



FUNGI: AS POTENTIAL BIOCONTROL AGENTS

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ABSTRACT

Biological control is the adjustment in cultural practices, which either terminates a niche or encourages competitions of the pathogens to fill the niche and also provide the crop with a better means to resist, tolerate or escape the pathogens. Several species of *Trichoderma*, *Gliocladium*, *Penicillium*, *Cylindrocarpon*, *Chaetomium*, *Brachysporium*, *Botrytis*, *Absidia* and *Fusarium* have greater biocontrol capabilities when mycelial preparation of germinated conidia is added in soil against plant pathogens. Fungal cell walls are complex structures constituted mainly of polysaccharides such as chitin, β -glucan and proteins. *Trichoderma* species is extensively used for controlling several soil and seed borne fungal plant pathogens. *Trichoderma* species is mainly preferred to other fungal biocontrol agents owing to the antagonistic properties include the competition for key nutrients and space, inducing host-plant resistance, production of antibiotics and hydrolytic enzymes, viz. hemicellulases, cellulases, chitinases, proteases and β -1, 3-glucanases that are involved in the process of mycoparasitism.

KEYWORDS: Fungi, Antagonist, Biocontrol Agents

When an alien microorganism is introduced into areas devoid of natural antagonists, the new microorganism may increase in population and other the original biological balance on the host surface. This potential problem is averted by introducing a second mechanism selected for its specificity to antagonise. Thus, any process, occurring naturally or done artificially which affects the relationship between microorganisms in such a way that the natural biological balance is advantageously altered, can be regarded as biological control (Nigam and Mukerji, 1988). According to Garrett (1956) defined biological control as "any condition under which, or practice where survival or activity of any pathogen is reduced through the agency of any other living organism (except man himself), with the result that there is reduction in the incidence of the disease caused by the pathogen". Biological control is the adjustment in cultural practices, which either terminates a niche or encourages competitions of the pathogens to fill the niche and also provide the crop with a better means to resist, tolerate or escape the pathogen or in other words biocontrol of disease provide the crop a better and healthy atmosphere for survival.

Now a major challenge is felt in the field of plant pathology to introduce or develop some new disease control strategies, as the more traditional chemical control measures have become obsolete. The release accumulation and persistence of chemicals (generally fungicides and pesticides) into the environment over a period of time especially in the soil and aquatic

ecosystem has hazardous effect on plant and animal life. The greatest overall potential for an increased role of biological control agents, but in exploiting or ever-increasing understanding of the role of various cultural practices on general biological activities in crop ecosystems. An appreciation of ecological intervention is a prerequisite for the success of any biocontrol undertaking. To control plant pathogens biologically, it is necessary to select the biocontrol agent, understand the mechanism involved in the control and optimize the delivery of the agent at or near the pathogen. An important factor to consider is the inoculum potential of the biocontrol agent. In some cases mycelium of the agent is more effective inoculum rather than the non-germinated conidia, eg. *Trichoderma harzianum* Rifai, *T. viride* Pers. Ex Fr., *T. atroviride* Karst., *T. koningii* Oudem, *T. pseudokoningii* Rifai, *T. hamatum* Rifai, *T. lignorum* (Tode) Harz, *T. reasei* E. G. Simmons, *T. virens* (Miller, Giddens & Foster) v. Arx, *T. aureoviride* Rifai, *T. citrinoviride* Bisset, *T. asperellum* Samuels, lieckfeldt & Nirenberg, *T. effusum* Bissett, Kubicek & Szakacs, *T. fertile* Bissett, *T. flavofuscum* Bissett, *T. fasciculatum* Bissett, *T. konilangbra* Samuels, Petrini & Kubicek. *T. longibrachiatum* Rifai, *T. piluliferum* Webster & Rifai, *T. polysporum* (Link ex Fries) Rifai, *T. strictipilis* Bissett *Gliocladium roseum* Bainer, *Penicillium notatum* Westling, *Penicillium nigricans* Thom, *Penicillium fuscum* (Sopp) n. comb, *Penicillium waksmanii* Zaleski, *Penicillium felutanum* Biourge, *Penicillium adametzii* Zaleski, *Penicillium harqueii* Bainer et Sartory,

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Penicillium canescens Sopp, *Penicillium charlesii* Smith, *Penicillium daleae* Zaleski, *Penicillium brevicompactum* Diercks, *Cylindrocarpon didymium* (Hartig) Woll., *Absidiopsis spinosa* Lendner, *Brachysporium obovatum* Hughes *Botrytis terrestris* Jensen and *Chaetomium aureum* Chivers have greater biocontrol capabilities when mycelial preparation of germinated conidia are added in soil (Lewis and Papavizas, 1983; Lewis and Papavizas, 1984a; 1984b). Use of chlamydospores of the fungal agent can also be advantageous as they can withstand unfavourable conditions for a long period. However, dormant resting spores in the soil are likely to be unacceptable in biocontrol, unless there are certain abiotic factors like moisture and temperature, which help the spores to germinate in soil. In the present review paper, the fungi are discussed and its role as potential biocontrol agents against soil-borne phytopathogen is reviewed and summarized.

MECHANISM OF BIOCONTROL AGENTS

Like all other organisms, fungi are influenced by changes in their environment. The microbial antagonism that is seen in biological control of plant pathogens is broadly based on the categories of competition for key nutrients and space, parasitism which may be by the production of volatile or non-volatile antibiotics and hyperparasitism. All of these mechanisms may operate together or independently and their activities can result in suppression of microbial plant pathogens. The non-pathogenic microorganisms which are already present in the soil play a major role in preventing pathogen from becoming established on the same site or substrate. Once these non-pathogenic colonize and pathogens are established in or on the substrate, they must rely on vigorous competition, hyperparasitism and antibiosis of potential plant pathogens to retain their grip on the substrate. Upper horizon of the soil profile has the greatest abundance of organic matter, microbes and plant roots. This is the most active zone of microbial activity. Some times lysis of vegetative fungal hyphae takes place by lytic enzymes produced by other soil microorganisms or by the germination lysis phenomenon. In latter phenomenon, dormant spores are stimulated for germination and subsequently the germ tubes are lysed by the action of soil microbiota eg. In *Phymatotrichum omnivorum*, *Thelaviopsis basicola*, *Macrophomina phaseolina* and *Fusarium solani* (Papavizas and Lumsden, 1980).

Nutrients usually are necessary for the pathogens before infection and if they are used up by other microorganisms. It will be difficult for the pathogen to

survive and infect the host. Fokkema and Lorbeer (1974) found that the growth of *Alternariaporrigerm tubes* on onion leaves was reduced by both *Aureobasidium pullulans* and *sporobolomyces roseus*. The lesion mycelial growth of *Septoria nodorum* on wheat leaves was reduced by *A. pullulans*, *S. roseus* and *Cryptococcus laurent* to less than half (Fokkema *et al.*, 1976). Plant roots continuously interact with the soil micro flora. The net positive or negative microbial interaction results in a selective microbial population in and on the root. The composition of the rhizosphere plays a key role in determining the susceptibility or resistance of a plant to infection. Root exudates play an important role in the selective stimulation of microbes around seedling roots.

Modes of Antagonism

The scientific evidence signifies that several soil and seed borne diseases of fungal pathogen can be controlled by many fungi. The various modes of antagonism include competition, mycoparasitism and antibiosis.

(A) Competition

Competition of the biocontrol agents with other microorganisms or fungal pathogens can be triggered by abiotic factors. The fungal pathogens (dormant propagules of fungi) living in soil and on plant surface which suffer the scarcity of certainly available nutrients which may ultimately result in nutrient competition between fungal pathogen and *Trichoderma* spp. (Adams and Ayers, 1982; Garrett 1956). Besides *Trichoderma* spp. releases compounds known as siderophores which would complex the micronutrient iron and render it as unavailable form, this leads to competition.

(B) Mycoparasitism

One of the mechanism involved in the antagonistic activity of *Trichoderma* spp. against a range of economically important pathogens is the mycoparasitism (Dennis and Webster, 1971b), where the production of fungal cell wall-degrading enzymes by *Trichoderma* spp. is believed to play a role. It was shown that extracellular lytic enzymes, β -1, 3-glucanases, chitinases and proteases excreted by *Trichoderma* spp. were involved in cell wall degradation of plant pathogens. *Trichoderma* directly attacks the plant pathogen by excreting lytic enzymes such as β -1, 3-glucanases, chitinases and proteases (Haran *et al.*, 1996 a; Hjeljord and Tronsmo, 1998). Because the skeleton of fungal cell wall contains chitin, glucan and protein, enzymes that hydrolyze these components have to be present in a successful antagonist in order to play a significant role in cell wall lysis of the pathogen (Lorito *et al.*, 1994;

Carsolio *et al.*, 1999). β -1, 3-glucanases play in nutritional role in saprophytes and mycoparasites (Sivan and Chet, 1989). Several chitinolytic enzymes have been reported in *T. harzianum* (De la Cruz *et al.*, 1992). These include endochitinases, exochitinases and 1, 4- β -N-acetylglucosaminidases. Enzymatic degradation of chitin is generally involved in many biological processes, such as autolysis (Vessey and Pegg, 1973), morphogenesis and nutrition (Griffin, 1994), and in addition to mycoparasitism plays also a role in relationships between fungi and other organisms such as plant-fungus interactions (Mauch *et al.*, 1988). In addition to chitin and glucans, fungal cell wall contains proteins. Thus, the production of proteases also plays an important role in lysis of cell wall of fungal pathogen during mycoparasitism (Flores *et al.*, 1997). There are several factors which directly or indirectly affect mycoparasitism such as:

(a) Age of the host

Sometimes the parasitism is restricted by mechanical barriers put forth by host mycelium eg. *Mucor recurvis* develops a protective layer outside the hyphae to protect itself against *Rhizoctonia solani* (Butler, 1957).

(b) Nutrition

Host susceptibility is mainly influenced by nutrients. Butler (1957) observed that the kind of carbohydrates supplied to the medium affected the degree of parasitism of *R. solani* on *M. recurvis*.

(c) Temperature

The optimum temperature for mycoparasitism is generally 25-30°C (Butler, 1957).

(d) Moisture

Water content of the soil influences some mycoparasites of *Phytophthora erythroseptica*. It is suggested that soil moisture conditions may influence the susceptibility of the fruit tree root stocks to *Phytophthora* attack. Low levels of oxygen occur during flooding or water saturation. This may increase the susceptibility of the host to infection by *P. eryptogea*. Thus water management has greater potential to minimize or avoid losses due to *Phytophthora* in fruit orchard (Broadbent and Baker, 1974: 1975).

(e) pH

The degree of interaction was more at pH 5.5 between *Pythiumdebaryanum* and *R. solani*.

(f) Light

Abd-El-Moity and Shatla (1981) noted that mycoparasitism of sclerotia of *Sclerotium cepivorum* by *Trichoderma harzianum* occurred in complete darkness.

(C) Antibiosis

This mechanism involves the secretion of both volatile and non volatile anti-microbial metabolites, which are portable in nature and thereby suppressing or killing the fungal pathogen (antibiosis) around the surrounding area (Corley *et al.*, 1994; Horvath *et al.*, 1995). Dennis and Webster (1971a) found that many isolates of *Trichoderma* spp. produce non volatile antibiotics, which were active against a range of pathogen. Biological activity of antagonistic fungi may partially be associated with production of antibiotic (Estebarian *et al.*, 2000; Faull *et al.*, 1994). The production of antibiotics; Trichodermin (Godfredsen and Vangedal, 1965), ergokonin (Kumeda *et al.*, 1994), viridin (Chet *et al.*, 1977; Grove *et al.*, 1996) and viridin fungin A, B and C (Harris *et al.*, 1993) by *Trichoderma* spp. have been reported. The principal functions of these antibiotics are cell membrane disruption, inhibition of metabolic activity and stimulation of plant defence system.

SOME IMPORTANT FUNGAL BIOCONTROL AGENTS

(A) *Trichoderma*

The genus *Trichoderma* is a cosmopolitan and saprophyte fungus with several mycoparasitic species (Cook and Baker, 1983). Most of the species of *Trichoderma* possess rapid growth rate, sporulation abundantly, compete well with other soil microorganisms and produce antibiotics (gliotoxin and viridin), lytic enzymes and volatile compounds (acetaldehyde). Elad *et al.* (1983) observed that chitinase and cellulase activity increase when *T. harzianum* attaches itself to *R. solani* or *S. rolfisii*, either through clamps or by coils. Thus, *Trichoderma* has both antibiotic as well as enzymatic properties useful for biocontrol.

Ridout *et al.* (1988) demonstrated that the extracellular chitinase, β -1, 3-glucanase and protease enzymes produced by *T. harzianum* are responsible for mycoparasitism against phytopathogen. *T. album*, *T. hamatum*, *T. harzianum*, *T. lignorum*, *T. polysporum*, *T. pseudokoningii* and *T. viride* are known to control growth of numerous fungi. Hyphae of *Trichoderma* can also attack rhizomorphs, sclerotia and other types of fruiting bodies of fungi (Papavizas, 1985). Weindling (1932) was the first to report *Trichoderma* as a mycoparasite of *R.*

solani, *S. rolfisii*, *Phytophthora* spp., *Pythium* spp. and *Rhizoctonia* hyphae. *Trichoderma* is able to act as a parasite of certain other fungi without necessarily making physical contact. An isolate of *T. harzianum* directly attack on the mycelium of *R. solani* (Hadar *et al.* 1979). The some strain of *T.harzianum* when applied in wheat bran culture of *R. solani* infested soil, effectively controlled damping-off of bean, tomato and other seedlings (Elad *et. al.*, 1980). Backman and Rodriguez-Kabana (1975) developed a system of mass culture and delivery of *T. harzianum* utilizing diatomaceous earth granules impregnated with molasses. They found that Southern blight of Peanuts caused by *S. rolfisii* was reduced by 42% when the inoculum was applied in fields after 70 to 100 days of planting, increased yield of the crop. *Trichoderma* spp. has also been used to control *Hetrobasidion annosum* decay in trees. Delivery systems for *Trichoderma* spp. affect their biocontrol ability. For instance, chitin was added with inoculum as a seed coating, it increased the efficiency of *T. hamatum* as a seed treatment of peas and radish (Harman *et al.* 1981). Nelson *et al.* (1983) demonstrated that composted hardwood bark was an excellent vehicle for growth and delivery of suppressive *Trichoderma* spp. Excellent results were obtained when lignite and still age were used as substrate and carriers for *Trichoderma* biocontrol agents of various pathogens (Jones *et al.*, 1984).

(B) *Fusarium* and Other Antagonists

Fusarium spp. is generally soil-borne plant pathogens but are antagonistic and mycoparasitic. Some species of *Fusarium* are mycoparasites of rust and smuts. Uredospores of *Puccinia graminis f. sp. tritici* show reduced germination and uredosori were slow to develop on wheat seedling (Prasad and Sharma, 1964). Ergot disease caused by *Claviceps purpurea* on *Lolium perrene* was controlled by *F. heterosporum* (Hornok and Weloz, 1983). *Rhizoctonia solani*, a well known plant pathogen of many economic crops is parasitized by *F. oxysporum*, *F. udum* and *F. semitectum* showing penetration and coiling of hyphae of the host (Arora and Dwivedi, 1980). Even lysis and chlamydospores formation in *R. solani* occur due to these parasites (Gupta *et al.*, 1979). *F. merismoides* is a mycoparasites on spores of *Pythium ultimum* in natural soil (Hoch and Abawi, 1979).

Gliocladium roseum parasitizes *Phomopsis sclerotoides* causal organism of black root rot of cucumber. The pathogen is killed by the toxic enzymatic activity of *G. roseum* (Moody and Gindrant, 1977). Damping-off of cotton seedlings caused by *Pythium ultimum* and *R. solani* can be controlled by direct

parasitism and production of antibiotics by *G. virens* (Howell, 1982). *Penicillium oxalicum* forms a positive layer on seeds against diseases caused by species of *Aphanomyces*, *Fusarium*, *Pythium* and *Rhizoctonia*. It also utilizes seed exudates so that colonization by other organisms is suppressed (Windels, 1981). *Peniophora gigantia* replaces the pathogen *Heterobasidion annosum* through competition for substrates (Rishbeth, 1978). *Pythium oligandrum* suppresses damping-off by *P. ultimum* on many plants through mycoparasitism (Martin and Hancock, 1982).

CONCLUSION

The extra cellular enzyme systems of *Trichoderma* and other antagonistic fungi important for competition and hyperparasitism can remain active even under environmental conditions unfavourable for mycelial growth, which suggests the possibility of species/ strains important for better stresstolerance. The application of hyperparasitic *Trichoderma* species/ strains with improved tolerance of unfavourable environmental conditions could increase the efficacy of biocontrol against fungal phytopathogen under a wide range of environmental conditions. Considerable work on biocontrol of various plant diseases has already been done and the results are promising for further exploring and useful products. Combinations of microorganism may be effective. It would also be beneficial, to better understand both the mechanism of biocontrol and the induced host response. The application methods for biocontrol agents should be efficient and further evaluated. The advantage of biological control is numerous and successful biological control of plant diseases has already been achieved.

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