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## Review

# Use of endophytes as biocontrol agents



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### ABSTRACT

Plant diseases, caused by various microorganisms, including viruses, bacteria, fungi, protozoa and nematodes, affect agricultural practices and result in significant crop losses. Fungal pathogens are the major cause of plant diseases and infect most plants. Agrochemicals play a significant role in plant disease management to ensure a sustainable and productive agricultural system. However, the intensive use of chemicals has adverse effects on humans and ecosystem functioning and also reduces agricultural sustainability. A sustainable agriculture is achieved through reduction or elimination of fertilizers and agrochemicals, resulting in minimal impact to the environment. Recently, the use of antagonistic endophytes as biocontrol agents is drawing special attention as an attractive option for management of some plant diseases, resulting in minimal impact to the environment. Endophytes that resides asymptotically within a plant, have the potential to provide a source of candidate strains for potential biocontrol applications. This review addresses biocontrol methods using endophytic fungi such as *Colletotrichum*, *Cladosporium*, *Fusarium*, *Pestalotiopsis* and *Trichoderma* species as an attractive option for management of some plant diseases. Potential endophytes are screened *in vitro* and *in vivo* to test their antagonistic actions by different mechanisms, including mycoparasitism, production of lytic enzymes and/or antibiotics and induction of plant defenses. Currently, efforts are being made to commercialize these biocontrol agents. A continued research pipeline consisting of screening, *in vitro* and *in vivo* testing, biomass production and commercialization of endophytes as biocontrol agents may contribute to sustainable agriculture.

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## 1. Introduction

### Global issues with fungal pathogens

Plant diseases affect crops and wild plants all over the world (Reuveni and Reuveni 1998; Anderson et al., 2004; Burketova et al., 2015). Approximately, 10–16% of global food production are reduced due to field and post-harvest plant diseases (Strange and Scott 2005; Ghini et al., 2008; Chakraborty et al., 2000; Chakraborty and Newton 2011, Lo Presti et al., 2015). Plant diseases are caused by various microorganisms including bacteria, fungi, nematodes, protozoa and viruses. Fungal pathogens are one of the major causative agents of plant diseases, that can infect most plant parts, including roots, stems, leaves, flowers and fruits (Hyde et al., 2009a, 2009b, 2014; Manamgoda et al., 2011; Maharachchikumbura et al., 2014; Udayanga et al., 2014).

Crop losses are a major threat to food security, especially in developing countries (Ghini et al., 2008; Chakraborty and Newton 2011). In history of plant diseases, fungi and oomycetes played major roles in some food crisis scenarios. Potato late blight caused by oomycetes *Phytophthora infestans* was marked as catastrophic event in history, causing an unprecedented threat to agriculture in 1845 in Europe and Ireland (Chakraborty et al., 2000; Brown and Hovmoller 2002; Strange and Scott 2005; Chowdappa et al., 2015). Karnal bunt is an important disease of wheat, durum, rye and triticale and is caused by the fungus *Tilletia (Neovossia) indica*. It was first recorded in South Asia and Iraq during 1931 and then spread to Mexico and USA (Anderson et al., 2004; Chakraborty and Newton 2011). In addition, fungus *Pyricularia oryzae*, causes rice blast disease, reducing 10%–30% of rice yield every year (Strange and Scott 2005). Other epidemics caused by fungi are wheat rust caused by *Puccinia graminis*, maize smut caused by *Ustilago maydis* and soybean rust caused by *Phakospora pachyrizi* (Fisher et al., 2013).

In addition to that forest plants were also infected by fungi. Chestnut blight is a devastating disease that spread through North America during 1904–1944 caused by *Cryphonectria parasitica* (Brown and Hovmoller 2002; Anderson et al., 2004; Weller et al., 2014). This fungus infected trees and caused cankers and ultimately caused trees to wilt and die (Coakley et al., 1999). Similarly, Dutch elm disease is another situation where fungus *Ophiostoma ulmi* eliminated *Ulmus* sp. throughout North America, Europe and southwest Asia during 20<sup>th</sup> century (Anderson et al., 2004). During 2012, ash dieback was recorded in ash trees in Britain. *Hymenoscyphus fraxineus* is the causative agent and causes leaf loss, crown dieback and bark lesions and death in affected trees (<https://www.forestry.gov.uk/>).

Current agricultural practices use different varieties of crop plants developed from wild varieties. These new modified plants are grown in geographic areas away from their center of origin. Therefore, new pathogens may evolve and sometimes cause disease epidemics, because these crop plants are unable to develop resistance against the new pathogens (Strange and Scott 2005, Lo Presti et al., 2015). The global spread of many diseases is facilitated by a limited diversity in monoculture crops and dispersal of fungal pathogens via numerous wind dispersed spores (Brown and Hovmoller

2002; Strange and Scott 2005, Lo Presti et al., 2015). On the other hand, less aggressive pathogens in natural forests may become more aggressive in monoculture crops growing nearby (Chakraborty et al., 2000). The coffee rust epidemic in the 1800s in Asia is such a situation, where *Coffea arabica* was infected by *Hemileia vastatrix* a native pathogen present in forests near the coffee plantation (Chakraborty et al., 2000; Weller et al., 2014).

Climate change effects plant diseases, depending on how particular climatic variation affects the host and pathogen physiology (Coakley et al., 1999; Chakraborty et al., 2000; Garrett et al., 2006, Lo Presti et al., 2015). Temperature, radiation, precipitation, humidity, wind speed, and circulation patterns are abiotic factors that significantly influence the life cycle of pathogens and development of diseases (Chakraborty et al., 2000; Rosenzweig et al., 2001). The virulence of various diseases can be increased by unfavorable environmental conditions, such as lack or excess of nutrients, moisture and light, and the presence of toxic chemicals in the air or soil (Anderson et al., 2004; Weller et al., 2014). Due to anthropogenic activities, the global climate is changing, which affects the sustainability of plants in natural and agricultural communities (Chakraborty et al., 2000; Rosenzweig et al., 2001; Suryanarayanan et al., 2016). Changing climatic conditions may reduce plant growth. For example, a larger reduction in plant growth was recorded due to reduction in net photosynthesis caused by Barley powdery mildew infection under elevated CO<sub>2</sub>, than in ambient CO<sub>2</sub> concentrations (Chakraborty et al., 2000). A similar finding in the woody weed rubber vine (*Cryptostegia grandiflora*) was recorded, when the rust pathogen *Maravalia cryptostegiae* was used as biological control of the weed. A reduction of growth in rust inoculated weed was found under twice ambient CO<sub>2</sub> concentration (Chakraborty et al., 2000). When temperatures increase under drought conditions, endophytic *Cryptostroma corticale* becomes more aggressive invading vascular and bark tissues of *Acer pseudoplatanus* (Chakraborty et al., 2000). Similarly, in China, rice and wheat crop damage by Rice blast (caused by *Pyricularia grisea*) and Wheat scab (caused by *Fusarium graminearum*) have increased since the 1970s, due to increase of temperature in spring and early summer (Coakley et al., 1999). It has also been reported that the number of oak trees in southern and Mediterranean Europe, declined due to *Phytophthora cinnamomi*, a soil borne oomycete that became more active with increasing temperatures (Coakley et al., 1999).

### Current methods used to control plant diseases

#### Agrochemicals

Modern agriculture should respond to the challenge of plant diseases that play a major limiting role of providing sufficient, safe and nutritious food (Reuveni and Reuveni 1998; Russell 2005; <http://www.apsnet.org/>). It is estimated that the world population will increase by 30% to nine billion people by 2050 (Lamberth et al., 2013; Weller et al., 2014). Therefore, plant disease management strategies should ensure a sustainable and productive system for the future (Russell 2005; Weller et al., 2014). Agrochemicals play a significant role in the

reduction of crop losses in current agriculture production systems (Ridgway *et al.*, 1978; De Waard *et al.*, 1993; Carvalho 2006). Agrochemicals can be divided in two large groups, chemical fertilizers and pesticides (Carvalho 2006). Chemical fertilizers contain minerals such as nitrogen, phosphorus and potassium (Carvalho 2006). Pesticides include insecticides, fungicides, and herbicides against insects, pathogens and weeds respectively (Yudelman *et al.*, 1998; Carvalho 2006; Weller *et al.*, 2014). Interestingly, fungi and oomycetes are the most prevalent causative agents of plant diseases and fungal toxins are able to cause acute or chronic health problems in humans and other animals (Weller *et al.*, 2014). For example, *Fusarium graminearum* (causes Fusarium head blight/wheat scab) not only reduces wheat yield, but also produces the toxin deoxynivalenol (DON), which is a potential health risk for humans and animals (Weller *et al.*, 2014). The development of these fungicides, their wide availability and increased effectiveness attribute to combat the wide variety of fungal pathogens and enhance crop productivity. Therefore, plant disease control is heavily dependent on fungicides (De Waard *et al.*, 1993; Reuveni and Reuveni 1998; Weller *et al.*, 2014).

It is believed that, fungicide usage was started with the discovery of Bordeaux mixture in 1885, which is considered as the first generation of fungicides. This chemical was used to control powdery and downy mildew in grape (De Waard *et al.*, 1993; Weller *et al.*, 2014). Organic chemicals such as dithiocarbamates are considered as a second generation of fungicides (De Waard *et al.*, 1993; Russell 2005). Development of second generation fungicides started in 1934 (De Waard *et al.*, 1993). These fungicides act on the surface of plants and do not penetrate plant tissues (De Waard *et al.*, 1993). A third generation of organic fungicides (1970–1980), were able to penetrate plant tissues and control infections (De Waard *et al.*, 1993). The fourth generation of fungicides (1980s to present) interfere with fungal penetration of the host plant and activate host-plant resistance (De Waard *et al.*, 1993; Russell 2005). Details of some of these chemicals are given in Table 1.

The intensive use of pesticides has adverse effects on humans and ecosystem functioning and also reduces agricultural sustainability (Ridgway *et al.*, 1978; Dumroese *et al.*, 1990; De Waard *et al.*, 1993; Widawsky *et al.*, 1998; Anderson *et al.*, 2004; Vinale *et al.*, 2008; Suryanarayanan *et al.*, 2016). Agrochemicals cause chemical pollution in the environment, food-chains, soil, reduces soil fertility and disturbs ecological balances (Chou 2010; Tranier *et al.*, 2014; Suryanarayanan *et al.*, 2016). Long term usage of chemical fertilizers containing nitrogen can cause ground water contamination and ammonium fertilizers might reduce soil pH levels and thereby increase Fusarium wilt disease (Bruggen 1995; Carvalho 2006). Some fungicides affect the soil microflora, especially saprobic fungi such as *Penicillium* sp. and *Trichoderma* species (De Waard *et al.*, 1993; Suryanarayanan *et al.*, 2016).

Development of fungicide resistance is the most serious negative impact of frequent use of the fungicides, leading to failures in disease control (De Waard *et al.*, 1993; Ishii 2006; Vinale *et al.*, 2008; Hahn 2014; Burketova *et al.*, 2015; Suryanarayanan *et al.*, 2016). Most of the first and second generation fungicides containing organic antifungal compounds are multisite inhibitors. Third generation fungicides

are site-specific, highly efficient and low toxicity compounds for the control of fungal diseases. However, these site-specific fungicides soon became less active because of resistance development by phytopathogens (Ishii 2006; Hahn 2014). *Botrytis cinerea* is a major pathogen that causes grey moulds on cultivated vegetables, fruits and ornamental flowers. A single-site mutation in the protein coding gene ( $\beta$ -tubulin) of *Botrytis cinerea* resulted in resistance development against benzimidazoles.

QoI fungicides (strobilurin fungicides) are presently the most important class of fungicides and azoxystrobin is the world's largest-selling fungicide (Ishii 2006). QoI fungicides generally have a broad spectrum of control activity against a large number of pathogens. QoI fungicides however induce resistance development in pathogens. Powdery mildew in cucumber and melon crops, as well as in downy mildew in cucumber crops in Japan developed fungicide resistant to QoI fungicides (Ishii 2006). Another example is development of fungicide resistant by the wheat pathogen *Mycosphaerella graminicola* to strobilurin fungicides (Mikaberidze *et al.*, 2014). DMI fungicides are used to control diseases on fruit trees, vegetables, cereals, and other crops (scab disease caused by *Venturia nashicola*) (Ishii 2006). The apple scab fungus (*Venturia nashicola* and *V. inaequalis*) have developed DMI fungicide resistance (Ishii 2006). Pesticide resistance requires higher levels of pesticides to achieve pest control (Widawsky *et al.*, 1998; Tranier *et al.*, 2014). This higher level of pesticide usage decreased the effectiveness of pesticides (De Waard *et al.*, 1993; Widawsky *et al.*, 1998). Since, excessive use of agrochemicals causes environmental pollution and development of pathogen resistance, alternative methods to combat pathogens become important and have received more attention in the past decade (Hasan *et al.*, 2013). A critical question is the alternative strategies with non-chemical products to achieve high-quality crops with optimal yields.

#### Other methods used to control plant diseases

A sustainable agriculture is achieved through reduction or elimination of fertilizers and agrochemicals (Vinale *et al.*, 2008; Chou 2010). Many countries have taken regulatory measures to minimize chemical pest-control and support in alternative methods to control agricultural pest and pathogens (Widawsky *et al.*, 1998). One effective alternative crop management strategy is the Integrated Crop Management system. The Integrated Crop Management system is defined as 'the economical production of high quality crops, giving priority to ecological safe methods of crop cultivation, minimizing the undesirable side effects and use of crop protection products' (Oerke 2006). Integrated crop management uses a combination of agrochemicals, organic fertilizers, biological control and improved soil and water management which leads to sustainable agriculture (Carvalho 2006; Oerke 2006; Suryanarayanan *et al.*, 2016). Organic farming is another alternative method to achieve sustainable agriculture (Chou 2010). Organic debris contains compounds such as phenolics, flavonoids, terpenoids, alkaloids and fatty acids, which can be utilized as natural bio-agrochemicals (Chou 2010).

Development of crop plants with pest resistance varieties is one non-chemical control method against pathogens

**Table 1 – Different fungicides used for plant pathogens.**

Time	Name of chemical	Country	Crop plant	Target pathogen	Reference
1800s	Sulfur, copper, and organic mercury <sup>a</sup>	France (wine)	Peaches	Powdery mildews	<a href="#">Russell 2005, Weller et al., (2014)</a>
First generation 1885	Bordeaux mix	France	Grapes	Powdery and downy mildews	<a href="#">Weller et al., 2014, De Waard et al., (1993)</a>
Second generation from 1934 to 60s'	Organic chemicals	Worldwide	Many crops	Powdery mildews, mold and rusts	<a href="#">De Waard et al. (1993)</a>
Third generation (1960–70s')	Dithiocarbamates	Worldwide	Potatoes and many crops	<i>Phytophthora infestans</i>	<a href="#">Caldas et al., 2001, Russell 2005, Weller et al., 2014</a>
	Organic chemicals	Worldwide	Vegetable and fruit crops	Powdery mildews, mold and rusts	<a href="#">De Waard et al.,1993,</a>
	Benzimidazoles, Carboxamides, Phenylamides	Worldwide	Vegetable and fruit crops	Broad spectrum of fungi	<a href="#">Weller et al., 2014, Torres-Padron et al., 2010</a>
	Dodemorph	Worldwide	Ornamental flowers (eg: Rose)	Rose powdery mildew ( <i>Sphaerotheca pannosa</i> )	<a href="#">Russell (2005)</a>
	Tridemorph	Worldwide	Cereals	Powdery mildews ( <i>Erysiphe graminis</i> ), rust diseases	<a href="#">Russell 2005, Lewis et al., (2016)</a>
Fourth (1980 to present)					
1981	Tricyclazole	Rice growing countries	Rice	Rice blast fungus ( <i>Magnaporthe grisea</i> )	<a href="#">De Waard et al., 1993, Russell 2005, Magar et al., 2015</a>
1981	Benalaxyl (Phenylamide)	Worldwide	Vegetables, grapes, ornamentals and flowers	Oomycetes	<a href="#">Russell 2005, Lewis et al., (2016)</a>
1981	Mepronil (Carboxamide)	Worldwide	Vegetables	Rust diseases	<a href="#">White and Thorn 1980, Russell 2005</a>
1990	Triazole DMI	Worldwide	Cereals, turfgrasses	Broad spectrum	<a href="#">Russell 2005, Mitkowski and Chaves 2013</a>
1998	Hydroxyanilide	Worldwide	Vegetables and fruits	Botrytis sp.	<a href="#">Russell (2005)</a>
2000	Qol	Worldwide	Vegetables, fruits and ornamental flowers	Broad spectrum	<a href="#">Russell (2005)</a>

a Not available at present.

and pests (Widawsky et al., 1998). Scientists are trying to introduce new cultivars which are able to withstand disease, while allowing higher yields (Carvalho 2006). The International Rice Research Institute (IRRI), in the Philippines has developed new rice varieties through hybridization and genetic engineering (Carvalho 2006). These hybrid crops also have negative impacts on agriculture. For example, Triticale is an artificial hybrid of wheat and rye that is resistant to the powdery mildew pathogen (*Blumeria graminis*) (Menardo et al., 2016). However, *Blumeria graminis* f. sp. *triticale*; a pathogen that grows on triticale and wheat has been described as a hybrid between wheat powdery mildew (*B. graminis* f. sp. *tritici*) and mildew on rye (*B. graminis* f. sp. *secalis*). This implies that a novel pathogen may arise from two pathogens from two different hosts and infect hybrid plants (Menardo et al., 2016).

Besides conventional breeding, genetic engineering deals with transgene-mediated resistance against plant pathogens (Sharad et al., 2015). In transgenic crop plants genes encoding for chitinases and glucanases are commonly used to achieve good resistance against fungal pathogens (Sharad et al., 2015). Chitinases digest the fungal cell wall and induce further defense reactions by releasing pathogen-borne elicitors (Sharad et al., 2015). These transgenic plants over-expressing chitinase genes, show enhanced diseased resistance and delayed disease symptoms (Sharad et al., 2015). Another important group of plant cell wall degrading enzymes are polygalacturonases (PGs) that are produced by pathogens (Kalunke et al., 2015). Plants can inhibit the pectin-depolymerizing activity of polygalacturonases by the activation of cell wall polygalacturonase inhibiting proteins (Kalunke et al., 2015). Transgenic tomato plants that over-express polygalacturonase inhibiting proteins are capable of inhibiting PGs, secreted by *Botrytis cinerea* and reduction of disease lesions (Kalunke et al., 2015). Recently, genetic engineering revealed *Lr67* gene shows partial resistance against three wheat rust pathogen species and powdery mildew pathogen (Moore et al., 2015). The *Lr67* resistance gene encodes hexose transporter (LR67res) that differ from the susceptible form of transporter (LR67sus). The LR67sus function as high-affinity glucose transporters whereas LR67res reduce glucose uptake. It might possible to use genetic engineering to alter these transporters to reduce glucose uptake by pathogens (Moore et al., 2015).

Scientific investigations of cloning of resistance (R) genes from different crops and wild relatives have been carried out to transfer these R genes to susceptible cultivars (Narusaka et al., 2014). As an example, a single NLR-type R protein, Mi-1.2 in tomato provides resistance against pests including root-knot nematodes, potato aphid, and sweet potato whitefly (Narusaka et al., 2014). However transferring this type of R genes between different plant families or related species in the same family was not successful (Narusaka et al., 2014). This is believed to be due to fail to function of R gene (either no responses or inappropriate auto-immune responses) (Narusaka et al., 2014). Another group of resistance genes are those of the nucleotide binding site-leucine-rich repeat (NBS-LRR) class (Marone et al., 2013). Resistance (R) genes of the host plants encodes R proteins that detect avirulence (Avr) proteins secreted by the pathogens and confer resistance

against pathogens (Marone et al., 2013; Narusaka et al., 2014). *Pseudomonas syringae* Avr gene *AvrPphB* encodes proteases that cleave the host plant protein kinase (Marone et al., 2013). This cleavage was detected by R protein; RPS5 that resistance to *Pseudomonas syringae* activation of R protein confer resistance to *Pseudomonas syringae* (Marone et al., 2013).

However, biosafety of genetically modified plants has received high concern because of human consumption. Many of these genetically modified plants contain a high amount of foreign proteins that might affect human health. Therefore, risk assessment of genetically modified plants is essential (Mintz 2017).

### Biocontrol as an alternative method of plant disease control

One of the tools to control plant pathogens with minimal impact to the environment is biocontrol (De Waard et al., 1993; Vinale et al., 2008; Larran et al., 2016). William Roberts did experiments for the first time in history concerning the antagonistic action between *Penicillium glaucum* and bacteria and introduced the term 'antagonism' (Foster and Raoult 1974). Biological control of plant disease management was first used by C. F. Von in 1914 (Baker 1987; Cook 1988). Early biocontrol strategies involved the parasitic nature of *Trichoderma lignorum* to several plant pathogens or antibiotic production of microbes in the soil by *Aspergillus*, *Penicillium*, *Trichoderma* and *Streptomyces* species. The importance of siderophore (complex iron (III)) production by *Erwinia carotovora* in soils resulting in iron becoming unavailable to pathogens, thus inhibiting their growth (Kloepper et al., 1980; Junaid et al., 2013).

Biological control is defined as the use of beneficial organisms, their genes, and/or products, such as metabolites, to reduce the negative effects of plant pathogens and promote positive responses by the plant' (Vinale et al., 2008; Tranier et al. 2014). International Biocontrol Manufacturers Association (IBMA) defines biocontrol as the use of agents or products that naturally affect crop pests and pathogens by limiting their propagation. These agents or products include macroorganisms, microorganisms, chemical mediators, and natural substances (Lecomte et al., 2016). Although, biocontrol is considered as environmentally friendly, there appears to have been few very successful products. Thus, the development of this technology has been limited (De Waard et al., 1993; Maloy 2005; Suryanarayanan et al., 2016).

In general, *Aspergillus* spp., *Gliocladium* spp., nonpathogenic *Fusarium* spp., *Petriella* spp. and *Trichoderma* spp., as well as *Bacillus* spp., *Enterobacter* spp., *Lysobacter* spp., *Pantoea* spp., *Pseudomonas* spp. and *Streptomyces* spp. have been identified as important biocontrol agents (Byrne et al., 2005; Estrella et al., 2013, Larran et al. 2016; Herrera et al., 2016). *Peniophora gigantea* has been inoculated to tree stumps to prevent infection of adjacent trees by the wood decay fungus *Heterobasidion annosum* (Maloy 2005). Another example is the application of the nonpathogenic (non-tumor producing) bacterium *Agrobacterium radiobacter* to prevent infection by the crown gall bacterium (*Agrobacterium tumefaciens*) (Maloy 2005).

The biocontrol bacterium is introduced to fruit trees before planting trees (Maloy 2005). Microbial control agents also have been isolated from organic waste based compost (Estrella

et al., 2013). *Acetobacter indonesiensis*, *Bacillus pumilus*, *Paecilomyces variotii*, *Streptomyces griseus* and *Acremonium chrysogenum* are considered as biocontrol agents that suppress the devastating phytopathogens *Xanthomonas campestris* and *Fusarium oxysporum* f.sp. *melonis*, in the Southeast of Spain (Tamietti and Valentino 2006; Estrella et al., 2013).

Currently, there are some biocontrol agents such as bacteria (*Agrobacterium* spp., *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp. (eg: *Pseudomonas chlororaphis*), *Streptomyces* sp.), *Paenibacillus* spp. and *Pantoea* spp. (Larran et al. 2016; Herrera et al., 2016) and fungi (*Ampelomyces* spp., (eg: *Ampelomyces quisqualis*), *Aureobasidium pullulans*, *Beauveria bassiana*, *Candida* spp., (eg: *Candida oleophila*), *Colletotrichum* spp., *Coniothyrium* spp., (eg: *Coniothyrium minitans*), *Gliocladium* spp., (eg: *Gliocladium catenulatum*), *Metarhizium anisopliae*, *Paecilomyces lilacinus*, *Phlebiopsis gigantea*, *Purpureocillium lilacinum*, *Trichoderma* spp., (eg: *Trichoderma asperellum*, *Trichoderma atroviride*, *Trichoderma gamsii*, *Trichoderma polysporum*) and *Verticillium* spp. (eg: *Verticillium lecanii*)) that are available as commercial products (Vinale et al., 2008; Tranier et al., 2014; Larran et al. 2016; Herrera et al., 2016).

### Endophytes

Endophytes are a hidden bioresource of fungal diversity that have the potential to provide an unexplored source of candidate strains for potential biocontrol applications (Ek-Ramos et al., 2013; Oono et al., 2015; Potshangbam et al., 2017). The brief overview of 'Endophytes' is given here for better understanding of the following sections of the review. Often it is defined that endophyte resides asymptotically within a plant for at least a part of their life cycle (Guo et al. 2001; Hyde and Soyong 2008; Kusari et al., 2012a; O'Hanlon et al., 2012; Clay et al., 2016; Solis et al., 2016). Endophytes grow inter- or intracellularly, systemically or locally within their hosts without causing visible manifestations of infection or disease (Kusari et al., 2012a; Lo Presti et al., 2015; Schulz et al., 2015). Busby et al. (2016) gave an additional advanced definition for endophytism as "...infections are inconspicuous, the infected host tissues are at least transiently symptomless, and the microbial colonization can be demonstrated to be internal...". It is suggested that every single plant harbors endophytes and the diversity depends on a variety of factors, including host plant species, plant density, nutrient availability, local environmental conditions and interaction with soil fungi and bacteria (Ek-Ramos et al., 2013; Mejia et al., 2014; Oono et al., 2015; Persoh 2015; Yan et al., 2015).

Endophytes are ubiquitous in various aboveground and belowground plant tissues of liverworts, hornworts, mosses, lycophytes, equisetopsids, ferns, and spermatophytes from natural forests and agricultural ecosystems (Kusari et al., 2012a; Jeewon et al., 2013; Doilom et al., 2017; Potshangbam et al., 2017). Fungal endophytes are a diverse, polyphyletic group of microorganisms that are broadly classified into two groups, the clavicipitaceous (C) and the non-clavicipitaceous (NC) endophytes based on evolutionary relatedness, taxonomy, host plant range and ecological function (Rodriguez et al., 2009; Kusari et al., 2012a; O'Hanlon et al., 2012; Santangelo et al., 2015). Clavicipitaceous endophytes (family Clavicipitaceae) including *Atkinsonella*, *Balansia*, *Balansioipsis*,

*Echinodothis*, *Epichloe*, *Myriogenospora*, *Neotyphodium* and *Parepichloe* species are commonly associated with grasses in the family Poaceae and rely on their host throughout their life cycle as mutualist species (Rodriguez et al., 2009; O'Hanlon et al., 2012; Hardoim et al., 2015; De Silva et al., 2016). Non-clavicipitaceous endophytes such as *Fusarium* sp., *Colletotrichum* sp., *Phomopsis* sp., and *Xylaria* sp. are found in most terrestrial plants and might not inhabit the plants for their entire life cycle inside the host (Promputtha et al., 2005, 2007; Hyde et al. 2009a, 2009b; Rodriguez et al., 2009; Delaye et al., 2013; De Silva et al., 2016; Jayawardena et al., 2016). Currently, considerable interest is directed towards non-clavicipitaceous endophytes in various scientific disciplines because of their ability to switch between endophytic, pathogen and saprobic life-styles (Rodriguez et al., 2009; Delaye et al., 2013; De Silva et al., 2016).

Clavicipitaceous endophytes exhibits vertical transmission from mother plant to offsprings via seeds (Yan et al., 2015; Santangelo et al., 2015). Clavicipitaceous endophytic fungi show systemic growth (through internal plant tissues) as they grow into seedlings from germinating seeds (Yan et al., 2015). It is suggested, clavicipitaceous endophytes (family Clavicipitaceae) that live as intercellular symbionts are transmitted vertically from maternal plants to offspring and grow systemically throughout leaves and stems (Arnold et al. 2003; Santangelo et al., 2015). In contrast, endophytes associated with woody angiosperms foliage are transmitted horizontally by sexual or asexual spores (Arnold et al., 2003).

Endophytes are able to alter interactions with pests and pathogens and they can act as potential biocontrol agents. The endophyte, *Acremonium alternatum* controls damage from the moth *Plutella xylostella*, in beans and induces resistance against *Leveillula taurica* (the powdery mildew pathogen) in tomatoes (Ek-Ramos et al., 2013). *Phomopsis* sp. is a fungal endophyte (isolated from cotton plant) that reduced caterpillar herbivory activity in cotton plants (Ek-Ramos et al., 2013). Occasionally one particular endophyte exhibit potential as a biocontrol agent and also the same endophyte species might boosts host plant growth that given the additional value. *Neotyphodium* species protect the host plant against pests and pathogens while they boost host plant growth, host plant fitness and stress tolerance (Oono et al., 2015; Clay et al., 2016; Larran et al., 2016; Solis et al., 2016). Moreover, endophytic *Penicillium citrinum* and *Aspergillus terreus* reduced pathogenic *Sclerotium rolfsii* and also induced the biomass yield of sunflower plants (Potshangbam et al., 2017). Above examples emphasize the importance of endophytic fungi as biocontrol agents of the environmental safety techniques and their effect of high productivity of plants. The next important question would be how endophytes reduce pathogens and pests. Before dealing with different biocontrol mechanisms, we elaborate on how endophytes maintain their intimate relationship with their host.

### Plant – endophyte interaction

Asymptomatic colonization of endophytes is explained by the hypothesis of 'balanced antagonism' with their host (Kusari et al., 2012a; Schulz et al., 2015). The balanced antagonism between the host and the endophyte is maintained by avoiding

activation of the host defenses and activating resistance against toxic metabolites of the host. When fungal virulence and plant defense are at equilibrium, the fungal colonization would apparently be asymptomatic and avirulent (Kusari et al., 2012a). However, if the plant defense mechanisms completely counteract the fungal virulence factors, the fungus will be unable to colonize plant tissues (Kusari et al., 2012a; Suryanarayanan et al., 2016). If the fungal virulence factors can act against plant defense mechanisms, a plant-pathogen relationship would lead to plant disease (Kusari et al., 2012a; Lo Presti et al., 2015; Suryanarayanan et al., 2016).

Some endophytes become pathogens when they are influenced by certain intrinsic or environmental factors to express factors that lead to pathogenicity (Kusari et al., 2012a; Lo Presti et al., 2015). Switching life-styles of endophytic and pathogenic strains of *Colletotrichum magna* is suggested by disrupting single genetic loci or closely linked genes that induce anthracnose in cucurbit plants (Freeman and Rodriguez, 1993; Rai and Agarkar 2016). Experimental evidences supported that a non-pathogenic mutant strain (Path-1) that obtained by UV mutagenesis from a pathogenic strain (CmL2.5) of *Colletotrichum magna* asymptotically colonize the roots and stems of cucurbit plant and suppress the virulent strain of *Colletotrichum magna* (Freeman and Rodriguez, 1993; Rai and Agarkar 2016). This common phenomenon of switching life-styles of *Colletotrichum* might possible because of excessive humidity or poor nutrient supply that alter host's susceptibility in natural conditions (Fisher and Petrini, 1992; Rai and Agarkar 2016). It has also been shown that endophytic *Epichloë festucae* express the stress- and mitogen-activated protein kinase gene (*sakA*) to maintain mutualistic association with the host *Lolium perenne* (perennial ryegrass). If the fungus is unable to express the gene, the endophyte will switch to being pathogenic and/or saprobic unfavorable environmental conditions (Delaye et al. 2013; De Silva et al., 2016).

Some endophytes secrete antifungal and antibacterial metabolites at low concentrations, thus inhibiting competitors (both endophytic and pathogenic bacteria and fungi) and maintaining a balance of antagonism with the competitors (Schulz et al., 2015; Suryanarayanan et al., 2016). For example, endophytic *Phialocephala* sp., from *Picea glauca* (white spruce) synthesizes the insecticidal metabolite rugulosin toxic to *Choristoneura fumifurana* (spruce bud worm) (Sumarah and Miller 2009, Schulz et al., 2015). Secondary metabolites play a crucial role in maintaining equilibrium of antagonisms among endophytes, competitors and plant hosts. Estrada et al. (2012) showed endophytic *Fusarium verticillioides* in *Zea mays* was able to reduce the aggressiveness of pathogenic *Ustilago maydis* and also able to degrade defensive chemicals produced by the plant against *U. maydis*. Additionally, multipartite symbiosis between endophytes, competitors and host plants might result in reduced pathogen growth as their growth and survival will be limited by secondary metabolites (Schulz et al., 2015; Suryanarayanan et al., 2016). Recently, it has been found that the secondary metabolite genes in fungi are silent in pure culture and can be activated in dual culture assays with antagonistic microbes (Ochi and Hosaka 2013; Schulz et al., 2015). In conclusion, it is clear that plant-endophyte interactions involve complex and precisely controlled interactions that control

the equilibrium of host defense, fungal virulence and secondary metabolites.

### Biocontrol mechanism

After discussing plant-endophyte interactions, this section will summarize different mechanisms that endophytes utilize in antagonistic mechanisms against various phytopathogens. Plant inhabitant endophytes are thought to influence plant pathogens and thereby modify disease severity (Freeman and Rodriguez 1993; Arnold et al., 2003; Busby et al., 2016). However, mechanisms of endophytes involvement in plant defense is underappreciated. It is therefore important to understand ecological, biochemical and evolutionary significance of endophytes with host plants and their contributions to plant disease severity (Busby et al., 2016). Endophytes can decrease pathogen antagonism or increase pathogen facilitation or sometimes neutral effect towards plant disease severity (Busby et al., 2016). In addition, plant disease severity may be influenced by other factors including plant susceptibility, pathogen virulence and an abiotic environment. Some fungal endophytes modulate host plant resistance (Busby et al., 2016). Fungi are known to act against pathogens by triggering host resistance via two mechanisms, systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Estrella et al., 2013; Busby et al., 2016).

It is important to understand components of the disease triangle (host, pathogen and environment) and pathogen's disease cycle starting from initial infection to colonization and reproduction of the pathogen to establish effective plant pathogen control (Punja and Raj, 2003; Junaid et al., 2013). Biocontrol strategies utilize some mechanisms that disrupt some stages of the disease or the life cycle of the pathogen including prevention of infection, reduction in colonization of host tissues, reducing sporulation and limitation of survival of the pathogen (Punja and Raj, 2003). Antagonism is the mechanism that inhibites phytopathogenic organisms by secreting substances that interfere with the life cycle of the target microorganism (Hasan et al., 2013; Busby et al., 2016). In general, different mechanisms of antagonistic actions of a biocontrol agent are described as mycoparasitism, production of lytic enzymes and/or antibiotics, induction of plant defense and competition for nutrients and ecological niches (Estrella et al., 2013; Yan et al., 2015; Busby et al., 2016; Lecomte et al., 2016; Schlegel et al., 2016; Cheong et al., 2017). Fungi including *Trichoderma*, *Aureobasidium*, *Fusarium* and *Penicillium* species antagonize plant pathogens via mycoparasitism and by triggering SAR (Arnold et al., 2003; Busby et al., 2016).

Mycoparasitism is the parasitism of a microorganism by another microorganism (Lecomte et al., 2016). Some strains of *Trichoderma* parasitize *Fusarium oxysporum* by developing haustoria and by the synthesis of enzymes or secondary metabolites to uptake nutrients from the pathogenic fungus (Lecomte et al., 2016). *Bacillus thermoglucosidasius* produces antibiotics that can inhibit and control growth of *Fusarium oxysporum* f. sp. *lilii* (Lecomte et al., 2016). Endophytic *Ampelomyces* species parasitize powdery mildews (Busby et al., 2016). As powdery mildews are biotrophs, their antagonists act mainly through antibiosis and mycoparasitism (Kiss, 2003; Busby et al., 2016). *Trichoderma asperellum* competes

pathogenic *Ganoderma boninense* via mycoparasitism in Oil palm (Cheong et al., 2017). *Trichoderma* sp. produce cell-wall degrading enzymes such as  $\beta$ -1,3-glucanase, N acetylglucosaminidases, chitinase, which degrade fungal mycelia (Cheong et al., 2017). *Paraconiothyrium* are also known for the production of antibiotics that inhibit growth of pathogens (Schlegel et al. 2016). This endophytic species can inhibit germinating ascospores of *Hymenoscyphus fraxineus* that causes dieback on native European ash trees (*Fraxinus excelsior*) (Schlegel et al. 2016).

In addition, competition for nutrients and/or space and induction of plant defense also responsible for antagonism (Kiss, 2003; Busby et al., 2016). As an example, *Ampelomyces* species penetrate via the hyphae of powdery mildews and produce pycnidia (Kiss, 2003). *Ampelomyces* can suppress the sporulation of powdery mildews and kill all the parasitized cells (Kiss, 2003). *Pseudozyma* species and *Tilletiopsis* species can inhibit powdery mildew causing plasmolysis through antibiosis (antifungal metabolites including fatty acids and hydrolytic enzymes) (Kiss, 2003). Phyllosphere fungi antagonize rust pathogens by inhibiting the rust's thigmotropic mechanism of locating stomata and thereby germinating rust spores are unable to locate stomata to penetrate the plant tissue (Busby et al., 2016). Further, some *Pseudomonas* species secrete siderophores to control *Fusarium oxysporum* f. sp. *dianthi* by increasing competition for nutrients (Lecomte et al., 2016). These evidences revealed microorganisms are capable of compete for microhabitats and resources when they inhabit the same ecological niche.

Endophytes can produce an array of secondary metabolites that may have potential biological activity against pests or pathogens (Kusari et al., 2012a; Yan et al., 2015). Some examples of bioactive metabolites include paclitaxel (also known as taxol), podophyllotoxin, deoxypodophyllotoxin, camptothecin and structural analogs (Kusari et al., 2012a). The endophyte *Aspergillus fumigatus* from the host plant *Juniperus communis* produced deoxypodophyllotoxin that acts against a number of pathogenic bacteria (Kusari et al., 2009). An endophytic fungus *Eupenicillium parvum* isolated from *Azadirachta indica* produced Azadirachtin that is a natural insecticides having antifeedant and insect growth-regulating properties (Kusari et al., 2012b). Further, endophytic *Phialocephala fortinii* from rhizomes of the plant *Podophyllum peltatum* was used to extract lignan podophyllotoxin that is clinically valuable precursor for anticancer drugs (Eyberger et al., 2006). And also, some of the important precursors such as Camptothecin and 10-hydroxycamptothecin for the anticancer drugs have been identified endophytic *Fusarium solaniform* (Shweta et al., 2010; Kusari et al., 2012b).

The fungal leaf endophyte *Colletotrichum tropicale* induces the expression of many host defense-related genes in *Theobroma cacao* (Mejia et al., 2014; Busby et al. 2016). *Colletotrichum tropicale* influences expression of genes that are related to change host's physiology, metabolism, anatomy, and resistance to pathogens and herbivores (Mejia et al., 2014). Some of these genes are involved in the ethylene signaling and defense response pathways, signaling proteins such as receptor kinases, peroxidases and components of the jasmonic acid defense pathway, pathogenesis related proteins (eg: PR4 protein) and synthesis, modification, and degradation of cell wall (eg: Proline rich proteins) (Mejia et al., 2014).

Some endophytes counteract the pathogens by utilizing a combination of mechanisms. For example, endophyte action against *Puccinia triticina* is thought to increase concentrations of phenolic metabolites against pathogen or express other defense-related compounds and express some of direct chemical and physical antagonism or reduce carbon availability to the pathogen (Dingle and Mcgee 2003). And also dark septate endophytic *Harpophora oryzae* inhabit rice roots utilizes different strategies to control rice blast disease caused by biotrophic *Magnaporthe oryzae*. *Harpophora oryzae* stimulates accumulation of  $H_2O_2$  inside the plant tissues after the infection.  $H_2O_2$  is a reactive oxygen species that diffuses via biological membranes (some epidermal and outer cortical cells), and leads to the programmed cell death. In addition, *Harpophora oryzae* blocks invasion of *Magnaporthe oryzae* by restricting its access to water and nutrients (Su et al., 2013). It is suggested that antioxidant enzymes act against reactive oxygen species for maintaining the optimum condition of plant cells (Su et al., 2013). Induced activity of ROS-detoxifying enzymes would trigger a systemic defensive response in rice against *Magnaporthe oryzae* (Su et al., 2013).

Fungal endophytic activities are altered by abiotic factors including temperature, pH, soil moisture, atmospheric conditions and air humidity (Busby et al., 2016). *Trichoderma* species parasitize sclerotia of *Sclerotinia sclerotiorum* and suppress spore germination and mycelial growth (Busby et al., 2016; Jones et al., 2016). Reduction of soil moisture enhances biocontrol activity of *Trichoderma* species and also reduced sclerotial viability (Busby et al., 2016; Jones et al., 2016). Biological control depends on handling and storage practices including temperature and controlled atmosphere (Usall et al., 2000). *Candida sake* (CPA-1) controls *Penicillium expansum* that is responsible for decay of apples (Usall et al., 2000). Usall et al. (2000) experimentally modified the atmosphere (3%  $O_2$ -3%  $CO_2$  atmosphere) and reduced decay of fruits by *Penicillium expansum* without any negative effects to the biocontrol agent *Candida sake*.

In natural ecosystems, endophytes exhibit different interactions with a plant pathogen within a plant pathosystem (Kurose et al., 2012; Busby et al., 2016). Kurose et al. (2012) isolated endophytes strains including, *Alternaria* W2374i, *Colletotrichum* TH-SZ2d, *Pestalotiopsis* TH-SZ1c, *Phoma* HND-Bc and *Phomopsis* HS-SZ1j strains from healthy leaves of *Fallopia japonica* (Japanese knotweed) on Kyushu Island in Japan, in December 2005. *Fallopia japonica* is a problematic invasive weed. *Puccinia polygoni-amphibii* var. *tovariae* (rust fungus) and *Mycosphaerella polygoni-cuspidati* (leaf-spot fungus) are two fungal pathogens that damage *Fallopia japonica* in its natural environment. Kurose et al. (2012) studied, interactions between fungal endophytes and the rust pathogen; *Puccinia polygoni-amphibii* var. *tovariae* were studied. Green-house experiments confirmed three types of interactions between endophytes and the rust pathogen on rust disease development ('suppressive fungi', 'promoting fungi', and 'non-effective or neutral fungi'). *Alternaria* W2374i and *Phoma* HND-Bc suppressed fungi that antagonize the rust pathogen. *Phomopsis* HS-SZ1j was a promoting fungus that significantly increased disease development. However, *Colletotrichum* THS22d, and *Pestalotiopsis* TH-SZ1c were non-effective or neutral fungi that did not cause any significant effects for

disease development. Kurose *et al.* (2012) suggested that further studies of interactions between endophytes and the rust pathogen will be able to develop biocontrol strategies of *Fallopia japonica*.

The black cottonwood (*Populus trichocarpa*) inhabit endophytes also exhibit variety of interactions with its pathogens (Busby *et al.*, 2015, 2016). The leaf endophytes comprising species of *Chaetomium*, *Cladosporium*, *Penicillium*, *Stachybotrys*, *Trichoderma*, *Truncatella* and *Ulocladium* can antagonize while the leaf endophyte *Epicoccum nigrum* can facilitate *Melampsora* rust disease severity caused by *Melampsora* species in a same plant pathosystem (Busby *et al.*, 2015, 2016). However root endophytic *Morchella* species can reduce *Melampsora* rust disease severity (Baynes *et al.*, 2012; Busby *et al.*, 2016). These incidents give insight that wild plant inhabitant endophytes are capable of modifying plant disease severity, important part of forest ecosystems and in future these endophytes will be potential sources of biocontrol agents in agriculture (Busby *et al.*, 2016).

### Current status of endophytes in biocontrol

The use of antagonistic endophytes as biocontrol agents is considered as an attractive option for management of some plant diseases. The first record of control of pathogens using fungal endophytes is by *Epichloë typhina* from Timothy-grass (*Phleum pratense*). This fungal endophyte was able to reduce susceptibility to disease by the fungus *Cladosporium phlei* as compared with non-endophytic plants (O'Hanlon *et al.*, 2012). The endophyte *Epichloë festucae* also reduces dollar spot disease caused by *Sclerotinia homoeocarpa*, when compared to plants without endophytes (O'Hanlon *et al.*, 2012).

It has been revealed that the rain forest tree, *Theobroma cacao* (cocoa), contains diverse fungal endophytic taxa that reduces foliar damage of black pod rot caused by *Phytophthora* species (O'Hanlon *et al.*, 2012; Clay *et al.*, 2016; Larran *et al.*, 2016). *Trichoderma* species have successfully been proven as antagonistic against diseases of *Theobroma cacao* (cacao) and *Fusarium* wilt of lentil (Larran *et al.*, 2016). Endophytic *Chaetomium* spp. (from healthy wheat leaves) reduce the number of pustules caused by *Puccinia recondita*. This reduction in the number of pustules resulted by use of some endophyte culture washings (Cell-free washings from endophyte culture plates were prepared by passing the solutions of spores and hyphal fragments through a sterile 0.2 mm filter) through activating plant defense mechanisms or due to direct inhibitory substances (Dingle and Mcgee 2003; O'Hanlon *et al.*, 2012; Larran *et al.*, 2016). Similarly, endophytic *Chaetomium globosum* and its culture washings suppressed the development of the tan spot pathogen, *Pyrenophora tritici-repentis* in wheat leaf segments (O'Hanlon *et al.*, 2012). An experiment confirmed accumulation of proteins in the presence of endophytes in leaves and no effect on pathogen growth *in vitro* due to culture washings. Therefore, it was suggested that induction of plant resistance is the mechanism responsible for suppressing pathogens (O'Hanlon *et al.*, 2012).

In a study performed on wheat, Larran *et al.* (2016) demonstrated successful in *in vitro* and greenhouse experiments, that endophytes from healthy leaves and stems of wheat cultivars

controlled *Drechslera tritici-repentis*, the causal agent of tan spot of wheat. The results suggested that *Trichoderma hamatum*, *Chaetomium globosum* and *Fusarium graminearum* significantly reduced tan spot disease severity as compared to the control. Larran *et al.* (2016) concluded, that *Bacillus* species and *Trichoderma hamatum* are good candidates as biocontrol agents against *Drechslera tritici-repentis*.

Another study was performed by Zheng *et al.* (2016) on the herbaceous medicinal and food plant, *Panax notoginseng* that is extensively used as therapeutic agents in China and has many pharmacological effects. The root-rot disease complex is a most destructive disease caused by *Alternaria panax*, *A. tenuissima*, *Cylindrocarpon destructans*, *C. didymum*, *Fusarium flocciferum*, *F. oxysporum*, *F. solani*, *Phoma herbarum*, *Phytophthora cactorum* and *Rhizoctonia solani*, resulting in reduced yield and low content of active ingredients. Forty-one endophytic morphotypes were isolated from healthy roots, stems, leaves, and seeds from a plantation during this study. In addition, phytopathogens were isolated from rotten root samples (*Alternaria panax*, *Fusarium oxysporum*, *F. solani*, *Phoma herbarum*, and *Mycocentrospora* sp.). *In vitro* antagonistic activity of endophytes was assayed against host phytopathogens and *Cladosporium oxysporum*, *Cladosporium* sp., *Pestalotiopsis cocculi*, *Trichoderma koningiopsis*, and *Penicillium chrysogenum* strains were able to antagonize all pathogens tested. Furthermore, *Cladosporium oxysporum*, *Pestalotiopsis cocculi*, *Colletotrichum gloeosporioides*, *Penicillium crustosum*, and *Trichoderma koningiopsis* strains showed relatively strong antagonistic activity against the pathogens *Fusarium oxysporum*, *F. solani* and *Phoma herbarum*. The study highlighted that endophytes that exhibit a broad spectrum or strong antagonistic activities might be potential biocontrol agents against root-rot disease.

*Sarocladium strictum* is a cosmopolitan fungal endophyte that can provide benefits to host plants in different ways (Clay *et al.*, 2016). This endophytic taxon reduces hyphal growth and sporulation of *Helminthosporium solani* *in vitro* which causes silver scurf disease in potatoes (Clay *et al.*, 2016). Moreover, *Sarocladium strictum* also suppresses the nematode, *Meloidogyne incognita* that causes root knot disease in tomato (Clay *et al.*, 2016). *Sarocladium strictum* has also been reported to produce secondary metabolites that stimulate host defenses (Clay *et al.*, 2016). Fungal endophytes inhabiting natural forest trees also act against pathogens (Schlegel *et al.*, 2016). Endophytes inoculated on *Theobroma cacao* seedlings showed a significant decrease of both leaf necrosis and mortality by a pathogenic *Phytophthora* species, as compared to non-inoculated controls (Schlegel *et al.*, 2016; Larran *et al.*, 2016). These studies indicated that most of the endophytes are good candidates for biocontrol of phytopathogens. However, a successful research pipeline for screening endophytes through *in vitro* or *in vivo* tests to determine their antagonistic activities and commercialization of these potential endophytes as commercial products for farmers to improve agricultural crop performance, needs to be developed.

### Screening potential microbial biological control agents

Different strategies are employed to identify potential microbial biological control agents (mostly fungi and bacteria) through *in vitro* or *in vivo* tests (Lecomte *et al.*, 2016). For

example, the dual culture assay which is done *in vitro* to test antagonistic activity of endophytes against pathogens (Fig. 1).

Experimental approaches are mainly performed in *in vivo* glasshouse assays. These tests evaluate biocontrol efficiency from the incidence and/or severity of the disease according to a defined disease index in the greenhouse (Busby et al., 2016; Lecomte et al., 2016). *In vivo* screening also includes physiological status of the plant through measurements of the water status (e.g. transpiration, stomatal conductance), changes in antioxidant activity (e.g. enzymatic activity levels), or the production of plant defense molecules (e.g. phytoalexins), plant growth parameters such as plant height, the dry or fresh weight of certain plant parts, or the flowering date (Lecomte et al., 2016). Stadler and von Tiedemann (2014) showed biocontrol activity of *Microsphaeropsis ochracea* against the soilborne pathogen *Verticillium longisporum* *in vitro* and *in vivo* laboratory conditions, but they were unable to confirm any activity under field trials. Therefore, it is recommended to evaluate biocontrol activity initially *in vitro* and *in vivo* laboratory conditions, followed by field trials to check the adaptability of biocontrol agents in the biotic and abiotic environment (Pandey et al., 1993; Busby et al., 2016).

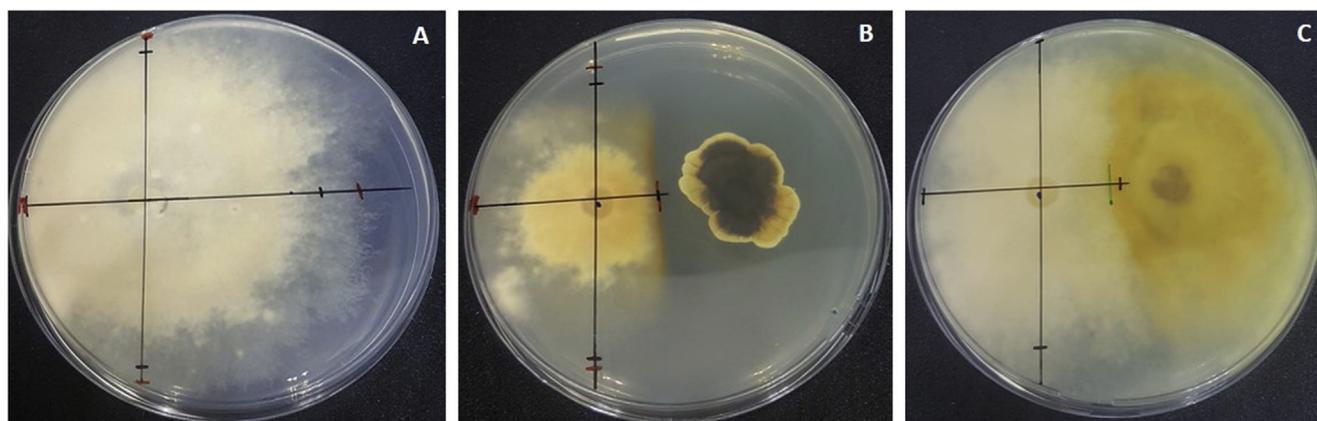
Different endophytic isolates exhibit different effects on different pathogens (Busby et al., 2016). Pandey et al. (1993) reported the antagonistic ability of 15 leaf endophyte isolates against *Pestalotia psidii* and *Colletotrichum gloeosporioides* in *Psidium guajava*. Fourteen isolates were antagonistic against *Colletotrichum gloeosporioides*, but only nine were antagonistic against *Pestalotia psidii* (Pandey et al., 1993). Perello et al. (2002) demonstrated different antagonistic activities of nine leaf endophyte species against four different necrotrophic wheat pathogens, including *Alternaria triticumaculans*, *Bipolaris sorokiniana*, *Drechslera tritici-repentis* and *Zymoseptoria tritici*. They found nine endophytes were antagonistic against *Zymoseptoria tritici* and *Drechslera tritici-repentis*, and eight were antagonistic against *Bipolaris sorokiniana*, and four were antagonistic against *Alternaria triticumaculans*. However, *Glomus* root endophytes (arbuscular mycorrhizal fungi) showed similar antagonistic effects against two pathogens (*Embellisia chlamydospora* and *Fusarium oxysporum*) of *Vulpia ciliata* (Newsham

et al., 1995). Busby et al. (2016) presented possible explanations for different antagonistic activity of endophytes. Biological differences of pathogens alter endophytic antagonistic activities. Biotrophs utilize living plant cells, whereas necrotrophs kill and feed on dead cells (Redman et al., 1999; Busby et al., 2016). Endophytes antagonize biotrophs, such as rusts and powdery mildews, via antibiosis and/or mycoparasitism and necrotrophs via competition for nutrients in the apoplast (Busby et al., 2016).

Endophytic antagonistic ability is also altered by different host plant species (Martin et al., 2015). Martin et al. (2015) stated that endophytic disease modification activity will change with different genotypes of Dutch elm. This is because of some endophyte species prefer to colonize particular plant genotypes and interact with pathogens more efficiently (Arnold and Lutzoni 2007; Martin et al., 2015).

### Commercialization of biocontrol agents

Some government organizations (agencies) are responsible to confirm biosafety of biocontrol agents and biopesticides (Tranier et al., 2014). The Environmental Protection Agency (EPA) is a U.S. government agency that assesses the risks of products for both human health and the environment (Junaid et al., 2013; Tranier et al., 2014). The first taxon, *Trichoderma harzianum* (strain ATCC 20476) was registered in the EPA for the control of plant diseases (Junaid et al., 2013). The Pest Management Regulatory Agency (ARLA) in Canada was established to promote, verify and ensure the efficiency of the product over several seasons and over all particular geographical areas of Canada (Tranier et al., 2014). Commercialization of bio-control products should undergo multi-step processes, including isolation of micro-organisms from the natural ecosystem, evaluation of bio-agent both *in vitro* and under glass house conditions, testing of the best isolate under field conditions, mass production, formulation, delivery, compatibility, registration and release (Junaid et al., 2013). Success of biocontrol, depends on the target pathogen, the crop and the field (Junaid et al., 2013). Endophytic fungi from various host plants have been shown to be effective



**Fig. 1** – Dual culture of an endophytic and a pathogenic fungus after 21 days. A) Single culture of pathogenic fungus. B) Endophytic fungus that show inhibition against pathogenic fungus. C) Endophytic fungus that did not show inhibition against pathogenic fungus.

**Table 2 – Different commercialized biocontrol agents.**

Biocontrol agent	Product	Target disease/organism	Crop	Manufacturer/Country	Reference
<i>Ampelomyces quisqualis</i> isolate M-10	AQ10	Powdery mildew	Fruits, ornamentals, vegetables	Ecogen, USA	<a href="#">Junaid et al. (2013)</a>
<i>Ampelomyces quisqualis</i>	AQ10	Powdery mildew	Wheat, barley, grapevines, apple, vegetables and ornamentals	Ecogen, Inc, USA	<a href="#">Kiss (2003)</a>
<i>Aspergillus flavus</i> AF36	Alfa guard	<i>Aspergillus flavus</i>	Cotton	Circleone globa, USA	<a href="#">Junaid et al. (2013)</a>
<i>Aspergillus flavus</i> NRRL 21,882	Afla-guard	<i>Aspergillus flavus</i>	Peanut, Corn	Circleone globa, USA	<a href="#">Fravel 2005, Sexton et al. (2016)</a>
<i>Candida oleophila</i> I-182	Aspire	<i>Botrytis</i> spp., <i>Penicillium</i> spp.	Citrus, pome fruit	Ecogen, Inc. cabot blvd. west, Langhorne PR China	<a href="#">Gardener and Fravel (2002)</a>
<i>Colletotrichum gloeosporioides</i> f. sp. <i>cuscutae</i>	Luboa 2	<i>Cuscuta chinensis</i> , <i>Cuscuta australis</i>	Soybeans	PR China	<a href="#">Butt et al. (2001)</a>
<i>Colletotrichum gloeosporioides</i> f. sp. <i>malvae</i>	Biomal	<i>Malva pusilla</i>	Wheat and lentils	Canada	<a href="#">Butt et al (2001)</a>
<i>Colletotrichum gloeosporioides</i> f. sp. <i>aeschynomene</i>	Collego	<i>Aeschynomene virginica</i>	Rice	Encore technologies, USA	<a href="#">Butt et al. (2001)</a>
<i>Coniothyrium minitans</i> CON/M/91-08	Contans WG; Intercept	<i>Sclerotinia sclerotiorum</i> and <i>Sclerotinia minor</i>	Agricultural soil	Prophyta biologischer pflanzenschutz GmbH, Germany	<a href="#">Fravel (2005)</a>
<i>Gliocladium catenulatum</i> strain JI446	Prima stop soil guard	Soil borne pathogens	Vegetables, herbs, spices	Kemira agro oy, Finland	<a href="#">Fravel 2005, Junaid et al. 2013</a>
<i>Gliocladium virens</i> GL-21	Soilgard	<i>Rhizoctonia solani</i> and <i>Pythium</i> spp.	Ornamentals, vegetables, cotton	Thermo trilogy corporation, USA	<a href="#">Gardener and Fravel 2002, Fravel 2005, Junaid et al. 2013</a>
<i>Myrothecium verrucaria</i>	DiTera	Plant parasitic nematodes	All food, fiber, and ornamental crops, grape, ornamentals, turf	Abbott laboratories, USA	<a href="#">Gardener and Fravel 2002, Fravel (2005)</a>
<i>Pseudozyma flocculosa</i>	Sporodex	Powdery mildew	Wheat, barley, grapevines, apple and vegetables	Ecogen, Inc, USA	<a href="#">Kiss (2003)</a>
<i>Trichoderma harzianum</i> ATCC 20,476	Binab T	Tree wound pathogens (fungi)	Wounds in ornamental, shade, and forest trees	Bio-Innovation, Sweden	<a href="#">Butt et al. 2001, Fravel (2005)</a>
<i>Trichoderma harzianum</i> complex: T-22 ( <i>T. afroharzianum</i> )	Root shield, plant shield	<i>Pythium</i> spp., <i>Rhizoctonia solani</i> , <i>Fusarium</i> spp	Ornamentals, cabbage, tomato, cucumber	Bio works, USA	<a href="#">Gardener and Fravel 2002, Junaid et al. 2013, Chaverri et al. (2015)</a>
<i>Trichoderma harzianum</i> T-39	Trichodex	<i>Botrytis cinerea</i> soil-borne	Most of the food crops	Bio works, USA	<a href="#">Fravel 2005, Junaid et al. 2013</a>
<i>Trichoderma afroharzianum</i>	Canna	fungal plant pathogens	Most of the food crops	makhteshim agan of North America	<a href="#">Degenkolb (2015)</a>
CBS 134709 (IBT 41409, G.J.S. 08-137)	Trichosan	( <i>Botrytis</i> spp.,	Ornamentals, food crops	USA Canna International	<a href="#">Degenkolb (2015)</a>
<i>Trichoderma simmonsii</i>	Promot WP	<i>Fusarium</i> sp., <i>Phytophthora infestans</i> )	Most of the food crops	BV, NL-Breda	<a href="#">Degenkolb (2015)</a>
CBS 134706 (IBT 41406, G.J.S. 08-134)		soil-borne fungal plant pathogens ( <i>Botrytis</i> spp., <i>Fusarium</i> sp.)		Vitalin Pflanzengesundheit GmbH, D-Ober-Ramstadt	
<i>T. guizhouense</i> CBS 134707 (IBT 41407, G.J.S. 08-135)		soil-borne fungal plant pathogens ( <i>Botrytis</i> spp., <i>Fusarium</i> sp.)		JH Biotech Inc., Ventura, CA, USA	

biocontrol agents that reduce disease severity of plant diseases. *Alternaria* spp., *Cladosporium* spp. and *Leptosphaeria* spp., isolated from wheat (Huang et al., 2016), *Chaetomium globosum*, *Fusarium* spp., *Penicillium* spp., *Trichoderma hamatum* and *T. hamatum*, isolated from *Theobroma cacao* (Larran et al., 2016), and *Alternaria alternata* isolated from grapevine leaves (Zhang et al., 2017) are good examples. Nowadays, tremendous efforts are being made to commercialize these biocontrol agents and some of those are given in the Table 2.

### Challenges and solutions

Endophytes are appropriate substitutes for chemicals to improve agricultural crop performance especially as biocontrol agents. One of the most vital issues of a biocontrol agent is the success or failure of commercial product (Owen et al., 2015; Murphy et al. 2018; Vurukonda et al. 2018). A commercial product should be comply with farmer's requirements such as repeated positive results, reasonable price, easy handling and prolong product shelf-life (Murphy et al. 2018; Vurukonda et al. 2018). However, a biocontrol product with microbial inoculants and/or microbial secondary metabolites have specific problems of loss of viability and reduced effectiveness of the product against pathogen or pest during storage (Vurukonda et al. 2018). A lack of understanding of usage of biocontrol methods, may end up with reduced confidence for usage and demand in the product. Therefore, a full understanding of the practical use of endophytes as biocontrol agents in agriculture is essential. Nevertheless, there is an urgent need to improve communication between the public and researchers for efficient usage of biocontrol methods. It is therefore, crucial to increase the awareness of farmers, regarding effective usage of products for a particular pathogen (Kemen et al. 2015; Van der Heijden and Hartmann 2016; Murphy et al. 2018; Vurukonda et al. 2018).

There has been a great deal of research to investigate endophytes as potential biocontrol agents. The majority of these studies, have however, been restricted to controlled environment and applicability has tested in field trials. Murphy et al. (2018) have provided a detailed layout of protocols that would be beneficial for understanding how to overcome future challenges and commercialize potential biocontrol agents. They have studied endophytes from wild Barley from diverse environments and established correlations between endophyte recovery from different varieties and different environments. This indicates that there is a need for continuous *in vitro* and *in vivo* experiments to derive efficient products that have a broad range of applications with varied crops. On the other hand, some potential biocontrol agents might be compatible with particular plants under a limited range of environmental conditions. The efficacy of biocontrol is altered by variations in agro-environmental conditions, such as soil organic matter, pH, nutrient levels, and moisture level (Murphy et al. 2018; Vurukonda et al. 2018). Comprehensive data analysis of field trials will lead to reliable biocontrol agents that are compatible with the crop and environment, as most of endophytes are unique to their host and the environment. Therefore, a the best approach is to identify the field problems and try to provide a solution to that particular problem with continuous *in vivo* and *in vitro* experiments for endophyte antagonistic

activity against pathogen and plant disease development. These products are supposed to have a higher demand in the market (Vurukonda et al. 2018).

## 2. Conclusion and future directions

Endophytes are an attractive alternative to chemical pesticides as they may provide alternatives for plant disease management that contribute to sustainable agriculture. It is interesting to note that endophytes such as *Trichoderma* species are already widely used as biocontrol agents against plant diseases. While ongoing attempts to screen putative endophytes, through *in vitro* experiments under standardized conditions are routinely carried out, field experiments under different environment conditions are needed to develop successful commercial biocontrol agents. Field experiments should investigate physiological and ecological aspects of biocontrol agents with host plant or crop plants and also environmental effects in the field. Further research is essential for commercialization of biocontrol agents, as these will minimize economic and environmental costs significantly. This could be achieved through novel approaches using molecular technologies (e.g., metagenomics), ecological dynamics and statistical advances. Future work could be integrated with screening, testing antagonistic ability in green house and field trials, coupled with biomass production, following commercialization of biocontrol agents that are ultimately essential to ensure global agricultural security.

### Conflict of interest

None.

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