

# Effects Of Indigenous Trichoderma Species On Faba Bean (*Vicia Faba*) Root Rot Caused By *Fusarium Solani* (Mart) Appel & Wollenw

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**Abstract:** Faba bean root rot caused by *Fusarium solani* is one of the most important biotic stresses in the major growing areas in Ethiopia. The effect of indigenous *Trichoderma* species applied on soil and seed on faba bean root rot development was studied in glass house pot experiments. Seven individual species (*T. viridea*, *T. hamatum*, *T. oblongisporium*, *T. longibrachiatum*, *T. atroviridea*, *T. harziunum* and *T. asperillum*) and three combinations of species (*T. asperillum* + *T. harziunum*, *T. oblongisporium* + *T. atroviridea* and *T. viridea* + *T. hamatum*) previously tested for in-vitro antagonistic effects to the pathogen were used as bio-control agents together with untreated control. Sterilized soil in each pot was artificially inoculated with *F. solani* multiplied on faba bean seeds. The *Trichoderma* species were mass produced on wheat bran and applied to the soil or dressed to the seeds one week after inoculation of the pathogen. Five faba bean seeds of a susceptible local variety "kassa" were planted per pot and observed for mortality. Only one treatment produced significant reduction in root rot incidence in each of seed dressing and soil treatment methods. Highest protection of seedlings (only 14% mortality) was obtained from seed dressing with combinations of *T. oblongisporium* + *T. atroviridea*. In the case of soil treatment, *T. hamatum* resulted in significantly low mortality (51%) of seedlings. All the other treatments were not significantly different from the control in terms of mortality. Field experiments consisting of similar treatments are recommended to confirm the efficacy of the bio-control agents for wider application.

**Key words:** *Fusarium solani*, *Trichoderma*, biological control.

## INTRODUCTION

*Fusarium solani* is considered as one of the most important causal agents of faba bean (*Vicia faba* L.) root rot alongside *Rhizoctonia solani* and *Sclerotium rolfsii* in growing areas [18],[1],[9]. The pathogen causes a highly destructive root rot in field grown beans [5]. The disease is also a major biotic stress in faba bean growing areas in Ethiopia, [23], [24]. Similarly wilt and root rots cause up to 70% yield loss in farmers fields in sever conditions [21], [15]. Management of root rots is a difficult task as most pathogens live near the rhizosphere and survive for a long period by forming resistant structures [12]. Chemical control of faba bean root rot is neither efficient nor economical. Management options are mostly agronomic practices such as crop rotation, good soil drainage and use of disease free or fungicide treated seeds that may help reduce losses and there are no adequate control measures for *Fusarium* rots in the field [4]. Biological control using antagonistic fungi such as *Trichoderma* spp. has shown promising results in controlling several diseases [6]. More effective control of soil-borne pathogens can be achieved by the use of these environmentally friendly biological control agents [20]. The mechanism of control of *Trichoderma* spp. include: competition, rhizosphere competence, mycoparasitism, antibiotic and enzyme production, induced resistance and promoting plant growth [10], [17], [16]. Limited attempts have been made in exploiting the use of indigenous *Trichoderma* spp. for use in biological control in Ethiopia. In addition to incurring foreign currency loss, imported exotic biological control agents may fail to establish in the local environment due to adaptation problems. In an attempt to develop indigenous biological control agents, isolates of seven *Trichoderma* spp. were tested *in-vitro* for inhibition potentials against the root rot pathogen *Fusarium solani* and showed The *Trichoderma* species were previously tested for their pathogenicity against *F. Solani in-vitro* and showed inhibition percentage ranging from 30- 50% on the pathogen [8]. The objective of these experiments was therefore to further evaluate these promising indigenous bio-

logical control agents in glass house pot experiments before they can be tested on field.

## Materials and Methods

### Preparation of soil and seeds

Sterilized black clay soil (1.75kg) was placed in each foam pot (20 cm diameter) and was left for a week to cool and to check for any growth of weeds. All pots were irrigated from bottom using tap water. A susceptible local variety (Kassa) was used for all the experiments. Germination test of the seeds revealed an average viability of 97%.

### Preparation and inoculation of the pathogen

The pathogen, *Fusarium solani* was isolated from infected faba bean roots grown in a well developed sick plot at Ambo Plant Protection Research Center. Roots were thoroughly washed using tap water and surface sterilized using 1% NaOCl solution for 30 seconds and rinsed with sterilized distilled water twice. Finally the root pieces were transferred into Petri dishes containing Potato Dextrose Agar and incubated at 25 °C. After four days of incubation, pure cultures were obtained by sub-culturing and identification was done by using morphological, cultural and microscopic characteristics following *Fusarium* identification key [7]. Flasks (250ml) were filled with 250gm of faba bean seeds and sterilized at 121°C at 15 PSI for 1hr covered with aluminum foil. For inoculation, 5ml of *F. solani* suspension containing  $1 \times 10^6$  conidia per ml was placed into the flasks containing the faba bean seeds with a sterile micro pipette. The flasks were kept at room temperature for 10 days. Finally the contents of the flasks were poured into each pot containing sterilized soil and incorporated thoroughly using sterilized metal spatula and left for one week to establish before the bio-agent was incorporated into the soil.

### Preparation and inoculation of the bio-agents

Seven *Trichoderma* spp. from culture collections of Ambo Plant Protection Research Center (APPRC) viz., *T. oblongisporium*, *T. longibrachiatum*, *T. atroviride*, *T. asperillum*, *T. hamatum*, *T. viride*, *T. harzianum*, which were isolated from soil samples collected from different parts of Ethiopia and identified at molecular level [22] were used for the experiments. The *Trichoderma* species were previously tested for their pathogenicity against *F. Solani in-vitro* and showed inhibition percentage ranging from 30- 50% on the pathogen [8]. Flasks (250ml) were filled with 250gms of wheat bran and 150ml of distilled sterilized water was added to moisten the wheat bran. The flasks were covered loosely with aluminum foil and sterilized at 121°C and 15 PSI for 1hr. The flasks were allowed to cool for 2hrs before being inoculated. They were then inoculated with 5ml of the respective *Trichoderma* spp. containing  $1 \times 10^6$ /ml of conidia with sterile micro pipettes and incubated for 15 days at room temperature. For soil treatment, each of the pots was inoculated with the *Trichoderma* spp. by pouring the contents of the flasks and incorporating using sterile metal spatula at a ratio of 7:1w/w (soil: inoculums). For seed treatment, the contents of the flasks on which the *Trichoderma* spp. grew were poured on to a sterilized glass bowl and mixed with Tween 20. In the case where two *Trichoderma* spp. were used, 125 gms of each of the species were proportionally mixed with Tween 20. Finally, the seeds to be planted were rolled on the bowl until fully covered with the wheat bran containing the *Trichoderma* spp. and five seeds were planted per pot.

**Treatments and experimental design**

For both soil treatment and seed dressing methods, seven individual and three combined *Trichoderma* spp. were used as treatments together with control treatments (Table 1). Controls were treated with sterilized wheat bran with no *Trichoderma*. The experimental design was completely randomized design (CRD) with four replications. The experiment was conducted in glass house at Ambo Plant Protection Research Center. The average temperature and relative humidity of the glass house during the experiments were 19.5 °C and 61.7% respectively. The experiments were conducted between September, 2010 and January, 2011.

**Table (1)** Individual and combined treatments used for the experiment

No	Treatment	No	Treatment	Remarks
1	<i>T.harzianum</i>	7	<i>T.hamatum</i>	
2	<i>T.oblongisporum</i>	8	<i>T. viride</i> + <i>T. hamatum</i>	Combination
3	<i>T.longibrachiatum</i>	9	<i>T. oblongisporium</i> + <i>T. atroviride</i>	Combination
4	<i>T.asperillum</i>	10	<i>T. asperillum</i> + <i>T. harziunum</i>	Combination
5	<i>T.viride</i>	11	Control	No bioagent

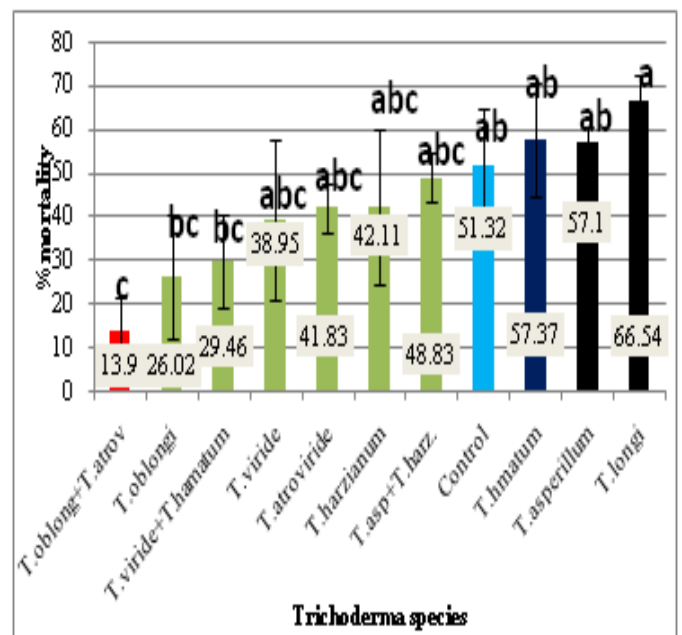
6	<i>T.atroviridea</i>			
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**Data collection and statistical analysis**

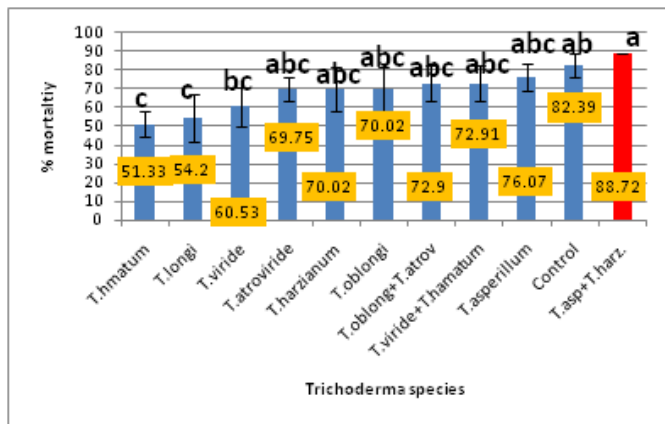
Percent mortality and emergence were recorded. Mortality was assessed every month by removing dead plants. Death due to *F. solani* was confirmed by isolating the pathogen from the roots of the dead plants. Mortality data were subjected to arcsine transformation to normalize the data and analysis of variance (ANOVA) was conducted using SAS software version 9.

**Results**

The results of the pot experiments (Figure 1) showed that seed treatment of faba beans with *Trichoderma* spp. had generally greater protection effect on faba bean root rot than soil treatment. Better protection of seedlings (13.93% mortality) was obtained from seed treatment of faba bean with combinations of *T. oblongisporium* and *T. atroviridea* followed by seed treatment with *T. oblongisporium* (26% mortality) and *T. harzianum* combined with *T. hamatum* (26.01% mortality) as compared to the control treatment. The experiment also revealed that seed treatment with combined rather than individual *Trichoderma* spp. gave better protection of faba bean seedlings. Seeds treated with three of the individual *Trichoderma* spp. viz., *T. asperillum*, *T. hamatum* and *T. longibrachiatum* showed 57.10%, 57.37% and 66.59% mortality respectively, which was above the control mortality (51.32%). In the case of soil treatment, the lowest mortality (51% mortality) of seedlings was recorded on pots treated with species of *T. hamatum* which was significantly different from the control. The rest of the treatments were not significantly different from the control. There was no significant difference in the emergence percent of all the treatments both in seed and soil treatments.



**Fig. 1.** Percent mortality of faba bean seedlings grown in pots from seeds treated with different *Trichoderma* spp. (LSD = 33.82, C.V. 55.43 %)



**Fig.2.** Percent mortality of faba bean seedlings grown in pots treated with different *Trichoderma* spp. (LSD= 26.33, C.V. 26.18)

## Discussion

In the present study it has been observed that the *Trichoderma* spp. which performed very well *in-vitro* failed to produce the same effect *in-vivo*. Seed treatment with *Trichoderma* spp. seemed to be more effective than soil treatment in this study. This may be attributed to the fact that the seeds are protected by the bio-agents at their immediate surrounding before the pathogen comes into contact with the seeds. Some of the mechanisms of *Trichoderma* spp. that may play major roles in the control of soil borne pathogens include; parasitism on hyphae of other fungi, production of extracellular lytic enzymes for cell wall degradation and competition [13], [11]. In contrast as low as 15% and 25% incidence of root rot due to *F. solani* was observed from soil treatment of pots with *Trichoderma viride* and *Trichoderma harzianum* respectively [1]. Abdel-Kader [1] also reported that *T. harzianum* incorporated to the soil significantly reduced root rot incidence of faba bean plants. Inconsistent results have been observed in bio-control experiments as local conditions highly influence the efficiency of *Trichoderma* spp. in antagonizing plant pathogens. In addition, microbiological agents commonly encounter widespread resistance from the biotic environment as compared with chemicals and this may include carriers used for the *Trichoderma* spp. which may lead to poor performance [19]. The current study has demonstrated the ability of *Trichoderma* spp to cause significant reduction on incidence of the root rot disease under greenhouse conditions further confirming the *in-vitro* results. Similar results have been reported by several researchers. For example, suppression of *Fusarium solani* by combination of *T. Viride* and *Bacillus subtilis* *in-vitro* was reported by [14]. Another report by [25], indicated effective control of *Fusarium* root rot of Okra by use of *T. harzianum* and *T. viride* in combination with three fungicides Benlate, Ridomil and Dithane M-45 resulting in increased yield. The results obtained are encouraging with respect to integrated management of the disease. Field experiments consisting of similar treatments are recommended to confirm the efficacy of the bio-control agents for wider application.

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