

## New paradigms in exploration of microbial endophytes in insect pest management

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**Abstract:** The strategic use of naturally occurring organisms to control pest populations and increase production of major crops represents a viable option to host-plant resistance in pest control. One group of biological control agents that provide a source for novel pest control is the mutualistic microbial symbionts, which are termed as “Endophytes”. Entomopathogenic endophytes play vital role in reducing pest load by imparting host defense mechanisms against various crop insect pests. In recent years, entomopathogenic endophytes have been explored for pest management in various countries. Unique endophytes could be used directly to treat seeds or transplants. The capability of colonizing internal host tissues has made endophytes valuable for agriculture as a tool to improve crop protection. In this paper, the major topics concerning the screening of microbial endophytes from different crop plants, induction of endophytes in to the plant, control of insect-pests by endophytic microorganisms *etc.*, have been reviewed and presented. Several examples of insect control are described, notably those involving the interactions between fungi and insect. The mechanisms by which endophytic fungi control insect attacks are listed and include toxin production as well as the influence of these compounds on plant and how their production may be affected by genetic and environmental conditions. The future use of biological–chemical combinations of endophytes in combination with commercial pesticides applied to the seed or seedling could lead to synergistic effects on one or multiple insect pests or disease causing agents. The chemicals could provide near instantaneous suppression on insect pests, while the biological agent could provide continuing control well into the crops production cycle.

**Keywords :** Biological control, Insect-pests, Microbial Endophytes

To economically feed an increasing world population, it is important that food production be increased while the cost of producing the food be decreased. The strategic use of naturally occurring organisms to control pest populations and increase production of major crops represents a viable option to host-plant resistance and pesticide-based pest control. One group of biological control agents that provide a source for novel pest control are the mutualistic microbial symbionts, which are termed as “Endophytes”.

Modern agriculture is facing new challenges in which ecological and molecular approaches are being integrated to achieve higher crop yields while minimizing negative impacts on the environment. In this context, enhancing plant growth and plant resistance by using beneficial microorganisms is currently considered as an important key strategy (Pineda *et al.*, 2010). Approximately 3,00,000 plant species growing in unexplored area on the earth are host to one or more endophytes (Strobel and Daisy, 2003; Strobel *et al.*, 2004) and the presence of biodiverse endophytes in huge number plays an important role on ecosystems with greatest biodiversity. Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthenes and others (Tan and Zou, 2001).

Plants are associated with a diverse community of microorganisms. The microorganisms residing within the plants or endophytes are unique in their adaptations to specific chemical environment of host plant. Endophytes are microorganisms (bacteria, fungi and unicellular eukaryotes)

which can live at least part of their life cycle inter or intracellularly inside of plants usually without inducing pathogenic symptoms. This can include competent, facultative, obligate and opportunistic endophytes. Endophytes can have several functions and these may change function during their lifecycle (Murphy, *et al.*, 2014).

The term “endophyte” was introduced by De Bary (1884) and was initially applied to any organism found within a plant that causes asymptomatic infections entirely within plant tissues but no symptoms of disease (Wilson, 1995). Endophytic fungi have been examined in conifers (Petrini *et al.*, 1992), including *Pinus* spp. (Sieber *et al.*, 1999), *Taxus* spp. (Fisher and Petrini, 1987) and *Juniperus* spp. (Petrini and Müller, 1979; Petrini and Carroll, 1982). They exist in a range of tissues types within a broad range of plants, colonizing the plant systemically with bacterial/fungal colonies and biofilms (Jalgaonwala, 2011). They are ubiquitous, colonize most of the plants and have been isolated from almost all plants examined till date. Endophytes live symbiotically within the plants. While growing inside the plant, the endophytes show no visible symptoms of infection and disease (Bacon and White, 2000). The close association of endophytes with internal tissues of host plant has increasingly gained them scientific and commercial interest due to their potential to improve plant quality and growth (Schulz *et al.*, 2006). Endophytic microorganisms can vary based on the plant source, age, type of tissue, season of sampling and environment. Generally, the concentration of the endophytic bacteria is more at the root than at shoot tissue (Zinniel *et al.*, 2002).

Endophytes exhibit complex interactions with their hosts which involves mutualism and antagonism. Their association can be obligate or facultative. Plants strictly limit the growth of endophytes and these endophytes use many mechanisms to gradually adapt to their living environments. In order to maintain stable symbiosis, endophytes produce several compounds that promote growth of plants and help them adapt better to the environment (Ulrich *et al.*, 2008). Some of the endophytes are known to protect their host from being attacked by fungi, insect and mammals by producing secondary metabolites (Zhang, 2007).

A screening for endophytes containing antimicrobial and herbicidal compounds showed that endophytes can also synthesize compounds directed against plants (Schulz *et al.*, 1999). Insects were also proposed to influence endophytic fungi producing bioactive compounds and host plants (Kusari *et al.*, 2013). Therefore, insecticidal substances can also be found among those produced by the endophytes. However, the scenario might become more complicated by observations that fungi contain their own symbionts.

#### Endophytes producing bioactive compounds

Mainly within the last decade endophytes have attracted greater attention due to their potential to synthesize a wide array of bioactive secondary metabolites (Tan and Zou 2001; Schulz *et al.*, 2002; Strobel 2003; Prado *et al.*, 2013). The endophyte derived compounds belong to diverse structural groups such as terpenoids, steroids, xanthenes, chinones, phenols, isocoumarins, benzopyranones, tetralones, cytochalasines and enniatines (Schulz *et al.*, 2002). They sometimes constitute variations of already known structures such as ergosterol, a fungal steroid, or the plant hormone indole-3-acetic acid (Lu *et al.*, 2000). The biosynthesis of such compounds is important for the endophyte when it is in competition with other organisms surrounding the plant (Schulz *et al.*, 1999). Therefore, in many cases these compounds possess antimicrobial activities. The protection of plants against pests can often be attributed to some of the compounds found in endophytes (Poling *et al.*, 2008). To isolate endophytes from the close vicinity of their hosts was a successful strategy in the identification of (novel) organisms with high antimicrobial and insect deterrent potential which could be of practical significance (Casella *et al.*, 2013).

The first studies with endophytes were conducted with host plants from temperate regions; however, more recent research was dedicated to the endophytic fungi and bacteria inhabiting the plants from tropical countries such as Brazil and India (Suryanarayanan and Vijaykrishina 2001; Mishra *et al.*, 2012); indeed, examples of endophytic species of fungi from tropical plant hosts were recently reviewed (Azevedo and Araujo 2007). Endophytic microorganisms have been used for the biological control of insect pests and plant diseases.

#### Fungal endophytes

Fungal endophytes are defined as fungi that during any of their growth stages, asymptotically colonize the internal

tissues or organs of plant. They are found ubiquitously in various plants; from mosses and ferns to various gymnosperms and angiosperms (Arnold and Lutzoni 2007; U Ren *et al.*, 2012), including aquatic plants (Li *et al.*, 2010). Their ability to co-exist and co-evolve with host plants is attributed to the balance achieved between the virulence of endophytes and the host's defenses (Schulz *et al.*, 2006). In this symbiotic relationship, fungal endophytes receive "shelter" and nutrients from the hosts while the hosts benefit from an array of beneficial attributes (White *et al.*, 2002; Debbab *et al.*, 2012) which include safeguarding against natural enemies of plants such as pathogens and herbivores (Vega *et al.*, 2008; Singh *et al.*, 2011).

Fungal endophytes have been detected in hundreds of plants, including many important agricultural commodities such as wheat (Larran *et al.*, 2002a), bananas (Pocasangre *et al.*, 2000; Cao *et al.*, 2002), soybeans (Larran *et al.*, 2002b) and tomatoes (Larran *et al.*, 2001). Several roles have been ascribed to fungal endophytes, including providing protection against herbivorous insects (Breen, 1994; Clement *et al.*, 1994), plant parasitic nematodes (Elmi *et al.*, 2000) and plant pathogens (Wicklow *et al.*, 2005).

Some endophytes belong to genera that include fungal entomopathogens such as *Beauveria* has been reported as an endophyte in maize (Arnold and Lewis, 2005; Kambrekar *et al.*, 2016), potato, cotton (Jones, 1994), in sorghum, chilli, sunflower and beans (Kambrekar *et al.*, 2016), tomato (Ownley *et al.*, 2004), in opium poppy (Quesada-Moraga *et al.*, 2009), on date palm (Go'mez-Vidal *et al.*, 2006), in banana (Akello *et al.*, 2007) and in coffee (Posada *et al.*, 2007). In addition, cocoa (Posada and Vega, 2005) and coffee seedlings (Posada and Vega, 2006) have been successfully inoculated with *Beauveria bassiana* (Bals.-Criv.) by depositing a spore suspension on the radicle shortly after germination. Referring to *B. bassiana*, Steinhaus (1949) wrote, "It also grows on corn and certain other plants but not so well as on insects." Fuller-Schaefer *et al.* (2005) have reported on the colonization of sugarbeet roots by the fungal entomopathogens, *B. bassiana* and *Metarhizium anisopliae* (Metschn.) Sorokin.

Other entomopathogenic fungi have also been reported as endophytes and they include *Lecanicillium lecanii* (Zimm.) in sorghum, cotton, wheat, chickpea, pigeonpea, mango and banana (Kambrekar *et al.*, 2016), in an Araceae (Petrini, 1981); *L. lecanii* and *Paecilomyces farinosus* (Holmsk.) Brown and Smith (= *Isaria farinosa*) in the bark of *Carpinus caroliniana* Gray (Bills and Polishook, 1991); *Paecilomyces* sp. in *Musa acuminata* (Cao *et al.*, 2002) and in rice (Tian *et al.*, 2004). On a wide variety of crops, fungal endophytes have been reported to deter feeding, oviposition and performance of stem boring, sap sucking, chewing and leaf mining insects (Jallow *et al.*, 2004; Cherry *et al.*, 2004; Qi *et al.*, 2011; Akutse *et al.*, 2013) (Table 1).

#### Techniques and procedure for isolation of endophytic fungi

The isolation procedure is a critical and important step in working with endophytic fungi. The living plant tissues are

subjected to a serial process of surface sterilization to remove all organisms from the surface of the plant. Only internal fungi are isolated by means of incubation of the plant samples on to nutrient plates.

Cultivation-dependent techniques generally include

- Thorough washing of the plant tissue under tap water to remove adhering soil particles, debris and major epiphytes.
- Surface sterilization of plant tissue to kill any microorganisms on the host surface, applying different protocols to different tissue types (Hallmann *et al.*, 2006).
- Isolation of endophytic fungi growing out from samples placed on nutrient agar.
- Purification and sporulation of endophytic isolates under various incubation conditions.
- Identification of the endophytic fungi based on morphological characteristics in cultures or through molecular detection.

In recent years, fungal endophytes have been explored for diverse applications. This is attributed to the vast range of valuable compounds produced such as enzymes and secondary metabolites. Chitinase, lipase and cellulase are common enzymes produced by various fungal endophytes such as *Beauveria*, *Lecanicillium*, *Metarhizium* and *Trichoderma* which contribute to their role in control of pest and diseases (Fang *et al.*, 2005; St Leger and Wang 2010; Sandhu *et al.*, 2012). Endophytes have also showed potential in the agriculture sector as plant growth

promoters (Silva *et al.*, 2012; Chujo and Scott 2014; Islam *et al.*, 2014) and biocontrol agents (Li *et al.*, 2008; Silva *et al.*, 2012; Kusari *et al.*, 2012).

### Bacterial endophytes

Endophytic bacteria can be defined as those bacteria that colonize the internal tissue of the plant showing no external sign of infection or negative effect on their host (Schulz and Boyle, 2006). Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents (Berg *et al.*, 2005). Indeed, numerous reports have shown that endophytic microorganisms can have the capacity to control insects (Azevedo *et al.*, 2000) and nematodes (Hallmann *et al.*, 1998).

### Isolation and biodiversity of bacterial endophytes

The endophytic niche offers protection from the environment for those bacteria that can colonize and establish in planta. These bacteria generally colonize the intercellular spaces and they have been isolated from all plant compartments including seeds (Posada and Vega, 2005). Endophytic bacteria have been isolated from both monocotyledonous and dicotyledonous plants, ranging from woody tree species, such as oak and pear to herbaceous crop plants such as sugar beet and maize. Classical studies on the diversity of bacterial endophytes have focused on characterization of isolates obtained from internal tissues following disinfection of plant surfaces with sodium hypochlorite or similar agents (Miche and Balandreau, 2001). Lodewyckx *et al.* (2002) highlighted the

Table 1. Fungal entomopathogens reported as endophytes and plants harboring them

Endophyte	Crop	Reference
<i>Beauveria bassiana</i>	Sorghum, Green gram, Sunflower, Chilli, Beans, Brinjal, Pomegranate, Cabbage and Cauliflower Cotton, Potato Maize Tomato Banana Coffee Date palm Cocoa	Kambrekhar <i>et al.</i> , 2016 Jones, 1994 Lewis <i>et al.</i> , 2001; Kambrekhar <i>et al.</i> , 2016 Ownely <i>et al.</i> , 2004; Kambrekhar <i>et al.</i> , 2016 Akello <i>et al.</i> , 2007 Posada <i>et al.</i> , 2007 Gomez – viadal <i>et al.</i> , 2006 Posada and Vega, 2005
<i>Beauveria brongniartii</i> (Saccardo)	Coffee	Vega <i>et al.</i> , 2008
<i>Lecanicillium lecanii</i> (Zimm.)	Araceae <i>C. caroliniana</i> (N) Sorghum, Cotton, Wheat, Sunflower, Chickpea, Tomato, Pigeonpea, Mango, Banana.	Petrini, 1981 Bills and Polishook, 1991 Kambrekhar <i>et al.</i> , 2016
<i>Lecanicillium psallioate</i> (Tres-Chew)	Date palm	Gomez – Vidal <i>et al.</i> , 2006
<i>Paecilomyces farinosus</i> (Holmsk)	<i>Carpinus caroliniana</i>	Bills and Polishook, 1991
<i>Paecilomyces</i> spp.	Banana and Rice	Cao <i>et al.</i> , 2002; Tian <i>et al.</i> , 2004
<i>Acremonium</i> spp.	Coffee	Vega <i>et al.</i> , 2008
<i>Acremonium alternatum</i>	Coffee	
<i>Cladosporium</i> spp.	Coffee <i>Avecennia officinalis</i> <i>Rhizophora mucronata</i> <i>Sonneratia caeseolaris</i>	Vega <i>et al.</i> , 2008 Ananda and Sridhar, 2002
<i>Clanostachys rosea</i>	Coffee	Vega <i>et al.</i> , 2008
<i>Paecilomyces</i> spp.	<i>Musa acuminata</i> Colla (N) (Banana) <i>Oryza sativa</i> L. (N) (Rice)	Cao <i>et al.</i> , 2002; Tian <i>et al.</i> , 2004
<i>Aspergillus</i> sp.	Maize, Safflower, Linseed, Citrus	Kambrekhar <i>et al.</i> , 2016

methods used to isolate and characterize endophytic bacteria from different plant species. A very comprehensive list of bacterial endophytes isolated from a broad range of plants is provided by Rosenblueth and Martinez-Romero (2006) and Berg and Hallmann (2006), which updates the groundwork laid by Hallmann *et al.* (1997) and Lodewyckx *et al.* (2002).

### Endophytes as potential biocontrol agents in insect pest control

In the early 80's the specialized literature published showed that endophytic microorganisms, particularly fungus could play an important role inside plants in the reduction of insect attacks. Landmark reports on the subject started in 1981, less than two decades ago. From 1981 to 1985, which may be considered a historical period to the field, it was demonstrated the existence of plant protection against herbivore insects given by endophytic microorganisms.

Webber (1981) was probably the first researcher to report an example of plant protection giving by an endophytic fungus, in which the endophyte *Phomopsis oblonga* (Desm.) protected elm trees against the beetle, *Physocnemus brevilineum* Say. It was suggested that the endophytic fungus, *P. oblonga* was responsible for reducing the spread of the elm Dutch disease causal agent *Ceratocystis ulmi* (Buisman) by controlling its vector, the beetle *P. brevilineum*. The author associated the repellent effect observed towards the insect to toxic compounds produced by the fungi. This was confirmed four years later by Claydon *et al.* (1985), who showed that endophytic fungi belonging to the *Xylariaceae* family synthesize secondary metabolites in hosts of the genus *Fagus* and that these substances affect the beetle grub.

Other observations on the control of insects-pests by endophytic fungi are those of Funk *et al.* (1983) showing protection of the perennial ryegrass, *Lolium perenne* L. against the sod webworm. Gaynor and Hunt (1983) observed in several ryegrasses that high fungi infection is correlated with a decrease in the attack frequency of the Argentine steem weevil, *Listronotus bonariensis* (Kuschel). These authors also showed that these associations may be complex and factors like the level of nitrogen fertilizers may as well affect attack frequency. With the same insect-plant interaction, in New Zealand, Barker *et al.* (1984) and Prestidge *et al.* (1984) observed that plants free of endophytic fungi of the genus *Acremonium*, now-a-days classified as *Neotyphodium* are severely attacked. Lasota *et al.* (1983) had also correlated in the white spruce *Picea glauca* Moench, death rate of the Homoptera, *Adelges abietis* L. when galls were infected with the endophytic fungus, *Cladosporium sphaerospermum*. In *L. perenne* and members of the genus *Cyperus*, weight gain and survival of the insect-pest, *Spodoptera frugiperda* (J.E. Smith), were affected by endophytic fungi like *Balansia cyperi* Edg. (Clay *et al.*, 1985a; 1985b; Hardy *et al.*, 1985). Studies related to insect-pest affecting different species of *Lolium* were also conducted by Latch *et al.* (1985b) with two endophytic fungi, *Acremonium lolii* Latch and a member of the genus *Gliocladium*. The authors discussed the benefits and risks resulting from the increase in

toxin production to insect control and resulting harm to domestic animals that would feed on these plants. Latch *et al.* (1985a) also observed that *Acremonium* affected aphids feeding on members of the genera *Lolium* and *Festuca*. Ahmad *et al.* (1985) verified similar effects of the same fungi over the grasshopper *Acheta domesticus*. Johnson *et al.* (1985) using choice tests showed that insects would feed on endophyte-free *Festuca* plants rather than on infected samples.

Lewis *et al.* (2000) conducted field and greenhouse studies to determine the proclivity of *Bacillus thuringiensis* (Berliner)-transgenic corn (*Zea mays* L.) to form an endophytic relationship with *B. bassiana* and to evaluate the corn for possible plant pathological effects associated with this relationship. *B. bassiana* was applied as a granular formulation to two separate lines of corn, expressing Bt events MON802 and MON810 and their corresponding isolines. There were no significant differences in levels of endophytism between transgenic events or their near-isolines. In greenhouse studies, *B. bassiana* was applied as a liquid seed treatment to Bt transgenic corn hybrids Pioneer 34R06 (event MON810) and Ciba Max 454 (event 176) and their near isolines at a rate of  $2 \times 10^{10}$  conidia per ml. There were no significant differences in seed germination or presence of root pathogens in transgenic or isolate seeds soaked in a *B. bassiana* suspension. The same lines of corn were used in field experiments with treatments of seeds soaked in a suspension of *B. bassiana*, a foliar application of a granular formulation of *B. bassiana* and corresponding untreated checks. Plants were sampled throughout the growing season and evaluated for growth of individual plant components, including sheaths, leaves, stem, husk, ear, plant leaf-to-stem ratio and overall plant growth. There were no significant differences in overall plant growth between the *B. bassiana* treatments or in the growth of each plant component. The results indicated that *B. bassiana* readily forms an endophytic relationship with both transgenic and non-transgenic corn.

Bing and Lewis (1991, 1992) isolated *B. bassiana* from maize and the fungus was used to control the European corn borer, *Ostrinia nubilalis* (Hübner). In Brazil, some laboratories isolated endophytic fungi those were obtained from plant hosts including sugarcane, maize and soybean (Stuart *et al.*, 2010). Some of the *Beauveria* strains isolated from maize were used against the insect pest, *S. frugiperda* Walker and the results showed that these endophytes were as good or even better biocontrol agents than the commercial entomopathogenic strains used in Brazil. Further, Tseng and Cheng (1998) applied *B. bassiana* conidia to whorl-stage maize by foliar application and stem injection against corn borer. The results showed that above 90 per cent of maize were colonized by *B. bassiana* although maize plants had various levels of colonization in their different tissues. Furthermore, the results indicated that there was a significant reduction in the density of *O. nubilalis* by endophytic *B. bassiana*. Wagner and Lewis (2000) observed long hyphal structures to follow the leaf apoplast in any direction from the point of penetration. A few hyphae were observed within xylem elements. Because, vascular bundles

are interconnected throughout the corn plant, this may explain how *B. bassiana* travels within the plant and ultimately provides overall insecticidal protection. Virulence bioassays demonstrated that *B. bassiana* does not lose virulence towards the European corn borer once it colonizes corn. This endophytic relationship between an entomopathogenic fungus and a plant suggests possibilities for biological control, including the use of indigenous fungal inocula as insecticides.

As novel pest management tools to replace chemical insecticides, the use of endophytes in rice cultivation is now on trial to test its feasibility. Use of the endophytes enabled us to reduce the cost and environmental risks of the insect pest management, while direct effects of the endophyte-infected rice plants on insect pests was less than traditional chemical pesticides. However, the use of the endophytes has some advantages such as safety for non-target organisms including natural enemies and long-lasting effectiveness. Therefore, its effectiveness must be discussed by taking indirect and durable effects into consideration. The rice brown planthopper (BPH) is one of the most injurious insect pests of rice plants in Japan. Sato *et al.* (2006) reported that endo-symbiotic bacteria (endophytes), *Harbaspirillum* sp. B65 and *Azospirillum* sp. B510a induce infected rice plants to have systemic resistance against insect pests. To discuss the usefulness of the endophyte application in managing BPH, its inclusive effects was investigated using simulation models of BPH population dynamics. The simulation results suggested that sole use of the endophyte-infected rice plants may not always regulate BPH density below the economic injury level successfully. It follows that additional control measures were needed in cases where immigrant BPH density is high.

Vega *et al.* (2008) conducted survey of fungal endophytes in coffee plants from Hawaii, Colombia, Mexico and Puerto Rico has revealed the presence of various genera of fungal entomopathogens, including *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys* and *Paecilomyces*. Two of these, *B. bassiana* and *Clonostachys rosea* (Link.) were tested against the coffee berry borer and were shown to be pathogenic.

Akello *et al.* (2008) studied the effect of endophytic *B. bassiana* in banana plants on the banana weevil, *Cosmopolites sordidus* (Germar) and reported dead mycosed insects in the rhizome of *B. bassiana*-inoculated plants, suggesting a direct mode of action through mycosis infection of the banana weevils by the fungus. Further, they reported that the presence of *B. bassiana* as an endophyte in banana tissues greatly reduced banana weevil populations and their damage to plants. Between 53.4 and 57.7 per cent of the banana weevil adults died because of *B. bassiana* infection, resulting in a reduction of plant damage by 29.1-62.7 per cent depending on plant part. Griesbach (2000) has also showed that the grub of the banana weevil obtained from endophyte-inoculated plants were smaller in size than those obtained from non-inoculated plants. Further, endophytic colonization of banana by *B. bassiana* significantly reduced the survivorship of grubs of banana weevil resulting in 42-87 per cent reduction in plant damage (Akello *et al.*, 2008).

Two-week old date palm seedlings were grown in petri-dishes and subjected to direct injection with two rates of *B. bassiana* spore suspension ( $0.5 \times 10^7$  or  $1.5 \times 10^7$  spores/ml). The endophytic fungus was recovered from sites distant from the point of inoculation six months after application. This indicated that the fungus has the potential to move throughout the plant tissues. Date palm pulp of the endophytically colonized seedlings was used in a laboratory diet for the grubs of the red palm weevil (RPW). The mortality of grubs during 14 days achieved 80.3 per cent, under laboratory conditions (Arab and Deeb, 2012).

Two formulations containing culture filtrates and conidial suspensions of the endophytic fungus *Cladosporium oxysporum* (Berk & Curt.), isolated previously from stems of *Euphorbia bupleuroides* subsp. *luteola* (Kralik) Maire, were experimentally tested for their aphicidal activity against the black bean aphid, *Aphis fabae* Scop. found in Algeria. It was shown that invert emulsions were more effective against aphids, than using aqueous suspensions. This was especially true for formulations containing culture filtrates. The relatively insignificant mortalities obtained by formulations containing conidial suspensions indicated a low infectious potential towards the aphids. The proteolytic activity seemed to be more important than the chitinolytic activity of the fungus against the black bean aphid, *A. fabae* (Bensaki *et al.*, 2015) (Tables 2 & 3). *Cladosporium* can include some naturally entomopathogenic members, experiencing biological control potentialities against several insect pests, particularly whiteflies (Aldeghairi *et al.*, 2013). Within the framework of the biological control of key crop pests, there have been few works which were devoted to the use of the culture filtrates of *Cladosporium* spp. By evaluating the effect of the culture filtrates of *Cladosporium echinulatum* (Berk.) G.A. de Vries and *Paecilomyces* sp. isolated from *Nerium oleander* L. against *Acanthoscelides obtectus* Say, Bensaki *et al.* (2013) obtained mortalities ranging from 39 to 84 per cent for concentrations of 25 and 100 per cent, respectively.

Saranya *et al.* (2010) showed that the conidial suspension of *C. oxysporum* generates a corrected mortality of 77.50 per cent in *Aphis craccivora* Koch individuals, when the suspension was used at a concentration of  $10^8$  conidia per ml incorporated to the teepol solution.

Table 2. Aphicid action and  $LT_{50}$  of bio-formulations containing culture filtrates of *Cladosporium oxysporum*, against *Aphis fabae* at 48 hours after treatment

Formulation	Concentration of culture filtrates (%)	Corrected mortality (%)	$LT_{50}$ (h)
Aqueous suspension	20	20.94	93.94
	40	44.94	64.34
	60	46.95	66.33
	80	50.44	62.17
Invert emulsion	20	58.44	57.08
	40	61.45	51.99
	60	82.44	43.74
	80	91.94	36.11

Table 3. Aphicid action and LT<sub>50</sub> of bio-formulations containing conidial suspension of *Cladosporium oxysporum*, against *Aphis fabae* at 120 hours after treatment

Formulation	Conidial concentration (ml <sup>-1</sup> )	Corrected mortality (%)	LT <sub>50</sub> (h)
Aqueous suspension	1 x 10 <sup>6</sup>	19.97	186.35
	1 x 10 <sup>6</sup>	25.47	175.64
	1 x 10 <sup>7</sup>	29.47	180.92
	1 x 10 <sup>8</sup>	36.47	167.04
Invert emulsion	1 x 10 <sup>6</sup>	40.96	161.12
	1 x 10 <sup>6</sup>	48.97	152.94
	1 x 10 <sup>7</sup>	61.46	141.96
	1 x 10 <sup>8</sup>	67.96	137.20

Muvea (2014) examined the effects of inoculated endophytes on *Thrips tabaci* Lindiger infesting onion. Seven fungal endophytes were able to colonize onion plants either by the seed or seedling inoculation methods. Seed inoculation resulted in 1.47 times higher mean percentage of post inoculation recovery in all the endophytes tested as compared to seedling inoculation. Fewer thrips were observed on plants inoculated with *Clonostachys rosea* (Link.) ICIPE 707, *Trichoderma asperellum* (Samuels, Lieckf. & Nirenberg) M2RT4, *T. atroviride* ICIPE 710, *Trichoderma harzianum* 709, *Hypocrea lixii* F3ST1 and *Fusarium* sp. ICIPE 712 isolates as compared to those inoculated with *Fusarium* sp. ICIPE 717 and the control treatments. Onion plants colonized by *C. rosea* ICIPE 707, *T. asperellum* M2RT4, *T. atroviride* ICIPE 710 and *H. lixii* F3ST1 had significantly lower feeding punctures as compared to the other treatments. Among the isolates tested, the lowest numbers of eggs were laid by *T. tabaci* on *H. lixii* F3ST1 and *C. rosea* ICIPE 707 inoculated plants. These results extended the knowledge on colonization of onions by fungal endophytes and their effects on *T. tabaci*.

Lefort *et al.* (2016) detected *B. bassiana* in seedlings which had not previously been exposed to the fungus, indicating a vertical mode of transmission. The fungus could colonize all parts of the pines, but did not always persist. They found that the presence of the fungus negatively affects the fitness of the below-ground insect feeding on the plant by reducing their survival by over 10 per cent and their weight by 5 per cent. They also showed that the mode of action of endophytic *B. bassiana* in pine is likely to be by feeding deterrence of insects induced locally by fungal metabolites, rather than by direct fungal infection of the insects. *Beauveria* can reduce pest damage (Gurulingappa *et al.*, 2010) by inhibiting insect

development and establishment (Cherry *et al.*, 2004; Ownley *et al.*, 2004; Vega *et al.*, 2008).

Zhou *et al.* (2016) reported that endophytic fungus, *Chaetomium globosum* Kunze inhibited root-knot nematode, *Meloidogyne incognita* (Kofoid & White) infection and reduced female reproduction below ground. To confirm the endophytic effect of *C. globosum* on root-knot nematode, a contact fungicide was applied to remove soil-borne and epiphytic *C. globosum*. Consistent inhibition of nematode activity was observed by a post-fungicide treatment, with positive *C. globosum* colonization confirmed within plant tissues. Above ground, endophytic *C. globosum* also negatively affected the fecundity of both cotton aphids (*Aphis gossypii* Glover) and beet armyworms (*Spodoptera exigua* Hub.) in cotton. Similarly reduction in feeding and reproduction by *Aphis gossypii* Glover has also been reported on cotton endophytically colonized by either *B. bassiana* or *L. lecanii* (Gurulingappa *et al.*, 2010).

Maragy *et al.* (2016) surveyed 30 samples (6 from wheat and 24 from tomato) for endophytic fungi. Leaves, shoots and roots of the two plants were examined. Thirty-three fungal species belonging to 17 genera were isolated as endophytes of the two plants. *Beauveria bassiana* isolated from tomato leaves was pathogenic on the fourth instar larvae of the greater wax moth, *Galleria melonella* (Lepidoptera: Pyralidae). Kambrekar *et al.* (2016) isolated entomopathogenic fungal endophytes from different agricultural and horticultural crops. As an endophyte, *Beauveria bassiana* was isolated from sorghum, sunflower, maize, cauliflower, cabbage, greengram, tomato, brinjal, chilli, beans and pomegranate. *Lecanicillium lecanii* was found as an endophyte in the leaves of banana, chickpea, wheat, sunflower, tomato, cotton, redgram, mango and sorghum. Further, the pathogenicity of these fungal endophytes was studied against the host specific target insect pests under laboratory condition. *B. bassiana* isolated from sorghum was found pathogenic to sorghum stem borer, *Chilo partellus* Swinhoe and sorghum armyworm, *Mythimna separate* Walker. Further, the same fungal endophyte isolated from maize was pathogenic to maize stem borer, *C. partellus* Swinhoe. Similarly, *B. bassiana* isolated from cabbage and cauliflower is pathogenic to Diamond back moth, *Plutella xylostella* L. Against *Helicoverpa armigera* Hub., *B. bassiana* isolated from chilli and tomato was found pathogenic. *Lecanicillium lecanii* isolated from the leaves of tomato, chickpea and cotton was pathogenic to leaf hoppers, Aphids, *Aphis craccivora* Koch and *Aphis gossypii* Glover under laboratory conditions (Table 4).

Table 4. List of fungal endophytes and their pathogenicity against the target pests

Endophytes	Plant /plant part	Target pests	Pathogenicity status
<i>Beauveria bassiana</i>	Sorghum stem & leaf	Stem borer, <i>Chilo partellus</i>	Pathogenic
	Sorghum leaf	Army worm, <i>Mythimna separata</i>	Pathogenic
	Cabbage & Cauliflower	DBM, <i>Plutella xylostella</i>	Pathogenic
	Maize leaf & stem	Stem borer, <i>Chilo partellus</i>	Pathogenic
	Tomato fruit	<i>Helicoverpa armigera</i>	Pathogenic
	Chilli leaf	<i>Helicoverpa armigera</i>	Pathogenic
<i>Lecanicillium lecanii</i>	Tomato leaf	Leaf hoppers	Pathogenic
	Cotton leaf & stem	Aphids	Pathogenic
	Chickpea leaf	Aphids	Pathogenic

## Endophytes and the decrease in the incidence of insects on host plants

Ahmad *et al.* (1986) showed that the blue grass billbug, *Sphenophorus parvulus* Gyllenhal is less frequent on plants infected with endophytic fungi. Further, Ahmad *et al.* (1987) verified the same results in grasses attacked by the Southern armyworm, *Spodoptera eridania* Stoll. Saha *et al.* (1987) studied endophytes in fine fescue and found that endophytic fungi from the genus *Acremonium* were associated with host resistance to *Blissus leucopterus hirtus* (Montandon) and the same observation was made by Mathias *et al.* (1990) in *L. perenne*-*A. lolii* interaction.

Kanda *et al.* (1994) reported the preference of larvae from the bluegrass webworm, *Parapediasia teterrella* Zincken for diets with endophyte-free plants of *Lolium perenne* L. and *Festuca arundinacea* Schreb, to a point that the larvae would starve to death if only plants infected with *Acremonium* were available. In the field, endophyte-free species were severely attacked by insects, whereas those infected with *Acremonium* stayed almost free of insect larvae. As it will be seen in more details, endophytic fungi (Clavicipitaceae, Ascomycetes) synthesize alkaloids during plant infection, reducing the survival and the development of *S. frugiperda* in Graminae and Cyperaceae. In the flour beetle, *Tribolium castaneum* (Herbst), diets composed of grinded seeds of *F. arundinacea* and *L. perenne* infected with endophytic fungi resulted in a reduction of infested seeds and insect growth, notably in *F. arundinacea* (Cheplick and Clay, 1988).

Although plants other than grasses can be protected by endophytes, the data on these cases are not so conclusive nor numerous. Sherwood-Pike *et al.* (1986) described in oak (*Quercus* sp.), the role of the endophytic fungus, *Rhizoctonia parkeri* against the insect *Contarinia* sp. Petrini *et al.* (1989) found that the endophytic fungi *Phyllosticta* sp. and *Hormonema dematioides* were dominant in the balsam fir, *Abies balsamea* (L.) Mill. In the black spruce *Picea mariana* (Mill.), Johnson and Whitney (1994) found that 21 hyphae extracts out of 100 were toxic to spruce bud worm, *Choristoneura fumiferana* (Clemens) and extracts of fungi isolated from older leaves were more effective than young ones in cell culture. The dry weight and development of larvae fed on hyphae extracts were reduced.

The results described above show the efficiency of some endophytic fungi in reducing host damage provoked by insect attack. The examples given so far include researches performed in field and lab conditions, as well as comparisons between field and lab experimental data. Choice tests on diets composed of endophytes-free and infected plants are often used along with several choice combinations.

## Mechanisms of insect control displayed by endophytic fungi

Since the pioneer works in the field, the capacity of endophytic fungus to repel insects, induce weight loss, growth and development reduction and even to increase pest death rate, was correlated with toxin production. In several cases, it

was shown that the mode of action of certain fungi was based on the capability to render the plant unpalatable to several types of pests like aphids, grasshoppers, beetles, *etc.* (Carroll, 1988; Clay, 1988a; 1988b). Bacon *et al.* (1977) established for the first time a correlation between an endophytic fungi, *Epichloë typhina* (Pers.) and the toxicity of its host, *F. arundinacea* to herbivorous domestic mammals. It is now a fact that several toxins are produced by endophytic fungi and that these substances confer host protection against different herbivorous.

In certain cases, the production of toxin by the endophytic fungi was a plausible explanation for interactions resulting in natural insect control. Thus, Clark *et al.* (1989) showed in *A. balsamea* and red spruce *Picea rubens* (Sarg.), that from 900 samples of fungal isolates, five produced toxic substances and three of those produced powerful toxins that, once extracted and given to insects, caused death and decreased development rate of *Choristoneura fumiferana* (Clemens). Many other cases of insect control involving toxins produced by endophytic microorganisms can be found in the literature. Siegel *et al.* (1990) verified the presence of the alkaloids N-formil, N-acetyl loline, peramine, lolitrem B and ergovaline during plant attack by aphids. Several grasses infected with *Acremonium* spp. and *E. typhina* were analysed. These fungi generally produce alkaloids, mainly peramine and ergovaline. Peramine, lolitrem B and ergovaline were found in *Lolium* and *Festuca* infected with *Acremonium coenophialum* (Morgan-Jones & Gams) and *A. lolii* and in *Festuca longiflora* Thuill. infected with *E. typhina*. Individuals of *Rhopalosiphum padi* L. and *Schizaphis graminum* (Rondani) did not survive in grasses containing the alkaloid loline. On the other hand, ergovaline did not affect both the insect species.

The methanolic extracts of *F. arundinacea* infected with *A. coenophialum*, contain lolines of fungal origin, which are able to alter feeding behaviour and weight of insects-pests. Diets amended with extracts containing loline derivatives reduced weight and altered certain behaviours of *S. frugiperda* and *O. nubilalis*, notably in the first insect (Riedell *et al.*, 1991).

Patterson *et al.* (1992) observed in *Lolium* and *Festuca* that the production of alkaloids by *Acremonium* reduced attacks of the Japanese beetle, *Popilla japonica* Newman. Other works find that ambient variations may affect toxin production by endophytic fungi. Breen (1992) verified that changes in temperature and seasons modify toxin levels in the plant. This is the case of the peramine toxin, produced in *L. perenne* by *A. lolii*. The antixenosis towards the aphid, *S. graminum* is dependent on peramine and the endophyte concentration. so, a natural consequence is that the effect on the insect-pest will also be modified. The specificity between certain endophytic fungi and host plants led Leuchtmann (1992) to suggest a further study on the occurrence of physiological races in endophytic fungi that could be important to the development of new biological controls.

Surveys have been carried out aiming to the discovery of new toxins useful to insect control. Two new active toxins against the Spruce budworm, *Choristoneura fumiferana*

(Clemens) were found in an unidentified endophytic fungus infecting the wintergreen *Gaultheria procumbens* L. (Findlay *et al.*, 1997). Alkaloids from *N. lolii* and *L. perenne* are capable of altering insect behaviour. Several of these alkaloids were added to the diet of adult individuals of the Coleoptera *Heteronychus aratur* Fab. Peramine, lolitrem B, lysergol-type alkaloids, festuclavine and lisergic acid showed no effects on the insect. Ergovine showed moderate effects whereas ergotamine, ergovaline from the ergot-type alkaloid family seem to be responsible for the plant resistance (Ball *et al.*, 1997). Miles *et al.* (1998) showed that endophytic isolates of *Neotyphodium* sp. produce N-formilonine and a paxiline analogous in the host *Echinopogon ovatus* (G. Forst). These compounds show insecticidal activity against *Listronotus bonariensis* (Kuschel) and other insects. The majority of works related to toxin production was performed in grasses. However, Calhoun *et al.* (1992) for the first time identified toxic products synthesized by endophytic fungi in woody plants and that were able to modify growth and death rates in larvae of the spruce budworm, *C. fumiferana* feeding on balsam fir. The endophytes in this case were identified as *Phyllosticta* and *Hormonema dematioides* Lagerberg & Melin. and the toxic compounds were mainly heptelidic acid and rugulosine. Bills *et al.* (1992) also detected the existence of tremorgenic toxins in tropical woody plant infected with an endophytic fungus from the genus *Phomopsis*.

Cherry *et al.* (1999, 2004) in Africa revealed the feeding deterrence/antibiosis hypothesis. They studied *B. bassiana* endophytism in Africa when attempting to control the maize stem borer (*Sesamia calamistis* Hampson) by treating maize seeds with dry *B. bassiana* conidia, by spraying conidial suspensions in the leaf axils and by injecting conidial suspension in the stem. Lomer *et al.* (1997) had previously reported that *B. bassiana* could be isolated as an endophyte in maize in Africa and Cherry *et al.* (1999, 2004) showed reduced tunneling of the stem borer in plants treated with *B. bassiana*, even though no attempt was made to re-isolate the fungus. In plants injected with *B. bassiana*, larvae were lighter than in the control plants suggesting they did not feed as much, supporting the deterrence/antibiosis hypothesis. None of the dead larvae exhibited symptoms of mycosis.

### Induction of entomopathogenic endophytic fungi into plants

It is widely known the existence of fungi and bacteria able to cause disease in insects. Fungal species like *Metarhizium anisopliae* (Metchnikoff) Sorokin, *B. bassiana*, *L. lecani* and others are often used in the biological control of agricultural insects-pests. If these microorganisms inhabit plant tissues as endophytes, they will obviously be able to act on insect control. Even in situations where they are not present in the plant, artificial inoculation techniques may circumvent this obstacle. As in this review, endophytic fungi that do not colonize insects and cause disease, may be used as a natural insect control as well as an effective insect control if they are artificially inoculated into plants and display characteristics against the pests.

Clay (1989) worked on the potential of insect control by endophytic fungi and stressed that insect bio-control may be improved by the development of artificial inoculation techniques, as those developed by Latch and Christensen (1988) and Leuchtman and Clay (1985). As endophytic fungi, notably members of the genus *Acremonium*, are able to control some insects-pests, it is important to know if they can be inoculated in endophyte-free plants and, if they can transmit to the host the capacity to resist to certain pests. That was done with success by Koga *et al.* (1997) by infecting *F. arundinacea* and *L. perenne* seeds with the endophytic fungus *Acremonium* and as a result, obtained plants resistant to the bluegrass webworm, *P. teterrella*. In the same manner, Pereira *et al.* (1999) using a tropical host, the banana tree inoculated artificially an endophytic fungus tagged with a genetic marker for resistance to fungicides. It was observed that not only the inoculation worked well but also the mutant fungus was kept in competition with wild type ones. These results demonstrated that inoculation of valuable strains for biological control will not only be used with success but, modified microorganisms, once inoculated into a host, will be able to coexist and even overcome wild type strains of the same species. Knoch *et al.* (1993) observed in *F. arundinacea*, in which the endophytic fungus *Acremonium coenophialum* (Morgan-Jones & W.Gams) is transmitted by seeds that ants often prefer to collect endophyte-free seeds, avoiding those infected. Even seeds infected with the fungus are later discarded, favouring seed dispersion and germination.

Entomopathogenic fungi can be placed inside plants. This procedure was done in corn (Bing and Lewis, 1992a; 1992b; 1991) to verify the potential of these fungi as endophytes to control the European corn borer, *O. nubilalis*. *B. bassiana* was inoculated by injection and aspersion and was detected endophytically, colonizing several corn tissues and blocking the insect attack in some of its developmental stages. Injection resulted in 95 per cent colonized plants and aspersion in 98.3 per cent. An interesting finding was that in 33.3 per cent of the control plants, therefore not treated, the fungus was found indicating that the fungus is a natural endophyte. It was not determined the process by which the fungus invades the plant, but the movement was shown to be passive, occurring in the vascular system. It was also showed that the infected insect does not transmit the fungus because plants free of attacks could contain the fungus in an endophytic state. Bing and Lewis (1993) also refer to the fact that the entomopathogenic fungus *B. bassiana* has been used for a long time in the control of the European corn borer and this fungus, as mentioned before, is isolated as endophyte from corn. This could explain the pest suppression in certain seasons, correlated with the presence of *B. bassiana* in the corn senescent phase (Bing and Lewis, 1991).

Several roles have been ascribed to fungal endophytes, including providing protection against herbivorous insects (Breen, 1994; Clement *et al.*, 1994), plant parasitic nematodes (Elmi *et al.*, 2000) and plant pathogens (Wicklow *et al.*, 2005).



Table 5. Establishment of endophytic entomopathogenic fungi and effects on herbivorous insect feeding or development

Entomopathogenic fungus	Plant species colonized	Plant parts treated -assessed for colonization	Herbivore species targeted	Effect	Mycosis	Reference
<i>Beauveria bassiana</i>	Maize	Leaves-stems	<i>Ostrinia nubilalis</i>	Larval tunnelling reduced	Not reported	Bing and Lewis (1991)
	Maize	Leaf sheets-no final assessment	<i>Sesamia calamistis</i>	Larval tunnelling reduced	Not reported	Cherry <i>et al.</i> (1999)
	Maize	Stems-no final assessment	<i>Sesamia calamistis</i>	Fewer larvae, less dead hearts per 20 stems	Not observed	Cherry <i>et al.</i> (2004)
	<i>Musa acuminata</i> x <i>M. balbisiana</i>	Roots, rhizomes	<i>Cosmopolites sordidus</i>	Reduced larval survivorship	< 5 % of eggs, < 60 % of adults	Akello <i>et al.</i> (2008)
	<i>Solanum lycopersicum</i>	Seeds, roots-stem, leaves, lateral shoots	<i>Helicoverpa zea</i>	No differences in acute larval mortality or longevity		Powell <i>et al.</i> (2009)
	Sorghum	Leaves-stems	<i>Chilo partellus</i>	Larval tunnelling reduced	Not reported	Reddy <i>et al.</i> (2009)
	<i>Papaver somniferum</i>	Leaves-leaves	<i>Iraella luteipes</i>	Reduced larval numbers	Not reported	Moraga <i>et al.</i> (2009)
	Cotton, Maize, Wheat, Tomato, <i>Cucurbita maxima</i> , <i>Phaseolus vulgaris</i>	Leaves-leaves	<i>Aphis gossypii</i> , <i>Chortoicetes terminifera</i>	Reduced aphid reproduction and locust growth rate	Not observed	Gurulingappa <i>et al.</i> (2010)
	<i>Vicia faba</i> , <i>Phaseolus vulgaris</i>	Leaves-leaves	<i>Helicoverpa armigera</i>	Reduced larval survivorship	Observed, but not in all isolates tested	Vidal and Iaber (2015)
	<i>Vicia faba</i>	Seeds-leaves	<i>Liriomyza huidobrensis</i>	Fewer pupation, reduced emergence and adult survival	Not observed	Akutse <i>et al.</i> (2013)
<i>Metarrhizium anisopliae</i>	<i>Phaseolus vulgaris</i> , <i>Vicia faba</i>	Seeds-roots	<i>Acyrtosiphum pisum</i> , <i>Aphis fabae</i>	Reduced population growth	Not observed	Akello <i>et al.</i> (2012)
	<i>Vicia faba</i>	Leaves-roots	<i>Acyrtosiphum pisum</i>	No effect on population increment	Not observed	Akello <i>et al.</i> (2012)
	<i>Brassica oleracea</i>	Leaves, leaf-petioles, stems	<i>Aphis fabae</i> , <i>Plutella xylostella</i>	Higher larval mortality	Not reported	Batta (2013)

Variable effects have sometimes been reported, e.g. Clement *et al.* (2005) reported different effects on two aphids (bird-cherry oat aphid, *Rhopalosiphum padi* (L.) and rose grass aphid, *Metopopophium dirhodum* Walker and the wheat stem fly, *Mayetiola destructor* (Say) exposed to different wild barleys infected with *Neotyphodium*. Jallow *et al.* (2004) reported drastic negative effects on larvae of *H. armigera* Hubner reared on tomato plants infected with a non-grass endophyte, *Acremonium strictum* (W.Gams). The reports collected from the few reviews on establishment of endophytic entomopathogenic fungi and their effects on herbivorous insect feeding or development are presented in Table 5.

The pioneering work on entomopathogenic endophytes was conducted using maize including *B. bassiana* and the European corn borer, *O. nubilalis* as a model system. Season-long suppression of insects, measured as reduced tunneling due to *O. nubilalis*, was achieved by applying *B. bassiana* in an aqueous (Lewis and Cossentine, 1986) or granular (Lewis and Bing, 1991) formulation on corn plants. Lewis and Bing (1991) suspected that *O. nubilalis* suppression was due to establishment of *B. bassiana* in the plant, based on prior reports of this phenomenon by Vakili (1990). In a subsequent paper, Bing and Lewis (1991) used granular formulations and injections of *B. bassiana* spore suspensions to obtain season-long suppression of *O. nubilalis* and concluded that this was due to *B. bassiana* becoming established as an endophyte in maize. Their methodology for assessing *B. bassiana* presence involved sterile techniques but does not report surface-sterilization (Bing and Lewis, 1991, 1992a), as is usually done when attempting to isolate endophytes (Arnold *et al.*, 2001). Nevertheless, in a subsequent paper Bing and Lewis (1992b) reported surface-sterilization of tissues and recovery of *B. bassiana*, thus providing evidence of an endophytic relationship. The fact that no mycosed insects were reported which suggests that the reduced tunneling reported in all the papers (Bing and Lewis, 1991; Bing and Lewis, 1992a, b) could be a result of feeding deterrence or antibiosis. Bing and Lewis (1993) reported that out of 1623 *O. nubilalis* larvae sampled from control maize plants and maize plants inoculated with *B. bassiana*, 1556 were negative for *B. bassiana*, based on mycosis.

Wagner and Lewis (2000) have showed that following conidia germination and germ tube development, *B. bassiana* enters maize tissues directly through the plant cuticle. Subsequent hyphal growth occurs within the apoplast, but only occasionally extending into the xylem elements. The introduction of endophytic *B. bassiana* in maize is compatible with other pest management strategies. It has been shown that endophytic *B. bassiana* is compatible with both *B. thuringiensis* and carbofuran applications used to suppress *O. nubilalis* (Lewis *et al.*, 1996). Use of Bt transgenic corn did not have any detectable effect on the establishment of *B. bassiana* as a corn endophyte (Lewis *et al.*, 2001). Endophytic *B. bassiana* caused no mortality to *Coleomegilla maculata* De Geer, a predator of *O. nubilalis* eggs and larvae (Pingel and Lewis, 1996).

Bing and Lewis (1993) showed that 2.5 per cent mycosis in insects feeding on endophytic plants (and 1.7% mycoses in insects feeding on plants with no *B. bassiana*). The lack of *B. bassiana* conidia in endophytic plants (Wagner and Lewis, 2000) also suggests a mode of action involving feeding deterrence or antibiosis rather than direct fungal infection.

Stefan and Lara (2015) tested the virulence of endophytic *B. bassiana* strains on third instar larvae of the American bollworm (*H. armigera*), using inoculated faba bean plants, a host plant species for this herbivore. All *H. armigera* larvae fed upon plants of the control treatment remained alive until pupation, larval mortality was observed on faba plants inoculated with the fungus in all other treatments (Table 6). However, only plants inoculated with the isolates ATP01, ATP02, and Bb03032 resulted in a significantly higher larval mortality compared to control plants (one-way ANOVA,  $P < 0.05$ ; Tukey's HSD test with Bonferroni correction). While none of the larval cadavers collected from plants inoculated with isolates/strains ATP01, ATP03 and BB1022 displayed BB mycosis, between 16.76 and 100 per cent of the cadavers recovered from plants inoculated with the remaining isolates/strains showed mycosis (Table 6). Survival time varied

significantly among larvae fed upon plants inoculated with different BB isolates/strains (oneway ANOVA,  $F_{12,99}=60.847$ ;  $P < 0.0001$ ). Isolate ATP02 followed by isolate ATP01 caused significantly earlier larval mortality compared to the remaining isolates/ strains (Table 6).

### Other endophytic interactions affecting insect control

It would be naive to suppose that endophytic fungi interact with the host and the insect-pest in a simple manner and independently of environmental conditions, genotypes and other factors mentioned before. Therefore, different indirect effects may occur and some of them will be addressed in the following discussions.

### Indirect effects in pest control: insect-vector elimination and gall formation by insects-pests

The first work showing the importance of endophytic fungi to the control of agricultural pests was curiously an indirect demonstration that the endophyte, by blocking insect gall formation, also was able to control a disease caused by a fungus (Carrol, 1986). Several other examples were analysed in which endophytic fungi block or alter insect's larvae installation and by doing so, repress potential vectors to disseminate diseases. Hata and Futai (1995) studied the endophytic mycobiota of *Tecodiplosis japonensis* galls in *Pinus*. The endophytic fungus *Phialocephala* sp. was the most frequent, however young galls did not contain the fungus, indicating that the endophytes was not inoculated by the insect. In this case, it was possible to verify that the insects do not behave as vector for endophytic fungi but on the other hand, do not avoid the transmission of plant pathogenic fungi. Other interesting effect of endophytes over insect galls was seen in oak, where apparently dormant endophytic fungi become active with gall formation and by destroying the leaves, also kill the insect, protecting, therefore, their hosts. This process was described in three associations between endophytes and insects, one of them involving the fungus *Gloeosporium quercinum* Westend and the insect *Neuroterus numismalis* Fourcroy (Butin, 1992).

Table 6. Virulence of 12 *B. bassiana* endophytic isolates/strains against third instar *Helicoverpa armigera* larvae fed on leaves of inoculated *Vicia faba* plants

Treatments <i>B. bassiana</i> isolate	Parameter sampled $\pm$ SE		
	Mortality (%)	Mycosis (%)	Survival time (days)
ATP01	70.00 $\pm$ 0.11 <sup>ab</sup>	00.00 $\pm$ 0.00 <sup>b</sup>	10.36 $\pm$ 0.82 <sup>b</sup>
ATP02	85.00 $\pm$ 0.08 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>	6.41 $\pm$ 0.58 <sup>a</sup>
ATP03	10.00 $\pm$ 0.07 <sup>cd</sup>	00.00 $\pm$ 0.00 <sup>b</sup>	23.5 $\pm$ 1.50 <sup>cd</sup>
ATP04	30.00 $\pm$ 0.11 <sup>bcd</sup>	16.76 $\pm$ 0.17 <sup>b</sup>	21.33 $\pm$ 0.67 <sup>cd</sup>
ATP05	40.00 $\pm$ 0.11 <sup>abcd</sup>	37.50 $\pm$ 0.18 <sup>b</sup>	20.63 $\pm$ 0.59 <sup>cd</sup>
Bb03032	55.00 $\pm$ 0.11 <sup>abc</sup>	54.55 $\pm$ 0.16 <sup>a</sup>	18.64 $\pm$ 0.64 <sup>c</sup>
EABb04/01-Tip	45.00 $\pm$ 0.11 <sup>abcd</sup>	66.67 $\pm$ 0.17 <sup>a</sup>	19.11 $\pm$ 0.63 <sup>c</sup>
Bb64	40.00 $\pm$ 0.11 <sup>abcd</sup>	50.00 $\pm$ 0.19 <sup>ab</sup>	20.25 $\pm$ 0.59 <sup>c</sup>
Bb135	25.00 $\pm$ 0.10 <sup>bcd</sup>	40.00 $\pm$ 0.25 <sup>ab</sup>	20.80 $\pm$ 0.66 <sup>cd</sup>
Bb1022	30.00 $\pm$ 0.11 <sup>bcd</sup>	00.00 $\pm$ 0.00 <sup>b</sup>	21.00 $\pm$ 0.97 <sup>cd</sup>
Bb1025	35.00 $\pm$ 0.11 <sup>bcd</sup>	28.57 $\pm$ 0.18 <sup>b</sup>	20.29 $\pm$ 0.67 <sup>c</sup>
Naturalis® (strain ATCC74040- based bioinsecticide)	25.00 $\pm$ 0.10 <sup>bcd</sup>	22.22 $\pm$ 0.15 <sup>b</sup>	21.11 $\pm$ 0.68 <sup>cd</sup>
Control	00.00 $\pm$ 0.00 <sup>d</sup>	00.00 $\pm$ 0.00 <sup>b</sup>	24.60 $\pm$ 0.83 <sup>d</sup>

Means (SE) followed by the same alphabet within a column are not significantly different at  $P < 0.05$  (Tukey's HSD test with Bonferroni correction for multiple testing)

## Conclusion

The role of microbial endophytes protecting plants against insects has been well documented in various countries. However, the research is based mainly on endophytes in few host plants. Apart from isolated studies, very recently, efforts are being directed to recognize the role of endophytes in insect control. This is a broad field of investigation that is almost entirely open to new findings. The results presented in this review shows that, as expected, great diversity has been found among endophytes isolated from different plants. Thus, it is expected that new ways of interactions between endophytes and their hosts, secondary metabolites produced by candidate endophytes, biotechnological approaches in enhancing the

efficacy of endophytes and at the end, the amount of inoculum required to protect the host from the herbivore is essential. This should also make the approach attractive to the biotechnology industry looking for alternatives to traditional pesticides, as they target precisely the zone of insect infestation. The future use of biological control in combination with endophytes along with commercial pesticides applied to the seed or seedling could lead to synergistic effects on one or multiple pests and disease causing agents. The chemicals could provide near instantaneous suppression on insect pests, while the biological agent could provide continuing control well into the crops production cycle. IPM involving endophytes reduces costs and environmental impact, while allowing the biological agent to build up momentum for insect pest control.

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