paecilomyces militaris*. *Journal of Natural Products*, **66**, 378-383. <https://doi.org/10.1021/np020430y>
- [37] Dymarska, M., Janeczko, T. and Kustrzema-Suslow, E. (2018) Glycosylation of Methoxylated Flavonoids in the Cultures of *Isaria fumosorosea* KCH J2. *Molecules*, **23**, Article 2578. <https://doi.org/10.3390/molecules23102578>
- [38] Krawczyk-Lebek, A., Dymarska, M., Janeczko, T. and Kostrzema-Suslow, E. (2020) Entomopathogenic Filamentous Fungi as Biocatalysts in Glycosylation of Methylflavonoids. *Catalysts*, **10**, Article 1148. <https://doi.org/10.3390/catal10101148>
- [39] Li, X.-Q., Xu, K., Liu, X.-M. and Zhang, P. (2020) A Systematic Review on Secondary Metabolites of *Paecilomyces* Species: Chemical Diversity and Biological Activity. *Planta Medica*, **86**, 805-821. <https://doi.org/10.1055/a-1196-1906>
- [40] Wu, H.-Y., Wang, Y.-L., Tan, J.-L., Zhu, C.-Y., Li, D.-X., Huang, R., Zhang, K.-Q. and Niu, X.-M. (2012) Regulation of the Growth of Cotton Bollworms by Metabolites from an Entomopathogenic Fungus *Paecilomyces catenibliquus*. *Journal of Agricultural and Food Chemistry*, **60**, 5604-5608. <https://doi.org/10.1021/jf302054b>

- [41] Hussain, A. and Mohammed Aljabr, A. (2020) Potential Synergy between Spores of *Metarhizium anisopliae* and Plant Secondary Metabolite, 1-Chlorooctadecane for Effective Natural Acaricide Development. *Molecules*, **25**, Article 1900. <https://doi.org/10.3390/molecules25081900>
- [42] Bojke, A., Tkaczuk, C., Stepnowski, P. and Gulebiowski, M. (2018) Comparison of Volatile Compounds Released by Entomopathogenic Fungi. *Microbiological Research*, **214**, 129-136. <https://doi.org/10.1016/j.micres.2018.06.011>
- [43] Kirkland, B.H., Eisa, A. and Keyhani, N.O. (2005) Oxalic Acid as a Fungal Acaricidal Virulence Factor. *Journal Medical Entomology*, **42**, 346-351. <https://doi.org/10.1093/jmedent/42.3.346>
- [44] Borges, D., Díaz, A.O., San Juan, A.N. and Gómez, E. (2010) Secondary Metabolites Produced by Entomopathogenic Fungi. *ICIDCA about Sugar Cane Derivatives*, **44**, 49-55.
- [45] Gao, Y., Feng, M.-G. and Ying, S.-H. (2018) Oxaloacetate Hydrolase Gene Links the Cytoplasmic Route of Oxalate Formation to Differentiation and Virulence of Entomopathogenic Fungus *Beauveria bassiana*. *Journal of Asia-Pacific Entomology*, **21**, 211-216. <https://doi.org/10.1016/j.aspen.2017.12.003>
- [46] Asaff, A., Cerda-García-Rojas, C. and De La Torre, M. (2005) Isolation of Dipicolinic Acid as an Insecticidal Toxin from *Paecilomyces fumosoroseus*. *Applied Microbiology and Biotechnology*, **68**, 542-547. <https://doi.org/10.1007/s00253-005-1909-2>
- [47] Luo, Z., Ren, H., Mousa, J.J., Rangel, D.E.N., Zhang, Y., *et al.* (2017) The PacC Transcription Factor Regulates Secondary Metabolite Production and Stress Response, but Has Only Minor Effects on Virulence in the Insect Pathogenic Fungus *Beauveria bassiana*. *Environmental Microbiology*, **19**, 788-802. <https://doi.org/10.1111/1462-2920.13648>
- [48] De Souza Daniel, J.F., Vinícius Scalco, A., De Souza, R.M., Marins Ocampos, F.M., Barison, A., Angeli Alves, L.F. and Oliveira Janeiro Neves, P.M. (2018) Susceptibility of *Alphitobius diaperinus* to *Beauveria bassiana* Extracts. *Natural Product Research*, **33**, 3033-3036. <https://doi.org/10.1080/14786419.2018.1514396>
- [49] Xiao, G., Ying, S.-H., Zheng, P., Wang, Z.-L., Zhang, S., Xie, X.-Q., Shang, Y., Leger, R.J.S., Zhao, G.-D., Wang, C. and Feng, M.-G. (2012) Genomic Perspectives on the Evolution of Fungal Entomopathogenicity in *Beauveria bassiana*. *Scientific Reports*, **2**, Article No. 483. <https://doi.org/10.1038/srep00483>
- [50] Cho, E.-M., Drion, B. and Keyhani, N.O. (2006) EST Analysis of cDNA Libraries from the Entomopathogenic Fungus *Beauveria (Cordyceps) bassiana*. II. Fungal Cells Sporulating on Chitin and Producing Oosporein. *Microbiology*, **152**, 2855-2864. <https://doi.org/10.1099/mic.0.28845-0>
- [51] Namara, L.M., Dolan, S.K., Walsh, J.M.D., Stephens, J.C., Glare, T.R., Kavanagh, K. and Griffin, C.T. (2019) Oosporein, an Abundant Metabolite in *Beauveria caledonica*, with a Feedback Induction Mechanism and a Role in Insect Virulence. *Fungal Biology*, **123**, 601-610. <https://doi.org/10.1016/j.funbio.2019.01.004>
- [52] De Carolina Sánchez-Pérez, L., Barranco-Florido, J.E., Rodríguez-Navarro, S., Cervantes-Mayagoitia, J.F. and Ramos-López, M.A. (2014) Entomopathogenic Fungal Enzymes, Advances and Knowledge. *Advances in Enzyme Research*, **2**, 65-76. <https://doi.org/10.4236/aer.2014.22007>
- [53] Tellez-Jurado, A., Cruz Ramírez, M.G., Mercado Flores, Y., Asaff Torres, A. and Arana-Cuenca, A. (2009) Mechanisms of Action and Response in the Relationship between Entomopathogenic Fungi and Insects. *Revista Mexicana de Micología*, **30**, 73-80.

- [54] Nowak, M., Bernat, P., Mrozinska, J. and Rózalska, S. (2020) Acetamidrid Affects Dextruxins Production but Its Accumulation in *Metarhizium sp.* Spores Increases Infection Ability of Fungi. *Toxins*, **12**, Article 587. <https://doi.org/10.3390/toxins12090587>
- [55] Kato, T., Nishimura, K., Suparmin, A., Ikeo, K. and Park, E.Y. (2021) Effects of Cordycepin in *Cordyceps militaris* during Its Infection to Silkworm Larvae. *Microorganisms*, **9**, Article 681. <https://doi.org/10.3390/microorganisms9040681>
- [56] Patocka, J. (2016) Bioactive Metabolites of Entomopathogenic Fungi *Beauveria bassiana*. *Military Medical Science Letters*, **85**, 80-88. <https://doi.org/10.31482/mmsl.2016.015>
- [57] Da Silva Santos, A.C., Gonvalves Diniz, A., Viera Tiago, P. and De Oliveira, N.T. (2019) Entomopathogenic *Fusarium* Species: A Review of Their Potential for the Biological Control of Insects, Implications and Prospects. *Fungal Biology Reviews*, **34**, 41-57. <https://doi.org/10.1016/j.fbr.2019.12.002>
- [58] Vivekanandhan, P., Swathy, K., Kalaimurugan, D., Ramachandran, M., Yuvaraj, A., Kumar, A.N., Shivakumar, M.S., Kweka, E.J., *et al.* (2020) Larvicidal Toxicity of *Metarhizium anisopliae* Metabolites against Three Mosquito Species and Non-Targeting Organisms. *PLOS ONE*, **15**, e0232172. <https://doi.org/10.1371/journal.pone.0232172>
- [59] Marín-Cruz, V.H., Rodríguez-Navarro, S., Barranco-Florido J.E. and Cibrián-Továr, D. (2017) Insectistatic and Insecticide Activity of *Beauveria bassiana* in *Bradysia impatiens* (Diptera: Sciaridae). *Revista Chapingo Serie Ciencias Forestales y del Ambiente*, **23**, 329-340. <https://doi.org/10.5154/r.rchscfa.2016.10.053>
- [60] Vivekanandhan, P., Karthi, S., Shivakumar, M.S. and Benelli, G. (2018) Synergistic Effect of Entomopathogenic Fungus *Fusarium oxysporum* Extract in Combination with Temephos against Three Major Mosquito Vectors. *Pathogens and Global Health*, **112**, 37-46. <https://doi.org/10.1080/20477724.2018.1438228>
- [61] Sharapova, I.E. (2019) Prospects of Using Entomopathogenic Fungus in Development of a Biopesticide Product with Nematicidal Activity. *Biocatalysis and Agricultural Biotechnology*, **19**, Article 101098. <https://doi.org/10.1016/j.bcab.2019.101098>
- [62] Zhang, X., Hu, Q. and Weng, Q. (2019) Secondary Metabolites (SMs) of *Isaria cicadae* and *Isaria tenuipes*. *RSC Advances*, **9**, 172-184. <https://doi.org/10.1039/C8RA09039D>
- [63] Dai, Z.-B., Wang, X. and Li, G.-H. (2020) Secondary Metabolites and Their Bioactivities Produced by *Paecilomyces*. *Molecules*, **25**, Article 5077. <https://doi.org/10.3390/molecules25215077>
- [64] El-Ghany, T.M., El-Sheikh, H.H., El-Rahman G.A. and El-Nasser A.M. (2012) Biodiversity of Entomopathogenic Fungi in New Cultivated Soils with Their Use for the Control of *Galleria mellonella*. *International Journal of Research and Review*, **4**, 17-31.
- [65] Rosas-García, N.M., Mireles-Martínez, M. and Villegas-Mendoza, J.M. (2020) Detection of Bassianolide and Beauvericin in Strains of *Beauveria bassiana* and Their Participation in the Pathogenic Activity towards *Spodoptera sp.* *Biotecnia*, **22**, 93-99. <https://doi.org/10.18633/biotecnia.v22i3.1060>
- [66] Abd-ElAzeem, E.M., El-Medany, W.A.Z. and Sabry, H.M. (2019) Biological Activities of Spores and Metabolites of Some Fungal Isolates on Certain Aspects of the Spiny Bollworms *Earias insulana* (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, **29**, Article No. 90. <https://doi.org/10.186/s41938-019-0192-y>
- [67] Erol, A.B., Abdelaziz, O., Birgücü, A.K., Morad, M., Oufroukh, A. and Karaca, I. (2020) Effects of Some Entomopathogenic Fungi on the Aphid Species, *Aphis goss-*

- ypii* Glover (Hemiptera: Aphididae). *Egyptian Journal of Biological Pest Control*, **30**, Article 108. <https://doi.org/10.1186/s41938-020-00311-3>
- [68] Han, J.H., Jin, B.R., Kim, J.J. and Lee, S.Y. (2014) Virulence of Entomopathogenic Fungi *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* for the Microbial Control of *Spodoptera exigua*. *Mycobiology*, **42**, 385-390. <https://doi.org/10.5941/MYCO.2014.42.4.385>
- [69] Lozano-Tovar, M.D., Ortiz-Urquiza, A., Garrido-Jurado, I., Trapero-Casas, A. and Quesada-Moraga, E. (2013) Assessment of Entomopathogenic Fungi and Their Extracts against a Soil-Dwelling Pest and Soil-Borne Pathogens of Olive. *Biological Control*, **67**, 409-420. <https://doi.org/10.1016/j.biocontrol.2013.09.006>
- [70] Espinoza, F., Vidal, S., Rautenbach, F., Lewu, F. and Nchu, F. (2019) Effects of *Beauveria bassiana* (Hypocreales) on Plant Growth and Secondary Metabolites of Extracts of Hydroponically Cultivated Chive (*Allium schoenoprasum* L. [Amaryllidaceae]). *Heliyon*, **5**, E03038. <https://doi.org/10.1016/j.heliyon.2019.e03038>
- [71] Weng, Q., Zhang, X., Chen, W. and Hu, Q. (2019) Secondary Metabolites and the Risks of *Isaria fumosorosea* and *Isaria farinose*. *Molecules*, **24**, Article 664. <https://doi.org/10.3390/molecules24040664>
- [72] Wang, H., Peng, H., Li, W., Cheng, P. and Gong, M. (2021) The Toxins of *Beauveria bassiana* and the Strategies to Improve Their Virulence to Insects. *Frontiers in Microbiology*, **12**, Article 705343. <https://doi.org/10.3389/fmicb.2021.705343>
- [73] Hugo, V.M.-C., Navarro, S.R., Florido, J.E.B., Sierra, R.T. and Tovar, D.C. (2018) Metabolites and Conidia of *Beauveria bassiana* for Control of a Darwinged Fungus Gnat under Greenhouse Conditions. *Southwestern Entomologist*, **43**, 691-703. <https://doi.org/10.3958/059.043.0315>