In-vitro Evaluation of Indigenous *Trichoderma* Species for Bio-control of Faba Bean Root Rot Caused by *Fusarium solani*

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Abstract

Eight *Trichoderma species viz., T. oblongisporum, T. longibrachiatum, T. asperillum, T. cerinum (tomentosum) T. harzianum, T. atroviride, T. viride* and *T. hamatum* (seven of which were identified at molecular level) were isolated following standard procedure from soil samples taken in different parts of Ethiopia and evaluated *in-vitro* for their antagonistic potential against root rot of faba bean caused by *Fusarium solani*. Dual culture technique was used for the investigations in which a 6mm diameter mycelial disc of each of the antagonists and the pathogen were confronted on opposite sides of 9cm Petri dishes. Radial growth of the pathogen and the antagonist were recorded. All but one isolate was highly antagonistic that they covered and colonized the colony of the pathogen. There was statistically significant difference (P=0.0005) among *Trichoderma* isolates in inhibiting the pathogen colony. The overall inhibition effect of the antagonists on the pathogen's colony ranged from none to 50.5 %. Isolate of *T. asperillum* had the highest inhibition effect (50.5%) followed by *T. harzianum* (48.1 %), *T. hamatum* (47.7%), *T. viride* (46.3 %) and *T. longibrachiatum* (43.6%) which were not significantly different one from each other. Therefore, these local isolates could be further studied in the glasshouse (greenhouse) and field experiments and used in the biological control of the disease as one component of the integrated root rot management.

Introduction

Out of 1.2 million hectares appropriated to pulses in Ethiopia, 411,719 ha (34.31%) are covered by Faba bean (*Vicia faba* L.) with annual production of 446,850 tons (CSA, 2006). However, its production is constrained by several biotic and abiotic factors and Black root rot caused by *Fusarim solani* (Mart) Appel &Wollenw is one of the most important biotic stresses in the major faba bean growing areas (Tesfaye, 1995; 1999). *Fusarium solani* is a highly destructive pathogen of field grown beans (Akrami *et al.*, 2009). Annual yield loss due to wilt and root rots reach up to 70% in severe conditions on farmers' fields (Stewart and Dagnachew, 1967; Habtu and Dereje, 1985). The disease can cause complete crop loss in severe infection when favorable conditions prevail (Negussie *et al.*, 2008)

Management options for the disease include; rotation with non-susceptible crops, good soil drainage and use of disease free or fungicide treated seeds that may help reduce losses. However, there are currently no adequate control measures for *Fusarium* rots in the field (Agrios, 2005). Use of environmentally friendly biological control agents can more effectively control soil borne phythopathogens (Saleem *et al.*, 2000).

The development of biological control agents as key component of integrated disease management has tremendous potential for application in the African context for the reduction of losses from plant diseases. Several biological agents can suppress diseases as effectively as fungicides, an input that is often prohibitively expensive to be of value to resource poor farmers (Neuenschwander *et al.*, 2003). *Trichoderma* species in particular have promising antagonistic potential against a diversity of soil borne pathogens (Bajwa *et al.*, 2004). *Trichoderma* species are useful avirulent plant symbionts that act as biocontrol agents against phythopathogenic fungi via mechanisms of competition, rhizosphere competence, mycoparasitism, antibiotic and enzyme production, induced resistance and promoting plant growth (Chet and Inbar, 1994; Howell, 2003; Harman *et al.*, 2004;). General environmental concerns and safety issues about possible adverse effects of chemicals also necessitate the use of additional control methods to strengthen integrated disease management tools such as host resistance and cultural practices.

However, except for some attempts done on some imported bio-agents, the use of indeginous *Trichoderma* species as potential biological agents of plant disease control in Ethiopia has been limited. Importing biological agents, in addition to being a long process, causes loss of foreign currency. Moreover, variation in antagonistic abilities of *Trichoderma* spp and in resistance of pathogens to antagonists requires choosing isolates for specific applications (Bell *et al.*, 1982). The objective of this research was therefore to evaluate the potential of locally available *Trichoderma* species to be used as biological control agents for the management of faba bean root rot disease.

Materials and Methods

Source of *Trichoderma* species and pathogen cultures

Eight *Trichoderma* species were used for the experiments (table 1). Seven of the *Trichoderma* species were isolated from soils of Jima, Wellega, and SNNP using the soil dilution plating method. For each sample, 1g of air-dried fine soil was suspended in 10 ml of sterile distilled water which was then serially diluted to get dilutions of 1/100, 1/1000, 1/10000 and 1/100000. One ml of the diluted suspension was then aseptically plated on PDA(Potato Dextrose Agar) media in three replications and incubated at 25°C for 4 days. After 4 days, *Trichoderma* were identified based on visual and microscopic observation and the putative *Trichoderma* colonies were purified by two rounds of sub-culturing on PDA. These were then identified at molecular level at Vienna University of Technology, Austria (Temesgen, 2005). One species was obtained from Ambo Plant Protection Research Center (PPRC) laboratory.

The pathogen, *Fusarium solani*, was isolated from roots of infected faba bean plants grown in well developed sick plot at Ambo PPRC and showing typical symptoms of the disease. Portions of infected roots were cut; cleaned with running tap water; disinfected with potassium hypochlorite solution for a minute; rinsed with sterile distilled water twice and air dried on filter paper. These were then placed on PDA plates and incubated for 3 days at 25°C. Pure cultures were obtained by sub-culturing from the plates. The isolated pathogen and the *Trichoderma* species were maintained as pure culture at 4°C in refrigerator until used.

In-vitro evaluation of *Trichoderma* isolates

All the *Trichoderma* isolates were evaluated *in-vitro* for their antagonistic and inhibition potential against the faba bean root rot causing pathogen *F. solani* using dual culture technique with direct confrontation test.

Each of the eight *Trichoderma* isolates and *F. solani* were inoculated on to PDA medium separately and incubated for 4 days at 25 °C. After 4 days, mycelial discs (6mm in diameter) of each of the *Trichoderma* isolates were placed separately on one edge of a Petri-dish containing 20ml of fresh PDA. Mycelial discs of *F. solani of* the same size were put on the opposite side of the Petri-dish. Plates containing the pathogen only and the antagonist only were also prepared to serve as controls. A Completely randomized design (CRD) was used. There were four replications per treatment and the plates were incubated at 25 °C for 5 days. Isolates were then scored for degree of

antagonism after five days using the rating system of Bell *et al.* (1982) on a scale of 1-5: where class1 = *Trichoderma* completely overgrow the pathogen and cover the entire medium surface; class 2 = *Trichoderma* overgrow at least 2/3 of the medium surface; class 3 = *Trichoderma* and *Fusarium* each colonize 50% of the medium surface and neither of them appear to dominate the other; class 4 = *Fusarium* colonizes at least two-thirds of the medium surface and appear to withstand encroachment by *Trichoderma* and class 5 = *Fusarium* completely overgrow the entire medium surface.

Table 1. Source of *Trichoderma* isolates used in the experiments

Trichoderma species	Sample No.	Date of isolation	Isolation place	**CPK No.
T. oblongisporum	J3	03/06/05	Jimma	1809
T. longibrachiatum	J9	of 09/07/05	Jimma	1815
T. asperillum	S2	of 03/06/05	SNNP	1820
T. cerinum	S10	of 27/06/05	SNNP	1892
T. harizianum	J6	of 09/07/05	Jimma	1812
T. atroviridae	S12	of 27/06/05	SNNP	1836
T. viridae	*	*	*	*
T. Hamatum	W3	of 03/06/05	Wellega	1827

* PPRC lab isolate, ** CPK: laboratory of Vienna University of Technology (Austria)

According to this rating system, a *Trichoderma* isolate is considered antagonistic, if the mean score was less or equal to class 2 and not antagonist if the number was greater than class 2. Another set of experiment was prepared for inhibition and colony growth tests. To determine the inhibition percentage of the pathogen by each of the tested antagonists, growth of *Fusarium solani* was recorded by measuring the diameter of the colonies. Percentage inhibition (I %) of its colony growth was then calculated using the following formula used by Whipps (1987);

$$I\ \% = \frac{(\text{I- average diameter of the } \textit{Treated}\ )}{\text{Average diameter of the } \textit{Control}} \times 100$$

Where I (%) represents the average inhibition percentage; *Treated*, indicates the average colony diameter of *F. solani* in the presence of the antagonist and *Control* is the average colony diameter of *F. solani* without the antagonist. Data were analyzed using SAS statistical tool version 9.

Results and Discussion

The antagonism test revealed that seven of the solates which were scored less than 2 were highly antagonistic to the root rot pathogen *F. solani* (Table 2). However, *T. cerinum/tomentosum*, which exhibited a score of 3, was not antagonistic at all. This isolate grew only up to 1cm in 5 days indicating that it was not antagonistic, whereas all the other isolates colonized and covered the colonies of *F. solani* on the respective plates. This isolate was, therefore, not tested further.

As shown in Table 2, only the seven species of *Trichoderma* which were found antagonistic were further tested and compared. The control plates in the inhibition test were used to calculate the percentage inhibitions of the respective *Trichoderma* species only.

The effect of the *Trichoderma* isolates on the colony growth of *F. solani* in dual culture test is shown in Table 2 a Figure 2. The results of the data recorded 5 days after inoculation showed that there was significant difference (P= 0.0001 and P = 0.0005 respectively) among the *Trichoderma* species in suppressing the colony growth of the pathogen and inhibition percentage (figure 2). Each of the antagonistic *Trichoderma* isolates limited the colony growth of the pathogen by overgrowing the pathogen colony. *Trichoderma* isolates are known to rapidly colonize medium surface and substrates (Kucuk and Kivance, 2004).

The mean colony growth of *F. solani* in plates containing *Trichoderma* isolates ranged from

2.1cm. (*T. asperillum)* (figure 1a) to 2.95cm. (*T. oblongisporum)*. In contrast, its colony growth reached 4.25 cm. in 5 days in control plates (figure 1b). *T. asperillum* showed much more influence on the pathogen. It inhibited the colony growth of *F. solani* by 50.45% followed by *T. harzianum* (48.06%), *T. hamatum* (47.74 %), *T. viride* (46.28 %) and *T. longibrachiatum* (43.60%) which were not significantly different from each other (table 3). Akrami *et al.* (2009) also found that *T. asperillum* and *T. harzianum* showed 43.2 % and 51.5% reduction in disease incidence respectively under greenhouse conditions. Up to 36% reduction of colony growth of *F. oxysporum* was obtained by using *Trichoderma harzianum* isolates (Sahi and Khalid, 2007).

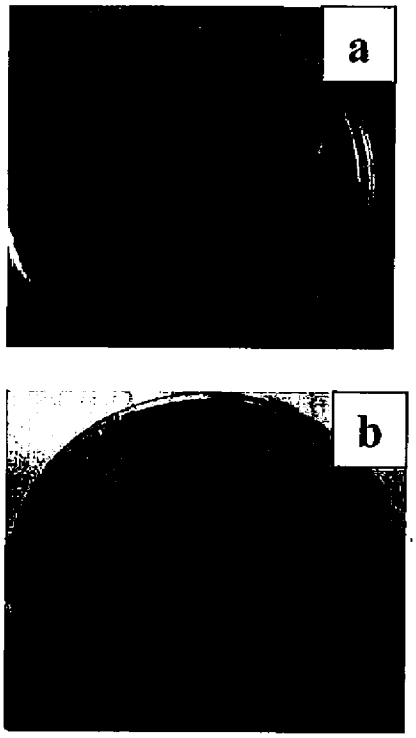

Figure 1. Dual culture *of T. Asperillum vs. F. solani on PDA (a) and* single culture of *F. Solani on PDA* (b)

In a similar study using culture filtrates of *T viride*, Tesfaye (1999) found that *F. solani* could grow only for 1.5 mm in 96 hrs (4 days). Tesfaye and Kapoor (2004) also found that *T.viridae* and *T. harzianum* inhibited the fungus *Botrytis gladiolorium* that causes corm rot on Gladiolus. Thus, *Trichoderma* species are among the most promising biocontrol agents against many fungal pathogens (Akrami *et al.*, 2009). Antagonistic interactions of *Trichoderma* species with other fungi and mechanisms involved in the biocontrol process are based on antibiosis, parasitism, induced resistance and competition (Hoitink *et al.*, 1997). Biocontrol agents also produce enzymes such as chitinase protease and cellulose that have been proved to be involved in the antagonistic activity (Howell, 2003). However, antagonistic fungi are specific in their antagonistic activity against specific fungi (Saleem *et. al.*, 2000). The mechanism of action of the *Trichoderma* isolates on the test pathogen in this study is most likely attributed to competition.

In-vitro tests are suitable for selecting antagonistic organisms with a particular mode of action, but are very poor predictors of the activity of the organisms in the field (Campbell, 1989). According to Mpika *et al.* (2009), efficiency of *Trichoderma* species in antagonizing plant pathogens is closely linked with local conditions. From the results of this study it is concluded that the tested indeginous *Trichoderma* isolates have high potential to inhibit the colony growth of *F. solani* which is the causal agent of Black root rot of faba bean. This indicates a good prospect for bio-control. However, further in-*vivo* studies are required to investigate the efficacy of the antagonistic isolates of *Trichoderma* to apply for bio-control of the disease as one component of integrated management under field conditions.

Table 2. Mean score of antagonism test of *Trichoderma* versus *F. solani* (Bell *et al.* 1982 scoring method) and mean colony growth of *F. solani* in the presence of *Trichoderma* species evaluated *in-vitro* 5 days after inoculation

Trichoderma species	Mean score(class) of antagonism	Mean colony growth of *F. solani* in presence of *Trichoderma* species (cm)
T. asperillum	1	2.10[c*]
T. harzianum	1.75	2.20[c]
T. hamatum	1	2.23[c]
T. viride	1	2.28[c]
T. longibrachiatum	1	2.40[c]
T. atroviride	1	2.88[b]
T. oblongisporum	2	2.95[b]
T.cerinum/tomentosum	3**	Not tested
Control (*F. solani* only)	----	4.25[a]
C.V.	----	8.69

*Means in a column followed by the same letter are not significantly different using LSD
**Not antagonistic

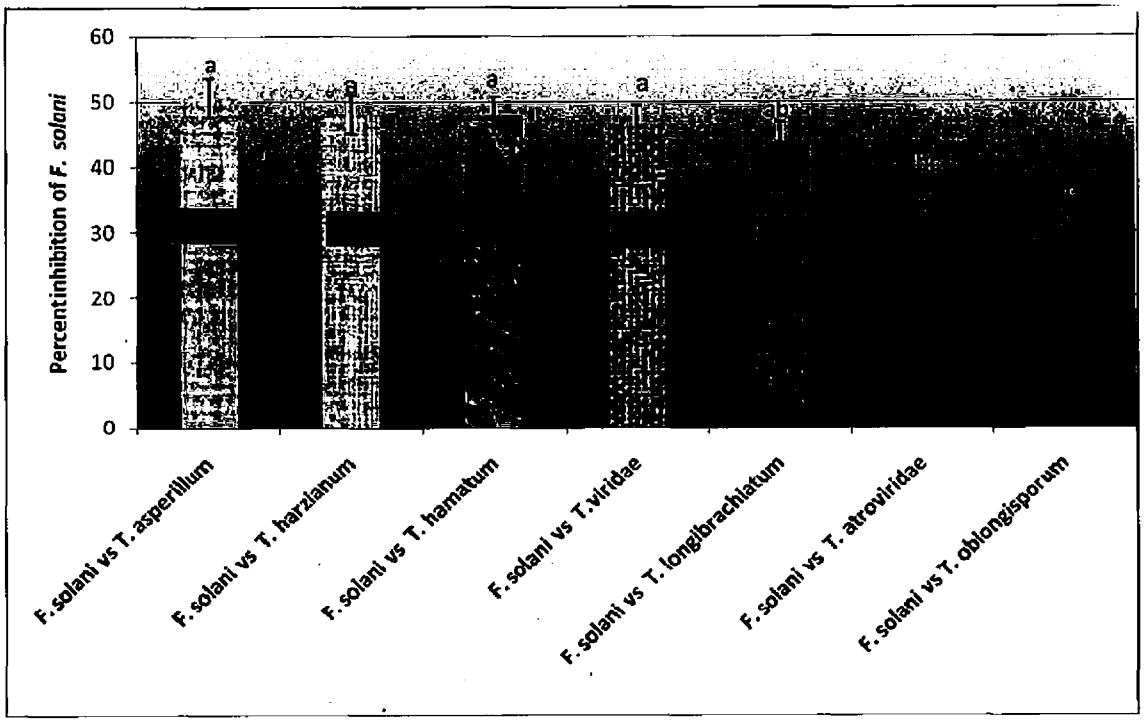

Figure 2. Percent inhibition of *F. solani* due to various *Trichoderma* isolates. Bars with different letters on their tops show significant difference (P=0.0005) as determined by t-test (LSD test)

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