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Hongos entomopatógenos en la provincia de Misiones: Revisión

Entomopathogenic Fungi in Misiones Province: A Review

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Resumen

Los hongos entomopatógenos (HEP) son microorganismos altamente específicos que se alimentan de insectos y por ello existe un gran interés en el uso de estos hongos como control biológico para cultivos de interés agrícola. El propósito de esta revisión fue actualizar la información disponible sobre HEP en Misiones e incrementar el conocimiento de la biodiversidad de este grupo y su uso potencial en biocontrol de plagas. La amplia y dispersa bibliografía disponible sobre este tema fue minuciosamente revisada, organizada y sintetizada. Se presenta una visión general sobre la información taxonómica de los HEP, y luego se realiza una recopilación y análisis de los estudios publicados. Como resultado de esta revisión se puede concluir que existen escasos estudios de HEP en la provincia de Misiones comparado con otras provincias de Argentina o Brasil. La expectativa de esta revisión de los estudios de HEP es fomentar la búsqueda y profundización del conocimiento científico en el área.

Palabras clave: Revisión; Hongos entomopatógenos; Biodiversidad; Control biológico; Misiones.

Abstract

Entomopathogenic fungi (EPF) are microorganisms with a high specificity and the ability to infect and kill insects and consequently there has been great interest in the use of these fungi as biocontrol agents against many crop pests. The purpose of the present review was to update the available information about EPF in Misiones in order to expand the knowledge of biodiversity of this group and the potential use in biocontrol of pest. The broad literature in this subject was reviewed and synthesized. First we present a general view of taxonomical information of EPF and then, noteworthy studies conducted by several authors. As a result of this review, it was concluded that there are scarce studies on EPF in Misiones province compared to other provinces in Argentina and Brazil. The goal of this overview is to encourage the search and in-depth examination of the scientific knowledge of EPF in Misiones province.

Keywords: Review; Entomopathogenic fungi; Biodiversity; Biological control; Misiones.

Introduction

The province of Misiones belongs to the Alto Parana Atlantic Forest, an ecoregion of the Atlantic Forest. It has a subtropical semi-deciduous forest with an annual rainfall of 1700-2200 mm, with no marked drought season (1). The biota of the Atlantic Forest is extremely diverse, and the assessment of the current state of this region´s biodiversity is still poorly known (2). Although current research data are significant, there are not equivalent to the huge amount of unknown diversity and every accurate research will lead to a beneficial contribution.

Entomopathogenic fungi (EPF) are microorganisms of the Fungi kingdom, mainly present in Ascomycota and Basidiomycota phyla, but there are also some strains belonging to Chytridiomycota and Zygomycota phyla (3).

Ascomycota is the major phylum in the Fungi kingdom, including many important entomopathogenic fungi in their asexual (anamorphic) phase like *Beauveria, Metarhizium, Lecanicillium, Nomuraea, or Paecilomyces = Isaria*; or the sexual (teleomorphic) state, *Cordyceps*. Also, many of the asexual fungi in the Hypocreales order are under intensive study due to several traits favoring their use as biological insecticides, owing their ease of mass production, storage, virulence, and application (4, 5).

Only a small number of members within the Basidiomycota phylum are true entomopathogens. This group of fungi is characterized by the development of reproduc-

tive spores, named basidiospores. Primarily *Septobasidium* and *Uredinella* genera are obligate pathogens of armored scale insects (Hemiptera). These fungi form a complex relationship with their hosts; they are parasitic on individual insects but may be mutualistic from the perspective of a population of insects (6, 7).

Chytrids are fungi in the phylum Chytridiomycota. They inhabit freshwater ecosystems and produce zoospores with a single flagellum. The best-known insect pathogenic species belong to the genus *Coelomomyces*; all are obligate parasites of mosquitoes and other Dipterans and require crustacean alternate hosts to complete their life cycles (8).

The zygomycetes, in phylum Zygomycota, are characterized by the formation of sexual spores called zygospores. Most entomopathogens within the Zygomycota occur within the order Entomophthorales. Many species have worldwide distribution and are often responsible for wide-scale epizootics, affecting mainly species listed in the orders Hemiptera, Lepidoptera, Orthoptera and Diptera. The most common genera include *Conidiobolus, Entomophaga, Entomophthora, Erynia, Pandora, Neozygites* and *Zoophthora*. Most are obligate parasites possessing restricted host ranges; however, some species, such as *Zoophthora radicans*, have relatively wide host ranges (3, 9).

The observation of major epizootics of fungal pathogens in natural populations of insects and other arthropods has encouraged intense interest in the use of EPF as biological control agents (10). Also, entomopathogenic Hyphomycetes have one of the widest spectra of host ranges among entomopathogens (11). Therefore, EPF can be employed in biological control strategies (12).

Biological control with EPF is a promising alternative to chemical control; also biocontrol with entomopathogenic fungi might provide long-lasting insect control without damage to the environment or non-target organisms (13).

The available literature of studies on entomopathogenic fungi in Misiones province is rather scattered. Despite the importance of entomopathogenic fungi, for biodiversity and agriculture, there has been little attempt to synthesize an overview of the diversity of these microbes in Misiones. The purpose of the present review was to collate and update the available information about this group of fungi in Misiones province (Argentina) in order to expand the knowledge of its biodiversity.

In order to survey the knowledge of investigations on entomopathogenic fungi in Misiones, Argentina, we reviewed the existing information and synthesized the scattered literature on this subject.

The bibliography used and cited here was carefully searched and compiled from researches and scientific data of fungal genera and families in Misiones province from major online bibliographic and academic databases.

Studies on entomopathogenic fungi in Misiones

Misiones has a rich but extensively underexplored diversity of fungi affecting arthropods; many of these fungi have an important potential use in biological control. The biodiversity in the Atlantic Forest is the richest on the earth, but microbial biodiversity in both Brazilian and Argentinean forests remains very poorly explored in comparison with the plant and animal biodiversity (14, 15).

One of the first investigations, involving EPF, cited for this region the teleomorphic fungi *Cordyceps martialis* (referred as *C. submilitaris*) in early last century (mentioned in Sosa-Gómez *et al.* [15]).

Trichomycetes is an interesting fungal group associated with living arthropods such as insects, millipedes, and crustaceans, growing extensively in the hind gut and some of them are reported to be of insects pathogens (16, 17, 18, 19). The term "trichomycetes" is derived from the Greek word "tricho", meaning hair, in reference to the hair-like appearance of *thalli* in the host gut (20). The fungal taxon Trichomycetes would include just two orders: Asellariales and Harpellales (Amoebidiales and Eccrinales were formerly included in this taxon) (21). Harpellales is the species-richest order in the Trichomycetes class. López-Lastra *et al.* (16) studied fourteen species of Trichomycetes (phylum Zygomycota) and found that five of them (*Genistellospora homothallica, Harpella tica, Smittium culisetae, Smittium* sp., and *Stachylina* sp.) came from Misiones. The importance of the increase knowledge of this group of fungi is to determine the potential use as biological control of some insect pest (20). Some records reported that *Smittium* spp. caused moderate or high larval mortality in *Anopheles*, *Aedes* and *Culex* genera in the laboratory, but these rates have never been found on mosquitoes in the field (22, 23). Regarding natural enemies of Culicidae (Diptera), these findings should be kept in mind, although further analyses are needed to evaluate the feasibility to use these fungi groups in biological control.

Entomophaga grylli, other member of Zygomycota, was isolated from the grasshopper *Ronderosia bergi* in Puerto Iguazú, Misiones (24). This was a new record of EPF from the Entomophthorales order isolated from grasshoppers in this province. Fungi of the *E. grylli* pathotype or species complex are parasites of grasshoppers and locusts (Orthoptera), and their host range includes many economically important Orthoptera species worldwide. The species of the *E. grylli* complex are highly pathogenic and can cause important field epizootics (5, 26).

Recent investigations related to pests in yerba mate (*Ilex paraguariensis* St. Hill.) and its biological control have studied and characterized different groups of EPF. Alves *et al*. (27) and Formentini *et al*. (28) emphasized the use of *Beauveria bassiana* and *Z. radicans* against some pests like *Thelosia camina*, *Hylesia* sp., *Oligonychus yothersi*, and *Gyropsylla spegazziniana*.

Table 1: Entomopathogenic fungi in Misiones province

Fungal species	Taxonomic location	Insect host	Taxonomic location of insect host	References
Beauveria bassiana	Cordycipitaceae	*Hedypathes betulinus; Thelosia camina, Hylesia sp., Oligonychus yothersi, and Gyropsylla spegazziniana*	Cerambycidae; Bombycidae; Saturniidae; Tetranychidae; Psyllidae	27, 28, 29.
Metarhizium anisopliae	Clavicipitaceae	Not specified	-	29.
Isaria sp	Cordycipitaceae	Not specified	-	29.
Paecilomyces lilacinus	Ophiocordycipitaceae	Not specified	-	31.
Genistellospora homothallica	Legeriomycetaceae	*Simulium auripellitum*	Simuliidae	16.
Harpella tica	Harpellaceae	*Simulium spp,*	Simuliidae	16.
Smittium culisetae	Legeriomycetacea	*Aedes aegypti*	Culicidae	16.
Smittium sp.	Legeriomycetacea	*Ceratopogonidae*	-	16.
Stachylina sp.	Harpellaceae	*Chironomidae*	-	16.
Zoophthora radicans	Entomophthoraceae	*G. spegazziniana*	Psyllidae	27.
Entomophaga grylli	Entomophthoraceae	*Ronderosia bergi*	Acrididae	24.
Cordyceps martialis	Clavicipitaceae	Not specified	-	15.

Soils of yerba mate plantations from Misiones were sampled and evaluated with respect to the presence of EPF. Schapovaloff *et al*. (29) found about 30 different strains of *B. bassiana*, *M. anisopliae* and *Isaria* sp., and proposed the evaluation of their potential as biological control against yerba mate pests. These authors considered *B. bassiana* against the main pest of yerba mate, *Hedypathes betulinus* (Coleoptera: Cerambycidae: Lamiinae) and found strains that caused mortality rates higher than 80%. These results agree with Leite *et al*. (30), who evaluated suspensions of *B. bassiana* against adults of *H. betulinus* under field conditions and found mortality rates of 76%.

Furthermore, Bich *et al*. (31) presented a survey about the hyphomycetes in soils of different types of yerba mate plantations. The presence of different fungal genera was observed, but just one strain of an EPF among all, was identified as *Paecilomyces lilacinus*, usually reported as pathogen of nematodes. However, its potential as biological control remains to be determined *a priori* using *in-vitro* assays.

In 2013 Bich *et al.* (32) evaluated the viability of spores of *Beauveria* sp. and *Metarhizium* sp. in three commonly used surfactants, and concluded that entomopathogenic formulations containing spore solutions with glycerin are considered preferable because they do not reduce spore viability and do not make foam.

These findings are important in the search of agents for biological control of pest in main crops of agronomical importance of Misiones.

Studies with EPF in places close to Misiones province

Several studies of EPF in other Argentinean provinces and Brazilian states near Misiones are discussed in this section.

The cicadellid leafhopper *Sonesimia grossa* is an important insect in agriculture because it causes considerable damage to crops as a vector of plant pathogens. These insects live in Tucumán and Misiones provinces (33). Toledo *et al*. (5) isolated *Clonostachys rosea* and considered it as an EPF of *S. grossa* in Argentina. These authors also evaluated the pathogenicity of *C. rosea* against two species of Hemiptera in Tucumán (other than *S. grossa*). Even though they confirmed mortality rates, the evaluation against *S. grossa* remains to be estimated in a search for a potential biological control of this leafhopper-pest in Misiones.

In Paraná State (Brazil), Dalla Santa *et al*. (34) analyzed *in-vitro* and in field conditions of *Beauveria* sp production and evaluated its pathogenicity against insects in *Ilex paraguariensis*. They found encouraging results that support that *Beauveria* sp can be considered to be an important biological control agent in yerba mate plantations.

Others species belonging to Entomophthorales order were reported in Corrientes province by Sosa-Gómez *et al*. (35). These authors reported that *Zoophthora radicans* can cause important disease outbreaks among susceptible hosts like psyllids. Also, *Z. radicans* affect satisfactory homoptera such as *Empoasca kraemeri* in Santa Catarina State (Brazil) (36).

Table 1 illustrates the published data on entomopathogenic fungi of Misiones, including the identification of hosts and publication references.

Conclusions

So far, eleven EPF genera affecting eight insect genera have been cited for Misiones, most of them jeopardize species of agricultural importance. It should also be noted that many outstanding investigations remain to be done because there were no studies corresponding to the Chytridiomycota phylum. All these EPF are important microorganisms that could be tested and probably used in biological control of harmful pests of crops in Misiones.

Also, this overview exposes the minor and fragmentary existence of EPF studies made in Misiones compared to other Argentinean and Brazilian provinces. The results compiled here are the most inclusive and current listing of fungi affecting arthropods for this province. This review contains most of the existing literature scattered among many local, national, and international publications in di-

verse languages, and this is the first attempt to compile and to synthesize the knowledge of EPF from this province.

The goal of this overview is to encourage the search and in-depth examination of the scientific knowledge of EPF in Misiones province.

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References

1. **Zamudio, F. and Hilgert, N.I.** Descriptive attributes used in the characterization of stingless bees (Apidae: Meliponini) in rural populations of the Atlantic forest (Misiones-Argentina). J Ethnobiol Ethnomed 8:9-19. 2012.
2. **Cardoso, J.M. and Casteleti, C.H.M.** Status of the biodiversity of the Atlantic Forest of Brazil. In: Galindo-Leal C, Câmara IG (eds). The Atlantic Forest of South America - Biodiversity Status, Threats, and Outlook. Island, Washington, 43-59. 2003.
3. **López-Lastra, C.C. and Scorsetti, A.C.** Revisión de los hongos entomophthorales (Zygomycota: Zygomycetes) patógenos de insectos de la República Argentina. Bol Soc Argent Bot 42:33-37. 2007.
4. **Gao, L.** A Novel Method to Optimize Culture Conditions for Biomass and Sporulation of the Entomopathogenic Fungus *Beauveria bassiana* IBC1201. Braz J Microbiol 42:1574-1578. 2011.
5. **Toledo, A.V.; Virla, E.; Humber, R.A.; Paradell, S.L. and López-Lastra, C.C.** First record of Clonostachys rosea (Ascomycota: Hypocreales) as an entomopathogenic fungus of Oncometopia tucumana and Sonesimia grossa (Hemiptera: Cicadellidae) in Argentina. J Invertebr Pathol 92:7-10. 2006.
6. **Henk, D.A.** New species of *Septobasidium* from southern Costa Rica and the southeastern United States. Mycologia 97:908–913. 2005.
7. **Humber, R.A.** Fungi: Identification. In: Lacey L (ed). Manual of techniques in insect pathology. Academic Presses, USA, 153-185. 1997.
8. **Wraight, S.P.** Inglis, G.D. and Goettel, M.S., Fungi. In: Lacey LA, Kaya HK (eds). Field Manual of Techniques in Invertebrate Pathology. Springer, The Netherlands, 223-248. 2007.
9. **Mascarin, G.M.; da Silveira, V.; Mendes, M. and Delalibera, I.** Natural occurrence of *Zoophthora radicans* (Entomophthorales: Entomophthoraceae) on *Thaumastocoris peregrinus* (Heteroptera: Thaumastocoridae), an invasive pest recently found in Brazil. J Invertebr Pathol 110:401–404. 2012.
10. **Smith, K.E.; Wall, R. and French, N.P.** The use of entomopathogenic fungi for the control of parasitic mites, Psoroptes spp. Vet Parasitol 92:97-105. 2000.
11. **Goettel, M.S. and Inglis, G.D.** Fungi: Hyphomycetes. In: Lacey L (ed). Manual of techniques in insect pathology. Academic Presses, USA, 213-249. 1997.
12. **Shah, P.A. and Pell, J.K.** Entomopathogenic fungi as biological control agents. Appl Microbiol Biotechnol 61:413-423. 2003.
13. **Sun, J.; Fuxa, J.R. and Henderson, G.** Sporulation of *Metarhizium anisopliae* and *Beauveria bassiana* on *Coptotermes formosanus* and in vitro. J Invertebr Pathol 81:78-85. 2002.
14. **Niveiro, N.; Popoff, O.F. and Albertó, E.O.** Contribución al conocimiento de los agaricales de la Selva Paranaense Argentina. Bol Soc Argent Bot 45:17-27. 2010.
15. **Sosa-Gómez, D.R.; López-Lastra, C.C. and Humber, R.A.** An Overview of Arthropod-Associated Fungi from Argentina and Brazil. Mycopathol 170:61-76. 2010.
16. **López-Lastra, C.; Scorsetti, A.C.; Marti, G.A. and Coscarón, S.** Trichomycetes living in the guts of aquatic insects of Misiones and Tierra del Fuego, Argentina. Mycologia 97:320–328. 2005.
17. **Labeyrie, E.S.; Molloy, D.P. and Lichtwardt, R.W.** An Investigation of Harpellales (Trichomycetes) in New York State Blackflies (Diptera: Simuliidae). J Invertebr Pathol. 68:293–298. 1996.
18. **Sweeney, A.W.** An undescribed species of *Smittium* (Trichomycetes) pathogenic to mosquito larvae in Australia. Trans Br Mycol Soc 77:55–60. 1981.
19. **Vojvodic, S. and McCreadie, J.W.** Morphological differences of symbiotic fungi *Smittium culisetae* (Harpellales: Legeriomycetaceae) in different Dipteran hosts. Mycol Res 113:967-972. 2009.
20. **Nelder, M.P.; Beard, C.E.; Adler, P.H.; Kim, S. and McCreadie, J.W.** Harpellales (Zygomycota: Trichomycetes) associated with black flies (Diptera: Simuliidae): world review and synthesis of their ecology and taxonomy. Fungal Divers 22:121-169. 2006.
21. **Lichtwardt, R.W.** Trichomycetes and the Arthropod Gut. In: Brakhage AA, Zipfel PF (eds). Human and Animal Relationships, 2nd Edition. The Mycota VI. Springer-Verlag, Berlin, 3-19. 2008.
22. **García, J.J.; Campos, R.E. and Maciá, A.** Prospección de enemigos naturales de Culicidae (Diptera) de la selva marginal de Punta Lara (Prov. De Buenos Aires, República Argentina). Rev Acad Colomb Cien 19:209-216. 1994.
23. **Scholte, E.J.; Knols, B.G.J.** Samson, R.A. and Takken, W., Entomopathogenic fungi for mosquito control: a review. J Insect Sci 4:1-24. 2004.
24. **Pelizza, S.A.; Cabello, M.N. and Lange, C.N.** Nuevos registros de hongos entomopatógenos en acridios (Orthoptera: Acridoidea) de la República Argentina. Rev Soc Entomol Argent 69:287-291. 2010.

25. **Méndez-Sánchez, S.E.; Humber, R.A. and Freitas, A.L.** The *Entomophaga grylli* complex (Fresenius 1856) Batko (Zygomycetes: Entomophthorales) infecting grasshoppers (Orthoptera: Acrididae) in Ilhéus (Bahia), Brazil: Notes and new. Entomotropica 24:71-81. 2009.

26. **Sánchez Peña, S.R.** In vitro production of hyphae of the grashopper pathogen *Entomophaga grylli* (Zygomycota: Entomophthorales): Potential for production of conidia. Fla Entomol 88:332-334. 2005.

27. **Alves, L.F.A.; Fanti, A.L.P.** Formentini, M.A and Schapovaloff, M.E., Los hongos entomopatógenos y las plagas de la yerba mate. XII Congreso Argentino de Micología, Posadas, Misiones, p. 67. 2011.

28. **Formentini, M.A.; Alves, L.F.A.; Schapovaloff, M.E.; Fanti, A.L.P.; Silva, R.N.C. and López-Lastra, C.C.** Comparación de hongos entomopatógenos aislados de Brasil y Argentina para el control de *Gyropsylla spegazziniana*. XII Congreso Argentino de Micología, Posadas, Misiones, p. 301. 2011.

29. **Schapovaloff, M.E.; Fanti, A.L.; Alves, L.F.; Medvedeff, M.G. and López-Lastra, C.C.** Evaluación de *Beauveria bassiana* para el control del taladro de la yerba mate *Hedypathes betulinus* (Klug) (Coleoptera: Cerambycidae). XII Congreso Argentino de Micología, Posadas, Misiones, p. 311. 2011.

30. **Leite, M.S.P.; Iede, E.T.; Penteado, S.R.C.; Zaleski, S.E.M.; Camargo, M.M. and Ribeiro, R.D.** Eficiência de *Beauveria bassiana* (Bals.) Vuill. formulado em óleo no controle de *Hedypathes betulinus* (Klug) (Coleoptera: Cerambycidae) em campo. IV Congreso Sudamericano de la Yerba Mate, Posadas, Misiones, p. 269-272. 2006.

31. **Bich, G.A.; Castrillo, M.L.; Bergottini, V.M.; Sosa, A.D.; Otegui, M.B.; Villalba, L.L. and Zapata, P.D.** Identificación de la diversidad de hongos filamentosos de suelos asociados a yerba mate. VIII Jornadas científico-tecnológicas de la Facultad de Ciencias Exactas, Químicas y Naturales, Posadas, Misiones, p. 56. 2011.

32. **Bich, G.A.; Castrillo, M.L. and Medvedeff, M.G.** Evaluación de la viabilidad de cepas de hongos entomopatógenos en suspensiones de esporas adicionadas con diferentes surfactantes. Jornadas Científico Tecnológicas de la Universidad Nacional de Misiones, 40 años. Posadas, Misiones, 3CA. 2013.

33. **Remes-Lenicov, A.M.M.; Paradell, S.; De Coll, O. and Agostini, J.** Cicadelinos asociados a citrus afectados por Clorosis Variegada (CVC) en la República Argentina (Insecta: Homoptera: Cicadellidae). Rev Soc Entomol Argent 58:211-225. 1999.

34. **Dalla Santa, H.S.; Sousa, N.J.; Pittner, E. and Dalla Santa, O.R.** Controle biológico em pragas de *Ilex paraguariensis* (A. St.- Hil.) com fungo *Beauveria* sp. Floresta 39:67-76. 2009.

35. **Sosa-Gómez, D.R.; Kitajima, E.W. and Rolon, M.E.** First Records of Entomopathogenic Diseases in the Paraguay Tea Agroecosystem in Argentina. Fla Entomol 77:378-382. 1994.

36. **Leite, L.G.; Alves, S.B.; Wraight, S.P.; Galaini-Wraight, S. and Robert, D.W.** Habilidade de infecção de isolados de *Zoophthora radicans* sobre *Empoasca kraemeri*. Sci. Agric 53:152-158. 1996.

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