English Version

Abstract

Biological control is considered a natural tool and an ecological alternative to overcome the problems caused by chemical methods for the protection of plants. From the fungi with potential of antagonism, the genus *Trichoderma* is one of the most researched and studied. This fungus is a microorganism naturally found in soil, which presents an important ecological function, and participates of several processes in this mean. The objective of the work is to develop a discussion about aspects of the biological control, specially in relation to effects of the fungus *Trichoderma* spp. in the biocontrol of pathogens of agricultural crops. With the discussions along the texts, it is considered that the biological control is a system which is being target of frequent and continuous investigation being an important tool for the control of several problems of phytossanity which occur in agriculture. Among the agents which are noteworthy in the investigations is the fungus *Trichoderma* spp., in which it was verified action as biocontrol agent and in some cases as growth promoter.

Aspects of the effects of the fungus *Trichoderma spp.* in biocontrol of pathogens of agricultural crops

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Introduction

Biological control is defined by Baker and Cook (1974) as "a redução da densidade de inóculo ou das atividades determinantes da doença causada por patógenos ou parasitas nos seus estados de atividade ou dormência, por um ou mais organismos antagônicos realizada tanto naturalmente como através da manipulação do ambiente, hospedeiro ou antagonista, e ainda, por introdução em massa de um ou mais antagonistas"[1]. Later, Santos (2008) report that these authors redefined the biological control as "a redução da soma do inoculo ou das atividades determinantes da doença provocada por um patógeno, realizada por ou através de um ou mais organismos que não o homem"[2].

Biological control is, still, defined as the use of specific microorganism, which interferes together with pathogenic organisms and plagues which cause diseases in plants, being a natural tool and an ecological alternative to overcome problems caused by chemical methods for the plant protection (CHET et al., 1997).

The principles of the mechanisms of biological control are based in antagonist relations as: competitions, predation, amensalism, parasitism, resistance induced or by the production of metabolites which inhibit the development of the other. Parasitism seems to be the most efficient mechanisms of antagonism in biological control, since hiperparasited depend on their hosts for survival and are subject to the same environmental variations (GRIGOLETTI Jr. et al., 2000). Amensalism is when a species harms the other trough substances which it produced and releases, without, apparently, have benefits in the process. This is a behavior which represents well the action of *Trichoderma* in soil.

Thus, the biological control of phytopathogens is noteworthy because it is not restricted to study only the relation between the phytoparasite and the host, but also because it considers the antagonists, the microflora adjacent to the place of infection and the relation of this complex in the entire ecosystem (BETTIOL, 1991).

1 The reduction of the density of inoculation or of the activities determinant of the disease caused by pathogens or parasites in their states of activity or dormancy, by one or more antagonist organisms performed either naturally or trough the manipulation of the environment, host or antagonist, and even, by introduction in mass of one or more antagonists.

2 The reduction of the sum of the inoculum or of the determining activities of the disease caused by a pathogen, performed by or trough one or more organisms but man.

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Best practices of soil management, according to Nazareno et al. (2001), promote the diversification of the microflora which inhabits the soil, increasing the efficiency of the biological control. It is verified that, in these systems more balanced ecologically, it is increased the population of fungi which control their equivalent plant pathogens, as *Rhizoctonia solani* Kuhn, and insects as the fungus *Metarhyzium* sp., among others.

According to Zilli et al. (2008), the difficulty of control of the pathogens present in soil by chemical, physical and cultural methods is a challenge which demands alternatives to the producer. In Brazil, *Sclerotium rolfsii* Sacc. and *R. solani* are widely disseminated, being considered important to several crops, since the control of these pathogens becomes hard due to their capacity in forming structures of resistance and survive as saprophyte. In this context, the biological control of these diseases is a promising strategy as compound of the integrated management, enabling even to add value to the product offered to the market.

The objective of the work is to develop a discussion about aspects of the biological control, specially in relation to effects of the fungus *Trichoderma spp*. in the biocontrol of pathogens of agricultural crops.

General aspects of the fungus *Trichoderma* spp.

From fungi with potential of antagonism, the genus *Trichoderma* is one of the most researched and studied. The fungus *Trichoderma* spp., according to Menezes et al. (2009), is a microorganism naturally found in soil, which presents an important ecological function, since it participates in the decomposition and mineralization of the vegetal residues, contributing with the availability of nutrients for the plants. It is considered, also, a natural fungicide, which reduces even until 100% the chances of any fungus to reach the crop. *Trichoderma* is a fungus with fast growth, and that is the great advantage of using it as agent of biocontrol in large scale.

According to Samuel and Hadavi (1996), the genus *Trichoderma* corresponds to the anamorphic phase of the genus *Hypocrea*, which belongs to the class of Mitosporic fungus, subclass Hyphomycetes, order Moniliales, family Moniliaceae. This genus is, according to Gams and Bisset (1998), one of the most interesting groups of antagonic fungi, since the species of *Trichoderma* are cosmopolitan, being found in most of the soils.

In study with potato crop cultivated in soil infested with *Trichoderma harzeanum*, the researchers of the Grupo de Pesquisa em Produção Vegetal (Group of Research in Vegetal Production) of the Universidade Estadual do Centro Oeste (State University of Mid West) – UNICENTRO (PR) verified images of the fungus in the reproductive phase in soil, according to Figure 1.

According to Domsh et al. (1980), the main morphological characteristic of the fungus *Trichoderma spp*. is the presence of mycelium, initially with white color and with fast growth. With the development, it becomes cottony and compact with green tufts. The color of the colony depends on the amount of conidia and of their pigments. These authors also observed that, in the culture mean, the colonies of *Trichoderma* spp. grow fast, presenting, initially, smooth and almost translucent surface, becoming later fuzzy or compact. The colony color exhibits several tones of green (sometimes, very light – ice color), and may be influenced by the pH of the crop mean.

The fungus *Trichoderma* spp is common in soil and its structures comprehend ramifications called hyphae, mycelium and one structure of air propagation called spore. It presents an important ecological function, since it participates in the mineralization of the rests of leaves, stems and roots which are already dead, helping to maintain the environmental balance (GAMS e BISSET, 1998).

Trichoderma spp. as pathogen biocontroller

Trichoderma spp. are fungi of free live, which have high interactivity in environment of roots, soil and leaves. This fungi are still noteworthy for producing a wide range of antibiotics and substances capable of parasitize other fungi SIVASITHAMPARAM and GHISALBERTI, 1998).

Besides that, they may compete with other organisms, for instance, for essential exudates,

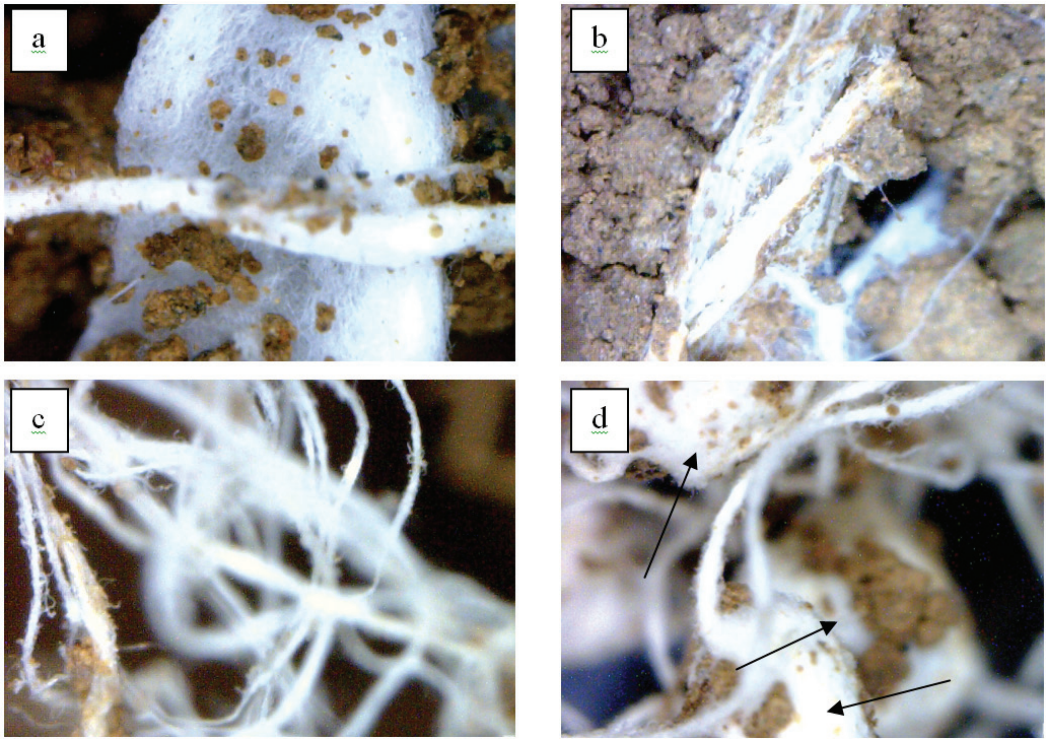

Figure 1. Images of the fungus *Trichoderma harzianum*, in reproductive phase 60 days after the inoculation in soil. Parts a, b, c are details of the mycelium, part d is the detail of the mycelium with reproductive structures. Images conceded by the Grupo de Pesquisa em Produção Vegetal – UNICENTRO-PR, 2009.

expelled by seeds, which are capable of stimulating, in soil, the germination of propagules of pathogenic fungus of plants (HOWELL, 2002) and, more frequently, may compete with microorganisms of the soil for nutrients and space (ELAD, 1996). These fungi are even capable of inhibiting or degrading pectinases and other enzymes which are essential to pathogenic fungi of plants, as *Botrytis cenerea* for the penetration in the leaf surface (ZIMAND et al., 2006).

Besides the hability of *Trichoderma* to prejudice or inhibit directly the growth of plant pathogens, recent discoveries indicate that they may also induce systemic and locate resistance to a variety of plant pathogens. Some strain have also substantial influence in the plant growth and development (YEDIDIA et al., 2001).

The efficiency of the fungus *Trichoderma* spp. have been demonstrated in laboratory work, green house and field, being efficient as biocontroller agent of pathogens in different situations. This antagonist has showed better acting together with pathogens which inhabit soil and which are less specialized, as *Pytium* sp., *Fusarium* sp., *Rhizoctonia* sp. and *Sclerotium* sp., since, as it is also a soil inhabitant, its characteristics of antagonism are better expressed in this environment (MELLO, 1991; MELO, 1996).

The fungus *Trichoderma* spp. competed for the exudates releases by seeds in the process of germination of propagules of phytopathogenic fungi (HARMAN et al., 2004). Ethur (2006) affirms that the competition is one of the main characteristics of isolates of *Trichoderma* spp. used as biocontrol agents, since only this way they will have capacity of developing in the rhizosphere.

According to Spiegel and Chet(1998) this fungus produced a variety of antifungal metabolites, including antibiotics and enzymes which degrade the cellular wall of the vegetables, different from those produced by bacteria. The action of these metabolites

combined with the one of benefic bacteria may expand the spectrum of action in the plague control.

The induction of localized and systemic resistance occurs in all or in most of the plants in response to the attack of microorganisms, physical damages due to insects or other factors, or due to the treatment with several chemical inductors and due to the presence of non pathogenic Rhizoctonia (KUC, 2001).

In relation to the induced resistance in plants by species of *Trichoderma,* De Meyer et al. (1998) emphasize that this is a characteristic that has not been studies so much if compares to what is already known of the induction of response by the rhizobacteria, since the researches with *Trichoderma* focused factors which are liked to direct effects in other fungi, specially micro parasitism and antibioses.

According to the same authors, these effects are probably the first clear demonstrations of induction of resistance by *Trichoderma* which was published by Birigama et al. (1997), who demonstrated that the soil treatment with the lineage T – 39 of *Trichoderma harzianum* produced leaves of bean plant resistant to diseases which are caused by pathogen fungi *B. cinérea* and *Colletotrichum lindemuthianum,* although T - 39 was present only in roots and not in leaves.

Similar studies were performed with a wide variety of plants, including either dicotyledonous or monocotyledonous, and with different species and vines of *Trichoderma*, according to which is verified in Resende et al. (2005). According to Aaltomare et al. (1999), the capacity of the lineage T – 22 of *T. harzianum* of inducing systemic resistance to pathogens in corn is particularly notable. There are no similar reports of resistance being induced in this crop by any other microorganism capable of association with roots in commensalism or symbiosis. Therefore, the capacity of inducing the resistance to a range of diseases – which are caused by several types of plant pathogens (including fungi, bacteria and virus) – in a variety of plants, seems to be a characteristic generalized in this type fungal genus.

This fungus also presents the capacity of inducing defense actions by the plant itself. According to Howell (1987) these actions Consist in the expression of a set of proteins known as RPs (resistance proteins) and in the release of phitoalexines, which protect plants against fungal infections. Plants pre-immunized with *Trichoderma* are capable to resist to diseases caused by pathogenic fungi.

When applied to the root system, *T. harzianum* - T39, was able to induce systemic resistance in dicotyledonous plants (De MEYER et al., 1998). Howell (1987) still emphasize that the genus *Trichoderma* seems to have the capacity of inducing resistance in a great variety of plants of species as tomato, tabacco, lettuce and cotton.

According to Lynch (1986), *Trichoderma harzanium* Rifai is a strong straw colonizer and, as other *Trichoderma* species, it presents a strong activity of cellulase, being, for this reason, of interest in the efficient control of several pathogens of the root. It is still necessary to consider that the vegetal residues can be substrate for soil organisms which are so benefic as antagonist to plant growth and that, when a substrate is induced in the soil, there is lower potential to develop the producers of pathogens and phytotoxines.

According to Harman et al. (2004), the mechanisms involved in the induction of plant resistance are still of low comprehension and, in order to increase its efficiency and reliability it is necessary more field research with this technique.

Growth promotion in plants by *Trichoderma* spp.

The growth promotion in plants caused by soil microorganisms occurs due to the action of several factors which are still unclear. It may involve production of vegetal hormones, production of vitamins or conversion of materials in a useful form to the plant, absorption and translocation of minerals and pathogen control (MELLO, 1991).

According to Howell (1987), besides their different characteristics, the presence of *Trichoderma* spp. in soil makes the nutrients soluble, enabling higher and faster absorption. Therefore soils containing *Trichoderma* spp. presented higher humus content, coming from lignin which is decomposed by this microorganism. Thus, it occurs the increase of the fresh matter in crops which are treated with *Trichoderma* spp.

According to Harman (2000), fungi are favored by the presence of high levels of roots, which are easily colonized. Some vines are highly capable of colonize and grow in roots in the same way they develop. The vines which colonize better the rhizosphere may be added to the soil or in seeds by any method. After they get in touch with the roots, they colonize the root surface or cortex, depending on the strains.

The growth promotion in plants promoted by the isolated T-22 of *T. harzianum* is in its ability to solubilise many nutrients important to the plant (ALTOMARE et al., 1999). In soil, macro and micronutrients suffer a complex dynamic balance of solubilization and insolubilization, strongly influenced by the pH and by microflora, which affect its accessibility to the absorption by plant roots.

With the application of nitrogen in soil together with the use of the isolate T-22 of *T. harzianum,* Harman (2000) verified that initially it did not occur differenced between areas with or without nitrogen, but in the presence of the nutrient, adult plants present higher averages of diameter of stalks and grain yield and silage.

Chang et al. (1986), when using soil treatment with suspension of conidia of *T. harzianum* observed promotion of plant growth trough the weight of dry matter superior to the control, in beans of 10%, in the radish of 8%, in tomato of 37%, in pepper of 42% and in cucumber of 93%.

Kleifeld and Chet (1992) also obtained positive results in the promotion of growth in cucumber (dry matter) by isolated of *T. harzianum*, both in autoclaved soil (26%) and in non autoclaved soil (43%). Ouley et al. (1993) observed that only one of the isolates of *T. harzianum* presented better result (5%) than the control concerning the growth (dry matter) in lettuce.

The isolated T-22 of *T. harzianum* was effective in the induction of formation of roots in tomato, as much as the commercial hormone, and it occurred an increase in soybean and corn roots treated with the referred isolate and higher productivity of pepper when compared to non treated control (HARMAN, 2000).

Sivan and Harman (1991), when treating corn and cotton seeds with the isolates T12, T95 and T22 (fusion of the earlier two) of *T. harzianum* observed that the isolate T22 promoted root growth, in relation to the control, of 31% in corn and 60% in cotton.

Isolates of *Trichoderma* spp. may act as growth promoters, even in the presence of mycohrrizal fungi. Calvet et al. (1993), when using treatments with application of the mycohrrizal fungi *Glomus mosseae,* antagonist *T. aureoviride* and the phytopathogenous *Pythium ultimum* in *Tagetes erecta,* observed that *G. mosseae* + *T. aureoviride* increased the weight of dry matter in 100% and leaf area in 55%, comparing with the control.

The contribution of *Trichoderma* spp. in the germination is also proved. Kleifeld e Chet (1992) observed positive action of isolated of *T. harzianum* in treatment of seed and soil in the germination (5 days after seeding) of bean in 77 and 100%, radish in 58 and 100%, tomato in 100 and 70%, pepper (12 days after seeding) in 90% and cucumber 90 and 100%, respectively, which evidenced that the variations in the results, in this experiment, come from the form of treatment and of the crop. Ousley et al. (1993) observe that some isolates of *T. harzianum* aided and others inhibited.

Rhizosphera is a zone with high microbial diversity both in number and in the activity of organisms and complex interaction of the microorganisms with the roots. Kennedy (1998) emphasizes that the plant development is influenced by the interaction microorganisms-roots, in which beneficial effects can be found: symbioses, antibioses, biocontrol, nitrogen fixation, promotion of growth, soil stabilization and availability of water; harmful effects: disease and phytotoxity; neutral or variable effects: nutrient flow, release of enzymes, allelopathy and competition.

Santos (2008) concluded also that the ability of the fungi of the genus *Trichoderma* in the promotion of development of plants may be related to its capacity of symbiotic association to plant roots, together with its decomposing action, enabling nutrients promptly absorbable by plants and, still, ability as agent of biological control, inhibiting the action of phytopathogens, which may interfere in an indirect way in the normal development of the plant. Altomare et al. (1999) postulated that the promotion of growth in plants, at least in the case of the isolated

T - 22 lies on their capacity of solubilization of nutrients which are important to the plant.

The interference of *Trichoderma* spp. in plant growth and in the increase in productivity occur, according to Harman et al. (2004), due to their capacity of colonizing roots. However, Louzada et al. (2009) add that this fungus develop direct action in the control of pathogens presenting only indirect action over the plant productivity and better soil exploitation by the root system.

Thus it is verified that, besides being used in control of pathogens the fungi of the genus *Trichoderma*, may also be used as agent promoter of the growth. This mechanism is referent to the plant development in general, including the beneficial effects in the seed germination, emergence and development of plantlets and production of grains and fruits (HARMAN, 2000). In relation to the effects of this fungus, Kleifeld and Chet (1992) described that the solubilized nutrients become available for absorption by roots and, thus, reduced the need of fertilization, phenomenon which according to Altomare et al. (1999), has been called increasing interest for research and practical application.

Final consideration

The biological control is a system which has been target of frequent and continuous investigation being an important alternative for the control of different problems of phytossanity which occur in agriculture. Among the agents which deserve emphasis in the investigations is the fungus *Trichoderma* spp., to which it was verified updates as agent of biocontrol and in some cases as growth promoter.

These characteristics make this fungus genus a potential alternative for the application in the crop management in agriculture, however, in a general way it is still necessary new studies aiming to deepen the knowledge about mechanisms of action and definition of the controlled and safe use of this kind of biological agent.

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