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Use and impact of endophytic entomopathogenic fungi: Their potential in the context of agricultural sustainability

Uso e impacto de hongos entomopatógenos endofíticos: Su potencial en el contexto de la sostenibilidad agrícola

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ABSTRACT

The use of entomopathogenic fungi (EF) as endophytes is an environmentally friendly alternative for sustainable food production, given that the current paradigm in crop protection is based on the use of organosynthetic pesticides, with more than two million tons per year worldwide; EF has the ability to live within plant tissues as endophytes, acting as biopesticides. Under this scenario, this review analyzes and discusses the global status of the endophytic entomopathogenic fungi (EEF), their potential in plant protection against plant diseases and insect pests and as plant growth promoters. Successes and failures, and prospects for field application are examined. More than 7000 studies on EEF have been published, with important success cases. However, it is necessary to understand that the agricultural production is based on the use of external inputs, mainly pesticides. While progressive changes occur, it is fundamental to investigate the effect of these substances on the efficacy and persistence of EEF, without neglecting that the lack of knowledge of the effect of biotic and abiotic factors on EEF is an important cause of failures. Future studies should be focused on clarifying aspects such as: application strategies, endophytic persistence and transmission routes to improve the sustainability of agricultural production.

Keywords: endophytes; growth promoters; integrated pest management; plant diseases.

RESUMEN

El uso de hongos entomopatógenos (HE) como endófitos constituye una alternativa para la producción sustentable de alimentos, dado que el paradigma actual en la protección de cultivos se basa en el uso de plaguicidas organosintéticos, con más de dos millones de toneladas anuales. Por estas razones, los HE tienen la capacidad de vivir dentro de los tejidos vegetales como endófitos los cuales actúan como bioplaguicidas. Esta revisión analiza y discute el estatus global de los hongos entomopatógenos endófitos (HEE), su potencial en la protección de plantas contra enfermedades y plagas de insectos y como promotores del crecimiento. Se examinan los éxitos, fracasos y perspectivas de aplicación en campo. Se han publicado más de 7000 estudios sobre HEE con importantes casos de éxito. Sin embargo, es necesario entender que la producción agrícola se basa en el uso de plaguicidas. Mientras ocurren cambios progresivos, es fundamental investigar el efecto de estas sustancias sobre la eficacia y persistencia de los HEE, considerando que el desconocimiento del efecto de los factores bióticos y abióticos sobre los HEE es una causa importante de fracasos. Estudios futuros deberán enfocarse en esclarecer aspectos como estrategias de aplicación, persistencia endófita y vías de transmisión para mejorar la sustentabilidad de la producción agrícola.

Palabras clave: endófitos; enfermedades de plantas; manejo integrado de plagas; promotores del crecimiento.

INTRODUCTION

Along the food production process, a high percentage is consumed by insects. Some of them are considered important pests in agricultural crops, causing losses of 10 to 25 % of world food production, with an estimated value of $ 470 billion (Deutsch *et al.*, 2018), with greatest losses (13 - 16 %) documented to occur in the field (Mantzoukas and Eliopoulos, 2020). Under these conditions, the environmentally healthy management of these pests represents a considerable challenge for world food security. Currently, pest management is based on the indiscriminate and extensive use of organosynthetic pesticides, with more than two million tons per year in the world. As a result, more than 500 species of pest insects have developed resistance to various active ingredients; furthermore, the elimination of biological control agents reduces the efficiency of natural control (Carvalho, 2017; Kumar and Kalita, 2017). This implies a strong demand for the development of environmentally friendly alternatives, but at the same time, profitable and reliable for a healthy and sustainable food production.

Biological control is presented as one of the safest strategies within the framework of Integrated Pest Management (IPM). A biological control strategy with high potential in the sustainable management of pests, is the use of fungal entomopathogens. In addition to insects, entomopathogenic fungi (EF) infect mites, nematodes, and even phytopathogenic fungi (Yun *et al.*, 2017; Devi, 2018). The species *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin 1912, *Isaria fumosorosea*

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Wize, 1904, *Hirsutella thompsonii* FE Fisher 1950, *Metarhizium anisopliae* Metschnikoff 1879, *M. brunneum* Petch, 1935 and *M. robertsii* Bisch, are some examples of them (Greenfield *et al*., 2016; Jaber and Enkerli, 2016; McKinnon *et al*., 2017; Ahmad *et al*., 2020; Sun *et al*., 2020). Although there are multiple studies on pest control via EF, its greatest use has been through the flood strategy (*e. g.* sprinkling). However, this strategy has been pointed out as one of the least efficient in pest control (Mora-Aguilera *et al*., 2017). This fact, together with the lack of knowledge in the implementation of microbial control as applied epidemiology has caused numerous failures in the use of entomopathogenic fungi in the field (Mora-Aguilera *et al*., 2017). The EF have the potential to be used through various application strategies (*e. g.* the autodissemination by the same target insect) (Kabaluk *et al*., 2015; Mfuti *et al*., 2015; Getahun *et al*., 2016; Gutiérrez-Cárdenas *et al*., 2019). A further advantage that has recently been elucidated for EF is their ability to live endophytically. Under these conditions, they do not harm the plant; on the contrary, they contribute to its protection against pests, growth promotion and antagonism against diseases triggered by phytopathogenic fungi (Barelli *et al*., 2016; Yun *et al*., 2017; Russo *et al*., 2019; Rivas-Franco *et al*., 2020). For example, it has been documented that some species of cultivated plants inoculated with EF, increase their growth (Jaber and Ownley, 2017; Russo *et al*., 2019).

The above has generated an enormous interest in demonstrating the potential of endophytic entomopathogenic fungi (EEF) in the stimulation and promotion of plant growth. However, there are still multiple questions to make the use of EEF more efficient, such as: What is the impact of pesticides on their persistence and effectiveness? What about transgenic plants? What is the best formulation and application technique in greenhouse and field? How do biotic and abiotic factors influence its persistence and effectiveness? This review analyzes and discusses the global status of entomopathogenic fungi as endophytes, their potential for use in agroecosystems, as well as their limitations and possible disadvantages under the current agricultural paradigm.

The discovery of the potential of endophytic entomopathogenic fungi: a historical overview

Insects, plants, and fungi have shared similar environmental conditions throughout their evolution (Khare *et al*., 2018). In the *Metarhizium* genera, its pathogenicity against insects is recognized since 1879 when E. Metchnikoff discovered that the fungus was the cause of death of some arthropods (Stone and Bidochka, 2020). However, it was not long ago that mycologists and entomologists observed in detail the influence that fungi have on insects, and motivated by the interest to understand these interactions, a great opportunity arose for their study and understanding. Thus, Quentin Wheeler, as a proponent of phylogenetic analysis, hoped to encourage entomologists and mycologists to work together, so he organized a Symposium with the participation of the Entomological Society of America, held in Syracuse and Ithaca, New York, USA in 1981 (Vega and Blackwell, 2005). This allowed the foundations to elucidate the mechanisms of these prominent biocontrolling agents. Later, Bing and Lewis (1992) reported the endophytic growth and colonization of *B. bassiana* in maize, in addition to observing for the first time that colonization of the plant by the fungus caused the mortality of the European corn borer *Ostrinia nubilalis* (Hübner 1796) (Lepidoptera: Crambidae). The findings documented by Bing and Lewis (1992), are now common to practically all plant species that have been analyzed for endophytic fungi and show that something common in EEF, is that they can colonize plant tissues without damaging the plant, while on the contrary, they bring benefits such as protection against pests and phytopathogens. In addition, it is currently known that EEF naturally colonize cocoa plants, beans, sorghum and wheat, among others (Behie *et al*., 2017; Sánchez-Rodríguez *et al*., 2018; Ambele *et al*., 2020; Mantzoukas and Grammatikopoulos, 2020).

Since then, more than 7000 scientific articles have dealed with a better understanding of the impact of environmental factors (*e. g.* humidity, temperature, presence or limitation of nutrients) in the colonization of plants by fungi, as well as their entomopathogenic role (Vega *et al*., 2009). However, much remains to be understood, which is why it is necessary to lead scientific research towards an approach based on a new paradigm, where EEF can be used massively under field conditions. Therefore, it is still necessary to unravel the full potential of these entomopathogenic agents, including investigations for their use as endophytes, antagonists of plant diseases, colonizers of the rhizosphere and plant growth promoting fungi. This will allow the use of EEF on a large scale as a healthy and sustainable alternative to conventional agrochemicals (Khare *et al*., 2018).

Potential of endophytic entomopathogenic fungi as promoter of plant growth and protection

The term endophytic refers to any organism that establishes a non-obstructive, asymptomatic, and transient cost-benefit relationship within the living tissues of the host plant (Jaber and Ownley, 2017). Endophytic microorganisms constitute a diverse group and can be found in a wide range of plants, including algae, mosses, grasses and other vascular plants (Bamisile *et al*., 2018b). In the case of EEF, they mainly belong to the Phylum Ascomycota, although those belonging to the Phylum Basidiomycota, Oomycota and Zygomycota are also important. An example as EEF use is the fungus belonging to the Basidiomycota phylum, *Leucocalocybe mongolica* (S. Imai) X.D. Yu & Y.J. Yao, 2011 (Agaricales: Clitocybaceae); Wang *et al*. (2022) documented that this fungus was able to promote the growth of *Arabidopsis thaliana* (Brassicaceae), wheat and cotton, showing that in these plants, when in the presence of *L. mongolica*, the fungus allowed the development of the bacterium *Bacillus pumilus* Meyer and Gottheil 1901 (Bacillales: Bacillaceae), which was able to solubilize phosphorus when the plants were placed under saline stress, thus promoting their growth.

Another more recent example was described by Andersen *et al*. (2024), who applied 10 mL of *Pythium oligandrum* (Pythiales: Pythiaceae) (fungus belonging to the phylum Oomycota) by foliar spray on table potato crops where they included four varieties (Kuras, Desirée, King Edward and Kuras Field) under field conditions and greenhouse. The results obtained revealed that the Kuras genotype responded to *P. oligandrum* treatment with significantly greater plant height and fresh weight of both shoots and roots compared to the untreated treatments, respectively.

Historically, the classification has grouped EEF both inside (colonize grasses), and outside (colonize angiosperms, conifers, ferns, and non-vascular plants) the family Clavicipitaceae (Bamisile *et al*., 2018b). One of the most important ecological functions of EEF as endophytes is the capacity that they confer on the host plant to take up nutrients. This is because the mechanism used by EEF to increase the plant's ability to absorb nutrients, is the plant-fungus symbiosis, which allows the plant to improve nutrient absorption when these fungi increase the effective areas of root absorption through the formation of dextramatrical hyphal length (EMH) (Chen *et al*., 2020; Ai-Tian *et al*., 2021). These hyphae constitute extensions of the fungal mycelium and extend beyond the plant root surface, thereby increasing significantly the volume of soil explored resulting in a greater capacity to absorb water and nutrients, including nitrogen and phosphorus (Mohamed *et al*., 2022; Zeng *et al*., 2024). The response of the plant is an increase in its growth, greater tolerance to stress and production of secondary metabolites, which protect the plant against insects and pathogens (Yun *et al*., 2017; Clifton *et al*., 2018; Ahmad *et al*., 2020).

It has recently been documented that, while some species of fungi function as natural endophytes in plants, others can be deliberately introduced into them using different inoculation methods. For the application of EEF various methods are available, but two of them constitute the majority of scientific studies developed by the scientific community, namely the promotion of plant growth and their impact as biological control agents of plant pathogens (Fontana *et al*., 2021). In this regard, pioneering studies document different application strategies: through known conidia suspensions (Garrido-Jurado *et al*., 2017; Jaber and Enkerli, 2017), direct inoculation of seeds (Ramakuwela *et al*., 2020), injection of inoculum in stems (Mantzoukas and Eliopoulos, 2020), inoculation of roots by immersion (Russo *et al*., 2015), and through irrigation with conidia suspensions (Sánchez-Rodríguez *et al*., 2018).

There are other mechanisms involved in promoting plant growth mediated by EEF; probably one of the most important is the absorption of nutrients (*e. g.* iron). This has been demonstrated in cabbage crops (*Brassica oleracea* L.) colonized by *B. bassiana* and *M. brunneum*, which promoted significantly longer shoot length (123 % for *B. bassiana* and 53.3 % cm for *M. brunneum*) in comparison with the control treatments (Dara *et al*., 2017). Behie *et al*. (2017), verified the ability of the fungus *M. robertsii* to fix nitrogen derived from corpses of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) insects and transfer it to bean plants (*Phaseolus vulgaris* L.), observing a significant increase in root growth (up to 31.8 %). These results have generated greater acceptance and popularity in the use of EEF in agricultural systems, with a significant reduction in agrochemicals, and with it, less damage to the environment and to human and animal health (Dara, 2019; Quesada-Moraga, 2020).

Recent studies conclude that the endophytism carried out by EEF and the close beneficial interactions with plants are a process like that of mycorrhizal fungi (Jaber, 2018). In addition, the EEF confer abiotic resistance to the plant against water stress (drought and waterlogging), salinity and mineral toxicity (Martinez-Medina *et al*., 2016; Wei *et al*., 2020). EEF has now been reported to have the ability to act as antagonists against phytopathogens. For instance, a study carried out with cotton plants (*Gossypium hirsutum* L.) under greenhouse conditions showed that soil inoculation with conidia of *B. bassiana* and *M. brunneum*, induced resistance in plants from 15 % to 57 % against the pathogen *Fusarium oxysporum* Schltdl., 1824 (Hypocreales: Nectriaceae), indicating the potential of EEF in plant protection (Dara *et al*., 2016).

Several capacities of EEF have also been verified (Table 1). Examples of this are observed in the potential of *B. bassiana* to be transmitted inside the soil by endophytically colonizing the roots of various plant species: cacao (*Theobroma cacao* L.) (Ambele *et al*., 2020), barley (*Hordeum vulgare* L.) (Veloz-Badillo *et al*., 2019), strawberry (*Fragaria x ananassa* Weston) (Canassa *et al*., 2020), beans (*Phaseolus vulgaris* L.) (Behie *et al*., 2017), corn (*Zea mays* L.) (Ahmad *et al*., 2020), cassava (*Manihot esculenta* Crantz) (Greenfield *et al*., 2016), soybean (*Glycine max* L.) (Russo *et al*., 2019), tobacco (*Nicotiana tabacum* L.) (Lee and Kim, 2019) and wheat (*Triticum durum* Desf., and *Triticum aestivum* L.) (Sánchez-Rodríguez *et al*., 2018; González-Guzmán *et al*., 2020). The above shows that EEF in natural environments colonize and promote plants development, being able to survive in or around them (even in the absence of their host insects) by obtaining nutrients directly from the plant without negatively affecting it. An important consideration within the potential of EF as endophytic is that they indirectly affect pest populations through non-entomopathogenic mechanisms, such as antibiosis and antixenosis (Dara, 2019), and induced systemic resistance (Wei *et al*., 2020). The suggested explanation is that secondary metabolites produced by fungi generate greater resistance to the plant against the attack of pest insects, reducing their appetite, fecundity, fertility, and longevity. Furthermore, the studies confirm that EEF not only affected the development of pests but were also able to promote a significant increase in plant growth (Sánchez-Rodríguez *et al*., 2018; Sun *et al*., 2020; Wei *et al*., 2020).

Effect of pesticides and genetic modified plants

Modern agriculture is based on crop production systems that require the use of agrochemicals to improve and protect

Table 1. Culture, type of endophytic entomopathogenic fungi, application and results obtained as antagonists, against phytophagous insects and for growth promotion.
Tabla 1. Cultivo, tipo de hongo entomopatógeno endófito, aplicación y resultados obtenidos como antagonista contra insectos fitófagos y para la promoción del crecimiento.

Crop	Fungal species	Inoculation type	Result on pest / or controlled disease / improved physiological appearance	Reference
Corn, soy	*Bb*	Si, Ri, Fs	Initial successful colonization (100 %) and persistence of 1.7-2.8 % of the inoculation in 28 days	Russo *et al.* (2015)
Cotton	*Bb, If, Mb*	Sd, Fs	Induction of 15-57 % resistance *vs Fusarium oxysporum*	Dara *et al.* (2016)
Cassava	*Bb, Ma*	Sd	Successful colonization (80-100 %). Persistence (40 %) of 47-49 days after inoculation	Greenfield *et al.* (2016)
Bean	*Bb, Hl, Ma*	Si	Successful inoculation (95-100 %) and significant decrease (25-28 %) in the levels of infestation by *Liriomyza* spp	Gathage *et al.* (2016)
Bean	*Bb, Mb*	Si	Growth promotion and persistence of 64 % for *Bb* and 58 % for *Mb* after 28 days after inoculation	Jaber and Enkerli (2016)
Corn	*Bb*	Fs	Successful colonization (66.66 %) and persistence for 60 days (33.33 %) *vs Chilo partellus*	Renuka *et al.* (2016)
Cabbage	*Bb, If, Mb*	Ss	The fungi *Bb* and *Mb* increased shoot length by 29 and 27.6 cm compared to 13 and 18 cm for controls	Dara *et al.* (2017)
Melon	*Bb, Mb*	Il	Successful colonization (40-98 %) and mortality of *Bemisia tabaci* nymph between 83.9 and 100 %	Garrido-Jurado *et al.* (2017)
Soy	*Bb, Mb*	Si	Successful colonization and 6.25 and 20.8 % of the fungi were recovered in stem and leaf samples	Clifton *et al.* (2018)
Wheat	*Bb, Mb*	Si	Growth promotion (26.9-28.3 *vs* 21.8 cm for control) and reduction of the incidence (50 %) of *Fusarium culmorum*	Jaber (2018)
Wheat	*Bb*	Sc y Sd	Increase in grain yield by 40 % and mortality of 30-57 % of *Spodoptera littora-lis* larvae	Sánchez-Rodríguez *et al.* (2018)
Cabbage	*Bb, Ll*	Il	Mortalities of 92-95 % of the aphid *Myzus persicae* after 10 days of the applica-tion of conidia	Javed *et al.* (2019)
Tobacco	*Ij*	Ri	Growth promotion (17 cm in height *vs* 12 cm for control) and 4 g increase for inoculated plants *vs* to 2 g for control	Lee and Kim (2019)
Soy	*Bb, Ma, Mr*	Fs, Si, Ri	The isolates of *Bb* showed 45-100 % of colonization, and *Ma* and *Mr* colonized 16-60 % 7 days after inoculation	Russo *et al.* (2019)
Corn	*Mr*	Si	Higher height *vs* control plants (91.54 *vs* 90.03 cm) and lower growth rate of *Agrotis ipsilon* in inoculated plants	Ahmad *et al.* (2020)
Cocoa	*Bb, Hl, Ma*	Si, Fs	Successful colonization (82.2-94.7 %) of *Hl* and 100 % of mortality of *Odon-totermes* spp	Ambele *et al.* (2020)
Strawberry	*Bb, Mr*	Ri	Reduction of *Tetranychus urticae* (225.6 ± 59.32) for *Mr*, and 206.5 ± 51.48 for *Bb vs* 534.1 ± 115.55 for control	Canassa *et al.* (2020)
Sorghum	*Bb, If, Mr*	Fs	Mortality of *Sesamia nonagrioides* larvae of 90-93 % and reduction in crop consumption (39-64 %) by the three fungi	Mantzoukas and Grammatiko-poulos (2020)
Walnut	*Bb*	Sc	Successful establishment of *Bb* in plants and mortality of 40-42 % of *Curculio caryae* 21 days after inoculation	Ramakuwela *et al.* (2020)
Eggplant	*Cf*	Si	Mortality between 28-33 % of *Bemisia tabaci* pupae	Sun *et al.* (2020)

Bb = *Beauveria bassiana*, *If* = *Isaria fumosorosea*, *Mb* = *Metarhizium brunneum*, *Ma* = *Metarhizium anisopliae*, *Hi* = *Hypocrea lixii*, *Ll* = *Lecanicillium lecanii*, *Ij* = *Isaria javanica*, *Mr* = *Metarhizium robertsii*, *Cf* = *Cordyceps fumosorosea*, Si = Seed inoculation, Ri = Root inoculation, Fs = Foliar spray, Sd = Soil drench, Il = Immersion leaves, Sc = Seed coating.

yields. Production of these substances increased by 11 % annually, from 0.2 million in the 1950 to more than 5 million tons in 2000. Herbicides, insecticides, and fungicides are the groups with the highest sales worldwide, with carbamates, organophosphates, pyrethroids, neonicotinoids and growth regulators being some of the most widely used substances (Carvalho, 2017).

The close association between endophytic fungi and their host plants suggests an important effect of agrochemicals in the former. However, there is still little knowledge in this regard (Zhou *et al*., 2016; da Costa Stuart *et al*., 2018). While several authors report that some pesticides did not affect the diversity of endophytic fungi (Zhou *et al*., 2016; Win *et al*., 2021), others have registered a reduction in diversity in treated crops (da Costa Stuart *et al*., 2018). However, there is consensus in the fact that pesticides can alter the composition of endophytic fungi, because where pesticides were applied, species that were not registered in the untreated crops usually appeared and predominated. It is argued that these changes could negatively influence the physiology of the plant, and probably the quality of the food we consume (Zhou *et al*., 2016; da Costa Stuart *et al*., 2018; Win *et al*., 2021). It is clearly appreciated that the previous studies were developed in different regions, crops and pesticides. Given the wide variety of pesticides (many with systemic activity), crops and varieties, and the complex interactions with the environment, the knowledge about the effect of chemical pesticides on endophytic fungi appears to be still incipient.

Additionally, the aforementioned studies have been focused on endophytic fungi in general, and only sporadically some species of entomopathogens are recorded. Although there is abundant knowledge on the impact of pesticides on entomopathogenic fungi *in vitro* (Pérez-González and Sánchez-Peña, 2017; Wari *et al*., 2020), there is a lack of knowledge about their effect endophytically.

Regarding the potential impacts of genetic engineering technologies on the well-being of farmers and the environment, there is much to analyze, highlighting that the economic effects of genetically modified crops are highly understood (Ganesan *et al*., 2017; Kumar *et al*., 2018; Araus *et al*., 2019). Cotton *Gossypium* spp (Malvaceae) is one of the most important crops worldwide that serves as raw material for textile industries, and has a high economic impact of approximately 600 billion dollars annually (Khan *et al*., 2020). In this sense, genetically modified cotton is in high demand and in its genome is the gene that encodes the *Cry1Ac* protein from *Bacillus thuringiensis* Berliner 1915 (Bacillales: Bacillaceae), which acts as a bioinsecticide against lepidopteran pests. This protein is expressed in these plants throughout their life cycle, and can interact directly with microorganisms associated with the crop, which could negatively affect the environmental functions of the endophytic fungal community (Bruinsma *et al*., 2003). Based on the above, De Souza Vieira *et al*. (2011) report in Brazil the evaluation of a transgenic Bt cotton (Acala 90B) and a natural non-Bt cotton (Acala 90) during different stages of crop development (pre-flowering, flowering, capsule formation and opening), to investigate the possible effects non-target in endophytic fungal communities. The results obtained revealed that the expression of the Bt *Cry1Ac* protein of the genetically modified cotton plants did not affect the degree of fungal colonization, which allowed the obtaining of a total of 17 genera of endophytic fungi, including the species *Acremonium* spp., *Cladosporium* spp., *Colletotrichum* spp., *Curvularia* spp., *Fusarium* spp., *Glomerella* spp., *Guignardia* spp., *Lecanicillium* spp., *Nigrospora* spp., *Pestalotiopsis* spp., *Phoma* spp., *Phomopsis* spp., *Rhizopus* spp., *Rhodotorula* spp., *Talaromyces* spp., *Tritirachium* spp. and *Xylaria* spp., respectively. In the case of EEF, in a study carried out by Morjan *et al*. (2002), is documented that herbicides used in genetically modified crops of soybeans and corn had negative effects on the fungi *B. bassiana*, *M. anisopliae*, *Nomuraea rileyi* (Farlow, 1974) (Hypocreales: Clavicipitaceae) and *Neozygites floridana* Weiser & Muma, 1966 (Neozygitales: Neozygitaceae), when exposed to seven standardized 0.96 % formulations (the lowest recommended dose for field applications) that include glyphosate as an active ingredient. In the study, the mycelial growth area and the spore density were estimated, finding that the four analyzed fungi were susceptible to glyphosate. These results are alarming, since spraying these herbicides in large areas and with 100 % coverage in transgenic crops can be detrimental to EEF and thus, negatively impact the natural epizootics necessary for the suppression of pest insects. Meanwhile, other studies mention that the flow of genes transferred horizontally in genetically modified crops is considered an important evolutionary force that alters gene frequencies through mutations, genetic drift and selection (Lu and Yang, 2009). In this sense, gene flow can negatively affect the environment by creating a reduction in the genetic variability of native populations, the alteration of biodiversity and the alteration of the soil microenvironment. For these reasons, the introduction of genetically modified plants in agroecosystems poses potential risks for the species that inhabit it, including EEF, with the premise that it is difficult to predict their consequences in the future, possibly leading to the development of superweeds, the evolution of new viral pathogens and the evolution of pest insects with resistance to new compounds (Tsatsakis *et al*., 2017). For these reasons, it is important to analyze and discuss the long term and large-scale impact of genetically modified crops on other non-target organisms in agricultural ecosystems before carrying out their approval and release into the environment. It is also necessary to make efforts that favor the design of new products, as well as contribute to the improvement of application devices. Besides, the growing public concern about the excessive use of agrochemicals and genetically modified organisms has triggered a social pressure for a transition towards agroecological pest management. Given this scenario, Lechenet *et al*. (2017) highlights that it is possible to achieve a significant decrease in the use of herbicides, fungicides, and insecticides (37, 47 and 60 %, respectively) through the adoption of new production strategies where biological control is of fundamental importance.

Considerations in the application of endophytic entomopathogenic fungi: the elucidation of its potential in sustainable agriculture

For its implementation in agricultural systems, EEF must be applied to plants, at least through three possible strategies: to the seed, to the foliage or to the roots. It is important to note that as research with EEF progresses, it has been discovered that their success on the capacity of colonization and growth promotion of host plants depends on the methodology used for inoculation (Jaber and Enkerli, 2016; Bamisile *et al*., 2018a) and some of the main EEF mechanisms of action involved in generating enhanced pest control or disease resistance, include the following: A) Toxin and enzyme production, an example of this is the fungus *Aspergillus sojae* Sakaguchi & K. Yamada ex Murakami, 1971, which was isolated from the oregano plant *Coleus amboinicus* Lour, 1790 (Lamiaceae) and determined the production of toxic enzymes such as 2-furancarboxaldehyde and levoglucosenone on the cotton leaf worm *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae), where when applied on L_3 larvae of *S. litura* by dipping, 57 % mortality was recorded (Elango *et al*., 2020); B) Competition for nutrients, this is due to the fact that EEF colonize the interior of plants and compete with phytophagous insects for available nutrients. Thus, *Acremonium alternatum* Link, 1809 caused L_1 larvae of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) feeding on leaves of previously inoculated cabbage plants to show mortality during the first 10 days of development (Raps and Vidal, 1998); C) Induction of resistance in the host plant, EEF has been shown to be able to biosynthesize "phytochemicals" originally thought to be produced only by their host plants, which induce defense responses in the host plant against diseases (Ancheeva *et al*., 2020). For example, in a study by Russo *et al*. (2015), it was shown that the foliar spraying of *B. bassiana* turned out to be more successful than the inoculation of seeds in tobacco, corn, wheat, and soybean plants which promoted the growth of these crops. In any case, it is important to standardize the methodologies for its inoculation, as well as to consider the concentration of conidia to be applied (Jaber and Enkerli, 2017; Javed *et al*., 2019). Besides, the implementation of EEF in agro-food production requires to a great extent, the understanding of the biotic and abiotic factors that interact in the plant-fungus relationship. Understanding the effect of these factors and inoculation methods, becomes a challenge for the development of sustainable agricultural production systems (Tall and Meyling, 2018; Vega, 2018). A possible explanation for the limited success obtained with EEF inoculation in seeds and roots, is focused on the competition with other soil-dwelling microorganisms that are transmitted in the same way as EEF: through water currents and wind (Bamisile *et al*., 2018a).

According to the above, it is necessary to identify and understand the effect of other microorganisms, and the soil characteristics that favors or limit the activity of EEF. For instance, Mayerhofer *et al*. (2017) investigated through ribosomal marker sequencing, the possible effects of Ascomycota, Zygomycota, Basidiomycota, Chytridiomycota, Glomeromycota, Blastocladiomycota, Proteobacteria, Actinobacteria, Chloroflexi and Acidobacteria naturally present in soils on the fungus *M. brunneum*. The study was carried out under field conditions for the biological control of *Agriotes* spp. in potato. The results revealed an efficiency of 77 % referring to decrease in tubers damaged by the insect compared to the control, as well as a successful establishment of the fungus in the crop, without negatively affecting the soil microbial communities described above. The foregoing will facilitate its manipulation and effectiveness for the development of technologies with a socially, economically, and ecologically viable approach (Jaber and Enkerli, 2016; Bamisile *et al*., 2018a; Vega, 2018). This approach must consider the ecology of EEF, enhancing their development and the availability of a greater number of products on a commercial scale for use in current agriculture (Jaber and Ownley, 2017). It should also be considered that among the desired characteristics of EEF strains, are a broad spectrum towards pest species, greater persistence, stimulation of plant growth, and the compatibility with other IPM (included chemical pesticides) strategies under greenhouse and field conditions (Gathage *et al*., 2016; Clifton *et al*., 2018; Mantzoukas and Grammatikopoulos, 2020).

The successful positioning of EEF as biological control agents widely used worldwide requires developing standard application methodologies under field conditions. In this regard, the factors that influence the development of fungi should be investigated (*e. g.* UV radiation and humidity). This will make possible to exploit all the beneficial capacities of such important microorganisms (Jaber and Ownley, 2017; Vega, 2018).

An important aspect to highlight is the compatibility of EEF with entomophagous insects (Gathage *et al*., 2016; González-Mas *et al*., 2019), which expands the possibilities in their use in IPM. For instance, the survival of the predator *Rhynocoris marginatus* (Fabricius, 1794) (Hemiptera: Reduviidae) was not affected when it fed on larvae of *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) infected with the fungi *I. fumosorosea* and *B. bassiana* (Ullah *et al*., 2019). These results suggest that EEF are compatible to be used together with the predator to promote the biological control of *S. litura*.

On the other hand, studies are needed to document the persistence of EEF in natural environments inside and outside their host plant. In one of them, it was documented that cotton seeds inoculated with the fungi *B. bassiana* and *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones and Samson 2011 (Hypocreales: Ophiocordycipitaceae), showed a successful endophytic colonization in the seedlings; its persistence fluctuated from 7 to 14 days after application under greenhouse conditions, and its presence limited the incidence of the aphid *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) (Lopez *et al*., 2014). It has been documented that in some cases the persistence of the fungi in endophytic form can be reduced with the age of the plant. For example, *B. bassiana* as endophyte of corn plants can persist up to 60 days in the culture. However, its persistence was reduced (44.44 ± 11.11 %) with the increase in the age of the plant, documenting that upon reaching 75-90 days of age, the persistence of the fungus was null. This was manifested in the protection against the corn stem borer *Chilo partellus* (Swinhoe, 1885) (Lepidoptera: Crambidae) (Renuka *et al*., 2016). Therefore, knowing in detail the persistence of EEF colonization within the plant will allow better results to be obtained.

Another important criterion to consider is the maintenance of shelf viability and pathogenicity. It is known that subcultures of entomopathogenic fungi in an artificial medium tend to reduce their pathogenicity (Jaber and Ownley, 2017). Given this fact, it is necessary to carry out continuous maintenance of the EEF isolates and to avoid the loss of these capacities, being recommendable to keep the isolates in tubes with sterile distilled water (Richter *et al*., 2016). For its part, current research on the potential of EEF as endophytic indicates the need to study the effects of the interactions between EEF strains genotype and plant genotype, with the aim of selecting the most compatible isolates to obtain higher levels of success in the plant colonization, considering the environmental conditions surrounding the fungus and its host, as well as the need to carry out studies under greenhouse and field conditions (Busby *et al*., 2016; Kumar *et al*., 2018).

PERSPECTIVES

One question we must ask ourselves is, if endophytic fungi have coevolved with plants, why is it that the crops have lost them? In other words, what will be the difference in endophytic fungi between crop wild relatives, landraces, and modern varieties? If the association between endophytic fungi-plants is natural as part of evolution, why is it necessary to induce colonization in crops? Experiences with native Mexican varieties of tomato, *Solanum lycopersicum*, have shown greater tolerance to pests than commercial varieties. Although it is presumed that a higher density of trichomes was the cause of the resistance (Nord *et al*., 2020), could endophytic fungi be involved? When the native varieties were grafted with the commercial variety, they transmitted resistance to the previously susceptible commercial variety. So, a good strategy to induce EEF colonization would be the grafting of commercial varieties in wild plants. EEF has become an ecologically alternative for crop protection by inducing their growth and resistance to pests and diseases. This lays the foundation for the generation of new EEF-based products (Bamisile *et al*., 2018a).

As detailed in this review, a great effort has been made to unravel the potential of EEF, carrying out recent studies that have made possible a better understanding of their behavior and mechanisms of action. However, only 1 to 2 % of known plant species have endophytic associations with EEF (Khare *et al*., 2018). Likewise, the current scenario indicates that it is also necessary to understand the biochemistry and physiology of EEF with the support of modern genomics, metabolomics, and proteomics techniques, given that there is little known research in these fields so far (Pathan and Deshpande, 2019; Putnoky-Csicsó *et al*., 2020). Therefore, knowing in detail the physiological functioning of EEF will be of fundamental importance to contribute to the solution of many of the problems that overwhelm current agricultural production. In this sense, the resistance that EEF induce in host plants against insect pests and pathogens, could potentially reverse the negative impacts of conventional agricultural production by emphasizing research in the development of commercial formulations based on these fungi (Khare *et al*., 2018). These formulations must address the lack of success in the inoculation of seeds and roots, solving the problem of microbial competition that occurs in the rhizosphere, which is considered the main cause of failure (Jaber and Enkerli, 2017; Javed *et al*., 2019).

Finally, to be more successful in the development of commercial formulations, it is necessary to understand the following premises: 1) To support the study of the genetic and molecular bases that regulate the communication between plant-endophyte (Bamisile *et al*., 2018a; Dara, 2019); 2) To promote the development of application methodologies to establish a successful association between EEF and host plant, as well as its survival (Lopez *et al*., 2014; Renuka *et al*., 2016; Mantzoukas and Eliopoulos, 2020); 3) To understand more deeply the mechanisms that influence the protection of the plant against insects and pathogens (Yun *et al*., 2017; Kumar *et al*., 2018; Ahmad *et al*., 2020); 4) To determine the impact of biotic and abiotic factors that mediate the success of the EEF (Tall and Meyling, 2018; Vega, 2018) and; 5) To understand in detail both, horizontal and vertical transmission routes, since it is recently known that EEF are capable of being transmitted through the reproductive tissues of plants, at least, to the next generation (Khare *et al*., 2018).

CONCLUSION

This review examined the integrative status of entomopathogenic fungi and their role as endophytes, as well as their limitations and disadvantages of their use in the current monoculture-based agricultural paradigm, where endophytic fungi and any other sustainable pest management strategy, experience a high challenge because modern agroecosystems are designed to express high productivity, and do not necessarily favor the presence and increase of beneficial organisms. Therefore, further research on this alternative is needed to promote healthier and more sustainable agricultural production.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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