Pathogenic fungi and Bio-control agents: Competitive bio-assay research

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Abstract

Fungi of the genus Trichoderma have a track record of being antagonist to quite of a number of agricultural important pathogens. Trichoderma have some unique characteristics that make it scientifically proven and suitable bio-control agents against varieties of pathogenic organism infecting economic food crops. Trichoderma has the advantage of being environment friendly and not hazardous to the health of human beings, livestock, soil and environment. Competitive bio-assay experiment was carried out in the laboratory on the effects of Trichoderma species (T. atroviride P1 isolates, T. harzianum T22 isolates, T. viride) on some crop pathogens (Phytophthora cinnanerium, Botrytis cinaria and Rhizoctonia solani). Pure culture of Trichoderma and pathogenic fungi were replicated four times and arranged in a complete block design. The result of the experiment shows that Trichoderma species are strong competitor of P. cinnanerium, B. cinaria and R.solani. Within 72 hours, the Trichoderma species were able to grow and completely overlap the P. cinnanerium, B. cinaria and R. solani. This strong competitiveness indicated that Trichoderma species would effectively inhibit the growth of P. cinnanerium, B. cinaria and R. solani on the infected crop; thus the application of Trichoderma species in the control of P. cinnanerium, B. cinaria and R. solani infected crops.

Introduction

Biological control of disease/ pathogen is the application of natural enemies in the control/ eradication of the pathogen population. Biological control is an environmentally friendly, scientifically proven and effective means of mitigating pathogens or pests through the use of natural enemies. A world estimated loss due to crop diseases was up to 12%, while a loss due to post-harvest food spoilage was between 10 and 50%. Effective control of crop losses due to pests (micro-organism, insect and weed) therefore holds the keys for steady and stable food supply of the world. Amongst all effective and recommended controls of the crop pests, biological control holds a great promise for the future. Basically, biological control has the advantages of being environmentally friendly and not hazardous to the health of human beings, livestock and wildlife; especially now that the whole world is clamoring for IPM methods of pest control (Lorito *et al*, 2006; Woo *et al*, 2006; Olabiyi, 2009).

Fungi of the genus *Trichoderma* have a track record of being antagonist to quite a number of agriculturally important pests. It had been most effective bio-pesticides applied for crop protection since the era of traditional farming and nascent organic agriculture. *Trichoderma* have some unique characteristics that make it scientifically proven and suitable bio-control agents against varieties of pathogenic organisms infecting economic food crops. These are: non-toxic to human beings, livestock and wildlife; non-pathogenic organism on crops; compatible with other control methods (physical, chemical, cultural, planting of resistance variety); effective at low concentrations; easy and cheap to culture or produce; could be bottled or prepared in another easily distributable pack; *Trichoderma* is ubiquitous (Lorito, 1998; Olabiyi, 2009). *Trichoderma* is capable of producing secondary metabolites with antibiotic activity. Of recent, *Trichoderma* composted hardwood bark isolates, was reported to produce a metabolite (Harzianic acid) with antifungal and plant growth promoting activity. *Trichoderma* species have been formulated and used as bio-pesticides, bio-protectants, bio-stimulants and bio-fertilizer on a large variety of crops (Reino *et al*, 2008; Vinale *et al*, 2009). Objective of this study is to determine in-vitro competition bio-assay between *Trichoderma* species (*Trichoderma harzianum* P 1 isolate, *Trichoderma harzianum* T 22 isolate, *Trichoderma viride*) and pathogenic fungi (*Phytophthora cinnanerium, Botrytis cinaria* and *Rhizoctonia solani*)

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Material and methods

Preparation of** Potato Dextrose Agar **(PDA)
Dissolve 27g of Potato Dextrose Broth (PDB) and 15g Micro Agar in 1 litre of deionised water in an Erlenmeyer conical flask (2 litre capacity). Sealed properly with cork, autoclaved at 121^{o}C, and 15psi for 20 minutes. Allow the autoclaved media to cool and thereafter pour small quantity (20-25mls) into Petri dish inside the Lamina flow (sterilized condition and working tools). Cover up the Petri dish after solidification process.

Source of Trichoderma and pathogenic fungi
Pure culture of *Trichoderma* species and pathogenic fungi used for the study were obtained from Istituto per la Protezione delle Piante, CNR, Portici Italy. The *Trichoderma* species were *T. harzianum* P 1 isolate, *T. harzianum* T 22 isolate and *T. viride*; while the pathogenic fungi were *Phytophthora cinnanerium*, *Botytis cinarea* and *Rhizoctonia solani*

Introduction of bio-control agents and pathogens to PDA

Bio-control agents and pathogenic fungi were carefully introduced onto the PDA. There were 15 treatments, replicated 4 times fitted into randomized complete block desin. The treatments were *T. harzianum* P 1 isolate; *T. harzianum* T 22 ATCC isolate; *T. viride*; *P. cinnanerium*; *B. cinarea*; *R. solani*; *T. harzianum* P 1 isolate and *P. cinnanerium*; *T. harzianum* P 1 isolate and *B. cinarea*; *T. harzianum* P 1 isolate and *R. solani*; *T. harzianum* T 22 ATCC isolate and *Phytophthora cinnanerium; Trichoderma harzianum* T 22 ATCC isolate and *B. cinarea*; *T. harzianum* T 22 ATCC isolate and *R. solani*; *T. viride* and *P. cinnanerium*; *T. viride* and *B. cinarea*; *T. viride* and *R. solani* The experiment was carried out under Lamina flow and immediately after the setting up of the competition bio-assay; they were arranged in the incubator at 25^{o}C for 72 hours. Records of growth of each bio-control and pathogenic organisms were taken at every 24 hours. Picture of each treatment and treatment combinations were also taken at the 72nd hour.

Results

The results presented revealed the competiveness of *Trichoderma* species and pathogenic fungi. Table 1 shows the time interval at which *Trichoderma harzianum* (T22 isolate) grew over pathogenic fungi - *R. solani, Botrytis cinarea* and *P. cinnanerium*. It was evident that within 72 hours (3 days), *T. harzianum* (T22 isolate) hindered the growth of *R. solani, B. cinarea* and *P. cinnanerium*. It was evident that *Trichoderma* species inhibit the growth of *R. solani.* During the competition between *Trichoderma* species and *R. solani, Trichoderma* species proved to be aggressive competitor over *Rhizoctonia*. *Trichoderma* species grew faster and overlay on the pathogenic fungi (*R. solani.*). Similar trend was observed in bio-assay competition between *Trichoderma harzianum* (T 22 and P1 isolates), *T. viride* and *Botrytis cinarea* (Table 2). *Trichoderma* species grew very fast and then hindered further growth of the pathogenic fungi (*Botrytis cinarea*).

Table 3 shows the time interval at which *Trichoderma viride* grew over pathogrnic fungi - *R. solani, B. cinarea* and *P. cinnanerium* in the laboratory. It was evident that within 3 days, *T. viride* prevented the growth and development of *R. solani, Botrytis cinarea* and *P. cinnanerium*. *Trichoderma* species grew faster, overlay on *P. cinnanerium* and prevented its further growth and development. *Trichoderma* species proved to be aggressive competitor over *P. cinnanerium*. Table 4 shows the competitive bio-assay between *T. viride* and pathogenic fungi (*R. solani, B. cinarea* and *P. cinnanerium*). *T. viride* grew faster to inhibit further growth of the pathogenic fungi (*R. solani, B. cinarea* and *P. cinnanerium*). Table 5 elicits the time interval at which *T. harzianum* T 22 isolate, *T. harzianum* P1 isolate and *T. viride* suppressed the growth of the *Botrytis cinarea*. It was evident that within 3 days, *Trichoderma* species prevented the growth and development of *B. cinarea*. It was evident that *T. harzianum* (P1 isolate) grew very faster to suppress the growth of the pathogenic fungi. *T. harzianum* (T22 isolate) proved to be an aggressive competitor over *R. solani, Botrytis cinarea* and *P. cinnanerium* (Table 6).

Table 1: Competition assay between *T. harzianum* T22 isolate and pathogenic fungi (Figures are in cm)

Time (in hours)	T22 alone	T22 versus *Rhizoctonia*	T22 versus *Botrytis*	T22 versus *Phytophtora*
24	1.7 x 2.0	1.5 x 1.0	1.2 x 1.4	1.0 x 1.5
48	5.5 x 5.5	4.5 x 5.5	4.0 x 5.0	4.0 x 5.0
72	5.5 x 5.5	5.5 x 5.5	5.5 x 5.5	5.5 x 5.5

Table 2: Competition assay between *T. harzianum* P1 isolate and pathogenic fungi (Figures are in cm)

Time (in hours)	P1 alone	P1 versus *Rhizoctonia*	P1 versus *Botrytis*	P1 versus *Phytophtora*
24	0.9 x 0.9	1.2 x 1.1	1.2 x 1.2	0.9 x 0.9
48	1.8 x 2.0	2.0 x 3.0	2.3 x 2.6	2.0 x 2.4
72	3.0 x 2.8	4.0 x 5.0	3.5 x 4.5	3.5 x 2.8

Table 3: Competition assay between *T. viride* and and pathogenic fungi (Figures are in cm)

Time (in hours)	*T. viride* alone	*T. viride* versus *Rhizoctonia*	*T. viride* versus *Botrytis*	*T. viride* versus *Phytophtora*
24	2.0 x 1.5	1.6 x 1.5	2.0 x 2.0	2.0 x 2.0
48	3.5 x 5.0	3.5 x 4.7	3.6 x 5.5	4.0 x 5.0
72	5.5 x 5.5	5.5 x 5.5	5.5 x 5.5	5.5 x 5.5

Table 4: Competition assay between *Rhizoctonia* and *Trichoderma* species (Figures are in cm)

Time (in hours)	*Rhizoctonia*	*Rhizoctonia* versus T22 isolate	*Rhizoctonia* versus P1 isolate	*Rhizoctonia* versus *T. Viride*
24	0.9 x 0.9	0.9 x 0.9	0.9 x 0.9	0.9 x 0.9
48	0.9 x 0.9	0.9 x 0.9	0.9 x 0.9	0.9 x 0.9
72	1.3 x 1.2	1.0 x 1.0	1.0 x 1.0	1.0 x 1.0

Table 5: Competition assay between *Botrytis* and *Trichoderma* species (Figures are in cm)

Time (in hours)	*Botrytis*	*Botrytis* versus T22 isolate	*Botrytis* versus P1 isolate	*Botrytis* versus *T. Viride*
24	1.0 x 1.0	0.9 x 0.9	0.9 x 0.9	1.0 x 1.0
48	1.5 x 1.3	1.3 x 1.2	1.5 x 1.6	1.8 x 1.7
72	4.0 x 4.0	3.0 x 2.5	2.5 x 2.8	2.0 x 1.8

Table 6: Competition assay between *Phythophtora* and *Trichoderma* species (Figures are in cm)

Time (in hours)	*Phythophtora*	*Phythophtora* versus T22 isolate	*Phythophtora* versus P1 isolate	*Phythophtora* versus *T. Viride*
24	1.2 x 1.0	1.1 x 1.1	1.0 x 1.0	1.0 x 1.0
48	1.8 x 2.0	2.0 x 2.0	2.0 x 2.0	1.8 x 2.0
72	2.4 x 2.2	2.4 x 2.2	2.5 x 2.4	2.2 x 2.1

Discussion

The application of bio-control agents and/ or their metabolites for plant diseases control is one of the promising ways to reduce the dependence on chemicals in agriculture, particularly in crop production/ crop protection. In particular, *Trichoderma* are among the most effective bio-control bio-pesticides recommended for plant disease protection against plant diseases under organic agriculture. *Trichoderma* is listed both in Europe and USA as a pesticide permitted for use in organic farming (Woo *et al*, 2006; Olabiyi, 2004).

In recent decades, many bio-control agents have been used in plant protection. However, *Trichoderma* species have been recognized for a long period of time as registered commercial products and biological control agents for the control of plant diseases. Couples with this, is the potency of *Trichoderma* species to increase plant growth and development (Lorito *et al*, 2006; Woo *et al*, 2006). *Trichoderma* species are known

to involve in complex interactions with host plants and soil microbes. The mechanisms involved in the antagonism of *Trichoderma* species on the pathogen were reported to be competition for nutrient, induction of systemic resistance to pathogen, cell wall-lytic enzyme activity, mycoparasitism and antibiosis (Marra *et al*, 2006; Vinale *et al*, 2008; 2004; Lorito, 1998). *Trichoderma* is capable of producing secondary metabolites with antibiotic activity. Of recent, *Trichoderma* composted hardwood bark isolates, was reported to produce a metabolite (Harzianic acid) with antifungal and plant growth promoting activity. *Trichoderma* species have been formulated and used as bio-pesticides, bio-protectants, bio-stimulants and bio-fertilizer on a large variety of crops (Reino *et al*, 2008; Vinale *et al*, 2009).

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