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, litchi, 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; Klinge 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).
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 m², 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).
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).

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*-value: 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*-value: 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*-value: 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.](image)

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*-value: 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*-value: 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.

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Cited literature


