

Evaluation of the Potential of *Trichoderma viride* as Biological Control Agent of Root Rot Disease, *Fusarium solani*, of Faba Bean

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Abstract

An experiment was conducted to determine the potential of *Trichoderma viride* as an antagonist to the pathogen *Fusarium solani* of faba bean. The radial growth of *F. solani* was significantly reduced on media as the concentration of *T. viride* and the exposure time increased. The statistical analysis indicates that factors such as concentration of the antagonistic pathogen, exposure time and interaction effects are highly significant at a probability level of 1%. When treatments were compared in the green house, soil treated by *T. viride* has shown the least percentage of mortality of faba bean by *F. solani* in all stages of the plant. There is highly significant difference at a probability level of less than 1% with corresponding coefficient of variation of 8.40%. So, in this laboratory and greenhouse studies the antagonist *T. viride* proved to be an effective control agent against *F. solani*.

Introduction

Diseases are one of the major production constraints of faba bean in Ethiopia. Root rots caused by *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp. acting singly or in combination, are considered to be the most important faba bean diseases in many parts of the country (Habtu 1985; Stewart & Dagnatchew 1967). Although these diseases could be controlled using host plant resistance, cultural practices, and chemicals, working only with these methods is not satisfactory and may also be expensive in some cases. Besides, the prices of pesticides are economically unaffordable to the poor Ethiopian farmers. Furthermore, general concern about possible adverse effects of chemical pesticides makes it desirable to seek additional control

methods. Thus, biological control is becoming increasingly important all over the world (Elad *et al.* 1981, Harder *et al.* 1984). These and other reasons necessitated the development of an alternative, cheap and safe methods of disease control.

As a component of integrated disease management strategy, there is a need to develop alternate, cheap and safe method of disease control. Hence, the objective of this study was to determine the efficacy of *Trichoderma* spp. on *F. solani*, the root rot pathogen of faba bean (*Vicia fabae*).

Materials and Methods

Pathogen Culture

The pathogen *F. solani* was isolated and multiplied on potato dextrose agar (PDA) medium (peeled and sliced potatoes 250g, agar 20g, dextrose 20g, in one l. distilled water). Spore concentration of the pathogen *F. solani* was adjusted to 1×10^6 conidia/ml (Elad et al. 1981).

Soil Sample Collection

Soil samples were collected from Ambo, Ginchi, Awassa, Arsi and Bako, areas where the pathogen, *F. solani*, has declined over the years. After crop harvest sampling was done diagonally. Soil samples were taken at depths of 5, 10, 20, and 30 cm. Ten grams of soil from each depth was collected in a flask and later 90 ml of sterilised water was added. The soil-water mixture was thoroughly mixed for 5min. Five ml of this solution was then taken and added to 50ml of distilled water. After shaking and mixing the solution thoroughly, 0.2ml of the mixed solution was plated on PDA. Trichoderma colonies that started to grow seven days later were sent to the International Mycological Institute (Elad et al. 1981) for identification purposes. The conidial spore suspension of *T. viride* was again grown on sterilised barley bran in bottles at 20°C (Elad et al. 1981).

In-vitro test

Each of the five isolates, collected from the five locations, was tested for its ability to inhibit *Fusarium solani* on agar plates of mixed nutrient broth with PDA. Colonies of *T. viride*, allowed to grow on nutrient broth for seven days, were filtered through sterilised filter. The filtered culture was mixed with PDA at the proportions of 0:1, 1:1, 2:1, and 3:1 culture to PDA ratio. Twenty ml of the media from each of the concentration, was transferred to each plate and allowed to solidify. Each plate was inoculated in the centre by transferring a 5 mm diameter block of 7 days old culture of *F. solani* grown on PDA incubated at 28°C. The experiment was replicated 4 times. Zone of inhibition was measured after every 24, 48, 72, and 96 hrs.

Greenhouse pot experiment

F. solani was allowed to grow on PDA for two weeks. The culture was then washed and suspended in a bottle. 300g of sterilized faba

bean seeds were later soaked in the suspended solution. The coated faba bean seeds were again mixed thoroughly and sown in a 15 cm diameter pot that contained 1kg of sterilized soil. Five faba bean seeds were sown on each of the pots. The antagonist agent, *T. viride* was isolated and purified on PDA. The isolated *T. viride* was added directly on *F. solani* inoculated pots. In the fourth treatment, prior to sowing faba beans, seeds were coated with *T. viride* and sown in an already *F. solani* inoculated pot. The treatments were replicated four times in a complete randomized block design. The temperature in the greenhouse was maintained at 27°C through out plant growth and development stages.

Results and Discussion

The radial growth of *F. solani* was seriously affected and was almost completely replaced by *T. viride*. The effect was more damaging with the increase in the concentration of culture filtrate. The pathogen, *F. solani*, varied in its tolerance against the varying concentrations of the mycoparasitic effect of *T. viride* (Table 1).

The highest mean diameter of the colony (82.5 mm) of the pathogen was recorded from the control treatment at 96 hrs and the lowest one was 1.5 mm recorded at the highest concentration level. The mean value revealed that as the concentration increased the radial growth of the fungi decreased. The mean radial growth of the fungi is 75.6, 26.7, 16.8, and 2.2 mm at concentration levels of 1, 2, 3 and 4, respectively. This shows that there is a mean difference of 73.4 mm between the lowest and highest concentration levels.

Concentration of the antagonistic pathogen, exposure time as well as their interaction showed highly significant difference in pathogen development at a probability level of less than 1% with the corresponding coefficient of variation of 5.4% (Table 2). With respect to time, the radial growth of the fungi increased with a decreasing rate as the time goes up from 48 to 96 hrs. The radial growth of the fungi was 22.7, 31.6 and 36.9 mm at 48, 72, and 96 hrs., respectively. The interaction of time and concentration levels was also statistically significant. The lowest radial growth which is 1.5 mm was found when the fungus was growing on concentration level 4 for 96 hrs, while the highest radial growth was recorded when the fungus was grown for 96 hrs at concentration level of 1. The mean value of the fungi growth at

this hr and concentration level was 82.4 mm.

When treatments in the greenhouse were compared with each other, the soil treated by *T. viride* had the least % of mortality by *F. solani* in all stages of the plant (Table 3). This is highly significant at a probability level of less than 1% with corresponding coefficient of variation of 8.4% (Table 4).

To conclude, the strain of *T. viride* fungus,

tested in the laboratory, possessed high antagonistic activity against *F. solani*, root rot pathogen of faba bean. From this laboratory and greenhouse experiments one can say that *T. viride* proved to be an effective antagonist and can be used as one of the components in the integrated management of *F. solani* in food legumes.

Table 1. Radial growth of *F. solani* in mm on PDA mixed with the filtrate of *Trichoderma viride* at different proportions and exposure time.

Concentration	Exposure Time (hr)		
	48	72	96
Filtrated <i>T. viride</i> :PDA			
0 : 1 (control)	65.3	79.5	82.5
1 : 1	15.5	29.5	35.6
2 : 1	7.5	14.2	28.5
3 : 1	2.0	3.0	1.5

Table 2. ANOVA table for the radial growth of *F. solani* on PDA.

Source of variation	Probability value
Replication	0.34 ^{ns}
Concentration of the inoculum (A)	0.0 ***
Time (B)	0.0 ***
A * B	0.0 ***

Note: *** significant at a probability level of less than 1%; ns-non-significant; Coefficient of variation- 5.39%.

Table 3. Influence of *Trichoderma viride* on the % of seedlings of faba bean inoculated with *Fusarium solani*.

Treatment	Pre-emergence mortality		Post-emergence mortality	
	Sterilized	Non-sterilized	Sterilized	Non-sterilized
Control without inoculum	0.0	9.2	2.6	4.6
Control with inoculum	44	43.5	33.1	34.7
<i>T. viride</i> with seed and soil inoculation	4.6	3.1	9.6	12.5
<i>T. viride</i> in soil and soil inoculation	3.9	4.8	8.4	5.9

Table 4. ANOVA table for the influence of *T. viride* on the seedling establishment of faba bean inoculated with *F. solani* on pots .

Source of variation	Probability value
Replication	0.367 ns
Inoculation treatment (A)	0.0 ***
Pre and post evaluation on sterilized and non sterilized soils (B)	0.001 ***
A * B	0.0 ***

Note:*** highly significant at a probability level of less than 1%; ns = non-significant. coefficient of variation: 8.40%

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