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REVIEW ARTICLES

Managing Plant Diseases and Promoting Sustainability and Productivity with *Trichoderma*: The Philippine Experience

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ABSTRACT

***Trichoderma* is a genus of asexually reproducing fungi that is present in all types of soils. *Trichoderma* species have been recognized as antagonists of soil-borne and foliage pathogens and as efficient decomposers of cellulosic waste materials. Moreover, they have the ability to increase plant growth and induce plant resistance. Along with mycoparasitism, antibiotics and competition, induced resistance is one of the most important mechanisms of *Trichoderma* action against fungal plant pathogens. Strategies to enhance biocontrol ability of *Trichoderma* include use of composts, UV irradiation and gene expression studies as applied to genetic engineering. Of over 50 research projects on *Trichoderma* in the Philippines as reported in this review, only less than 10% have been published at full length in scientific journals. *Trichoderma* have been often used in the control of rice and solanaceous crops and vegetables. Most projects have been conducted both *in vitro* and *in vivo* including the laboratory and greenhouse but rarely in the field. Most strains reported were not identified up to the species level especially by molecular techniques, an essential requirement for product commercialization. Of the several strains of *Trichoderma* that have been isolated and screened for biocontrol of plant diseases in the Philippines, only one strain has reached product commercialization. This, however, has led to a promising technology to improve management of vegetable diseases and increase farmers' income. The continuity in sustaining these programs is vital in maintaining sustainability and productivity of agricultural crops with *Trichoderma*.**

Keywords: Biological control, Enhancement, Commercialization, Mode of action

INTRODUCTION

Trichoderma is a genus belonging to the filamentous Class Deuteromycetes. The members are generally found in all soils (Chet 1987; Samuels, 1996). The fungus is a valuable source for the commercial production of enzymes and helpful in recycling cellulosic waste materials while producing useful by-products (Samuels, 1996). *Trichoderma* received the most attention as fungal antagonists not only of soil-borne pathogens (Amin *et al.*, 2010) but also of foliage pathogens such as *Botrytis cinerea* (Elad, 1994). This is because of the ability of some of its species to produce enzymes which inhibit other fungi (Lima *et al.*, 1997; Mohamed *et al.*, 2010). *Trichoderma* can function at the same time both as microbial antagonists and plant symbionts (Lorito *et al.* 2006). For these reasons, close to 20 fungal biocontrol preparations abroad are based on *Trichoderma* (Table 1).

Looking back at the developments of *Trichoderma* research in the Philippines, the fungus has been tested as a potential biocontrol agent over the last three decades. How far have we gone through in the advancement of biological control of plant diseases using *Trichoderma* in the Philippines? Nowadays, we need to appreciate the importance of biological diversity of living organisms in the context of sustainable plant protection systems. Because of environmental pollution brought about by

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Table 1. Commercial biocontrol products of *Trichoderma* worldwide.

Product	Strain	Company, Country
Topshield, Rootshield	*T. harzianum T-22*	Bioworks, Geneva, N.Y.
T-22G, T-22B	*T. harzianum T-22*	TGT Inc., New York
T35	*T. harzianum*	Makhteshim-Agan Chemicals, Israel
Harzian 20, Harzian 10	*T. harzianum*	Natural Plant Protection, Noguerres, France
F-stop	*T. harzianum*	Eastman Kodak Co., United States TGT Inc., New York
Supraavit	*T. harzianum*	Bonegaard and Reitzel, Denmark
Solsain, Hors-solsain, Plantsain	*Trichoderma spp.*	Prestabiol, Montpellier, France
ANTI-FUNGUS	*Trichoderma spp.*	Grondontsmettingen De Ceuster, Belgium
Ty	*Trichoderma spp.*	Mycontrol, Israel
GlioGard and SoilGard	*T. virens (Gliocladium virens)*	Grace-Sierra Co., Maryland
Bip T	*T. viride*	Poland
Promot PlusWP Promot PlusDD	*Trichoderma spp.* *Trichoderma koningii* *Trichoderma harzianum*	Tan Quy, Vietnam
TRiB1	*Trichoderma spp.*	National Institute of Plant, Vietnam
TRICÔ-ĐHCT	*Trichoderma spp.*	Can Tho University, Vietnam
Vi – ĐK	*Trichoderma spp.*	Pesticide Corp., Vietnam
NLU-Tri	*Trichoderma virens*	Ho Chi Minh University of Agriculture and Forestry, Vietnam
Biobus 1.00WP	*Trichoderma viride*	Nam Bac, Vietnam
Bio – Humaxin Sen Vàng 6SC, Fulhumaxin 5.15SC	*Trichoderma spp.*	An Hung Tuong, Vietnam
BioSpark Trichoderma	*T. parceramosum, T. pseudokoningii, and Ultraviolet irradiated strain of T. harzianum*	BioSpark Corporation, Philippines

pesticides, alternative pest management strategies are being developed. Some fungicides were taken off the market because of toxicity problems and pathogens developed resistance (Bruton, 1994). These and many other factors portend a renewed emphasis on biocontrol. Based on the increasing number of papers presented at the Pest Management Council of the Philippines (PMCP) in the last 20 to 30 years and the number of BS, MS theses and PhD dissertations on the topic of *Trichoderma* (see Table 2), there has been a substantial commitment of resources on this area. It is also imperative to emphasize the importance of correct identification of *Trichoderma* by molecular approaches as a valuable tool for studies of diversity and genetic structure of populations of these fungi, including determining whether a certain species possessed a unique mode of action and in an effort to provide accurate information on the active ingredients of the biocontrol product.

The general objective of this review paper is to introduce the current status and advances in *Trichoderma* as a biocontrol agent of plant diseases in the Philippines and in the world. The specific objectives are as follows: (1) to present data on the modes of action of *Trichoderma* and its asexual genetics; and (2) to describe the strategies for the enhancement of biocontrol ability of *Trichoderma* from formulation to genetic engineering.

Modes of Action of *Trichoderma*

Agar test is the simplest method of determining the mode of action of a biocontrol agent *in vitro*. This method has several fundamental drawbacks. First, agar test can be unreliable if it is used only as a primary screen for determining the mode of action of biocontrol strains of *Trichoderma* (Merriman and Russell, 1990). Although this method is the fastest way to screen for antibiotic production and/or mycoparasitism, it does not select strains which act by competition, avirulence and cross protection (Merriman and Russell, 1990). Second, the plant and soil are not accounted for in the assay. Since results had almost no predictive value for biocontrol efficacy, most laboratories prefer more effective and predictive methods such as plant–pathogen interaction

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Table 2. Examples of biocontrol research using *Trichoderma* in the Philippines.

Antagonist	Pathogen	Host	Condition	Site	Reference
T. viride	*Fusarium solani f.sp. phaseoli*	mungbean	*in vitro, in vivo*	greenhouse	Catedral and Halos (1977)
T. aureoviride, T. hamatum, T. harzianum, T. koningii ,Trichoderma sp.	*Rhizoctonia solani*	rice	*in vivo*	greenhouse	Usmani and Mew (1984)
Trichoderma spp.	*Rhizoctonia solani*	rice	*in vitro*	greenhouse	Imolehin, *et al.* (1989)
Trichoderma spp.	*Rhizoctonia solani*	rice	*in vitro*	greenhouse	Mew *et al.* (1980)
Trichodema sp.	*Fusarium oxysporum f.sp. vasinfectum*	cotton	*in vivo*	field	Cano and Catedral (1995)
T. aureoviride, *T. glaucum*	*Pythium* and *Phytophthora*	mungbean, cowpea, soybean, maize	*in vivo*	greenhouse	De la Peña *et al.* (1986)
T. harzianum	*Sclerotium rolfsii*	rice	*in vivo*	laboratory, greenhouse, field	Paderes *et al.* (1995); Paderes (1994)
T. aureoviride, T. harzianum	*Rhizoctonia solani*	maize	*in vivo*	greenhouse	Dalmacio *et al.* (1990)
T. harzianum, *T.viride*	*Rhizoctonia solani*	rice	*in vitro, in vivo*	laboratory and greenhouse	Cumagun and Ilag (1997a); Cumagun and Ilag (1997b)
Trichoderma sp.	*Botrytis cinerea*	strawberry	*in vivo*	greenhouse	Saclangan (2008)
Trichoderma sp.	*Plasmodiophora brassicae*	cabbage	*in vivo*	greenhouse	Cuevas and Kebasen (2005)
T.viride, Trichoderma sp.	*Sclerotium rolfsii*	sweet pepper	*in vitro, in vivo*	greenhouse and field	Paningbatan (1994)
Trichoderma sp.	*Plasmodiophora brassicae*	cabbage	*in vivo*	field	Nagpala and Palengleng (2008)
T. viride, Trichoderma sp.	*Rhizoctonia solani*	cabbage	*in vitro, in vivo*	laboratory and greenhouse	Acabal (2004)
Trichoderma sp.	*Phytophthora colocasiae*	taro	*in vivo*	field	Palomar *et al.* (2004)
Trichoderma sp.	*Lasiodiplodia theobromae*	sweet potato	*in vivo*	greenhouse field	Palomar and Palermo (2004)
Trichoderma sp.	*Colletotrichum, Fusarium*	onion	*in vivo*	greenhouse	Santiago *et al.* (2008)
T. harzianum, T. viride, T. koningii	*nematodes*	sugarcane	*in vivo*	greenhouse and field	Recuenco (1997)
T. piluliferum, T. reesei, *T. harzianum*	*Rhizopus stolonifer, Fusarium* sp,. *Phytophrthora* sp.	papaya	*in vitro, in vivo*	laboratory and greenhouse	Cruz (1995)
Trichoderma sp.	*Rhizoctonia solani*	rice	*in vitro, in vivo*	laboratory and greenhouse	Castro (1990)
T. viride	*Plasmodiophora brassicae*	cabbage	*in vitro, in vivo*	laboratory and greenhouse	Cueto (1983)
Trichoderma spp.	*Fusarium moniliforme*	rice	*in vitro, in vivo*	greenhouse	Bacolod (1988)
Trichoderma spp.	*Sclerotium* sp.	*Microsorium polypodium*	*in vitro, in vivo*	laboratory and greenhouse	Peñamante (2000)
Trichoderma sp.	*Pythium, Fusarium*	corn, cowpea	*in vitro, in vivo*	laboratory and greenhouse	Torres (2000)
T. glaucum	*Sclerotium rolfsii*	wheat	*in vitro, in vivo*	laboratory and greenhouse	Neypes (1986)
T. harzianum	*Rhizoctonia solani*	rice	*in vitro, in vivo*	laboratory and greenhouse	Rosales (1985)
Trichoderma spp.	*Sclerotium rolfsii*	tomato	*in vivo*	greenhouse	Legado and Ganoy (1994)
Trichoderma spp.	*Sclerotium rolfsii*	cucumber	*in vivo*	greenhouse	Paracha and Evangelista (1994)
T. harzianum	*Fusarium* sp.	ginger	*in vivo*	greenhouse	Samoy and Tangonan (1991)
Trichoderma sp.	*Sclerotium rolfsii*	cotton	*in vivo*	greenhouse	Sollorin and Tangonan (1990)

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Table 2. Continued

Antagonist	Pathogen	Host	Condition	Site	Reference
T. viride	*Ralstonia solanacearum*	tomato	*in vivo*	greenhouse	Parungao *et al.* (2008)
T. viride	*Diplodia natalensis*	mango	*in vivo*	laboratory	Moreno (1995)
T. aureoviride, T. glaucum, T. harzianum, T. pseudokoningi, T. viride	*Alternaria brassicae*	vegetables	*in vitro*	laboratory	Alcantara (1987)
T. aureoviride, T. glaucum, T. harzianum, T. pseudokoningi, T. viride	*Fusarium oxysporum f. sp. lycopersici*	vegetables	*in vitro*	laboratory	Alcantara (1987)
T. aureoviride, T. glaucum, T. harzianum, T. pseudokoningi, T. viride	*Cercospora canescens*	vegetables	*in vitro*	laboratory	Alcantara (1987)
T. aureoviride, T. glaucum, T. harzianum, T. pseudokoningii, T. viride	*Alternaria solani*	vegetables	*in vitro*	laboratory	Alcantara (1987)
T. harzianum, T. viride, T. glaucum, T. pseudokoningii	*R. solani*	rice	*in vitro, in vivo*	greenhouse and field	Sinohin and Plete (1998)
T. harzianum, T. viride	*Lasiodiplodia theobromae*	banana	*in vitro, in vivo*	laboratory	Mortuza and Ilag (1999)
T. viride	*Fusarium oxysporum*	abaca	*in vitro, in vivo*	laboratory and greenhouse	Bastasa and Baliad, 2005
Trichoderma sp.	*Phytophthora capsici*	black pepper	*in vitro, in vivo*	laboratory and greenhouse	Noveriza and Quimio (2004)
Trichoderma sp.	*Fusarium verticillioides*	banana	*in vitro, in vivo*	laboratory	Alvindia (2008)
Trichoderma sp.	*Fusarium sp., R. solani, Phoma terrestris, Pseudomonas solanacearum*	onion, rice, vegetables	*in vitro*	laboratory	Alvindia and Natsuaki (1998)
T. viride	*Aspergillus flavus*	maize	*in vitro, in vivo*	laboratory	Caasi (2003)
T. harzianum	*Radopholus similis, Meloidogyne incognita, Helicotylenchus multicinctus*	banana	*in vivo*	field	Generalao (1998)
Trichoderma spp.	*Fusarium* spp.	garden pea	*in vitro*	greenhouse	Nagpala (1999)
T. viride	*Sclerotium rolfsii, Rhizoctonia. solani, Pythium. debaryanum, F. oxysporum, Phytophthora infestans, Pseudomonas solanacearum*	wheat, rice, tomato, cowpea, potato and tomato	*in vivo*	laboratory and greenhouse	Molina (1985)
T. harzianum, T. aureoviride, T. glaucum	*Sclerotium rolfsii*	vegetables	*in vitro, in vivo*	laboratory	Fernandez and Gapasin (1990)
Trichoderma spp.	*Sclerotium rolfsii, Phytophthora sp*	vegetables	*in vitro, in vivo*	laboratory and greenhouse	Gonzales (1982)
Trichoderma spp.	*Pythium*	vegetables	*in vitro, in vivo*	greenhouse and field	Cuevas *et al.* 2005
Trichoderma ghanense, T. harzianum	*Plasmodiophora brassicae*	cabbage	*in vivo*	field	Cuevas *et al.* 2011

assays (Harman *et al.*, 1989). No single mode of action of *Trichoderma* species against fungal pathogens is known. As a rule, the greater the number and diversity of methods used by the organism to inhibit the pathogen, the more successful biological control is expected (Cook and Baker, 1983). The following are the modes of biocontrol action of *Trichoderma*:

Mycoparasitism

Mycoparasitism (*in vitro*) and enzyme-mediated antibiosis (*in vivo*) were the principal mechanisms of biocontrol of *T. harzianum* against *Rhizoctonia solani* causing sheath blight of rice (Cumagun and Ilag, 1997a). Mycoparasitism was evident with the coiling of the hyphae of *T. harzianum* along the hyphae of *R. solani*. Parasitism of sclerotia was very evident in an agar plate environment but the phenomenon was questionable in natural soil environment (Cumagun and Ilag, 1997b). Mycoparasitism expressed by species often results in nutrient rich media but in some cases these species are probably not mycoparasitic in nature (Rudakov, 1978). A recent study by Alvindia and Natsuaki (2008) examined the potential of *T. harzianum* as a biocontrol agent of crown rot pathogens isolated in the Philippines. Their results showed that *T. harzianum* directly parasitized and killed *Thielaviopsis paradoxa, Colletotrichum musae, Fusarium verticillioides, Clonostachys byssicola, Curvularia pallescens* and *Penicillium oxalicum in vivo.*

Antibiotic production

Trichoderma known for its mycoparasitic activity against several fungal plant pathogens is aided by the production of different chitinases, ß-1,3-glucanases and proteases and cellulase. These extra cellular enzymes such as ß-1,3-glucanase, chitinase and cellulase are effective in disrupting the mycelium of plant pathogenic fungi (Elad *et al.*, 1982; Samuels, 1996). Production of volatile compounds was not detected on the four isolates of *T. harzianum* that were tested *in vitro* against *R. solani* (Cumagun *et al.*, 1997b). Coconut smell is typical of *T viride* isolates (Rifai, 1969; Bissett, 1991), suggesting the presence of volatile compounds that are inhibitory to pathogen growth (Dennis and Webster, 1971).

Competitive saprophytic ability

Competition for substrates is the most important factor for fungi as is competition for light in the case of evolution of plants (Garrett, 1956). Rice and rice-based cropping systems involve growing two or three crops of rice or upland crops in the same field in rotation with rice. Unspecialized pathogens of rice and other crops such as *R. solani* causing sheath blight in rice-based cropping systems are usually present in stubbles during fallow and pose a threat to the succeeding component crop. The pathogen carry-over capacity of the cropping systems depends upon the survival ability of the pathogen in stubbles after rice in the soil (Mew and Rosales, 1985). Certain studies have shown that microbial degradation of crop residues or stubbles may lead to the control of this type of pathogen.

Trichoderma is capable of degrading straw with mycoparasitic ability against several plant pathogenic fungi (Chet and Henis, 1985; Chet 1987). In rice, *Trichoderma* is responsible for the reduction of inoculum potential of *R. solani* by decomposing rice straw and stubbles after rice harvest. Since *R. solani* also infects crops after rice, i.e., mungbean and corn, *Trichoderma* could also be efficient in controlling diseases inflicted on these crops. Mew and Rosales (1985) found that *Trichoderma* colonized rice straw pieces in dry land soil but not those in irrigated soil. Moisture content of the soil plays an important role in the activity and survival of *Trichoderma* (Kredics *et al.*, 2003). Cumagun *et al.* (2009) also found that the higher the amount of inoculum of *T. harzianum* strain no 94-016, the higher percent colonization of rice straw in the soil. Further tests were carried out to compare the decomposition of rice straw on the surface and buried in the soil and the effect of moisture on soil decomposition. The depth of rice straw and moisture content are factors identified as significant in affecting crop residue decomposition. Buried rice straw inoculated with *T. harzianum* strain no 94-016 with watering on a daily basis provided better decomposition (Cumagun *et al.* (2009). Knowledge gained from this study could help better understand and design field application of the beneficial microorganisms for disease management.

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Induced resistance

Induced systemic resistance is believed to be one of the most important mechanisms of biocontrol effects of *Trichoderma* (Harman *et al.*, 2006). The mechanism for increased plant growth could be due to inhibition of minor pathogens (Salt, 1978) and the production of a growth stimulating factor (Windham *et al.*, 1986). Some *Trichoderma* can induce a systemic response in plants through the JA/ethylene signaling pathway, potentiating the expression of several plant disease related genes and enabling the treated plant to be more resistant to pathogen infection (Viterbo *et al.*, 2002). Some strains of *Trichoderma* induce resistance on plants against pathogens. Cucumber roots treated with *T. harzianum* showed higher activities of chitinase, ß-1,3-glucanases and peroxidase (Yedidia *et al.*, 2000). *Trichoderma* in commercial form (BINABTF-WP and BINABT vector) induced systemic acquired resistance in strawberry against *B. cinerea* (Ricard and Jorgensen, 2000).

According to Harman *et al.* (2004), there are three recognized pathways of induced resistance in plants using *Trichoderma*. Two of these pathways involve the direct production of pathogenesis-related (PR) proteins. The first pathway which involves the production of PR proteins is the result of the attack by pathogenic microorganisms. In the second pathway, PR proteins are produced as a result of wounding or necrosis-inducing plant pathogens. The third pathway is considered as being induced by non-pathogenic, root-associated bacteria (Harman *et al.*, 2004).

In the Philippines, a few studies on growth promoting effects of *Trichoderma* were conducted. Cuevas (2006) showed that the presence of the fungus in the soil in sufficient population resulted in the uptake of more mineral nutrients especially P and Zn available for plant use that increased crop growth and yield in the screenhouse and farmers' field. The same author and a co-worker also found out that the application of *Trichoderma* as a soil inoculant significantly increased rice yield by 20% (Cuevas and Bacalangco, 2005) .

In the 1980's, *Trichoderma* has not been found as endophytes of living plants (Petrini, 1986). Typically, *Trichoderma* species are thought of as being soil fungi, but Evans *et al.* (2003) discovered many *Trichoderma* species and other soil fungi such as *Clonostachys*, *Fusarium*, and *Cylindrocarpon* as well as unidentified basidiomycetes existing as endophytes in healthy tree bolls and pods of the cacao relative *Theobroma gileri*.

Enhancement of Biocontrol Ability of *Trichoderma*

There are constraints in using *Trichoderma* as biocontrol agents. *Trichoderma* colonizes in the spermosphere effectively but they do not survive well in the rhizosphere (Deacon, 1994). The same author observed that *Trichoderma* spp. are active only in some types of soil and season thus achieving only transitory localized dominance of the rhizosphere. For this reason, another constraint is the quiescent and inactive nature of *Trichoderma* spores in the soil and because of this, *Trichoderma* cannot be added as spores (Vidhyasekaran, 2004). Furthermore, numerous mechanisms were discovered *in vitro*, but the key current problem is whether these actually function in biocontrol systems.

Identification and asexual genetics of Trichoderma

The taxonomy of *Trichoderma* is problematic because of a lack of reliable morphological characters. Rifai's classical keys for the identification of the taxon (Rifai, 1969) recognized nine species which were considered to be aggregates of morphologically very similar but genetically heterogenous species (Bissett, 1991). Even with the revised morphological approach, difficulties in recognizing differences between species remain (Samuels, 1996). A population of *Trichoderma* (N= 42) isolated by molecular markers from rice fields in different provinces of Luzon was identified and characterized. By rDNA-ITS1 and Universally Primed-PCR (UP-PCR), the population that was identified

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morphologically into seven species had been differentiated only into two species: *T. harzianum* and *T. viride* with 40 isolates identified as *T. harzianum* (Cumagun *et al.*, 2000). This concurs with the report of Nagamani and Mew (1987) that *T. harzianum* is a dominant species in upland rice and a common species in rainfed and irrigated - lowland rice.

Most strains are not encountered in association with sexual stages and are considered to be strictly mitotic, clonal fungi. This apparent lack of sexuality is a hindrance to understanding interrelationships within and among *Trichoderma* species (Samuels, 1996). To improve biocontrol ability, asexual hybridization via protoplast fusion is the method of choice. The trait of biocontrol ability which is polygenically inherited will be more difficult to improve than the trait of pesticide resistance which is usually monogenically inherited. Sexual reproduction is known in *Trichoderma* and the only known teleomorphs are species of *Hypocrea* Fr. and closely related genera under Order Hypocreales.

Currently, the number of recognized *Trichoderma* species has tripled; reaching 100 during the past decade. This has caused difficulty in *Trichoderma* taxonomy and species identification. To identify all known *Trichoderma* species based on sequence analysis, Druzhinina *et al.*, (2006) developed modern tools for *Trichoderma* species identification: the oligonucleotide barcode program *TrichO*KEY version 1.0, and *Tricho*BLAST, the multilocus database of vouchered sequences powered by a similarity search tool including the application of the Genealogic Concordance Phylogenetic Species Recognition approach. These advances make it possible to identify all known *Trichoderma* species based on sequence analysis. The species *Hypocrea rufa* and its anamorph *T. viride* have been re-described and epitypified using phylogenetic analyses of the translation-elongation factor 1 α gene (Jaklitsch *et al.* 2006.)

Composts and Trichoderma

Addition of organic amendments that have no selective stimulatory effect on the pathogen increases suppressiveness. For example, densities of *Phytophthora* and *Pythium* propagules in soil were lower while those of *Trichoderma* were higher in soils amended with various organic materials (composted cotton in trash, composted yard waste, or cattle manure) than with synthetic fertilizer (Bulluck *et al.*, 2002).

There is a great need to develop alternative management systems that would enhance biocontrol ability of *Trichoderma*. Biological system management is a term proposed by Vilich and Sikora (1998) to describe the concept of managing relationships between the biological elements of the agroecosystem and the biological elements of the crop production system to develop new or altered crop production techniques that contribute to a self-regulating system. Soil suppressivity is an important concept in biological systems management. The antagonistic potential of microorganisms against soil-borne pathogens is an active component of a suppressive soil. Organic amendments such as green manures and stable manures have long been recognized to enhance biological control of soil-borne plant pathogens if applied before planting (Baker and Cook, 1974). The use of composts and plant residues or green manures for biofumigation is a potential strategy to provide biological control of plant diseases caused not only by root pathogens but even foliar pathogens as well.

Composts produced from biosolids are used widely as peat substitutes to reduce production costs in horticulture (Hoitink and Grebus, 1994). Beneficial effects of composts include increased plant yield and vigor, improved food quality, and improved soil fertility including suppression of diseases caused by plant pathogens (Hoitink and Keener, 1993). The natural disease suppressive effects of composts are due to increase in microbial biomass and activity not only of *Trichoderma* but other beneficial microorganisms as well. As an ideal food base for biocontrol agents, it aids in their introduction and establishment into the soil for sustained biocontrol activities of soil microbiota (Hoitink and Boehm, 1999).

Composting with the fungus *Trichoderma* as an activator is mainly utilized in rice as

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organic fertilizer in the Philippines (Cuevas, 1997). The use of this technology resulted in reduction of fertilizer use by 30-50% and an increase in rice and corn yield by 20% (Cuevas, 1997). Strains of *Trichoderma* with good cellulolytic and competitive saprophytic abilities are suitable for crop residue decomposition in rice-based cropping systems under Philippine conditions (Cumagun *et al.*, 2009). *Trichoderma* spp. play an important role both as biocontrol agents and decomposers (Papavizas, 1985). However, there is a competition between cellulose and chitinase produced by *Trichoderma*. As long as cellulose is present, *Trichoderma* would readily prefer to degrade it to chitin, the degradation of the latter being more complex (Kubicek *et al.*, 2001). Thus, in the presence of raw organic material, the biocontrol activity of *Trichoderma* spp. is usually greatly reduced. For this reason, decomposition of raw organic materials such as straw is crucial. Microbial degradation of crop residues or stubbles in fields planted after or before rice could lead to potential control of unspecialized chitin containing pathogens such as *R. solani. T. asperellum* (strain-34) when added to cork compost rendered the soil highly suppressive against *Rhizoctonia* damping-off of cucumber plants due to minimal levels of biodegradable substances (Trillas *et al.*, 2006). *T. asperellum* strain T34 provided protection of tomato plants against *Fusarium oxysporum* and iron toxicity through inhibition of the fungus' siderophore synthesis (Segarra *et al.*, 2010).

Ultra-violet irradiation

The drawbacks in biocontrol have led to efforts to enhance the biocontrol ability of *Trichoderma*. The simplest method of genetic modification of biological control agents is mutation by irradiation or chemical mutagenesis. This is done to induce fungicide resistance or to develop rhizosphere competent mutants (Abd-El Moity *et al.* 1982; Ahmad and Baker, 1987). This technique is however limited to random point modification of single genes and therefore not capable of large changes in the genome. Cumagun (2006) studied the effect of irradiation of *T. harzianum* on its biocontrol ability. Reduction in growth of *R. solani in vitro* due to volatile compounds produced by the irradiated mutant isolate was not significant compared to the wild type. Recently, a UV-irradiated strain of *T. harzianum* in pellet form comparable to the chemical fungicide Mancozeb against damping-off of vegetables was applied 2 weeks before planting in a farmers' field in Laguna province, Philippines. (Cuevas *et al.*, 2005)

Chitinase protein and genes expression of Trichoderma

Little is also known about the enzymatic mechanism of the fungus effect on the pathogen. Chitin was demonstrated by Cumagun and Ilag (1997a) as an enhancer of biocontrol ability of *T. harzianum* when added in sterile and natural soil. The fungus excreted chitinase when grown on chitin. The endochitinase gene of *Trichoderma* confers resistance to the transgenic plants (Lorito, *et al.*, 1998). Some reported the role of chitinase in the antagonistic action of *Trichoderma* on *R. solani* and *S. rolfsii* (Carsolio *et al.*, 1999). Woo *et al.* (1999) demonstrated that a strain of *T. harzianum* deficient in the ability to produce endochitinase provided better control of *R. solani. Trichoderma* chitinase genes may be used to improve the defence mechanism of plants. Lorito *et al.*(1998) transferred the *ecb42* gene of *T. atroviride* into tobacco and potato.This resulted in an almost complete resistance to *Alternata solani, A. alternata*, *Botrytis cinerea* and *R. solani*.

Production and formulation

In general, biological control agents are very fragile to hostile environmental conditions. Attempts have been made to overcome this problem through proper formulation. Pelleting using common starch (gaw-gaw) and rice bran at 1:3 ratio is the most practical and economical binding method for *Trichoderma* in managing sheath blight of rice in the Philippines (Cumagun and Lapis, 1993). The pelletized *Trichoderma* was found to be viable for more than three months under room conditions (Cumagun and

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Lapis, 1993). The pelletized *Trichoderma* has been used by Cuevas *et al.* (2001) in managing seedling diseases of vegetables caused by *S. rolfsii*. The same formulation was employed by Llaguno *et al.* (2008) in rapid decomposition of different types of garbage. The development of a stable, cost-effective and easy-to-apply biocontrol formulation is critical for the advancement of biological control of plant pathogens with introduced antagonists (Lisansky, 1985). The addition of soil amendments like peat and wheat bran is to increase the nutrient status of the soil and possibly for mycoparasitism to occur due to the fact that *Trichoderma* is a facultative or opportunistic mycoparasite which means that the fungus attacks living mycelium when furnished with an external food base (Henis, 1984). The addition of food base also prolongs the viability of stored biocontrol preparations. For example, Jensen *et al*. (1996b) recommended the temperature of 4^{o}C for the storage of *Gliocladium roseum* in a mixture of peat and wheat bran since the viability was stable for 23 weeks. Etebarian (2006) also added wheat bran with *Trichoderma* to better control charcoal stem rot in melon. Other types of formulations used under Philippine conditions include granules and liquid formulations (Cumagun and Ilag 1997a and 1997b).

Biocontrol Studies, Product Commercialization and Monitoring of *Trichoderma*

Studies on biocontrol with *Trichoderma* in the Philippines

A large amount of work on *Trichoderma* comprising of over 50 research projects over the last 30 years has been conducted in the Philippines (Table 2). Only a few of these (<10%) have been published at full length in scientific journals. All outputs have shown the potential of *Trichoderma* as a biocontrol agent. *Trichoderma* have often been used in the management of diseases of rice and solanaceous crops. Most isolates of *Trichoderma* used are not identified up to the species level. The majority of experiments were conducted both in the laboratory and greenhouse and both *in vitro* and *in vivo* but rarely in the field. This indicates that *Trichoderma* has not yet reached the farmers at the field level.

The control of damping-off pathogens of vegetables caused by *Pythium* spp., *Sclerotium rolfsii* and *Rhizoctonia solani* using *T. parceramosum, T.pseudokoningii* and a UV-irradiated strain of *T. harzianum* in pellet form at a rate of 100g/sq m 2 wk before seed sowing in beds or direct seeding in the field was demonstrated in a farmers' field in Laguna province, Philippines. The performance of the biocontrol agent was comparable with the fungicide mancozeb. Along with the reduction in damping-off disease of *Brassica chinensis* (pechay) and *Lycopersicon esculentum* (tomato) seedlings in *Trichoderma* treated plots is the increase in seed germination thus providing more seedlings for transplanting in the field. Field application of *Trichoderma* pellets in Benguet, a high altitude province in northern Philippines not only reduced damping-off disease caused by *Rhizoctonia solani* but also showed growth enhancement in *Apium graveolens* (celery) and increased fruit size of *Solanum melongena* (eggplant) compared with the use of mancozeb (Cuevas *et al.* 2005). Recently, three farmers in Buguias, Benguet, Northern Luzon, Philippines used *Trichoderma* microbial inoculant to control club root of crucifers. There was an immediate control of the disease in treated plots with *Trichoderma* and new infections were prevented resulting in yield increase whereas the club root incidence remained at high levels in plots treated with pesticides. The use of the biocontrol product also tripled the farmers' partial gross income (Cuevas *et al.*, 2011).

Basic research to product commercialization

Although basic research provides the backbone for the future, the issue of technological discontinuity between discovery and application has been raised frequently (Marshall, 1985). Once an effective biocontrol system has been found, identified and confirmed by a molecular technique, the next step should be the commercialization of the product. As a biocontrol researcher, it has become clear that solving the technological problems is perhaps the easiest part of developing biocontrol agents and systems. Legal aspects are also important to be

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considered for the development of the technology including protection of intellectual property. If the technology cannot be protected, few companies will be interested in developing it further (Harman, 2006). A list of *Trichoderma*-based biocontrol products is shown in Table 1. Seven commercial products come from Vietnam alone (Ha, 2010). Only a single case has been commercialized in the Philippine market for biocontrol of plant diseases. The formulated product is called "Biocon Microbial Inoculant" produced by Tribio Technologies Corp. as a biofertilizer for fritzie palm, eggplant, papaya, banana, pechay and tomato (Monsalud, 2008) but was passed on to another company BioSpark Corporation under the trade name Trichoderma BioSpark effective against damping-off of vegetables caused by *Pythium* spp., *Sclerotium rolfsii* and *Rhizoctonia solani* mango gummosis and durian dieback caused by *Phytophthor*a.

Monitoring of Trichoderma released into the environment

In the development of biocontrol agents, apart from risk assessment and product approval, there is a need for methods that facilitate monitoring of introduced microorganisms (Jensen *et al.* 1996a). Biological control of *Rhizoctonia* sheath blight of rice and the genetic diversity of the fungal biocontrol agent *Trichoderma* using molecular methods have been initiated in the Philippines (Sinohin and Plete, 1998; Cumagun and Ilag 1997a and 1997b; Cumagun *et al*, 2000). Monitoring of promising strains of *Trichoderma* upon release in the field by molecular markers is an essential requirement for a biocontrol product to be commercialized in the market (Jensen *et al.* (1996a). *Trichoderma* isolates by UP-PCR and rDNA-ITS1 analysis from four provinces in the Philippines have been characterized (Cumagun *et al*, 2000). One of the *T. harzianum* isolates with good cellulolytic and competitive saprophytic abilities was analyzed using single and pair-wise combination of UP primers in order to distinguish it from the forty isolates of *T. harzianum*. A suitable diagnostic marker was identified and this marker will be valuable in monitoring the isolate in field tests (Cumagun *et al*, 2000).

CONCLUSIONS

A substantial amount of work on *Trichoderma* comprising of over 50 research projects over the last 30 years has been conducted in the Philippines. Only a few of these (<10%) have been published at full length in scientific journals. *Trichoderma* have often been used in the management of diseases of rice and solanaceous crops. Most isolates of *Trichoderma* used are not identified up to the species level. Most experiments are conducted in the laboratory and greenhouse and rarely in the field. Similarly both *in vitro* and *in vivo* experiments have been conducted. The modes of action of *Trichoderma* are mycoparasitism, antibiosis, competition and induced resistance. Enhancement of biocontrol ability of *Trichoderma* in the form of composting, use of UV irradiation, proper product formulation and gene expression for the development of genetically modified biocontrol agent are essential strategies for full realization of biocontrol as an important component of plant disease management. Furthermore, accurate strain identification by molecular approach is a prerequisite to commercialization to safeguard intellectual property rights. Although many studies have been conducted on biocontrol in the Philippines, there is no continuity in sustaining these programs. Little work has been done on the mechanism of action of *Trichoderma*. To my knowledge, only one case of biocontrol agent has been commercialized in the Philippine market. The product is called Trichoderma BioSpark effective against damping-off of vegetables and some tropical fruit diseases. However, this technology has been documented as a success story not only in managing crop diseases but improving the income of farmers particularly in vegetable growing areas in the Philippines. There is a need to sustain the biocontrol programs using *Trichoderma* whose beginnings were promising in order to maintain the sustainability and productivity of Philippine agriculture.

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مدیریت بیماری‌های گیاهی و افزایش پایداری وبهره وری با *Trichoderma*: تجربه فیلیپین

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چکیده

Trichoderma جنسی از قارچ‌های دارای زادآوری غیرجنسی است که در همه انواع خاک یافت می‌شود. گونه‌های *Trichoderma* به عنوان آنتاگونیست‌های خاکزی و عوامل بیماری‌زای شاخ وبرگ و همچنین تخریب‌کنندگان موثر ضایعات سلولزی شناخته شده اند. افزون بر این، آنها دارای توانایی افزایش رشد گیاه و القای مقاومت نیز می‌باشند. همراه با میکوپارازیتیسم، آنتی بیوتیک‌ها و رقابت، مقاومت القا شده یکی از مهمترین مکانیسم‌های عمل *Trichoderma* بر علیه عوامل بیماری-زای قارچی گیاه می‌باشد. استراتژی‌های مورد استفاده برای تقویت توانایی بیوکنترل *Trichoderma* عبارتند از استفاده از کمپوست، تابش UVو مطالعات بیان ژن در مهندسی ژنتیک. از بین بیش از ۵۰ پروژه تحقیقی در فیلیپین که در این مقاله مروری به آنها اشاره شده است، تنها کمتر از ۱۰٪ آنها به صورت مقالات کامل در مجلات علمی به چاپ رسیده اند. از*Trichoderma* عمدتا برای کنترل در برنج و محصولات و سبزیجات خانواده سولاناسه استفاده شده است. بیشتر مطالعات به صورت in vitroو in vivo در آزمایشگاه و گلخانه و به ندرت در مزرعه انجام شده‌اند. عمده سویه‌های گزارش شده تا سطح گونه به ویژه توسط تکنیک‌های مولکولی شناسایی نشده اند که این کار برای تجاری کردن محصول ضروریست. در بین سویه‌های متعددی از *Trichoderma* که در فیلیپین برای بیوکنترل بیماری‌های گیاهی جداسازی شده اند، تنها یک سویه تجاری شده است. با این حال همین کار به ایجاد فناوری برای بهبود مدیریت بیماری‌های گیاهی و افزایش درامد کشاورزان انجامیده است. پیوستگی در حفظ این برنامه ها برای دستیابی به پایداری و افزایش بهره وری تولیدات کشاورزی با استفاده از *Trichoderma* ضروری می‌باشد.

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