Article

Abundance and distribution of entomopathogenic fungi in different locations and environments of southern Tamaulipas

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Abstract

In order to generate a register of entomopathogenic fungi with the potential for pest control in the agricultural area of the state of Tamaulipas. In this study, abundance was quantified and the distribution of entomopathogenic fungi determined in different localities and environments of southern Tamaulipas, Mexico. In 2016, entomopathogenic fungi were collected in the Corpus Christi Breccia, Villa Cuauhtémoc, Esteros and Miradores. In each locality the following environments were selected: plots cultivated with grasses (sorghum and corn), fabaceae (soybean, bean and jicama), fruit trees (lemon, papaya, litche, mango and orange), vegetables (onion, chili, tomato and chard) and uncultivated plots (natural environment). In each environment, soil samples were collected. Later, in the collected soil, entomopathogenic fungi were tricked with larvae of *Tenebrio molitor* L. In total, 134 isolates of the genera were collected: *Beauveria* sp., *Lecanicillium* sp., *Metarhizium* sp., *Paecilomyces* sp., *Trichoderma* sp. and *Isaria* sp. of which, *Beauveria* sp. presented the greatest abundance and distribution. While, the other genera were collected in specific locations and environments. This result indicates the possibility that the genera of the fungi found are strongly adapted to the biotic and abiotic factors of the environment.

Keywords: *Beauveria* sp., *Lecanicillium* sp., *Metarhizium* sp., *Paecilomyces* sp., *Trichoderma* sp.

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Introduction

Entomopathogenic fungi are present in different environments (Klingen and Haukeland, 2006; Jaronski, 2010). Currently, 90 genera and 700 species are reported in forest, agricultural, scrub, desert and urban areas (Chandler *et al*., 1997; Onofre *et al*., 2001; Meyling and Eilenberg, 2007). In these environments, entomopathogenic fungi participate in the regulation of insect pests (Chandler *et al*., 1997). This has maximized the interest in studying the presence, abundance and distribution of fungi in different parts of the world (Meyling and Eilenberg, 2007).

Currently, the most studied are: *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin (Chandler *et al*., 1997; Bidochka *et al*., 1998; Klingen *et al*., 2002; Keller *et al*., 2003; Meyling and Eilenberg, 2006b; Zimmermann, 2008; Meyling *et al*., 2009; Ormond *et al*., 2010). According to Toledo *et al*. (2008) and Zimmermann (2008), both fungi are hosted in a wide range of insects and have a cosmopolitan distribution. Likewise, they have a preference for a particular environment. For example, *B. bassiana* occurs in greater abundance in uncultivated soils and forest environments. On the other hand, *M. anisopliae* is more abundant in cultivated soils and orchards of fruit trees (Bidochka *et al*., 1998; Bruck, 2004; Meyling and Eilenberg, 2006b; Quesada-Moraga *et al*., 2007; Fisher *et al*., 2011; Wyrebek *et al*., 2011).

The knowledge of the abundance and distribution of entomopathogenic fungi in an environment allows selecting the best adapted species (Meyling *et al*., 2011). Also, it helps to predict the success of fungi in the biological control of pests (Meyling and Eilenberg, 2007). In Mexico, *Beauveria* sp., *Metarhizium* sp. and *Paecilomyces* sp. has recently been registered in different environments (Lezama-Gutiérrez *et al*., 2001; Sánchez-Peña *et al*., 2011). This result shows the adaptation of fungi to different geographical areas and provides basic information for the development of future research focused on their ecology and biology. Considering the above, in the present study the abundance was quantified and determined the distribution of the entomopathogenic fungi in localities and environments of southern Tamaulipas, Mexico.

Materials and methods

Sampling sites

The study was conducted from August to November 2016, in the following locations: Corpus Christi Breccia, Villa Cuauhtémoc, Esteros and Miradores, belonging to the municipality of Altamira, Tamaulipas, Mexico (Figure 1).

In the study area a humid warm climate predominates, with rainfall regime from June to September, with the direction of the winds from southeast to northeast. The average annual temperature and precipitation is 16 °C and 1 000 mm. To the north and west of the localities, the soil is Pelic vertisol and in the southeastern part it is Calcium cabisole and Calcaric. With regard to the possession and use of land, the localities are ejidales focused on agricultural production (INEGI, 2018).

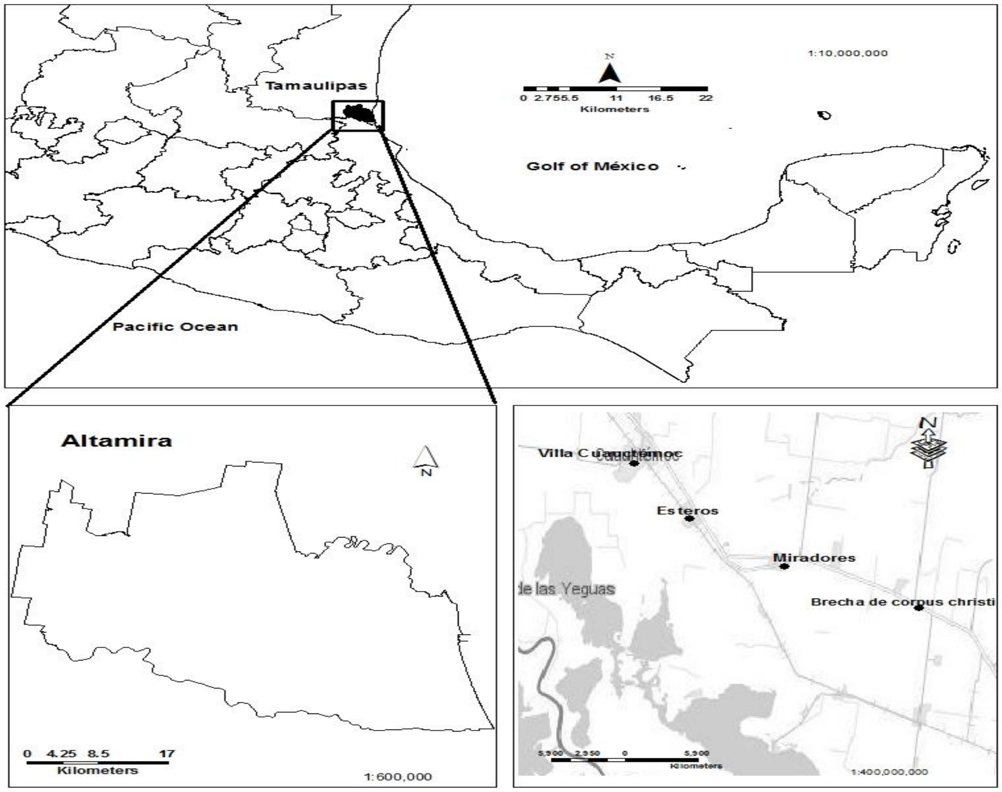

Figure 1. Distribution of the localities sampled in the present research work.

Collect soil samples

In each locality the following environments were selected: plots cultivated with grasses (sorghum and corn), fabaceae (soybean, bean and jicama), fruit trees (lemon, papaya, litche, mango and orange), vegetables (onion, chili, tomato and chard) and uncultivated plots (Natural environment). In each environment, five sampling points were selected based on the 'cinco de oros' method (Rendón, 1994; Infante and Zárate 2003). Each point was made up of 10 m^2, in this area a soil sample was collected from five randomly selected sites. Before harvesting the sample, weeds or crop residues were removed from the soil surface.

Then, at each point, 500 g of soil were collected at 20 cm depth with the help of a garden spade. The samples were placed in bags of polyethylene labels and were transported to the entomology laboratory of the experimental field 'Las Huastecas' belonging to the National Institute of Forestry, Agriculture and Livestock Research (INIFAP). The tool used was disinfested with 70% alcohol in each sample taken. Likewise, the sites were georeferenced with a satellite geolocator (GPS, Brand: Garmin, Model: Corp Etrex®).

In total, 348 samples were collected, distributed as follows: 96 in plots cultivated with grasses (sorghum and corn), 96 samples in plots planted with fabaceae (soybean, bean and jicama), 54 soil samples in uncultivated plots (Natural environment), 54 soil samples in orchards cultivated with fruit trees (lemon, papaya, litche, mango and orange) and 48 samples in plots cultivated with vegetables (onion, chili, tomato and chard). The number of samples was based on the availability of the agricultural plots of the cooperating producers.

Processing of soil samples for mushroom harvesting

The five samples collected at each site were mixed. Subsequently, six samples of 60 g were obtained. These, independently, were poured into 155 ml plastic containers. Then, three milliliters of distilled water were added and five larvae of the *Tenebrio molitor* L. coleopter were placed (Sánchez-Peña *et al*., 2011). The *T. molitor* insect was breeding in the laboratory until obtaining the third generation in order to minimize the presence of fungi and bacteria in them.

Finally, the containers were sealed with perforated caps and incubated in a climatic chamber (Thermo Scientific®) at 24 °C and 12 h light and 12 h of darkness for 30 days. The containers were inverted every 24 h for 10 days in order to put *T. molitor* larvae in contact with the fungi (Mietkiewski *et al*., 1997). At the end of the incubation period, the dead larvae were washed with 70% alcohol and 1% sodium hypochlorite. They were also washed twice with distilled water and placed on absorbent paper for three minutes in order to minimize humidity (Chandler *et al*., 1997). Next, the larvae were placed independently in 20 ml containers containing wet cotton and Whatman filter paper number 1. Finally, the containers were sealed and incubated at 25 °C for seven days. At the end of the incubation period, a sample of the fungus was seeded in a Petri dish with SDA culture medium (Sabouraud Dextrose Agar). The Petri dishes were incubated at room temperature until the sporulation of the fungi.

Taxonomic identification of fungi

The fungi developed in the culture medium were planted in the medium of Sabouraud Dextrose Agar (SDA) until obtaining monosporic cultures. Later, they were identified with taxonomic keys (Barnett and Hunter, 1999; Humber, 2012).

Statistical analysis

The average number of isolates was analyzed by gender, location and environment. Likewise, the same parameters were analyzed for the genera with the highest number of isolates and this was done with the nonparametric statistical test of Kruskal-Wallis followed by the comparison of means of Bonferroni with an alpha of 0.05. The analyzes were performed with the statistical software of SAS version 9.4 (SAS, 2012).

Results and discussion

In the localities and environments sampled, six genera of entomopathogenic fungi were collected. Of which, the abundance of these was different (*P*-value: 0.01). Of the fungi collected, *Beauveria* sp., showed the highest abundance followed by *Lecanicillium* sp., *Metarhizium* sp., *Paecilomyces* sp., *Trichoderma* sp. and *Isaria* sp. (Figure 2).

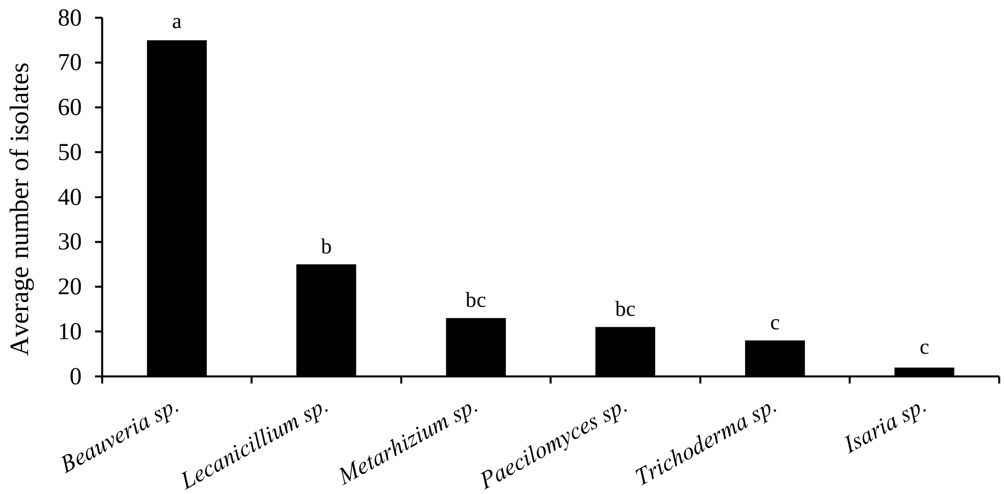

Figure 2. Analysis of the abundance of the genera of the entomopathogenic fungi collected in the study area.

The entomopathogenic fungi collected were distributed in the four environments, where the abundance of each of the fungi was different (*p*- value: 0.003). The greatest abundance of the isolates was observed in the orchards of fruit trees and in the plots cultivated with grasses and fabaceae, followed by the natural areas and the vegetable crops (Figure 3).

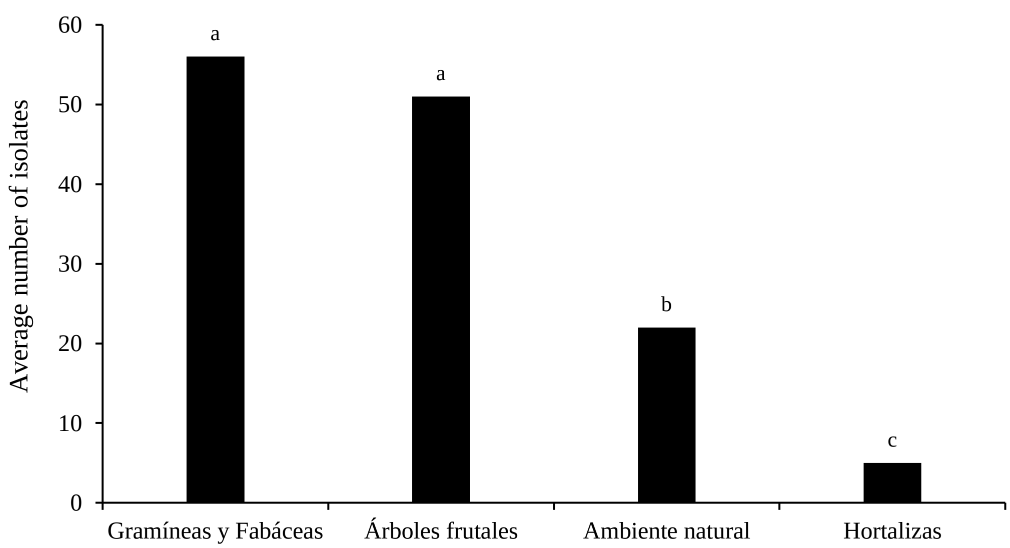

Figure 3. Analysis of the abundance of entomopathogenic fungi by type of environment.

On the other hand, the fungi were distributed in three of the four localities sampled. In localities, the fungal abundance was different (*p*- value: 0.001). The highest abundance of the isolates was recorded in the common of Villa Cuauhtémoc and in the Breccia of Corpus Christi with respect to the abundance observed in the common Miradores and Esteros, in the latter site no isolates of the entomopathogenic fungi were obtained (Figure 4).

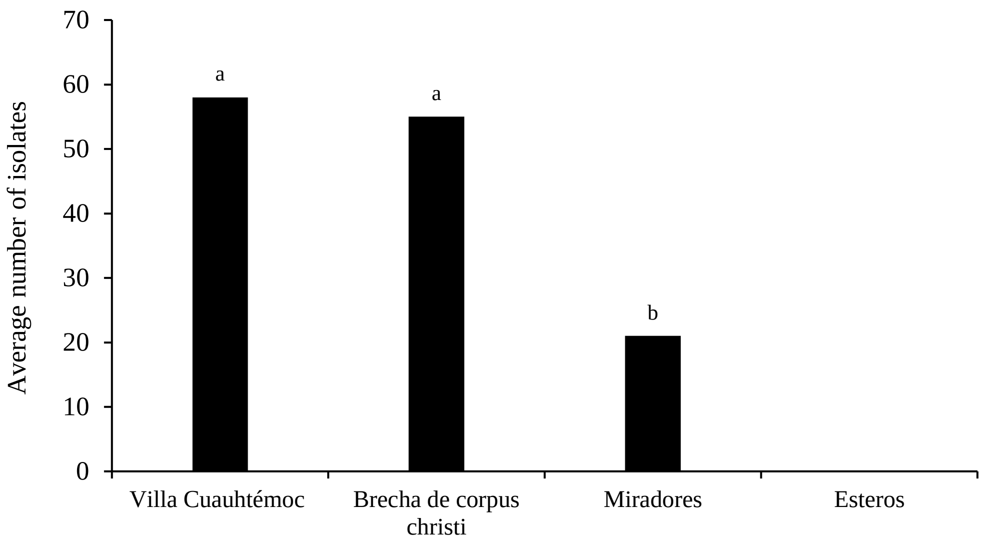

Figure 4. Analysis of the abundance of entomopathogenic fungi by type of location.

On the other hand, statistical analysis made between the types of genres and environments showed significant statistical difference (Figure 5). In the case of the genus *Beauveria* sp., The abundance of this fungus was different in the environments (*p*- vaule: 0.003). The greatest abundance was collected in the orchards with fruit trees and in the plots cultivated with grasses and fabaceae. The previous thing, with respect to the abundance of the fungus observed in the natural areas and in the plots cultivated with vegetables.

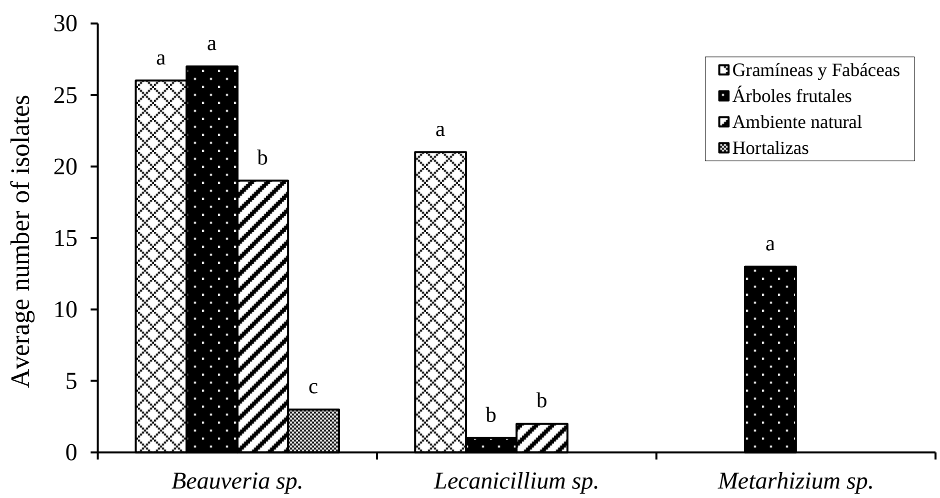

Figure 5. Analysis of the abundance of entomopathogenic fungi by type of environment.

While, *Lecanicillium* sp. it was distributed in three of the four environments. Among the environments, the abundance of the fungus was different (*p*- vaule: 0.027). The greater abundance of the fungus was observed in the environments cultivated with grasses and fabaceae, followed by the abundance determined in the orchards with fruit trees and in the natural areas. Conversely, this genus was not collected in the plots cultivated with vegetables.

Finally, statistical difference was observed in the number of isolates of the fungus *Metarhizium* sp., Between the environments. The genus *Metarhizium* sp., was only collected in the orchards of fruit trees.

On the other hand, the statistical analysis performed between the abundance of the genders and the types of locality showed statistical difference (Figure 6). To the respect, *Beauveria* sp., was distributed in three of the four locations sampled. In these, the abundance of the fungus was different (*p*- vaule: 0.002). The greatest abundance of *Beauveria* sp., was observed in the common Villa Cuauhtémoc followed by the abundance of the fungus determined in the Breach of Corpus Christi and in the common Miradores. While, in the Esteros common, the fungus was not collected.

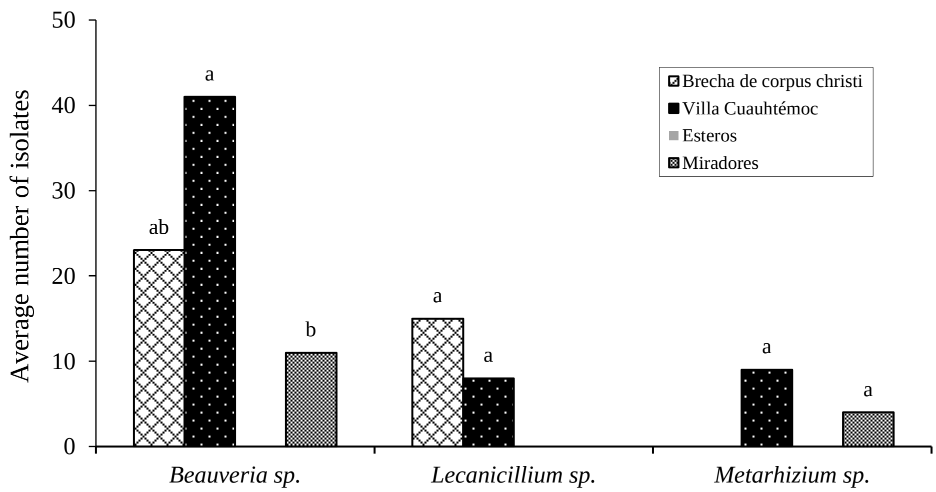

Figure 6. Analysis of the abundance of entomopathogenic fungi by type of location.

In the case of *Lecanicillium* sp. This fungus was distributed in two of the four locations. In which, the abundance of the fungus was different (*p*- vaule: 0.0001). The greatest abundance of the fungus was observed in the Corpus Christi Breccia. The above, with respect to the abundance of the fungus in the common Villa Cuauhtémoc. In contrast, this was not collected in both the common Miradores and the common Estero.

Similarly, *Metarhizium* sp. it was distributed in two of the four locations sampled. In these, the abundance of the fungus was different (*p*- vaule: 0.0001). The greatest abundance was observed in the common Villa Cuauhtemoc. The above, with respect to the abundance of the fungus determined in the common Miradores. While, in the Corpus Christi breccia and in the common Esteros, the fungus was not collected.

Entomopathogenic fungi are commonly isolated from the soil (Jaronski, 2010). In the present work, it was collected to the genera *Beauveria* sp., *Metarhizium* sp., *Lecanicillium* sp., *Paecilomyces* sp., *Trichoderma* sp. and *Isaria* sp. Of which, *Beaveria* sp. It was the most abundant. In Mexico, this fungus was collected in greater abundance in Guanajuato and Coahuila (Sánchez-Peña *et al*., 2011, Pérez-González *et al*., 2014). While, worldwide, it has been collected in greater abundance in the Czech Republic, Finland, Germany, Japan, Italy, Poland and Spain (Kleespies *et al*., 1989; Tarasco *et al*., 1997; Shimazu *et al*., 2002; Landa *et al*., 2002; Asensio *et al*., 2003; Sapieha-Waszkiewicz *et al*., 2003).

On the other hand, *Metarhizium* sp., *Lecanicillium* sp., *Paecilomyces* sp. and *Isaria* sp., were less abundant. *Metarhizium* sp., has been collected in lower abundance in Coahuila and Guanajuato, Mexico (Sánchez-Peña *et al*., 2011; Pérez-González *et al*., 2014). In contrast, in Canada and the USA UU., Bidochka *et al*. (1998); Shapiro-Ilan *et al*. (2003) report a greater abundance of this fungus in different environments. In the case of *Paecilomyces* sp., Sánchez-Peña *et al*. (2011) reports the shortage of this genus in Coahuila, Mexico. This same result has been obtained with *Lecanicillium* sp., *Paecilomyces* sp. and *Isaria* sp. in various parts of the world (Steenberg, 1995; Chandler *et al*., 1997; Keller *et al*., 2003; Tkaczuk, 2008). On the other hand, *Trichoderma* sp. is an opportunistic and anaerobic fungus present in the soil as a saprophyte or parasite of phytopathogenic fungi (Infante *et al*., 2009).

According to the distribution, entomopathogenic fungi are reported in aquatic forests, agricultural areas, pastures, deserts and urban areas (Sánchez-Peña, 1990; Lacey *et al*., 1996; Chandler *et al*., 1997). In the present work, six genera of fungi were collected in greater abundance in the orchards of fruit trees and in the areas cultivated with grasses and fabaceae followed by natural areas and vegetable crops. Like these results, Sánchez-Peña *et al*. (2011), reported higher abundance of entomopathogenic fungi in the soil with oak and shrub trees than in cultivated soils.

This same result was recorded in the tropical and temperate forests of Mexico and throughout the world (Evans and Samson, 1982; Sánchez-Peña, 1990; Wongsa *et al*., 2005). Ali-Shtayeh *et al*. (2002); McCoy *et al*. (2007) report that trees harbor a diversity of microorganisms, because their canopy provides shade, maintains moisture and minimizes the entry of UVB rays to the ground. In contrast, crops such as sorghum release allelochemicals that inhibit the development of living organisms in the soil (Dayan *et al*., 2010). This contrasts with the results of this research, due to the fact that in the cultivated areas with grasses and fabaceae, possibly due to the use of entomopathogenic fungi in the control of pests and the rotation of sorghum and soybean crops.

On the other hand, another factor that affects the presence of microorganisms in an environment is the use of chemical products and the agronomic management of the crop (Tkaczuk *et al*., 2013). According to several authors, entomopathogenic fungi are severely affected in vegetable crops by the amount of agrochemicals applied (Klingen and Haukeland, 2006; Quesada-Moraga *et al*., 2007; Jabbour and Barbercheck, 2009; Oliveira *et al*., 2013). Similar result was observed in the present work in the areas cultivated with sampled vegetables.

In another sense, fungi were collected in greater abundance in Villa Cuauhtemoc and in the Breach of Corpus Christi followed by Miradores. While, in Esteros they were not collected. According to Quesada-Moraga *et al*. (2007); Vega *et al*. (2012), the microclimate of the locality, the variety of crops, the type and the agronomic management of the soil play an important role in the presence and abundance of fungi in a locality. In relation to the variety of crops and agronomic management of the soil, the common Villa Cuauhtemoc and the Breach of Corpus Christi are localities with high productivity of soy, sorghum, cotton, safflower, corn and vegetables. While, Miradores and Esteros are common localities with scant agricultural activity.

Regarding the abundance and distribution of fungal genera collected, *Beauveria* sp., was collected in the four environments. The highest abundance of this fungus occurred in the orchards with fruit trees and in the areas cultivated with Gramineae and Fabaceae. It was also determined that *Beauveria* sp., was distributed in three of the four localities sampled. The greatest abundance of *Beauveria* sp., was recorded in the common Villa Cuauhtémoc. In contrast, in the Esteros common the fungus was not collected. Several authors indicate that *Beauveria* sp. it is capable of adapting to a wide range of environments and locations in various parts of the world (Sevim *et al*., 2010; Imoulan *et al*., 2011; Pérez-González *et al*., 2014).

The above is attributed to the wide range of hosts and the number of specialized cryptic species or adapted to hosts and specific environments of the study area (Pérez *et al*., 2014). In another sense, the absence of this fungus in the common Esteros may be related to the physicochemical properties of the soil. In this regard, Shimazu and Sato (2002); Quesada-Moraga *et al*. (2007); Karthikeyan *et al*. (2008); Medo *et al*. (2011) report that soil pH affects the development of the *Beauveria* genus. In addition to the above, also the lack of host insects due to the low agricultural activity of the locality affects the presence of the fungus. Because, *Beauveria* sp., requires frequent infection of insects to survive in an environment (Vänninen, 1996).

With regard to the genus *Lecanicillium* sp., it was collected in greater abundance in the areas cultivated with grasses and fabaceae. Oliveira *et al*. (2013); Tkazuk *et al*. (2014) reported this fungus in lower abundance in cultivated areas in Poland and Portugal. While, in China it was collected in greater abundance in natural environments (Sun and Liu, 2008). In the present work, *Lecanicillium* sp. it was not collected in the cultivation of vegetables. One of the main causes could be the number of pesticide applications. However, the presence of this fungus was observed in the localities with greater agricultural activity (Breach of Corpus Christi and Villa Cuauhtémoc). In this regard, Wraight *et al*. (2000) mentions that this may occur due to the use of the fungus as a bioinsecticide in the control of insect pests.

Whereas, the genus *Metarhizium* sp. it was only collected in the orchards with fruit trees in the common Villa Cuauhtemoc and Miradores. In several studies indicate that *Metarhizium* sp. it is found in agricultural areas because it is tolerant to insecticides (Vänninen, 1996; Bruck, 2004; Quesada-Moraga *et al*., 2007). However, in the present work it was not collected in the cultivation of vegetables and in the areas cultivated with grasses and fabaceae. Recently, it has been reported that some species of *Hypocreales* fungi can interact with the roots of plants and survive in the soil without the presence of host insects (Klingen *et al*., 2015). In this regard, Wyrebek *et al*. (2011) reported two species of the genus *Metarhizium* sp. in the rhizosphere of trees.

While, Fisher *et al*. (2011), determined the presence of *M*. *brunneum* Petch in the rhizosphere of strawberry and cranberries trees. As well as the presence of *M*. *guizhouense* Kepler, SA Rehner & Humber and *M*. *robertsii* SA, Rehner & Humber in the roots of conifers. In the present work, it was clear the presence of this fungus in the orchards with fruit trees. In this regard, Bidochka *et al*. (2001) indicates that *Metarhizium* sp., adapts to UV radiation and climatic conditions of the environment. This allows it to persist and have a greater probability of contact with host insects (Nishi *et al*., 2017). While, Fisher (2011) mentions that the roots of the trees favor the development of *Metarhizium* sp. and Bruck (2010) indicates that this genus can grow between the roots using plant carbon.

Conclusions

In conclusion, *Beauveria* sp., *Metarhizium* sp., *Lecanicillium* sp., *Paecilomyces* sp., *Trichoderma* sp. and *Isaria* sp., are found in different locations and environments of southern Tamaulipas. Of which, the genus *Beauveria* sp. presented greater abundance and distribution. While, the other genera were collected in specific locations and environments. According to this result, it is possible that the genera are formed by a cryptic complex of species. Due to the above, it is essential to carry out other studies focused on ecology, biology and taxonomy in order to have a better understanding of the role played by this group of pathogens in the regulation of plague insect populations.

Acknowledgments

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Cited literature

Ali, S. M. S.; Mara, A. B. and Jamous, R. M. 2002. Distribution, occurrence and characterization of entomopathogenic fungi in agricultural soil in the Palestinian area. Mycol. Appl. 156(3):235-244.

Asensio, L. T.; Carbonell, J. A.; López, J. and López, L. L.V. 2003. Entomopathogenic fungi in soils from Alicante province. Span. J. Agric. Res. 3(1):37-45.

Barnett, H. L. and Hunter, B. B. 1999. Illustrated genera of imperfect fungi. APS Press, American Phytopathol. Soc. 4th (Ed.). St. Paul, MN. 218 p.

Bidochka, M. J.; Kamp, A. M.; Lavender, T. M.; Dekoning, J. and De Croos, J. N. A. 2001. Habitat association in two genetic groups of the insect-pathogenic fungus Metarhizium anisopliae: uncovering cryptic species? Appl. Environ. Microbiol. 67(3):1335-1342.

Bidochka, M. J.; Kasperski, J. E. and Wild, G. A. M. 1998. Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. Canad. J. Bot. 76(7):1198-1204.

Bruck, D. J. 2004. Natural occurrence of entomopathogens in Pacific Northwest Nursery soils and their virulence to the Black Vine Weevil, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae). Environ. Entomol. 33(5):1335-1343.

Bruck, D. J. 2010. Fungal entomopathogens in the rhizosphere. Biol. Control. 55(1):103-112.

Chandler, D.; Hay, D. and Reid, A. P. 1997. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Appl. Soil Ecol. 5(2):133-141.

Dayan, F. E.; Rimando, A. M.; Zhiqiang, P.; Baerson, S. R.; Gimsing, A. L. and Duke, S. O. 2010. Sorgoleone. Phytochem. 71(10):1032-1039.

Evans, H. C. and Samson, R. A. 1982. Cordyceps species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems. I. The *Cephalotes (Myrmicinae)* complex Trans. Br. Mycol. Soc. 79(3):431-453.

Fisher, J. J.; Rehner, S. A. and Bruck, D. J. 2011. Diversity of rhizosphere associated entomopathogenic fungi of perennial herbs, shrubs and coniferous trees. J. Invertebr. Pathol. 106(2):289-295.

Humber, R. A. 2012. Identification of entomopathogenic fungi. *In*: Lacey, L. A. (Ed.). Manual of techniques in insect pathology. Academic Press, Inc. 2nd Ed. California, USA. 151-187 p.

Imoulan, A.; Alaoui, A and El Meziane, A. 2011. Natural occurrence of soil-borne entomopathogenic fungi in the Moroccan Endemic forest of *Argania spinosa* and their pathogenicity to *Ceratitis capitata*. World J. Microbiol. Biotechnol. 27(11):2619-2628.

INEGI. 2018. Encuesta nacional de Agropecuaria 2018. México, DF. https://www.inegi.org.mx/temas/agricultura/.

Infante, D.; Martínez, B.; González, N. and Reyes, Y. 2009. Mecanismos de acción de Trichoderma frente a hongos Fitopatógenos. Rev. de Protección Veg. 24(1):14-21.

Infante, G. S. y Zárate, L. 2003. Métodos Estadísticos. Un enfoque multidisciplinario. 2a (Ed.). Trillas. México, DF. 643 p.

Jabbour, R. and Barbercheck, M. E. 2009. Soil management effects on entomopathogenic fungi during the transition to organic agriculture in a feed grain rotation. Biol. Control. 51(3):435-443.

Jaronski, S. T. 2010. Ecological factors in the inundative use of fungal entomopathogens. Biol. Control. 55(1):159-185.

Karthikeyan, A.; Shanthi, V. and Nagasathya, A. 2008. Effect of different media and pH on the growth of *Beauveria bassiana* and its parasitism on leaf eating caterpillars. Res. J. Agric. Biol. Sci. 4(2):117-119.

Keller, S.; Kessler, P. and Schweizer, C. 2003. Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metarhizium anisopliae*. Biol. Control. 48(3):307-319.

Kleespies, R.; Bathon, H. and Zimmermann, G. 1989. Untersuchungen zum natürlichen vorkommen von entomopathogenen pilzen und nematoden in verschiedenen Böden in der umgebung von darmstadt. Gesunde Pflanzen. 41(10):350-355.

Klingen, I. and Haukeland, S. 2006. The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes. *In*: Eilenberg, J. and Hokkanen, H. M. T. (Ed.). An ecological and societal approach to biological control. Springer, Dordrecht. The Netherlands. 145-212 p.

Klingen, I.; Eilenberg, J. and Meadow, R. 2002. Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. Agric. Ecosyst. Environ. 91(1):191-198.

Klingen, I.; Westrum, K. and Meyling, N. V. 2015. Effect of norwegian entomopathogenic fungal isolates against *Otiorhynchus sulcatus* larvae at low temperatures and persistence in strawberry rhizospheres. Biol. Control. 81:1-7.

Lacey, L. A.; Fransen, J. J. and Carruthers, R. 1996. Global distribution of naturally occurring fungi of Bemisia, their biologies and use as biological control agents. *In*: Gerling, D. and Mayer, R. editors. Bemisia: 1995. Taxonomy, biology, damage, control and management. Andover: Intercept. 401-433 p.

Landa, Z.; Hornak, P.; Charvatova, H. and Osborne, L. S. 2002. Distribution, occurrence and potential use of entomopathogenic fungi in arable soils in Czech Republic. ISTRO-Conference, Brno, Session. 2:195-201.

Lezama-Gutiérrez, R. and Hamm, J. J. 2001. Occurrence of entomopathogens of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Mexican states of Michoacán, Colima, Jalisco and Tamaulipas. Fla. Entomol. 84(1):23-30.

McCoy, C. W.; Stuart, R. J.; Duncan, L. W. and Shapiro-Ilan, D. 2007. Application and evaluation of entomopathogens for the control of citrus pests. *In*: Lacey, L. A.; Kaya, H. K. (Ed.). Field manual of techniques in invertebrate pathology. Springer. The Netherlands. 567-581 p.

Medo, J. and Cagáň, L. 2011. Factors affecting the occurrence of entomopathogenic fungi in soils of Slovakia as revealed using two methods. Biol. Control. 59(2):200-208.

Meyling, N. V. and Eilenberg, J. 2006b. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric. Ecosyst. Environ. 113(1):336-341.

Meyling, N. V. and Eilenberg, J. 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol. Control. 43(2):145-155.

Meyling, N. V.; Lübeck, M.; Buckley, E. P.; Eilenberg, J. and Rehner, S. A. 2009. Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. Mol. Ecol. 18(6):1282-1293.

Meyling, N. V.; Thorup-Kristensen, K. and Eilenberg, J. 2011. Below- and aboveground abundance and distribution of fungal entomopathogens in experimental conventional and organic cropping systems. Biol. Control. 59(2):180-186.

Nishi, O.; Iiyama, K.; Yasunaga-Aoki, C. and Shimizu, S. 2017. Species associations and distributions of soil entomopathogenic fungi *Metarhizium* spp. In Japan. Mycology. 8(4):308-317.

Oliveira, I.; Pereira, J. A.; Quesada-Moraga, E.; Lino-Neto, T.; Bento, A. and Baptista, P. 2013. Effect of soil tillage on natural occurrence of fungal entomopathogens associated to *Prays oleae* Bern. Sci. Hortic. 159(11):190-196.

Onofre, S. B.; Miniuk, C. M.; de Barros, N. M. and Azevedo, J. L. 2001. Pathogenicity of four strains of entomopathogenic fungi against the bovine tick *Boophilus microplus*. Am. J. Vet. Res. 62(9):1478-1480.

Ornmond, E. L.; Thomas, A. P. M.; Pugh, P. J. A.; Pell, J. and Roy, H. E. 2010. A fungal pathogen in time and space: the population dynamics of *Beauveria bassiana* in a conifer forest. FEMS Microbiol. Ecol. 74(1):146-154.

Pérez-González, V. H.; Guzmán-Franco, A. W.; Alatorre-Rosas, R.; Hernández-López, J.; Hernández-López A.; Carillo-Benítez, M. G. and Baverstok, J. 2014. Specific diversity of the entomopathogenic fungi *Beauveria* and *Metarhizium* in Mexican agricultural soils. J. Invertebr. Pathol. 119(5):54-61.

Quesada-Moraga, E.; Navas-Cortés, J. A.; Maranhao, E. A. A.; Ortiz-Urquiza, A. and Santiago-Álvarez, C. 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological Res. 111(8):947-966.

Rendón, S. G. 1994. Muestreo. Aplicación en la estimación simultanea de varios parámetros. Departamento de Parasitología Agrícola. Universidad Autónoma Chapingo. 246 p.

Sánchez-Peña, S. R. 1990. Some insect- and spider-pathogenic fungi from Mexico with data on their host range. Fla. Entomol. 73(3):517-522.

Sánchez-Peña, S. R.; San-Juan, J. L. and Raúl, M. 2011. Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in Saltillo, México, and their virulence Towards Thrips and Whiteflies. J. Insect Sci. 11(1):1-14.

Sapieha-Waszkiewicz, A.; Mietkiewski, R. and Marjanska-Cichon, B. 2003. Occurrence of entomopathogenic fungi in soil from apple and plum orchards. IOBC/wprs Bulletin. 26(1):113-116.

SAS, Institute. 2012. SAS Software for Microsoft Windows Version 9.2. Cary, NC.

Sevim, A.; Demir, M.; Höfte, I.; Humber, R. A. and Demirbag, Z. 2010. Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. Biol. Control. 55(2):279-297.

Shapiro-Ilan, D. I.; Gardner, W. A.; Fuxa, J. R.; Wood, B. W.; Nguyen, K. B.; Adams, B.; Humber, R. A. and Hall, M. J. 2003. Survey of entomopathogenic nematodes and fungi endemic to pecan orchards of the Southeastern United States and their virulence to the pecan weevil (*Coleoptera: Curculionidae*). Environ. Entomol. 32(1):187-195.

Shimazu, M.; Sato, H. and Maehara, N. 2002. Density of the entomopathogenic fungus, *Beauveria bassiana* Vuillemin (*Deuteromycotina: Hyphomycetes*) in forest air and soil. Appl. Entomol. Zool. 37(1):19-26.

Steenberg, T.; Langer, V. and Esbjerg, P. 1995. Entomopathogenic fungi in predatory beetles (*Col.: Carabidae and Staphylinidae*) from agricultural fields. Entomophaga. 40(1):77-85

Sun, B. D. and Liu, X. Z. 2008. Occurrence and diversity of insect-associated fungi in natural soils in China. Appl. Soil Ecol. 39(1):100-108.

Tarasco, E.; De Bievre, C.; Papierok, B.; Poliseno, M. and Triggiani, O. 1997. Occurrence of entomopathogenic fungi in soils in Southern Italy. Entomologica (Bari). 31(1):157-166.

Tkaczuk, C. 2008. Występowanie i potencjał infekcyjny grzybów owadobójczych w glebach agro-cenoz i środowisk seminaturalnych w krajobrazie rolniczym. Numero 94 (Ed.). Rozprawa Naukowa-Akademii Podlaskie. 160 p.

Tkaczuk, C.; Król, A.; Majchrowska-Safaryan, A. and Nicewicz, Ł. 2014. The occurrence of entomopathogenic fungi in soils from fields cultivated in a conventional and organic system. J. Ecol. Eng. 15(4):137-144.

Tkaczuk, C.; Majchrowska-Safaryan, A. and Mietkiewski, R. 2013 Wplyw wybranych fungicydów oraz wyciagów glebowych na wzrost owadobójczego grzyba Metarhizium anisopliae. Prog. Plant Prot. 53(4):751-756.

Toledo, A.; De Remes, L. A. and López-Lastra, C. 2008. Host range findings on *Beauveria bassiana* and *Metarhizium anisopliae* (*Ascomycota: Hypocreales*) in Argentina. Bol. Soc. Argent. Bot. 43(3):211-220.

Vanninen, I. 1996. Distribution and occurence of four entomopathogenic fungi in Finland: effect of geographical location, hábitat Types and soil type. Mycol Res. 100(1):93-101.

Vega, F. E.; Meyling, N. V.; Luangsa-ard, J. J. and Blackwell, M. 2012. Fungal entomopathogens. *In*: Vega, F. E. and Kaya, H. K. (Ed.). Insect Pathol. 2(1):171-220.

Wongsa, P.; Tasanatai, K.; Watts, P. and Hywel-Jones, N. 2005. Isolation and in vitro cultivation of the insect pathogenic fungus Cordyceps unilateralis. Mycol. Res. 109(8):936-940.

Wraight, S. P.; Carruthers, R. I.; Jaronski, S. T.; Bradley, C. A.; Garza, C. J. and Galaini-Wraight, S. 2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentifolii*. Biol. Control. 17(3):203-217.

Wyrebek, M.; Huber, C.; Sasan, R. K. and Bidochka, M. J. 2011. Three sympatrically occurring species of *Metarhizium* show plant rhizosphere specificity. Microbiology. 157(10):2904-2911.

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 Sci. Techn. 18(9):865-901.