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A Novel Strain for the Managements of Plant Diseases: *Trichoderma* Spp.

Madhu Prakash Srivastava
Centre of Excellence, Department of Botany,
University of Lucknow, Lucknow-226 007. (Uttar Pradesh), India

(Corresponding author: Madhu Prakash Srivastava)
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ABSTRACT: The development of biological control agents as a key component of integrated disease management has tremendous potential for application context for the reduction of losses from plant diseases. Several biological control agents can suppress diseases as effectively as fungicides, an input that is often prohibitively expensive to be of value to resource-poor farmers. In India, fungal biological control agents, such as species of *Trichoderma* is efficacious in reducing damage caused by pathogens on maize and cowpea in research station trials. *Trichoderma koningii* and *T. harzianum* were effective in controlling damping-off of cowpea caused by *M. phaseolina* and effective dosage and application methods have been standardized in greenhouse trials to control the disease. *F. verticillioides* is an endophytic fungus that enhances growth of maize, but becomes pathogenic to cause root and stalk rot, damping off and ear rot when the plants undergo stress. Two strains of *T. harzianum* and *T. pseudokoningii* have been shown to reduce the stalk rot phase caused by the pathogen. These two *Trichoderma* species can penetrate the plant, move systemically within the stalk to occupy the same niche as *F. verticillioides*, and competitively exclude the pathogen. In greenhouse trials, the two species reduced stalk rot either when introduced into the stalk through injured sites or after seed treatment. These are few examples that reveal biological control as an effective adjunct in integrated disease management. However, much more work needs to be done to demonstrate field efficacy of biological control agents, their persistence, safety, and commercial feasibility, before practical application of biological control agents for plant disease control in India becomes a reality.

Key words: Biological control, Disease management, Mechanisms, *Trichoderma s*p

I. INTRODUCTION

The world today is characterized by an exponential growth in world population industrialization, pollution, food production and depletion of our natural resource. If this trend continues unchanged, there is almost a unanimous consensus that the limits to growth on this planet will be reached sometime within the next one hundred years. The most probably result will be a rather a sudden and uncontrollable decline in both, population and industrial capacity, However. This doomsday scenario will materialize only if our present way of doing thing will not change. Since there are ample evidence of mankind's ingenuity and social flexibility, we can safely assume that it is possible to alter these growth trends and to establish a condition of ecological and economic stability that is sustainable far into the future. The introductions of new technologies hold the promise to raise the limits to growth.

Economics of crop production, economics of losses caused by a disease in a specific situation, and ease and cost of applying disease management methods determine the level of intervention that a farmer is willing to commit to realize gains from farming. Usually, the primary foundation for disease control is manipulation of the physical environment and utilizing host resistance. Biological control and synthetic fungicides provide further support to disease management. However, use of fungicides is being discouraged due to economic reasons and growing concern for environment and safety issues. Biological control is potentially a sustainable solution of plant diseases in African agriculture since its effect is long-term with few, if any, undesirable side effects

Bio-control, or Biological Control, can be defined as the use of natural organisms, or genetically modified, genes or gene products, to reduce the effects of undesirable organisms to favour organisms useful to human, such as crops, trees, animals and beneficial microorganisms.

This strategy of control is ecologically clean and compatible with different models of agriculture: organic, biological and integrated pest/pathogen management (IPM) programmes.

Biological control agents act against plant pathogens through different modes of action. Antagonistic interactions that can lead to biological control include antibiosis, competition and hyperparasitism (Cook and Baker, 1983). Competition occurs when two or more microorganisms require the same resources in excess of their supply. These resources can include space, nutrients, and oxygen. In a biological control system, the more efficient competitor, i.e., the biological control agent out-competes the less efficient one, i.e., the pathogen. Antibiosis occurs when antibiotics or toxic metabolites produced by one microorganism have direct inhibitory effect on another. Hyperparasitism or predation results from biotrophic or necrotrophic interactions that lead to parasitism of the plant pathogen by the biological control agent. Some microorganisms, particularly those in soil, can reduce damage from diseases by promoting plant growth or by inducing host resistance against a myriad of pathogens (Kerry, 2000). Efficient biological control agents often express more than one mode of action for suppressing the plant pathogens.

Several naturally occurring microorganisms have been identified as biological control agents of plant pathogens. Several microorganisms have been evaluated as sources of microbial-based products for use in agriculture, such as biofertilizers and biopesticides. Among the biofungicides, during the past 20 years, several yeast species have been widely investigated for control of postharvest fungal pathogens of different host species (Spadaro and Gullino 2004).

This paper deals with biological control of fungal diseases of crops with fungal species belonging to the genera *Trichoderma*.

Trichoderma

Trichoderma is a fungal genus that was described in 1794, including anamorphic fungi isolated primarily from soil and decomposing organic matter. Strains within this genus include a wide spectrum of evolutionary solutions that range from very effective soil colonizers with high biodegradation potential, to non-strict plant symbionts that colonize the rhizosphere. Species concepts within *Trichoderma* are very wide, which has resulted in the recognition of many infraspecific groups. Some groups of biotypes within this conglomerate are able to antagonize phytopathogenic fungi by using substrate colonization, antibiosis and/or mycoparasitism as the main mechanisms.

This antagonistic potential is the base for effective applications of different *Trichoderma* strains as an alternative to the chemical control against a wide set of fungal plant pathogens (Harman and Björkman 1998). As a consequence of the variety of activities displayed by the *Trichoderma* strain conglomerate, a large range of applications have been developed: the antagonistic potential is the basis for the effective control of a wide set of phytopathogenic fungi and the biodegradative capacity is a source of useful enzymes in different industrial sectors (Harman and Kubicek 1998).

Biodiversity of *Trichoderma*

Most of the *Trichoderma* species are morphologically very similar and were considered for many years as a single species: *T. viride* (Bisby 1939). Since new species were discovered, a consolidated taxonomical scheme was needed and Rifai (1969) proposed and defined nine morphological species aggregates. DNA methods brought additional valuable criteria to the taxonomy of *Trichoderma* which are being used today for studies that include identification (Lubbock *et al.* 2000) and phylogenetic classification (Lieckfeldt and Seifert 2000). Most isolates of the genus *Trichoderma* that were found to act as mycoparasites of many economically important aerial and soil-borne plant pathogens, have been classified as *T. harzianum* Rifai (Gams and Meyer, 1998). Due to the fact that the species *"harzianum"* is generally considered as a group made of mycoparasitic and biocontrol strains, and there is large morphological plasticity that results in character overlaps with other species, the identification of the species may be difficult. Several authors have reported a large genetic variability among *T. harzianum* isolates (Grondona *et al.* 1997). In fact, it has been demonstrated that at least four distinct species are present within the biocontrol *T. harzianum* aggregate: *T. harzianum s.str., T. atroviride, T. longibrachiatum* and *T. asperellum* (Hermosa *et al.* 2000). Coevolution of organisms antagonistic to pathogens results in many *Trichoderma* strains being inactive against fungi other than those against which they were originally selected. This is strongly advantageous in that they are less likely to act against non-target organisms, but it does mean that a new selection process must take place for each crop/pathogen combination (Grondona *et al*., 1997).

The use of *Trichoderma* species as biological control agents has been investigated for over 70 years but it is only relatively recently that strains have become available commercially.

Many *Trichoderma* strains, mainly *T. harzianum*, *T. viride* and *T. virens* (formerly *Gliocladium virens*), have been identified as having potential applications in biological control and a partial list of genera of plant pathogenic fungi affected by *Trichoderma* includes: *Armillaria, Botrytis, Chondrostereum, Colletotrichum, Dematophora, Diaporthe, Endothia, Fulvia, Fusarium, Fusicladium*, *Helminthosporium, Macrophomina*, *Monilia, Nectria, Phoma, Phytophthora, Plasmopara, Pseudoperonospora*, *Pythium, Rhizoctonia, Rhizopus, Sclerotinia, Sclerotium, Venturia, Verticillium,* and wood rot fungi (Monte, 2001).

Biocontrol agents are widely regarded by the general public as "natural" and therefore non-threatening products, although risk assessments must clearly be carried out on their effects on non-target organisms. Moreover, knowledge concerning the behaviour of such antagonists is essential for their effective use.

T. viride *T. harzianum*

Fig. 1. Different spp of *Trichoderma* found in India.

Mechanisms' of action

The choice of active *Trichoderma* strains is important in designing effective and safe biocontrol strategies. Many species of *Trichoderma* have multiple strategies for fungal antagonism, and indirect effects on plant health (such as plant growth promotion effects and fertility improvements) also vary. Some strains are potent antibiotic producers, and their suitability for use in biocontrol systems must be carefully assessed. However, many other active strains have no antibiotic capacity, and these are likely to be more useful in food production systems. *Trichoderma* biocontrol strains have evolved numerous mechanisms for both attacking other fungi and enhancing plant and root growth (Harman 2000). The colonization of the root system by rhizosphere competent strains of *Trichoderma* results in increased development of root and/or aerial systems and crop yields (Harman and Kubicek 1998). Other activities, like the induction of plant systemic resistance and antagonistic effects on plant pathogenic nematodes (Sharon *et al*. 2001), have also been described.

These facts strongly suggest that during the plant-*Trichoderma* interactions, the fungus participates actively in protecting and improving its ecological niche. The dual roles of antagonistic activity against plant pathogens and promotion of soil fertility make *Trichoderma* strains appealing alternatives to soil fumigation technologies such as methyl bromide. Strains of *Trichoderma* may also be aggressive biodegraders (Wardle *et al*. 1993) and act as competitors to fungal pathogens in their saprofitic phases, especially when nutrients are a limiting factor (Simon and Sivasithamparam 1989). Strains have been reported as promoting activities of nonpathogenic bacteria (Vrany *et al*. 1990) and mycorrhizal fungi (Calvet *et al*. 1993). In the 1990s, the ability of *Trichoderma* strains to synthesize substances inducing SAR-like responses in plants was shown (Enkerli *et al*. 1999). Molecules produced by *Trichoderma* and/or its metabolic activity also have potential for promoting plant growth (Yedidia *et al*. 1999). Application of the species *T. harzianum* to plants resulted in improved seed germination, increased plant size, and augment of leaf area and weight (Altomare *et al.* 1999). The scenario of combined systemic biofungicides and plant growth promoters has great market potential if the molecular basis of the activities can be identified.

The strong biodegradation and substrate colonization performances of *Trichoderma* strains is the result of an amazing metabolic versatility and a high secretory potential which leads to the production of a complex set of hydrolytic enzymes. Similarly, the mycoparasitic process is based on the secretion of a rich cocktail of cell wall degrading enzymes (CWDEs) able to hydrolize the cell wall of various hosts (Kubicek *et al.* 2001)

Among others, chitinases (de la Cruz *et al*. 1992), b-1,3- glucanases (Noronha and Ulhoa 1996), b-1,6-glucanases (de la Cruz and Llobell 1999), a-1,3-glucanases (Ait-Lahsen *et al.* 2001) and proteases (Suárez 2001) have been described as important components of the multi-enzymatic system of *Trichoderma* strains. Some of these proteins display strong antifungal activities when are applied *in vitro*, alone and/or combined, against plant pathogens (Harman 2000). Some lytic enzymes can be involved in both antagonistic and saprophytic processes providing an evolutionary advantage to strains with both biodegrading and antagonistic potential, for the efficient colonization of different ecological niches in soil. A principal role in mycoparasitism has been attributed to chitinases (Lorito 1998) and glucanases (Benítez *et al*. 1998). However, fungal proteases may also be significantly involved in cell wall degradation, since fungal cell walls contain chitin and glucan polymers embedded in and covalently linked to a protein matrix (Kapteyn *et al*. 1996).

The production of secondary metabolites by *Trichoderma* strains also shows great variety and application potential. *Trichoderma* strains seem to be an inexhaustible source of antibiotics, from the acetaldehydes gliotoxin and viridin (Dennis and Webster 1971), to alpha-pyrones (Keszler *et al*. 2000), terpenes, polyketides, isocyanide derivatives, piperacines, and complex families of peptaibols (Sivasithamparam and Ghisalberti 1998).

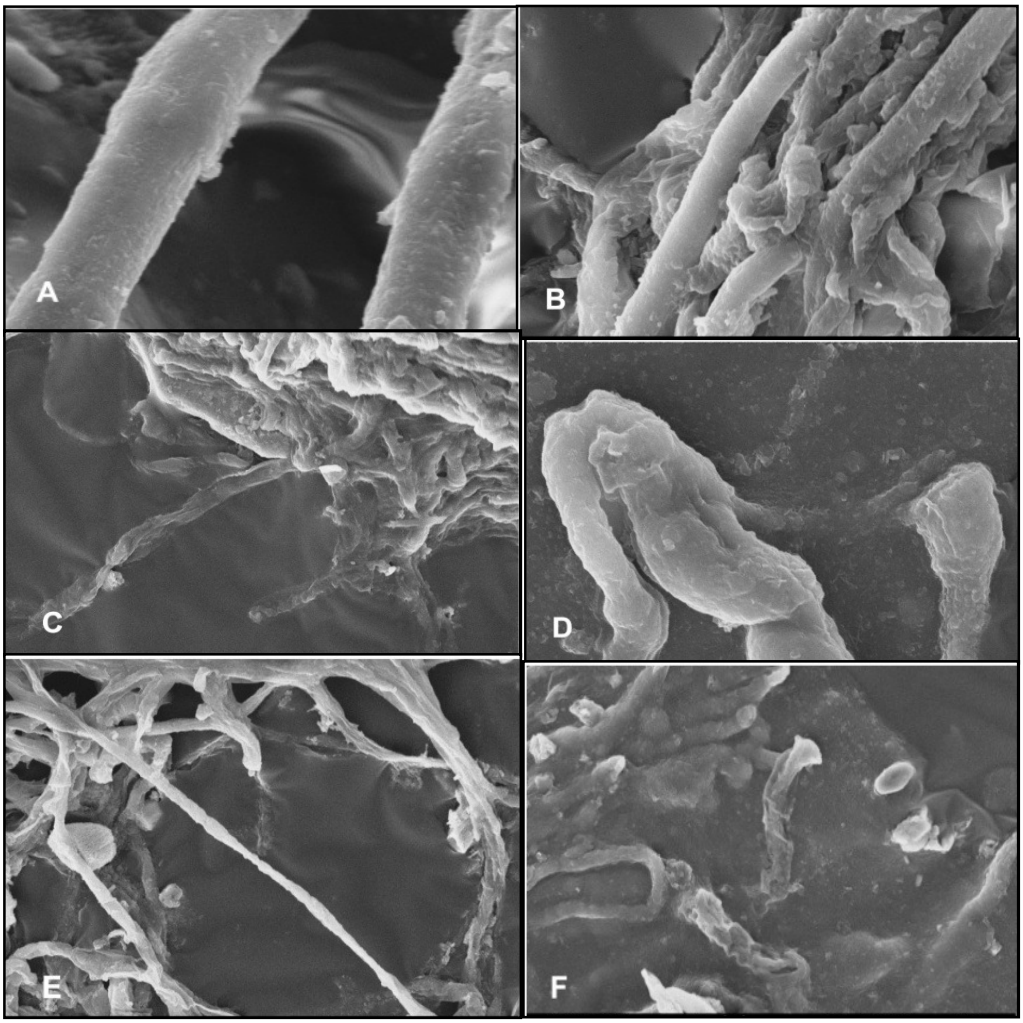

Fig. 2. Scanning electron micrographs (SEM) showing changes in mycelial structure of *Fusarium oxysporum* due to antibiotic effect of *Trichoderma* LUNS1 (A) Control,(B) Starting of lysis, (C) Cells started loosing turger, (D) Mycelium showing swollen tips, (E) Trifurcation of tips, (F) Distorted and disrupted hyphae**.**

All these compounds produce synergistic effects in combination with CWDEs, with strong inhibitory activity on many fungal plant pathogens (Lorito *et al.* 1996;). The potential of genes involved in biosynthetic pathways of antibiotics [e.g. polyketides, Sherman (2002) and peptaibols (Wiest *et al.* 2002)] with applications in human and veterinary medicine is not been explored yet.

Trichoderma is not only a good biocontrol agent, but also a general fertility promoter. In the absence of pathogens, application of appropriate *Trichoderna* formulations (following solarization and/or preceding fumigation with authorized and environmentally-friendly chemicals) can also serve to promote plant growth and crop precocity, increase fruit production and reduce chemical treatments.

Selection of *Trichoderma* strains

Once active strains have been identified with the *in vitro* assays, a further selection must be done by studying other factors such as: 1) activity *in vivo* using experimentally induced diseases on plants, 2) tolerance of high or low temperatures (necessary to survive other IPM treatments), 3) suitability for formulation as foliar sprays and/or soil enhancements (e.g. high sporulation levels, rapid growth in bulk conditions), 4) specificity (strains should be inactive against beneficial organisms and plant crops), 5) long-term survival in field conditions, 6) interactions with other *Trichoderma* strains already present in the cropping systems, 7) compatibility with agrochemicals used in the crop, or 8) shelflife and inoculum efficacy under commercial conditions.

***Trichoderma* Protein formulations**

Trichoderma protein extracts with high glucanase and chitinase activities, directly obtained from wild type strains, have been demonstrated to be effective as biofungicides. They can also be combined with chemicals (carbendazim, iprodione) with synergistic effects, and are stable enough to be considered for commercial application. We have investigated the antifungal properties of the proteins produced by *Trichoderma* species in laboratory and field conditions, defining the concentration of protein necessary to produce fungicide effects. It is recommended that any protein formulations contains at least one enzyme from each of the following classes: endochitinase, exochitinase, endoglucanase, exoglucanase (ß-1,3 plus ß-1,6), proteases and cellulalase (endocellulase). More than two enzymes from each class did not provide additional antifungal effect. In the field trials carried out with *Trichoderma* protein extracts, increased average weight of both roots and fruit per plant was detected in plots treated with *Trichoderma* proteins. The protein filtrates increased the total useful fruit weight by increasing the number of fruits of commercial size. These tests showed that *Trichoderma* chitinases and glucanases have no effect on the plant even if relatively large quantities are injected into plant tissues. CWDEs are not harmful to humans and animals, as indicated by eco-toxicological tests for registration of strains of *Trichoderma* for use as biocontrol agents in USA and the EU, and degrade into environmentally friendly residues. CWDEs can be effectively combined with whole-organism *Trichoderma* control, with considerable opportunities for synergism. CWDEs are particularly suited to post-harvest control. The genes coding for protein production can be introduced into suitable organisms to be used as cell factories for large-scale production of CWDEs.

***Trichoderma* genes**

Several methods for applying both biocontrol and plant growth promotion exerted by *Trichoderma* strains have recently been demonstrated and it is now clear that hundreds of separate genes and gene products are involved in the processes of mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance and inactivation of enzymes produced by pathogens (Monte 2001). Some of these genes have been identified, cloned from *Trichoderma* spp. (that offer great promise as transgenes to produce crops that are resistant to plant diseases since transgenic expression of high levels of chitinolytic and glucanolytic *Trichoderma* enzymes do not affect plant morphology, development or yield, or infection by arbuscular mycorrhizal fungi), patented and used to transgenically increase plant disease resistance (Lorito *et al.* 1998), but most of them are still unexploited for developing new biotechnologies.

Disease control

The antagonistic ability of *Trichoderma* species was discovered 70 year ago (Weindling, 1932). *Trichoderma* spp. are now the most common fungal biological control agents that have been extensively researched and deployed throughout the world. The primary mechanism of antagonism in *Trichoderma* is mycoparasitism. Lytic activity is the key feature responsible for the expression of mycoparasitism against several fungal pathogens. *Trichoderma* spp. are also good competitors in soil, and producers of volatile and non-volatile antibiotics to suppress target pathogens.

Due to their effectiveness and ease of production for commercial application, at least nine commercial biological control products based on *Trichoderma* species are manufactured and marketed in Belgium, Sweden, Israel, USA, Denmark, India, and New Zealand for use on several crops (Navi and Bandyopadhyay, 2002). In India too, considerable research has been done on biological control potential of *Trichoderma* spp. against several fungal pathogens that attack seeds, seedlings, roots, stems, and leaves of several crops. Some of the diseases that can be potentially controlled by *Trichoderma* species are listed in Table 1. Two specific examples are highlighted below to illustrate the potential biological control of seed and seedling blight of cowpea and stalk and ear rot of maize.

Fig. 3. Chickpea (*Cicer arietinum* (L)) seed treated with *Trichoderma* spp treatments (L2), Control: not treated (L1).

Table 1: Evidence for successful experimental use of *Trichoderma* spp. as a biological control agent of various crop diseases.

Host	Disease	Pathogen	Species of *Trichoderma*
Cowpea	Damping-off	*Macrophomina phaseolina*	*T. harzianum*, *T. koningii*
Cowpea	Web blight	*M. phaseolina*	*T. koningii*
Cowpea	Leaf smut	*Protomycopsis phaseoli*	*T.* spp.
Maize	Storage seed rot	*Gibberella fujikuroi* and *Aspergillus flavus*	*T.* spp.
Soybean	Brown stem rot	*Phialophora gregata*	*T. harzianum*
Potato	Stem canker and black scurf	*Rhizoctonia solani*	*T. harzianum*, *T. koningii*
Potato	Leak	*P. aphanidermatum*	*T. harzianum*
Tomato	Southern blight	*S. rolfsii*	*T. koningii*
Tomato	Basal stem rot	*S. rolfsii*	T. viridae
Lucerne	Damping-off, wilt and root rot	*R. solani* and *Fusarium oxysporum*	*T. harzianum*
Strawberry	Grey mould rot of fruits	Botrytis cinerea	*T. harzianum*
Cucumber	Damping-off	*R. solani*	*T.* spp.
Sugar beet	Damping-off and root rot	Several fungi	*T. harzianum*
Table beet	Damping-off	*Pythium aphanidermatum*	T. harzianum
Avocado	Root rot	*Phytophthora cinnamomi*	*T. harzianum T. hamatum*
Garlic	White rot	*Sclerotium cepivorum*	*T. harzianum*
Tobacco	Damping-off and root rot	*R. solani* *F. solani*	*T. harzianum*

Seed and seedling blight of cowpea

Several diseases affect cowpea (*Vigna unguiculata* Walp., Papilionaceae) during its growth and development from the time the seed germinates in soil to the time when seeds are produced and harvested. Some of these diseases are amenable to biological control while others are not. Seed decay and seedling damping-off cause serious losses in cowpea (Emechebe and Shoyinka, 1985).

Among the several pathogens associated with these seed and seedling diseases, *Macrophomina phaseolina* (Tassi.) Goid is prevalent in the Sudano-Sahelian areas where cowpea frequently suffers from moisture stress. In addition to seedling diseases, the pathogen also causes ashy stem blight or charcoal rot. *M. phaseolina* is extremely plurivorous and causes diseases in more than 300 plant species. This soil-inhabiting fungus survives for several years as free sclerotia in soil and in infected plant debris. Under favourable infection conditions, the pathogen propagules around the spermosphere and hypocotyl colonize the seed, hypocotyl and epicotyl leading to pre- and post-emergence damping-off of seedlings. In other words, seed decay and damping-off appear early during plant growth in a localised part of the plant. Therefore, disease control methods targeting the seeds have been useful in managing the disease. Seed treatment with systemic fungicides such as benzimidazole compounds is effective in controlling the disease (Kataria and Sunder, 1985). However, these fungicides are not generally available to resource-poor farmers who are the major cowpea producers.

Biological control of seed decay and damping-off of cowpea has been demonstrated using species of *Trichoderma* as antagonists (Adekunle *et al.*, 2001). *T. harzianum* Rifai, *T. koningii* Oudem and an unknown species of *Trichoderma* were tested at different doses to determine the efficacy of the antagonists. The plant stand was significantly improved when seeds were treated with *T. harzianum* and *T. koningii* compared to untreated seeds. Although the protection with the antagonists was lost over time, *T. harzianum* was more effective than *T. koningii* since the protection with the former lasted longer than the latter. Several formulations of the antagonists were also evaluated. The antagonists were grown in liquid culture, harvested, dried in an oven at 30^{o}C for 48 hours, and powdered in a blender. The powdered antagonist was suspended in water, and the aqueous suspension used to prepare two formulations: suspension with a sticker (Tween 20) and with cassava starch as an adhesive. The powdered antagonist was also transformed into concentrated slurry with water and uncooked cassava starch powder. Seeds were treated for different duration with each of these formulations. Generally seed treatment with the slurry formulation was not effective in reducing the disease, while soaking the seeds for 10-40 minutes in the aqueous suspension of the antagonists amended with cassava starch significantly reduced the disease.

Seed application requires only a small quantity of a biological control agent and can be easily combined with fungicidal seed dressing to enhance the efficacy of both for controlling diseases (Cook, 2000), as has been suggested for cowpea-*M. phaseolina* system (Alagarsamy and Sivaprakasam, 1988). Of course, the fungicide added in the biological control formulation should not be toxic to the biological control agent nor should it be expensive.

Seed dressing is a technology appropriate for African farming systems, and cottage industry production units have been shown to be economically feasible for meeting local or small-scale demands (Cherry *et al.*, 1999). The feasibility of a local biopesticide with *Trichoderma* depends on several factors. The raw materials, adhesive and production substrates need to be plentiful and cheap. The *Trichoderma* isolate would have to be quite robust and grow quickly on local substrate such as rice hulls or coconut shells. The risk of inadvertently increasing potential human pathogen along with the biological control agent must be very low. The dose response cannot be too stringent or safeguards would have to be developed for 'under-dosing' or 'over-dosing'. If these conditions can be met, then development of *Trichoderma*-based seed treatment in Africa will be attractive. *Trichoderma* populations in soil would probably increase with external introduction, particularly in acidic soils in West Africa.

Stalk and ear rot of maize

Species of *Fusarium* belonging to the section *Liseola* can cause seedling diseases, root rots, stalk rots and ear rots of maize in the field, as well as post-harvest storage rots. *Fusarium verticillioides* (Saccardo) Nirenberg (*F. moniliforme* J. Sheldon) and other anamorphs belonging to the teleomorph *Giberella fujikuroi* (Sawada) Ito in Ito and K. Kimura, are most frequently isolated from maize plants. Interest in the disease stems from the concern that infection of grain by *G. fujikuroi* can lead to loss of grain quality and potential production of fumonisin and other harmful mycotoxins (Munkvold and Desjardins, 1997). *G. fujikuroi* is comprised of several mating populations. Among these, those belonging to mating population A are considered as *F. verticillioides*. Other species infecting maize are *F. proliferatum* (Mats.) Nirenberg ex Gerlach and Nirenberg and *F. subglutinans* (Wollenw. and Reinking) Nelson, Toussoun and Marasas.

Members belonging to mating population A are more potent producers of fumonisin and are found more frequently on maize compared to mating population F (e.g., *F. thapsinum* Klittich, Leslie, Nelson and Marasas), which produces little or no fumonisin (Leslie *et al.*, 2001). *F. verticillioides* is closely associated with maize throughout the plant's life living as an endophyte within the plant right from seedling to grain harvest, often without causing any visible symptoms. While many infected plants remain free of symptoms, damage in others can be dramatic.

The fungus is transmitted through seed infection that results from vertical spread of the endophytic phase from stalk to the grain. Seed infection cannot be controlled by fungicide sprays since it is transmitted internally through the plant. The fungus also survives in plant debris on the soil surface, and free ambient spores can infect the stalk through the adventitious roots and the ear via the silk channel. Insects play an important role in moving the fungus and opening infection sites in maize stalks and ears (Munkvold and Desjardins, 1997). At the same time, *F. verticillioides* has been shown to attract insects to the plant (Schulthess *et al.*, 2002) resulting in a critical feed-back loop of infection and damage. Thus, control of endophytic *F. verticillioides* may exert a collateral effect of reducing attractiveness and susceptibility of the maize plant to insects.

Fumonisin related restrictions for trade have led to a renewed interest in finding strategies to reduce the levels of contamination of maize with the toxin. Currently, host-plant resistance, insect-pest control, and good storage practices are the major strategies for stalk rot and ear rot management. Biological control of stalk rot (Sobowale, 2002) and storage rot (Bacon *et al.*, 2001) by means of *Trichoderma* spp. has also been explored in order to reinforce other management tactics. Sobowale (2002) isolated 52 fungi from different parts of maize plants and tested these against *F. verticillioides* initially in *in vitro* tests. Seven of these fungal isolates, all belonging to *Trichoderma* spp., were further tested against *F. verticillioides* in artificially-infested stalks. *T. harzianum* and *T. pseudokoningii* Rifai were found to occupy the same niche as *F. verticillioides* and were able to competitively displace the pathogen. These two antagonists were able to move within the stalk to internodes further away from the point of introduction to the sites where *F. verticillioides* existed. Significantly, it appeared as if the antagonists sensed and tracked *F. verticillioides* in the stalks. Recovery of the pathogen from stalks co-inoculated with antagonists was significantly lower than from stalks in which the antagonists were absent. However, introduction of the antagonists into stalks was ineffective and did not protect against accumulation of fumonisins in grains.

The potential of *T. harzianum* and *T. viride* Persoon: Fries to reduce mycotoxin-producing potential of *F. verticillioides* in grain store has been further explored by Bacon *et al.* (2001) and Calistru *et al.* (1997). The latter authors suggested that the aggressive behaviour (towards *G. fujikuroi*) demonstrated by *Trichoderma* spp. could be partly explained by the liberation of extracellular enzymes by these fungi. An isolate of *T. viride* showed amylolytic, pectinolytic, proteolytic and cellulolytic activity. Although management of *Fusarium* stalk rot and grain spoilage in storage are potentially amenable to biological control, more work is required to test the biological activity of various agents, the different potential delivery mechanisms for biological control agents, and the practical feasibility and economy of this approach

Impact and prognosis for the future

The early beliefs that biological control agents offer more variable and less effective protection than fungicides have been refuted (Harman and Taylor, 1990). However, to achieve successful biological control, good knowledge of the host-pathogen-environment interaction is required in specific agroecosystems in which the biological control agent has to act. The interactions between microbial biological control agents, the target species to be controlled, the host and the environment can be complex and require a good research foundation prior to attempting formulation. The development of stable, cost-effective, easy-to-produce and easy-to-apply formulations of biological control agents is another critical research step in order to achieve successful biological control of plant diseases. This is particularly true for resource-constrained situations under which agriculture is practiced in Africa. Commercial use of biological control agents for plant disease control is not yet a reality in Africa, unlike the situation with biological control of insect pests that has seen spectacular successes. The final step in the development of microbial biological control agents for disease control in Africa will be to identify and define the economic and policy environment needed for successful increase and deployment of each agent. Depending on the agent, the options could be 1) cottage industry i.e. village/regional production as private or public enterprise; 2) nationally organised production at central laboratories, subsidised by public funding; 3) internationally organised production either as a one time, donor funded program, or as a business enterprise picked up and exploited by existing private sector companies. For the latter to occur, it is often necessary to obtain patents for the formulation or the isolate, thereby stabilising the proprietary status and securing the agent as a viable investment against the costs of commercialisation. It is important that development of biological control options for plant diseases does not stop at the research laboratory. Commitment to development of this technology for deployment in Africa is needed at policy level, requiring that as research laboratories enter into biological control agent testing, a conceptual framework for moving the agent to the field be part of the development agenda.

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