Reviews, Critiques and New Ideas

Mycofungicides and fungal biofertilizers

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Mycofungicides and fungal biofertilizers have been promoted for agricultural use because of their ability to control plant diseases and their ability to increase crop production in an environmentally friendly manner. In recent years several mycofungicides have been patented and registered for plant disease control, while fungal biofertilizers have also been registered for application in crop production. Several effective mycofungicides and fungal biofertilizers have been formulated for commercial production. Formulation of mycofungicides includes wettable powders and granules; these being applied to seeds, seedlings and mature plants. Examples are Ketomium®, formulated from *Chaetomium globosum* and *Ch. cupreum*, Promote® formulated from *Trichoderma harzianum* and *T. viride*, SoilGard® formulated from *Gliocladium virens*, and Trichodex® from *T. harzianum*. Fungal biofertilizers include plant growth stimulating fungi e.g. *Trichoderma*, mycorrhizal fungi (ectomycorrhiza e.g. *Pisolithus tinctonus* and arbuscular mycorrhizae e.g. *Glomus intraradices* which form mutualistic associations with plants), enzymatic producing fungi for compost production and P-solubilizing fungi and K-solubilizing fungi. Fungal biofertilizers play an important role in promoting plant growth, health, productivity and improving soil fertility.

Key words: mycofungicide, fungal biofertilizer, biological control agent

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Introduction

Fungi are ubiquitous; some having beneficial effects on plants, while others may be detrimental (Anderson and Cairney, 2004; Ipsilantis and Sylvia, 2007). A decrease in crop yield as a result of a plant disease caused by a pathogen is a negative effect. Some fungi are the main pathogens responsible for plant disease and they may cause high yield losses (Park *et al*., 2005; Pereira *et al*., 2007; Shenoy *et al*., 2007; Soares and Barreto, 2008; Than *et al*., 2008a,b). There are many ways to reduce yield losses caused by fungal disease, with the application of chemical fungicides presently being the most common method (Rosslenbroich and Stuebler, 2000; Than *et al*., 2008a). Chemical fungicides however, have a negative effect on human health and on the environment (Voorrips *et al*., 2004; Soytong *et al*., 2005; Gavrilescua and Chisti, 2005; Calhelha *et al*., 2006; Haggag and Mohamed, 2007). The application of chemical fungicides over a long period may result in plant pathogenic fungi developing resistance (Benítez *et al*., 2004, Agrios, 2005; Kim and Hwang, 2007). When this happens the chemical fungicides become ineffective and other fungicides must be used for effective disease control. The use of microorganisms as biological control agents to control plant disease is a potentially powerful alternative method (Kulkarni *et al*., 2007). Because of their rich diversity, complexity of interactions and numerous metabolic pathways,

microbes are an amazing resource for biological activity (Emmert and Handelsman, 1999; Alabouvette *et al*., 2006; Tejesvi *et al*., 2007; Mitchell *et al*., 2008; Raghukumar, 2008). Over the past 30 years, microorganisms have been described, characterized, and tested for their use as biocontrol agents against diseases caused by soil borne plant pathogens. Biocontrol agents and especially antagonistic fungi have been used to control plant diseases with 90% of applications being formulated using different strains of *Trichoderma* e.g. *T. harzianum, T. virens, T. viride* (Benítez *et al*., 2004). Many species of *Chaetomium* e.g. *Chaetomium globosum, C. cochlioides, C. cupreum* can also be antagonistic against various soil microorganisms (Soytong *et al*., 2001; Kanokmedhakul *et al*., 2002, 2006). A wide range of biological control agents have been developed as commercial mycofungicide products (Benítez *et al*., 2004; Kim and Hwang, 2004; Fravel, 2005). The initial stage of mycofungicide development is the collection of fungal isolates and screening for the effective strains against target plant pathogens, both in the laboratory, greenhouse, and in the field (Khetan, 2001). One of the most important considerations in mass production is compatibility of the product with both formulation and application techniques (Jenkins *et al*., 1998; Khetan, 2001). The USA and France are the main biofungicide users, although other countries are promoting biological control of agents because of bans on synthetic chemical pesticide residues on the agricultural products (Ricard and Ricard, 1997; Ecobichon, 2001; Spadaro and Gullino, 2005; Wesseling *et al*., 2005).

An alternative way to increase the crop yield besides using chemical fertilizers is biofertilizers. Biofertilizers promote increased absorption of nutrients in plants (Vessey, 2003; Hart and Trevors, 2005; Chen, 2006). Biofertilizers include materials derived from living organisms and microbial sources (Rola, 2000; Chen, 2006). Biofertilizers have various benefits, such as increased access to nutrients, providing growth-promoting factors for plants, and composting and effective recycling of solid wastes (Gaur and Adholeya, 2004; Das *et al*., 2007). Biofertilizers, commonly known as microbial inoculants are produced from cultures of certain soil organisms that can improve soil fertility and crop productivity such as mycorrhizae (Malik *et al*., 2005; Marin, 2006). Mycorrhizae are fungi which form mutualistic relationships with roots of 90% of plants (Gaur and Adholeya, 2004; Das *et al*., 2007; Rinaldi *et al*., 2008). Mycorrhizae promote absorption of nutrients and water, control plant diseases, and improve soil structure **(**Rola, 2000; Zhao *et al*., 2003; Chandanie *et al*., 2006; Rinaldi *et al*., 2008). Plants colonized by mycorrhizae grow better than those without them (Yeasmin *et al*., 2007, Singh *et al*., 2008) and are beneficial in natural and agricultural systems (Adholeya *et al*., 2005; Marin, 2006).

In this review, we focus on the advantages of using mycofungicides for plant disease control and fungal biofertilizers to increase crop production.

Mycofungicides

Microbial antagonists can suppress plant diseases and organisms that suppress pathogens can be referred to as biological control agents (BCA) (Alabouvette *et al*., 2006; Pal and Gardener, 2006). Various fungal species can be used as biological control agents and may provide effective activity against various pathogenic microorganisms. Examples are *Trichoderma harzianum* - a species with biocontrol potential against *Botrytis cineria, Fusarium, Pythium* and *Rhizoctonia* (Khetan, 2001); *Ampelomyces quisqualis,* - a hyperparasite of powdery mildew (Liang *et al*., 2007; Viterbo *et al*., 2007); *Chaetomium globosum* and *C. cupreum,* - having biocontrol activity against root rot disease caused by *Fusarium, Phytophthora* and *Pythium* (Soytong *et al*., 2001); *Gliocladium virens* - effective biocontrol of soil born pathogens (Viterbo *et al*., 2007); *Coniothyrium minitans* - a mycoparasite of *Sclerotinia* (Whipps *et al*., 2008); and *Fusarium oxysporum* (nonpathogenic species) - having biocontrol potential against *Fusarium oxysporum* (Fravel, 2003).

An effective biological control agent should be genetically stable, effective at low concentrations, easy to mass produce in culture on inexpensive media, and be effective against a wide range of pathogens (Wraight *et al*., 2001; Irtwange, 2006). The fungal biological control agent should also occur in an easily distributed

form, be non-toxic to humans, have resistance to pesticides, be compatible with other treatments, and be non-pathogenic against the host plant (Fravel, 2005; Irtwange, 2006). Several fungal taxa have been reported to be antagonist against plant pathogens and have been successfully formulated as mycofungicides or biological control products e.g. *Ampelomyces quisqualis*, *Aspergillus niger*, *Candida oleophila*, *Chaetomium cupreum*, *Ch. globosum*, *Coniohyrium minitans*, *Cryptococcus albidus*, *Gliocladium virens*, *G. catenulatum*, *Fusarium oxysporum*, *Phlebiosis gigantean*, *Pythium oligandrum*, *Rhodotorula glutinis*, *Trichoderma harzianum*, *T. polysporum*, *T. viride*, (Boyetchko *et al*., 1999; Butt *et al*., 1999; But, 2000; Hofstein and Chapple, 1999; Khetan, 2001; Soytong *et al*., 2001; Ghisalberti, 2002; Fravel, 2005; Ezziyyani *et al*., 2007) as seen in Table 1.

In this review, we highlight some of the important biological control agents used as mycofungicides.

Ampelomyces

Ampelomyces quisqualis is the mycoparasitic anamorphic ascomycete that reduces the growth and kills powdery mildews. It can affect the pathogen through anti-biosis and parasitism (Kiss, 2003; Viterbo *et al*., 2007). The fungus *A. quisqualis* was the first organism reported to be a hyperparasite of powdery mildew and it can be easily found associated with powdery mildew colonies (Paulitz and Belanger, 2001). Hyphae of *Ampelomyces* penetrate the hyphae of powdery mildews and grow internally then kill all the parasitized cells (Kiss, 2003). *Ampelomyces quisqualis* isolate M-10 has been formulated as AQ10 Biofungicide, developed by Ecogen, Inc, USA. This mycofungicide contains conidia of *A. quisqualis* and formulated as water-dispersible granules for the control of powdery mildew of carrot, cucumber and mango (Khetan, 2001; Paulitz and Belanger, 2001; Shishkoff and McGrath, 2002; Kiss, 2003; Viterbo *et al*., 2007).

Chaetomium

Chaetomium species are normally found in soil and organic compost (Soytong *et al*., 2001). The genus *Chaetomium* was first established in 1817 by Gustav Kunze (Soytong and Quimio, 1989a). The application of *Chaetomim* as a biological control agent to control plant pathogens first commenced in about 1954 when Martin Tviet and M.B. Moor found *Ch. globosum* and *Ch. cochliodes* occurring on oat seeds and that these taxa provided some control of *Helminthosporium victoriae* (Tviet and Moor, 1954). *Chaetomium* species have been reported to be potential antagonists of various plant pathogens, especially soil-borne and seedborne pathogens (Soytong and Quimio, 1989b; Dhingra *et al*., 2003; Aggarwal *et al*., 2004; Park *et al*., 2005). Many species of *Chaetomium* with potential to be biological control agents suppress the growth of bacteria and fungi through competition (for substrate and nutrients), mycoparasitism, anti-biosis, or various combinations of these (Marwah *et al*., 2007; Zhang and Yang, 2007). *Chaetomium globosum* and *Ch. cupreum* in particular have been extensively studied and successfully used to control root rot disease of citrus, black pepper, strawberry and have been shown to reduce damping off disease of sugar beet (Soytong *et al*., 2001; Tomilova and Shternshis, 2006). These taxa have been formulated in the form of powder and pellets as Ketomium®, a broad spectrum mycofungicide. Ketomium® has been also registered as a biological biofertilizer for degrading organic matter and for inducing plant immunity and stimulating plant growth (Soytong *et al*., 2001). The mycofungicide Ketomium® which comprises a *Chaetomium* spore suspension has been evaluated for its effect on Siberian isolates of the phytopathogenic fungi *Botrytis cinerea*, *Didymella applanata*, *Fusarium oxysporum* and *Rhizoctonia solani*. It was found that Ketomium-mycofungicide was most efficient in suppressing raspberry spur blight caused by *Didymella applanata* and could also reduce potato disease caused by *R. solani*, increasing potato yield (Shternshis *et al*., 2005). After 2-years in storage, this mycofungicide was still capable of inhibiting the growth of phytopathogens but at higher doses (Tomilova and Shternshis, 2006). Other species of *Chaetomium* which can act as biological control agents include *Ch. globosum* isolate CgA-1 which can reduce soybean stem canker disease caused by *Diaporthe phaseolorum* f. sp.

meridionalis (Dhingra *et al*., 2003) and *Ch. cochliodes* CTh05 and VTh01 which has activity against *Fusarium oxysporum* f. sp. *lycopersici* causing tomato wilt, while isolate CTh05 showed activity against *Phytophthora parasitica* causing citrus root rot (Phonkerd *et al*., 2008). *Chaetomium* species are reported as a broad spectrum mycofungicide that is not only used for protection but also for curative effect as well (Soytong, 2001). Moreover, a new strain of *Ch. cupreum* RY202 has preliminary proved to be antagonistic against *Rigidoporus microporus* which causes white root disease of rubber trees variety RRIM600. This promising strain is being investigated as a potential biological control agent against *R. microporus* (Saithong, pers comm.).

Gliocladium

Gliocladium species are common soil saprobes and several species have been reported to be parasites of many plant pathogens (Viterbo *et al*., 2007), for example, *Gliocladium catenulatum* parasities *Sporidesmium sclerotiorum* and *Fusarium* spp. It destroys the fungal host by direct hyphal contact and forms pseudoappressoria (Punja and Utkhede, 2004; Viterbo *et al*., 2007). *Gliocladium catenulatum* (Strain JI446) has also been used as a wettable powder named Primastop® by Kemira Agro Oy, Finland. This product can be applied to soils, roots, and foliage to reduce the incidence of damping-off disease caused by *Pythium ultimum* and *Rhizoctonia solani* in the greenhouse (Paulitz and Belanger, 2001; Punja and Utkhede, 2004). *Gliocladium virens* has been used as a biological control agent against a wide range of soil borne pathogens such as, *Pythium* and *Rhizoctonia* under greenhouse and field conditions (Hebbar and Lumsden, 1999; Viterbo *et al*., 2007). *Gliocladium virens* isolate GL-21 was formulated as an alginate prill named GlioGard® by W.R. Grace Co. and a granular formulation with the trade name SoilGard® produced by the Thermo Triology Corp., Columbia, MD. SoilGard® was developed for greenhouse application (Paulitz and Belanger, 2001; Punja and Utkhede, 2004). *Gliocladium virens* produces anti-biotic metabolites such as gliotoxin which have anti-bacterial, anti-fungal, anti-viral and anti-tumor activities. Recently, molecular evidence indicates that *G. virens* is more closely related to *Trichoderma* than those *G. virens*. This supports suggestions that this taxon should be referred to as *Trichoderma virens* (Hebber and Lumsden, 1999; Paulitz and Belanger, 2001; Punja and Utkhede, 2004).

Trichoderma

Trichoderma species are common in soil and root ecosystems and are ubiquitous saprobes (Harman *et al*., 2004; Thormann and Rice, 2007; Vinale *et al*., 2008; Kodsueb *et al*., 2008) and they are easily isolated from soil, decaying wood, and other organic material (Howell, 2003; Zeilinger and Omann, 2007). There are several reports on the use of *Trichoderma* species as biological agents against plant pathogens (Harman *et al*., 2004; Zeilinger and Omann, 2007). *Trichoderma* species have been used as biological control agents against a wide range of pathogenic fungi e.g. *Rhizoctonia* spp., *Pythium* spp., *Botrytis cinerea*, and *Fusarium* spp. *Phytophthora palmivora, P. parasitica* and different species can be used, e.g. *T. harzianum, T. viride, T. virens* (Benítez *et al*., 2004; Sunantapongsuk *et al*., 2006; Zeilinger and Omann, 2007). Among them, *Trichoderma harzianum* is reported to be most widely used as an effective biological control agent (El-Katathy *et al*., 2001; Szekeres *et al*., 2004; Abdel-Fattah *et al*., 2007). *Trichoderma harzianum* strain T-22 was produced by protoplast fusion between *T. harzianum* T-95 and T-12 and this strain was formulated as granular named RootSield® and powder named PlantShield® by Biworks, Geneva, NY. *Trichoderma harzianum* T-22 has efficacy against a wide range of plant pathogenic fungi including, *Botrytis cinerea, Fusarium, Pythium, Rhizoctonia* in many crops such as, corn, soybean, potato, tomato, beans, cotton, peanut, and various trees (Khetan, 2001; Paulitz and Belanger, 2001). *Trichoderma harzianum* strain T-39 is marketed as TRICHODEX, 20P by Makhteshim Ltd. for control of pink rot and stem rot of tomato caused by *Phytophthora erythroseptica* (Etebarian *et al*., 2000) and control of blight disease caused by *Botrytis cinerea* (Paulitz and Belanger, 2001).

The biocontrol mechanism in *Trichoderma* is a combination of mechanisms (Howell, 2003; Benítez *et al*., 2004; Zeilinger and

Omann, 2007). The main mechanism is mycoparasitism and anti-biosis (Howell, 2003; Vinale *et al*., 2008). Mycoparasitism relies on the recognition, binding and enzymatic disruption of the host fungus cell wall (Woo and Lorito, 2007). *Trichoderma* species have been very successfully used as mycofungicides because they are fast growing, have high reproductive capacity, inhibit a broad spectrum of fungal diseases, have a diversity of control mechanisms, are excellent competitors in the rhizosphere, have a capacity to modify the rhizosphere, are tolerant or resistance to soil fungicides, have the ability to survive under unfavorable conditions, are efficient in utilizing soil nutrients, have strong aggressiveness against phytopathogenic fungi, and also promote plant growth (Tang *et al*., 2001; Benítez *et al*., 2004; Vinale *et al*., 2006). Their ability to colonize and grow in association with plant roots is known as rhizosphere competence. The taxonomy of *Trichoderma* species is very complex and has been the subject of many recent taxonomic studies (Tang *et al*., 2001; Woo *et al*., 2006; Samuels, 2006). They also have a high level of genetic diversity (Harman *et al*., 2004; Harman, 2006). Thus it is likely that only a few of the species available have been utilized as mycofungicides. However, *Trichoderma* species are the most common fungal biocontrol control agents and are commercially formulated as biofungicides, biofertilizers, and soil amendments (Harman *et al*., 2004, Vinale *et al*., 2006; Harman, 2006).

Other fungi as mycofungicides

Coniothyrium minitans is an anamorphic coelomycete (Gong *et al*., 2007) which has been reported to be a mycoparasite of *Sclerotinia* species such as *Sclerotinia minor*, *S. sclerotiorum*, *S. trifoliorum* and *S. cepivorum* (Yang *et al*., 2007; Viterbo *et al*., 2007; Whipps *et al*., 2008). It has been applied successfully to control disease in many crops including lettuce (Jones *et al*., 2004), oil oilseed rape (Li *et al*., 2006), peanut (Partridge *et al*., 2006) and alfalfa (Li *et al*., 2005). The conidia of *Coniothyrium minitans* has been formulated as Contans® by Prophyta Biologischer Pflanzengchutz GmbH, Germany (Paulitz and Belanger, 2001; Gavrilescu and Chisti, 2005; Yang *et al*., 2007; Whipps *et al*., 2008) and has been registered for disease control in Germany, Switzerland, Norway and USA (Partridge *et al*., 2006;Yang *et al*., 2007). The main biological control mechanism of *C. minitans* is mycoparasitism which uses sclerotia of *S. sclerotiorum* as the source of food for survival (Yang *et al*., 2007; Whipps *et al*., 2008). The products of *C. minitans* can be applied to soil or can be sprayed on foliage (Shi *et al*., 2004; Li *et al*., 2005) and they can survive in soil for several years (Jones and Whipps 2002; Whipps *et al*., 2008). The efficiency of *C. minitans* can be improved by combinations with *Trichoderma* species (Li *et al*., 2005; Whipps *et al*., 2008).

The genus *Fusarium* includes both plant-pathogenic and non-pathogenic races (Larkin and Fravel, 1999). The non-pathogenic species are known to have effective biocontrol activity (Whipps; 2001; Harman *et al*., 2004; Kvas *et al*., 2009). Mechanisms of action include competition and induction of host defenses (Paulitz and Belanger, 2001; Whipps, 2001; Fravel *et al*., 2003; Agrios, 2005). The use of non-pathogenic strains of *Fusarium oxysporum* to control Fusarium wilt has been reported for many crops, but there has been little commercial production, because of lack of understanding of their genetics, biology and ecology (Fravel *et al*., 2003; Kvas *et al*., 2009). Non-pathogenic *F. oxysporum* strain Fo47 was marketed as liquid formulation named as Fusaclean® by Natural Plant Products, Nogueres, France for soil less culture (Khetan, 2001; Paulitz and Belanger, 2001). Similarly the genus *Rhizoctonia* contains both plant-pathogenic and non-pathogenic species and the latter can act as biocontrol agents (Harman *et al*., 2004).

Pythium oligandrum has shown ability to control soil-borne pathogens both in the laboratory and in the field. *Pythium oligandrum* oospores have been applied as seed treatments which reduce damping-off disease caused by *P. ultimum* in sugarbeet (Lewis *et al*., 1989; Khetan, 2001). *Pythium oligandrum* has been formulated as a granular or powder product named as Polygangron® by Vyskumny Ustav of Slovak Republic (Khetan, 2001). This fungus has indirect effects by controlling pathogens in the rhizosphere and/or direct

effects by inducing plant resistance. It also induces plants to respond more rapidly and efficiently to pathogen infections and increase phosphorus uptake (Le Floch *et al*., 2003). *Pythium nunn* is also an antagonistic fungus being a mycoparasite of pathogens such as *Rhizoctonia solani, Phytophthora cinnamomi, P. parasitica, Pythium aphanidermatum, P. ultimum* and *P. vexans*. The hyphae coil around the host, forming appressoria-like structures and then penetrate and parasitize the "host" hyphae (Khetan, 2001; Viterbo *et al*., 2007).

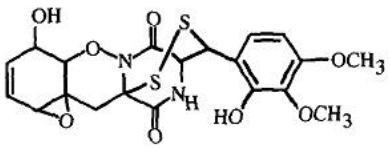
Other fungi that can be used as mycofungicides are *Aspergillus* and *Penicillium* species. *Aspergillus* species are effective against the white-rot basidiomycetes (Bruce and Highley, 1991). The fungal antagonists *Aureobasidium pullulans*, and *Ulocladium atrum* have also been tested for the control of *Botrytis aclada* which causes onion neck rot (Köhl *et al*., 1997). *Clonostachys rosea* is also reported as a biological control agent. The application of *Clonostachys rosea* as a single strain and mixture of strains against *Moniliophthora roreri* in cocoa crops has been tested by using motorized mist blowers and a directional hydraulic spray technique. Both mycofungicides reduced sporulation of the pathogen and the motorized mist blower technique gave better results than those by the directional hydraulic spray technique (Hidalgo *et al*., 2003).

Mechanisms of biological control

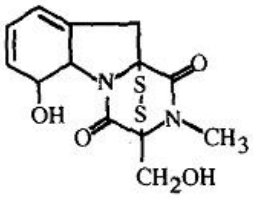
Biological control may result from direct or indirect interactions between the beneficial microorganisms and the pathogen (Alabouvette and Lemanceau, 1999; Benítez *et al*., 2004; Viterbo *et al*., 2007). A direct interaction may involve physical contact and synthesis of hydrolytic enzymes, toxic compounds or anti-biotics as well as competition. An indirect interaction may result from induced resistance in the host plant, the use of organic soil amendments to improve the activity of antagonists against the pathogens (Benítez *et al*., 2004; Pal and Gardener, 2006; Viterbo *et al*., 2007). The mechanisms of biocontrol agents and reaction with the pathogen are many and complex. Mechanisms are influenced by soil type, temperature, pH and moisture of the plant and soil environment and also by the presence of other microorganisms (Howell, 2003). There are four principle mechanisms of biological control anti-biosis, competition, mycoparasitism or lysis and induced resistance (Renwick and Poole, 1989; Chet *et al*., 1990; Fravel *et al*., 2003; Irtwange, 2006; Viterbo *et al*., 2007). These are detailed below.

Antibiosis: Antibiosis is defined as the inhibition or destruction of the microorganism by substances such as specific or nonspecific metabolites or by the production of anti-biotics that inhibit the growth of another micro-organism (Benítez *et al*., 2004; Irtwange, 2006; Viterbo *et al*., 2007; Haggag and Mohamed, 2007). Many biological control agents produce several types of anti-biotics (Lewis *et al*., 1989; Handelsman and Stabb, 1996). Some anti-biotics have been shown to play role in disease suppression (Lewis *et al*., 1989; Handelsman and Stabb, 1996) either impede spore germination (fungistasis), or kill the cells (anti-biosis) (Benítez *et al*., 2004; Haggag and Mohamed, 2007).

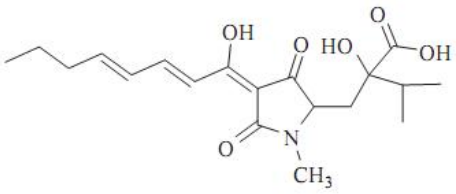
Gliocladium and *Trichoderma* species are well known biological control agents which produce a wide range of anti-biotics and suppress disease by diverse mechanisms (Handelsman and Stabb, 1996; Whipps, 2001; Harman *et al*., 2004). Gliovirin (Fig. 1A) produced by *Gliocladium virens* can kill *Pythium ultimum* by causing coagulation of the protoplasm (Whipps, 2001; Howell, 2003; Viterbo *et al*., 2007). Many anti-biotics are produced by *Trichoderma* species. These include gliotoxin (Fig. 1B), harzianic acid (Fig. 1C), trichoviridin (Fig. 1D), viridin (Fig. 1E), viridiol (Fig. 1F), and alamethicins. These anti-biotics are synergistic when combined with various cell wall degrading enzymes thus producing a strong inhibitory effect on many plant pathogens (Benítez *et al*., 2004; Woo and Lorito, 2007; Vinale *et al*., 2008). Trichotoxin A50 (Fig. 1K) produced by *T. harzianum* PC01 can inhibit the mycelial growth and sporangial production of *Phytophthora palmivora* (Suwan *et al*., 2000). *Chaetomium globosum* produces the anti-biotic chaetoglobusin C (Fig. 1G) which suppresses the growth of plant pathogens such as *Colletotrichum gloeosporioides, C. dematium, Fusarium oxysporum, Phytophthora palmivora, P. parasitica, P. cactorum, Pyricularia oryzae, Rhizoctonia solani* and



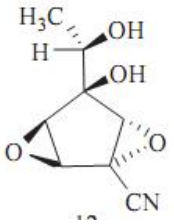
A: gliovirin



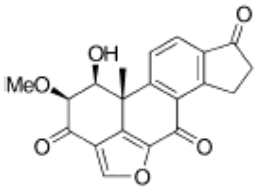
B: gliotoxin



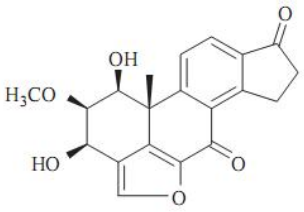
C: harzianic acid



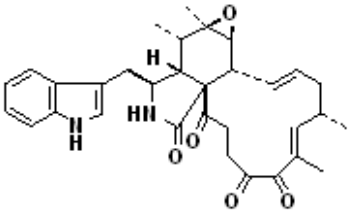
D: trichoviridin



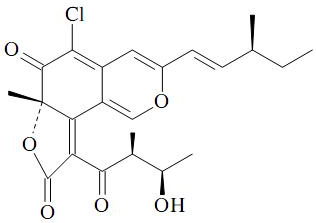
E: viridin



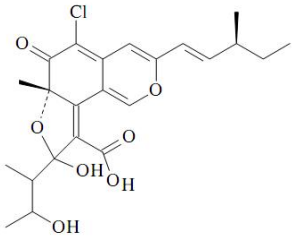
F: viridiol



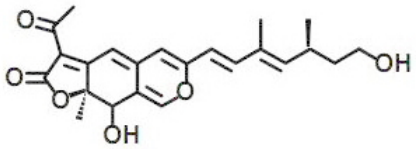
G: chaetoglobusin C



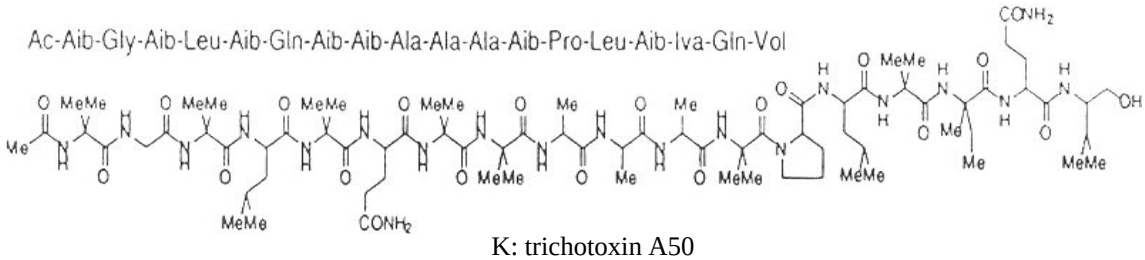
H: chaetoviridins A



I: chaetoviridins B



J: rotiorinol



K: trichotoxin A50

Fig. 1. Structure of some antibiotics produced by fungal biocontrol agents; gliovirin (**A**) produced by *Gliocladium virens*, gliotoxin (**B**), harzianic acid (**C**), trichoviridin (**D**), viridin (**E**) and viridiol (**F**) produced by *Trichoderma* species, chaetoglobusin C (**G**), chaetoviridins A (**H**), and chaetoiridins B (**I**) produced by *Chaetomium globosum*, rotorinol (**J**) produced by *Chaetomium cupreum* and trichotoxin A50 (**K**) produced by *Trichoderma harzianum* PC01.

Sclerotium rolfsii (Soytong *et al*., 2001) and chaetoviridins A (Fig. 1H) and B (Fig. 1I) which inhibit the mycelial growth of various plant pathogenic fungi such as, *Pyricularia oryzae*, *Magnaporthe grisea*, *Pythium ultimum* (Park *et al*., 2005). *Chaetomium cupream* produces rotiorinol (Fig. 1J) which can inhibit the growth of pathogens (Kanokmedhakul *et al*., 2006).

***Competition*:** Competition occurs between microorganisms when space and nutrients are a limiting factor (Lewis *et al*., 1989; Howell, 2003; Benítez *et al*., 2004; Viterbo *et al*., 2007). The rhizosphere is a major concern where competition for space and nutrient occurs (Whipps; 2001; Howell, 2003; Viterbo *et al*., 2007). Competition can be divided into saprobic competition for nutrients in the soil and rhizosphere, and competition for infection sites on and in the root (Fravel *et al*., 2003). Competition between the biocontrol agent and the pathogen can result in displacement of the pathogen. Biological control agents can compete with other fungi for food and essential elements in the soil and around the rhizosphere (Chet *et al*., 1990; Irtwange, 2006) and can complete the space or modify the rhizosphere by acidifying the soil, so that pathogens cannot grow (Benítez *et al*., 2004). For example, *Trichoderma harzianum* T-35 control of *Fusarium* species on various crops occurs via competition for nutrients and rhizosphere colonization (Viterbo *et al*., 2007).

***Mycoparasitism*:** In addition to antibiosis and competition, biological control agents also reduce plant disease by mycoparasitism (Benítez *et al*., 2004; Irtwange, 2006). Mycoparasitism involves the complex process that includes the following steps: (1) the chemothophic growth of the antagonist to the host; (2) recognition of the host by mycoparasite; (3) attachment; (4) excretion of extracellular enzymes; (5) lysis and exploitation of the host (Whipps, 2001; Benítez *et al*., 2004; Viterbo *et al*., 2007). Biological control agents are able to lyse hyphae of pathogens by release the lytic enzymes and this is an important and powerful tool for control of plant disease (Chet *et al*., 1990; Flores *et al*., 1997; Viterbo *et al*., 2007) such as chitinases, proteases, and β-1, 3 glucanases (Whipps, 2001). These enzymes lyse pathogen hyphal cell walls during mycoparasitic activity (Cruz *et al*., 1992; Schirmbock *et al*., 1994; El-Katathy *et al*., 2001; Khetan, 2001). β-1, 3 glucanases have properties for degrading cell walls, inhibiting mycelium growth and spore germination of plant pathogenic fungi (Benítez *et al*., 2004; Lin *et al*., 2007). For example, β-1, 3 glucanases produced from *Chaetomium* sp. can degrade cell walls of plant pathogens including *Rhizoctonia solani*, *Gibberella zeae*, *Fusarium* sp. *Colletotrichum gloeosporioides*, and *Phoma* sp. (Sun *et al*., 2006) and β-1, 3 glucanases produced from *Periconia byssoides* can degrade cell walls, inhibit mycelium growth and spore germination in *Fusarium* sp. and *Rhizoctonia solani* (Lin *et al*., 2007). Chitinases play important roles in the degradation of chitin, the main cell wall structure component of fungi (Cruz *et al*., 1992; Whipps, 2001). Proteases produced by *Trichoderma harzianum* T-39 are involved in the degradation of pathogen hyphal membranes and cell walls. They can deactivate the hydrolytic enzymes, endo-polygalacturonase and exo-polygalacturonase produced by *Botrytis cinerea* causing agent of grey mold, which results in reduction of disease severity (Elad and Kapat, 1999).

***Induced resistance*:** Induced resistance occurs in most plants in response to infestation by pathogens (Harman *et al*., 2004). Induced resistance of host plants can be localized and/or systematic, depending on the type, source, and amount of stimuli (Pal and Gardener, 2006). Induced resistance by biocontrol agents involves the same suite of genes and gene products involved in plant response known as systematic acquired resistance (SAR) (Handelsman and Stabb, 1996; Whipps, 2001). *Trichoderma* strains are capable of establishing interaction induced metabolic changes in plants that increase resistance to a wide range of plant-pathogenic fungi (Harman *et al*., 2004). Strains of *Trichoderma* added to the rhizosphere protect plants against many pathogens including viruses, bacteria, and fungi, because of the induction of resistance mechanisms similar to the hypersensitive response (HR), systematic acquired resistance (SAR), and induced systematic resistance (ISR) in plants (Harman *et al*., 2004; Benitez *et al*., 2004; Haggag, 2008). This concept is supported by Yedidia *et al*. (1999) who treated cucumber seedlings in a

hydroponic system with *T. harzianum* T-203 and found that plant defense responses had occurred in roots and leaves and the plant response was marked by an increase in peroxidase activity and chitinase activity. Howell (2003) also reported that peroxidase activity was induced by *T. virens* in cotton seedlings more than in the control experiment. Other fungal taxa can also induce resistant responses in plants, for example; *Chaetomium globosum* produces chaetoglobosin C and can induce a localized and sub-systemic oxidative burst in carrots, potatoes, sweet potatoes, tomatoes, and tobacco and this substance can act to induce plant immunity for disease resistance (Soytong *et al*., 2001; Kanokmedhakul *et al*, 2002). A non-pathogenic strain of *Fusarium*, *Pythium ultimum*, and *Rhizoctonia* could induce plant resistance to pathogenic stains (Harman *et al*., 2004).

Resistance may be the result in an increase in the concentration of metabolites and enzymes related to defense mechanisms, such as phenyl-alanine ammonio-lyase (PAL) and chalcone synthase (CHS) (Viterbo *et al*., 2007). These enzymes are involved in the biosynthesis of phytoalexins, chitinases and glucanases (Benitez *et al*., 2004; Viterbo *et al*., 2007). The metabolites produced by *Trichoderma* may act as elicitors of plant resistance (Benitez *et al*., 2004). There are at least three groups of substances that elicit plant defense responses and these include proteins, peptides, and low-molecular-weight compounds (Harman *et al*., 2004; Viterbo *et al*., 2007).

Production of mycofungicides

The use of fungal biological control agents to control plant pathogens has been investigated for more than 70 years, however research in this area has increased dramatically only in the past 20 years. Over 40 biological control products have been introduced into the market within the past ten years (Table 1), but these are used on a very small scale as compared to chemical fungicides (Paulitz and Belanger, 2001; Kim and Hwang, 2007). There is a little investment into research and development of biological control agents as compared with chemical fungicides because mycofungicides usually have narrow host ranges and mycofungicides have tended to provide inconsistent or poor control in the field (Kim and Hwang, 2004, 2007). Therefore, research into mycofungicides has emphasized on fungi with broad spectrum effects and on improvements in their associated production, formulation and application (Butt, 2000). The others reasons for the limited commercial formula is the high cost of production which may be due to high cost of substrate, low biomass productivity, or limited economics of scale (Spadaro and Gullino, 2005; Fravel, 2005) however, the starch industry wastewater may be used for antagonist production (Verma, 2007).

The commercial development of myco-fungicides has increased significantly in recent years because of the progress in isolation and characterization of antagonistic fungal strains (Hofstein and Chapple, 1999; Spadaro and Gullino, 2005). Mycofungicides have shown potential for disease control in the laboratory, greenhouse, and field studies and they can be cultured for mass production by standard fermentation (Lumsden and Lewis, 1989; Hofstein and Chapple, 1999; Khetan, 2001; Spadaro and Gullino, 2005). Two common methods used for producing inocula of biological control agents are liquid and solid fermentation (Tang *et al*., 2001; Spadaro and Gullino, 2005). Low cost and capacity to control disease following processing and storage are also important considerations (Alabouvette and Lemanceau, 1999; Spadaro and Gullino, 2005).

The development of the high-quality mycofungicides relies on the biological properties of the isolates. The factors that need to be considered when selecting isolates for potential biological control agents are as follows: laboratory virulence, field performance, genetic stability, productivity, stability of conidia in storage, stability in formulation, field persis-tence (tolerance to environmental factors such as UV, temperature, extremes and desiccation), mammalian safety, low environmental impact, and capacity to persist in the environment (Jenkins *et al*., 1998; Spadaro and Gullino, 2005). The important characteristics of a suc-cessful commercial product are good market potential, simplicity in production and appli-cation, adequate product and stability, shelf life during transport and storage, efficacy over a long term, guaranteed propagule viability,

Table 1. Some of the biological control products available in the market.

Products	Fungus	Target pathogen	Formulation	Producer
AQ10 Biofungicide	*Ampelomyces quisqualis* M-10	Powdery mildew	Water-dispersible granule	Ecogen, Inc. Langhorne, PA www.groworganic.com
Binab T WG Bineb T Pellet Bineb T Vector Bineb TF WP	*Trichoderma harzianum* *Trichoderma polysporum*	Fungi causing wilt, root rot wood decay	Wettable powder Granules pellets	Bio-Innovation, Sweden www.algonet.se/~binab/index2.html
Bioderma	*Trichoderma viride*	*Sclerotinia*, *Rhizoctonia*	Wettable powder	Biotech International Ltd., India www.biotech-int.com
Bioderma-H	*Trichoderma harzianum*	*Phytophthora*, *Fusarium* *Pythium* spp., *Cercospora*, , *Colletotrichum*, *Alternaria*, *Ascochyta*, *Macrophomina*, *Myrothecium*, *Ralstonia*	Wettable powder	Biotech International Ltd., India www.biotech-int.com
Biofox C	*Fusarium oxysporum* (nonpathogenic)	*Fusarium oxysporum* *Fusarium miniliforme*		SIAPA, Italy
Biofungus	*Trichoderma* spp.	*Sclerotinia*, *Phytophthora*, *Rhizoctonia solani*, *Pythium* spp., *Fusarium*, *Verticillium*	Granule Wettable powder	Grondortsmettingen deCuester n. v., Belgium
Cotans WG	*Coniothyrium minitans*	*Sclerotinia* spp.	granules	Prophyta, Germany www.prophyta.de
Fungi-Killer	*Trichoderma harzianum*	*Phytophthora*, *Fusarium*	Powder	Bangkok Organic Compost Ltd. Thailand
Fusaclean	*Fusarium oxysporum* Fo47 (nonpathogenic)	*Fusarium oxysporum*	Spores, microgranule	Natural plant Protection, France
Ketocin	*Chaetomium cupreum*	*Fusarium oxysporum f.sp. lycopersici*	Powder	Neoworld Ltd., Thailand
Ketomium	*Chaetomium globosum* *Chaetomium cupreum*	*Phytophthora palmivora* *Phytophthora parasitica* *Colletotrichum gloeospoiodes* *Fusarium oxysporum f.sp. lycopersici* *Fusarium moniliform* *Pyricularia oryzae* *Sclerotium rolfsii* *Drechsiera maydis*	Pellets, Powder	Guangxi Guilin Green Harvest Fertilizer Factory, China Nova Science, Thailand
Koni	*Coniothyrium minitans*	*Sclerotinia* spp.		BIOVED Ltd., Hungary www.bioved.hu
Novacide	*Chaetomium cupreum*	*Fusarium oxysporum f.sp. lycopersici*	Powder	Nova Science, Thailand
Polygandron Polyversum	*Pythium oligandrum*	*Pythium ultimum*	Granule or powder	Plant Protection Institute, Slovak Republic

Table 1 (continued). Some of the biological control products available in the market.

Products	Fungus	Target pathogen	Formulation	Producer
Prestop Primastop	*Gliocladium catenulatum* Strain JI446	*Pythium* spp. *Rhizoctonia solani* *Botrytis* spp. *Didymella* spp.	Wettable powder	Kemira Agro Oy, Fingland
Promote	*Trichoderma harzianum* *Trichoderma viride*	*Pythium* *Rhizoctonia* *Fusarium*		JH Biotech Inc., Ventura, CA, USA www.jhbiotech.com
RootShield PlantShield	*Trichoderma harzianum* Strain T-22	*Pythium, Rhizoctonia, Fusarium, Sclerotina*	Granules and Wettable powder	Bioworks, Inc. NY. USA www.bioworksbiocontrol.com
Sentinel®	*Trichoderma* spp. Strain LC52	*Botrytis cinera*	Wettable powder	Agrimms Technologies Ltd, www.vinevax.com
SoilGard (GlioGard)	*Gliocladium virens* GL21	Several plant diseases Damping-off and root pathogens	Granules Alginate prill	Thermo Triology, USA.
Trichodex	*Trichoderma harzianum* T-39	Fungal diseases e.g. *Botrytis cinerea* *Colletotrichum*, *Monilinia laxa*, *Plasmospara viticola* *Rhizopus stolonifer*	Wettable powder	Makhteshim-Agan, DeCeuster, Belgium
Vinevax™	*Trichoderma* spp.	Wood-infecting fungal pathogens of vineyard, orchard, ornamental trees, and vines	Wettable powder	Agrimms Technologies Ltd, www.vinevax.com

economic, suitable and appropriate action, and compatibility with agronomic practices and equipment (Boyetchko *et al*., 1999; Spadaro and Gullino, 2005).

An efficient formulation is essential to transfer the biological control agent from laboratory to the field. The formulation must be compatible to preserve the biological control agent activities. The living organisms must remain inactive whilst in storage, but rapidly become active when applied in the field (Butt *et al*., 1999). To achieve this, a drying process is necessary, for example air drying, freeze drying, atomization, bed-fluid drying or lyophilization (Butt *et al*., 1999; Spadaro and Gullino, 2005).

There are many types of formulation for fungal antagonists, for example, alginate prill formulation, fluid-bed granulation including dextrin as a binder, liquid formulation, water dispersible granule formulation, wettable powder formulation, dusts, granular or powder products (Khetan, 2001; Soytong *et al*., 2001). Some formulation types of commercial mycofungicides are shown in Fig. 2. The formulations can be applied to seeds, tubers, cuttings, seedlings, transplants, mature plants and soil (Boyetchko *et al*., 1999). However, liquid formulation is preferred with drip irrigation, granular formulations are more appropriate for combining with potting mix, while a wettable powder is more appropriate for root dips or sprays (Spadaro and Gullino, 2005). Biological control agents should be capable of application through standard hydraulic nozzles or application equipment with few special application requirements (Butt, 2000).

Mycofungicides comprise many ingredients such as active ingredients (microorganism or spores), adjuvants, dilution agents, bulking additives, membrane stabilizers, growth and contaminant suppressants, buffers, binders, dispersants, lubricants, activators, food sources, and coatings. These ingredients are added for various proposes such as follows: maintenance viability of biological control agents, manipulating bulk for handing and delivery, promoting the activity of biological control agents, protecting the inocula from unfavorable environmental conditions, and

Fig. 2 Some formulation types of mycofungicides. **A** Ketocin® in powder formulation, **B** Ketomium®in pellet formulation, **C** Fungi Killer® in pellet formulation and **D** Novacide® in powder formulation.

impressive growth of contaminants (Hynes and Boyetchko, 2005; Spadaro and Gullino, 2005). Presently, there are many mycofungicides worldwide in the market as show in Table 1 and Figs 2 and 3.

Fungal biofertilizers

Biofertilizers comprise microbial inocula or assemblages of living microorganisms which exert direct or indirect benefits on plant growth and crop yield through different mechanisms (Fuentes-Ramirez and Caballero-Mellado, 2005) These microorganisms are able to fix atmospheric nitrogen or solubilize phosphorus, decompose organic material, or oxidize sulfur in the soil properties (Marin, 2006) that are beneficial to agricultural production in terms of nutrient supply (Malik *et al*., 2005). One type of biofertilizer are the arbuscular mycorrhizal fungi, which are probably the most abundant fungi in agricultural soil (Marin, 2006; Khan, 2006). The inocula improve crop yield because of increased availability or uptake or absorption of nutrients, stimulation of plant growth by hormone action or antibiosis and by decomposition of organic residues (Wani and Lee, 2002). Selected fungal species which are used as biofertilizers are mentioned below.

Mycorrhizal fungi used as biofertilizers

Mycorrhizae form mutualistic symbiotic relationships with plant roots of more than 80% of land plants including many important crops and forest tree species (Smith and Zhu, 2001; Gentili and Jumpponen, 2006; Rinaldi *et al*., 2008). There are seven types of mycorrhiza: arbutoid mycorrhiza, ectomycorrhiza, endomycorrhiza or arbuscular mycorrhiza, ect-endomycorrhiza, ericoid mycorrhiza, monotropoid mycorrhiza, and orchidoid mycorrhiza (Raina *et al*., 2000; Gentili and Jumpponen, 2006; Das *et al*., 2007; Tao *et al*., 2008; Zhu *et al*., 2008). The two dominant types of mycorrhizae are ectomycorrhizae (ECM) and arbuscular mycorrhizae (AM) which can improve water and nutrient uptake and provide protection from pathogens but only a few families of plants are able to form functional associations with both AM and ECM fungi (Haskins and Gehring, 2005; Siddiqui and Pichtel, 2008). However, AM fungi are most commonly found in the rhizosphere roots of a wide range of herbaceous and woody plants (Das *et al*., 2007; Rinaldi *et al*., 2008). In this review, we focus

Fig. 3. Some of mycofungicides; (**A**) Fungi Killer, (**B**) Ketomium, (**C**) NovacideE, and (**D**) KONI® WG.

on ectomycorrhizal fungi and arbuscular mycorrhizal fungi because they are the most widespread and economically important types of mycorrhizal fungi. Ectomycorrhizal (ECM) fungi form mutualistic symbioses with many tree species (Anderson and Cairney, 2007). Most ECMfungi do not penetrate the living cells in the roots, but only surround them (Raina, 2000; Gupta *et al*., 2000; Das *et al*., 2007). ECM fungi occur naturally in association with many forest trees, for example, pine, spruce, larch, hemlock, willow, poplar, oak birch and eucalyptus (Dahm, 2006; Raja, 2006; Rinaldi *et al*., 2008). Most ECM fungi that are associated with forest trees are basidiomycetes, such as *Amanita* sp., *Lactarius* sp., *Pisolithus* sp., and *Rhizopogon* sp. and many of these are edible (Le *et al*., 2007; Buyck *et al*., 2008; Rinaldi *et al*., 2008) Some ascomycetes also form mycorrhizae such as, *Cenococcum* sp., *Elaphomyces* sp., and *Tuber* sp. (Dahm, 2006; Das *et al*., 2007; Rinaldi *et al*., 2008). The importance of ECM fungi to trees is in their ability to increase the tree growth due to better nutrient acquisition (Gentili and Jumpponen, 2006). ECM fungi help the growth and development of trees because the roots colonized with ectomycorrhiza are able to absorb and accumulate nitrogen, phosphorus, potassium, and calcium more rapidly and over a longer period than nonmycorrhizal roots. ECM fungi help to break down the complex minerals and organic substances in the soil and transfer nutrients to the tree. ECM fungi also appear to increase the tolerance of trees to drought, high soil temperatures, soil toxins, and extremes of soil pH. ECM fungi can also protect roots of trees from pathogens (Dahm, 2006). The most commonly widespread ectomycorrhizal product is inoculum of *Pisolithus tinctorius* (Schwartz *et al*., 2006; Gentili and Jumpponen, 2006). *Pisolithus tinctorius* has a wide host range and their inoculum can be produced and applied as vegetative mycelium in a peat vermiculite carrier. These fungus inocula are applied to nursery or forestry plantations (Gentili and Jumpponen, 2006). *Piriformospora indica* (Hymenomycetes, Basidiomycota) is another ECM fungus used as a biofertilizer. This taxon can promote plant growth and biomass production and help plant tolerance to herbivory, heat, salt, disease, drought, and increased below- and above-ground biomass (Waller *et al*., 2005; Tejesvi *et al*., 2007)

Endomycorrhizae from mutually symbiotic relationships between fungi and plant roots (Ipsilantis and Sylvia, 2007). The plant roots provide substances for the fungi and the fungi transfer nutrients and water to the plant roots (Adholeya *et al*., 2005; Chen, 2006). Endomycorrhizal fungi are intercellular and penetrate the root cortical cells and form structures called arbuscular vesicles and known as vesicular arbuscular mycorrhiza (VAM) but in some cases no vesicles are formed and they known as arbuscular mycorrhiza (AM) (Gupta *et al*., 2000). The agriculturally produced crop plants that form endomycorrhizae of the vesicular-arbuscular mycorrhiza type are now called arbuscular mycorrhizal (AM) fungi (Raja, 2006). AM fungi belong to nine genera: *Acaulospora*, *Archaeospora*, *Enterophospora*, *Gerdemannia*, *Geosiphon*, *Gigaspora*, *Glomus*, *Paraglomus*, and *Scutellospora* (Das *et al*., 2007). AM fungi are a widespread group and are found from the arctic to tropics and are present in most agricultural and natural ecosystems. They play an important role in plant growth, health, and productivity (Douds,

2005; Marin, 2006). AM fungi help plants to absorb nutrients, especially the less available mineral nutrients such as copper, molybdenum, phosphorus and zinc (Yeasmin *et al*., 2007). They increase seedling tolerance to drought, high temperatures, toxic heavy metals, high or low pH and even extreme soil acidity (Gupta *et al*., 2000; Kannaiyan, 2002; Chen, 2006). AM fungi can also affect plant growth indirectly by improving the soil structure, providing antagonist effects against pathogens and altered water relationships (Smith and Zhu, 2001). AM fungi can reduce the severity of soil-borne pathogens and enhance resistance in roots against root rot disease (Azcon-Aguilar and Barea, 1996; Kannaiyan, 2002; Chen, 2006; Akhtar and Siddiqui, 2008a,b). This results because of competition for colonization sites or nutrients in the same root tissues and production of fungistatic compounds (Johansson *et al*., 2004; Marin, 2006). AM fungi have been shown to have benefits to host plants including increasing herbivore tolerance, increasing pollination, increasing soil stability, and heavy metal tolerance (Hart and Trevors, 2005). The use of AM fungi as biofertilizers is not new, they have been produced for use in agriculture, horticulture, landscape restoration, and soil remediation for almost two decades (Hart and Trevors, 2005). Mass production of AM fungi has been achieved with several species such as *Acaulospora laevis*, *Glomus*. *clarum*, *G. etunicatum*, *G. intraradices*, *G. mosseae*, *Gigaspora ramisporophora and Gigaspora rosea* (Schwartz *et al*., 2006) but *Glomus intraradices* is the most common inoculum of endomycorrhizae products (Adholeya *et al*., 2005; Wu *et al*., 2005; Schwartz *et al*., 2006; Akhtar and Siddiqui, 2008b). Effective management of AM fungi involves increasing populations of propagules such as spores, colonized root fragments and hyphae using host plants and also by adoption soil management techniques (Smith and Zhu, 2001; Tiwari *et al*., 2004; Kapoor *et al*., 2008).

Production of mycorrhizal fungi as fungal biofertilizers

AM fungi are obligate symbiotic microorganisms since they cannot be grown without the plant host on synthetic media (Hart and Trevors, 2005). Therefore AM fungal inocula must be produced in association with the host plant and therefore there are many constraints to large scale commercial production. Mass production is by pot culture either in the greenhouse or in growth chambers is the most commonly used production method (Bagyaraj *et al*., 2002; Raja, 2006; Gentili and Jumpponen, 2006; Marin, 2006; Kapoor *et al*., 2008). AM fungal inocula have to be prepared by multiplication of the selected fungi in roots of susceptible host plants growing in the sterilized soil or substrates for example perlite, vermiculite, peat, sand, or mixture of them (Naqvi and Mukerji, 2000). The inocula of AM fungi can be applied as spores, or fragments of colonized roots. The spores and hyphae can be isolated from the soil rhizosphere and mixed with carrier substrates (Gentili and Jumpponen, 2006). Spore inocula are the most resistant and can survive unfavorable environmental conditions for a long period, but they colonize new root systems more slowly than other preparations. Therefore both types of inocula, e.g. spores and fragments of colonized roots should be combined in commercial products (Marin, 2006).

Root-based bulk inoculum production technology utilizes mass produced seedlings grown in sterilized soil infected with selected AM fungi using spores from fruiting bodies from cultivated plants. This technology results in seedlings with infected root systems and the roots and adhering soil are chopped up and used as the starter inoculum for scale up production. The inocula are produced in bulk by infecting fresh seedling of selected plants (Singh and Tilak, 2002; Gentili and Jumpponen, 2006). The root inocula are kept in polythene bags and used for pelleting seeds or in the preparation of granules for seed bed inoculation (Singh and Tilak, 2002). The others methods such as soil-free aeroponic, nutrient film, and root organ culture system have been used for production of AM, but these methods are costly and preclude commercial mass production (Gentili and Jumpponen, 2006). It may be possible to mass produce plants in tissue culture in sterile agar media and induce mycorrhizal associations using spores from fruiting bodies of selected mycorrhizal fungi. The dried root tissues and fungal mycelia could then be developed into mycorhizal seeding products (Hyde, *pers*. *comm*.).

Some steps are essential for development of a commercial fungal biofertilizers. They include selection, large scale production, carrier selection and preparation, mixing and curing, maintenance of appropriate numbers of inocula, and strong quality control (Malik *et al*., 2005). The criteria for selecting AM fungi will depend on details of the local environment, soil conditions, and host plants. The AM fungi must 1) colonize roots rapidly after inoculation, 2) absorb phosphate from the soil, 3) transfer phosphorus to the plant, 4) increase plant growth, 5) persist in soil and reestablish mycorrhizal symbiosis during the following seasons, and 6) form propagules that remain viable during and after inoculum production (Tanu *et al*., 2006).

The success of a formulation depends on whether it 1) is economically viable to produce, 2) does not alter the viability and function of the inoculum, 3) is easy to carry and enhance dispersal during application. The inoculum formulation may comprise one or more AM fungi and other organisms which together enhance the ability of the inoculum to form mycorrhizal associations with the target plant. The formulations are available in the form of powder, tablets/pellets or granules, gel beads and balls (Adholeya *et al*., 2005; Tiwari and Adholeya, 2005). There are many ways to apply the AM inocula (Adholeya *et al*., 2005; Schwartz *et al*., 2006) including: scattering by hand, in-furrow application, seed coating, root dipping, and seedling inoculation. The efficacy of the application of AM inocula depends on the product, environmental condition, delivery method, and other variables. The success of AM fungi inoculation depends on crop species, size and effectiveness of indigenous AM fungi populations, fertility of the soil, and cultural practices (Adholeya *et al*., 2005; Tiwari and Adholeya, 2005).

The production of commercial mycorrhizal inoculum has evolved considerably in recent years (Douds *et al*., 2000). There are various types of microbial cultures and inoculants available on the market today and these have rapidly increased because of the advances in technology (Raja, 2006). There are more than 30 companies worldwide marketing mycorrhiza products (some of them shown in Table 2 and Fig. 4) comprising one or multiple mycorrhizal fungal inocula. These products are plant growth promoters and to be used in horticulture, agriculture, restoration and forestry (Schwartz *et al*., 2006).

Other fungi used as biofertilizers

Other fungal biofertilizers which have been used to improve plant growth are *Penicillium* species. They are phosphate solubilizing microorganisms that improve phosphorus absorption in plants and stimulate plant growth (Wakelin *et al*., 2004; Pradhan and Sukla, 2005). *Penicillium bilaiae* has been formulated as a commercial product named Jumpstart® and was released to the market as a wettable powder in 1999 (Burton and Knight, 2005). *Penicillium bilaiae* is applied to increase dry matter, phosphorus (P) uptake and seed yield in canola (*Brassica napus*) (Grant *et al*., 2002; Burton and Knight, 2005). *Penicillium radicum* and *P. italicum* are also phosphate-solubilizing taxa (Whitelaw *et al*., 1999; Wakelin *et al*., 2004; El-Azouni, 2008). *Penicillium radicum* isolated from the rhizosphere of wheat roots, has shown a good promise in plant growth promotion (Whitelaw *et al*., 1999) while *P. italicum* isolated from the rhizosphere soil was tested for its ability to solubilize tri-calcium phosphate (TCP) and could promote the growth of soybean (El-Azouni, 2008).

Several species of *Aspergillus* have been reported to be involved in the solubilization of inorganic phosphates such as *A. flavus*, *A. niger* and *A. terreus*, (Akintokun *et al*., 2007). These fungi are able to solubilize of inorganic phosphate through the production of acids for example citric, gluconic, glycolic, oxalic acids, and succinic acid (Barroso *et al*., 2006). *Aspergillus fumigatus* which isolated from compost *has been reported to be* potassium releasing fungus (Lian *et al*., 2008).

The product of *Chaetomium* species can be fungal biofertilizers for example Ketomium® which is formulated from *Ch. globosum* and *Ch. cupreum* is not only a mycofungicide but also plant growth stimulant because tomato, corn, rice, pepper, citrus, durian, bird's of paradise and carnation treated with Ketomium® have a greater plant growth and high yields than non-treated plants (Soytong *et al*., 2001).

Trichoderma species can not only reduce the occurrence of disease and inhibit pathogen growth when used as mycofungicides, but they also increase the growth and yield of plants (Elad *et al*., 1981; Harman *et al*., 2004; Vinale *et al*., 2008). They are also increase the survival of seedlings, plant height, leaf area and dry weight (Kleifeld and Chet, 1992). *Trichoderma* species improve mineral uptake, release minerals from soil and organic matter, enhance plant hormone production, induce systematic resistance mechanisms, and induced root systems in hydroponics (Yedidia *et al*., 1999). For these reasons *Trichoderma* species are known as plant growth promoting fungi (Hyakumachi and Kubota, 2004; Herrera-Estrella and Chet, 2004) or are increasing plant growth (biofertilization) (Benítez *et al*., 2004). *Trichoderma* species have therefore, successfully been used as biofungicides and biofertilizers in greenhouse and field plant production (Harman *et al*., 2004; Vinale *et al*., 2008). There are many *Trichoderma* products as fungal biofertilizers available in the market (some of them shown in Table 2 and Fig. 4). Their applications are however related to their ability to control plant diseases and promote plant growth and development (Harman *et al*, 2004; Vinale *et al*, 2006). *Trichoderma* also has various applications and important sources of antibiotics, enzymes, decomposers and plant growth promoters (Daniel and Filho, 2007).

Future trends in mycofungicides and fungal biofertilizers

The use of fungi as fungicides and biofertilizers is not new although most have been developed in the last two decades. There are numerous reports stating the success in the ability of fungi to control plant diseases and promote plant growth as biofertilizers. Mycofungicides and fungal biofertilizers help to minimize the use of synthetic chemical fungicides and chemical fertilizers. This is beneficial since synthetic chemical compounds have probable detrimental affects on humans and the environment (Calhelha *et al*., 2006; Haggag and Mohamed, 2007).

Mycofungicides and fungal biofertilizers are presently used on a very small scale as compared to chemical compounds (Fravel, 2005). There has been little investment in the research and development of fungal products because they may have poor effect in the field (Tang *et al*., 2001). There is still a wide gap between the considerable, often unpublished research carried out in laboratories as compared to development for use in the field. Future research should therefore develop fungal products which have significant effects in field applications and that are also stable when stored. Aspects which should be considered include 1) which strains of beneficial fungi should be used; 2) whether they are reliable and cheap to produce on a large scale; 3) whether they are detrimental to the environment; 4) whether they are safe to humans and to the environment, and 5) whether patentability of the formulation is possible. Greater communication is needed between researchers and industry in the early stages of development. Integration or combination of inocula or combinations with other beneficial fungi should be considered since combinations may be more effective than a single inoculum. The production of mycofungicides and fungal biofertilizers should be directed to a new focus that will search for commercial properties through the use of biotechnologies of the inoculation of fungi and the benefits should clearly be demonstrated to the growers, both through extension and proven field trials. Commercial interest will then increase**.**

Although there are many biocontrol products (Table 1), there are still many problems to overcome to achieve successful commercialization of other potential biocontrol products. Some biocontrol agents work well in the laboratory but do not work well in the field (Tang *et al*., 2001). Biological control of plant diseases by fungal antagonists remains a challenge for future research and development (Spadaro and Gullino, 2005). Several private companies have been involved in the development of mycofungicides. There are many species of fungal antagonists that have been formulated and registered as commercial products. Unfortunately, these products have been used on a small scale due to their capacity to control plant diseases in the field which is often not as good (or perceived to be less effective) than synthetic fungicides (Paulitz and Belanger, 2001; Tang *et al*., 2001).

Table 2. Some fungal biofertilizers available globally.

Products	Fungi	Companies
AgBio-Endos	Ectomycorrhizal fungi	AgBio Inc, Westminter, USA
AgBio-Ectos	Endomycorrhizal fungi	Agbio-inc.com
AM120	Mycorrhizal fungi	Reforestation Technologies International, USA www.reforest.com
Bioorganic Plus	*Trichoderma harzianum* *Trichoderma hamatum*	NovaScience Co. Ltd, Thailand.
BioVam	Mycorrhizal fungi *Trichoderma* spp.	T&J Enterprises, USA www.tandjenterprises.com
BuRize	AM fungi	BioScientific Inc, Arizona, USA www.biosci.com
Diehard™ mycorrhizal inoculant	Mycorrhizal fungi *Trichoderma* spp.	Horticultural Alliance, Inc, Fl, USA www. horticulturalalliance.com
Endomycorrhizal inoculant (BEI), inoculant micronized (BEIM), Mycorrhizal root dip	Endomycorrhizal fungi	Bio-Organics, Oregan, USA www.bio-organics.com
MycoApply® Endo MycoApply® Endo/Ecto MycoApply® Maxx Plant Success ™	Ectomycorrhizal fungi Endomycorrhizal fungi	Mycorrhizal application Inc, Oregon, USA www.mycorrhizae.com
Mycogrow™	Ectomycorrhizal fungi Endomycorrhizal fungi	Fungi perfecti, LLC, WA., USA www.fungi.com
Mycomax	AM fumgi (*Glomous intraradices*)	JHBiotech Inc. California, USA www.jhbiotech.com
Myke Myke® Pro Mycorise®	Mycorrhizal fungi	Premier Tech Biotechnologies, Canada www.premiertech.com
PLantmate®	*Trichoderma* spp.	Agrimms Technologies Ltd, www.vinevax.com
Promote®	Ectomycorrhizal fungi (*Pisolithus tinctorius*)	JHBiotech Inc. California, USA www.jhbiotech.com
Rhizanova	Mycorrhizal fungi	Becker-Underwood Inc., USA www.beckerunderwood.com
Rootgrow, Rootgrow Professional	Mycorrhizal fungi	PlantWorks Ltd., United Kingdom www.plantworksuk.co.uk
SoilMoist™	Ectomycorrhizal fungi Endomycorrhizal fungi	JRM chemical, Inc. Ohio, USA www.soilmoist.com
Superzyme	*Trichoderma* spp.	JH Biotech, Inc., Ventura, CA. USA www.jhbiotech.com
Tricho®	*Trichoderma* spp.	Agrimms Technologies Ltd, www.vinevax.com

Many biological control agents produce secondary metabolites which have properties to control plant diseases. The inoculum therefore not only can be used as mycofungicides, but also the secondary metabolites can be developed as mycofungicides. The secondary metabolites, however, should be tested and studied and must be harmless to humans and the environment. Recent advances in the study of molecular genetics of biological control agent strains have provided a powerful tool that will help to improve the effectiveness of biocontrol activity and exploitation of the genetic potential of fungal antagonists (Irtwange, 2006; Paterson, 2006; Haggag and Mohamed, 2007). There should be further research on the application of fungal biofertilizers to the soil because they help to increase crop yield and improve soil quality (Tanu *et al*., 2006). Fungal biofertilizers help to enhance crop yield and promote sustainable agricultural production and are safe to the environment (Smith and Zhu, 2001). Fungal biofertilizers have advantages in terms of nutrient supply, soil quality and crop growth and yield. Development in the effectiveness of fungal species for formulation as biofertilizers should be considered. New strains of fungi should 1) improve nutrient uptake, 2)

Mycomax | Endomycorrhizal inoculant micronized (BEIM) | Mycorrhizal Root Dip | Bioorganic plus

Myke | Myke Pro | Superzyme

Fig. 4. Some of fungal biofertilizers available in the market.

be resistance for biotic and abiotic stresses, 3) be fast growing and 4) have high productivity. The large scale inoculum production cost should be low. A future prospect in fungal biocontrol agents may be obtained by using transgenic fungi (Marin, 2006).

Research into other ecological fungi should be pursued to find novel biofungicides and biofertilizers. For example, endophytic fungi which are symptomless colonizers of plants (Oses *et al*., 2008; Hyde and Soytong, 2008) and some, especially grass endophytes are symbionts (Sánchez Márquez *et al*., 2007; Tejesvi *et al*., 2007; Wei *et al*., 2007; Sánchez Márquez *et al*., 2008) could be exploited. Endophytes play an important role in ecosystem processes such as decomposition and nutrient cycling, and thus may be utilized as biofertilzers. Endophytes also have beneficial symbiotic relationships with the seeds and roots of many plants, such as orchids (Tao *et al*., 2008; Zhu *et al*., 2008) and could be use to improve orchid seed germination and orchid growth. Endophytic fungi may also have roles in plant growth and survival (Mitchell *et al*., 2008) by enhancing nutrient uptake and producing growth promoting metabolites such as gibberellins and auxins which are plant hormones (Khan *et al*., 2008). They have been shown to benefit the host plant, including tolerance to herbivory, heat, salt, disease, drought, and increased below- and above-ground biomass (Waller *et al*., 2005; Tejesvi *et al*., 2007). Moreover, they may have potential biocontrol properties to inhibit pathogen infection within the host via antibiosis, competition, mycoparasitism, inducing resistance to the host plant (Mejia *et al*., 2008), or by producing bioactive secondary metabolites (Evans *et al*., 2003). Rungjindamai *et al*. (2008) are searching for endophytes that can reduce white rot decay of *Elaeis guineensis*. Endophytic fungi are also known to be a rich source of bioactive metabolites (Tejesvi *et al*., 2007; Pongcharoen *et al*., 2008; Raghukumar, 2008; Rungjindamai *et al*., 2008).

The use of mycorrhizal fungi as biofertilizers is often limited due the fact that they will not grow in artificial culture. Ways should be sort by which we can grow these fungi in culture and produce inocula. As mentioned above plate cultivation of these fungi with tissue culture plants may be a solution. *Phlebopus portentosus*, the black bolete, is purportedly mycorrhizal and forms associations with several fruit trees (e.g coffee, mango, jack fruit). Lumyong *et al*. (2009) have successfully grown this species on artificial media may be good for *in vitro* cultivation. This fungus is a perfect target for a biofertilizer since it should enhance tree growth but also produce an annual crop of the expensive Black Bolete which is a sort after fungus which demands a good price. *Phlebopus portentosus* is an unusual bolete in that it has clamp connections and therefore its close relatives should also be examined to

establish whether they can be utilized in a similar way (Ji *et al*., 2007).

The moves towards safe food production and organic food should increase biofungicide and biofertilizer use and thus result in environmental and ecosystem savings. Reduction in the use of chemical fungicides and fertilizers is necessary to maintain ecosystem function and develop sustainable agriculture. Research and development on mycofungicides and fungal biofertilizers should therefore emphasize on improving effective stable strains for disease control or for promoting plant growth though traditional and molecular techniques.

Conclusion

The benefits of using fungi as mycofungicides and biofertilizers include decreasing the occurrence of plant diseases by inhibiting the growth of pathogens, suppressing the amount of inocula of pathogens, increasing in uptake of nutrient from the soil or atmosphere, and producing bioactive compounds, hormones and enzymes which stimulate plant growth. These benefits maintain and increase the crop production. There are many commercial mycofungicides and fungal biofertilizers available worldwide. Using mycofungicides and fungal biofertilizers offer more environmentally friendly alternatives than chemical fungicides and chemical fertilizers. There are however, some limitations in using these products. Their success can be affected by environment conditions, while application difficulties, limited shelf life, and slow action as compared to chemical products may discourage farmers to utilize them. Research on the development of mycofungicides and fungal biofertilizers needs to be carried out so that more effective products are produced.

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