
*Journal of Biological Control,* 30(3): 169-176, 2016, DOI: 10.18311/jbc/2016/15593

**Research Article**

# Biocontrol of faba bean black root rot caused by *Fusarium solani* using seed dressing and soil application of *Trichoderma harzianum*

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**ABSTRACT:** Black root rot of faba bean is one of the most destructive diseases that causes up to 100% yield loss on farmers' fields under severe conditions. As use of chemical fungicides for control of the disease is neither efficient nor economical, alternative options such as biological control need to be exploited. The antagonistic fungus *Trichoderma harzianum* was used as seed dressing or soil application treatment in combination with three varieties *viz*. Kasa (susceptible), Wolki (moderately resistant) and Wayu (resistant) in two consecutive cropping seasons in a sick plot. Each variety was sown with and without *T. harzianum*. There were a total of six treatments. The experimental design was randomized complete block design with three replications in a 2 x 3 factorial arrangement. Results showed that in the first year highly significant variations in the percentage of dead plants at harvesting stage were observed only due to varietal differences for soil treatment ($P = 0.0001$) and seed dressing ($P < 0.0001$) respectively. Percentage of dead plants of variety wayu was 26.1% and 30.8% in soil application and seed dressing treatments respectively. Similarly, 92.1% and 95.2% dead plants were observed for the susceptible variety Kassa in soil application and seed dressing treatments respectively. In the second year there was a significant interaction between varieties and *Trichoderma* treatments ($P$=0.0047, F=8.66, df= 2) with variety Wayu showing the least percentage of dead plants (56.58%) significantly differing from Kassa (93.86%) and Wolki (77.08%) in the soil treatments. It is concluded that use of *T. harzianum* in combination with a resistant variety such as Wayu is effective to reduce the incidence of faba bean root rot caused by *Fusarium solani*. Future research should focus on studying the effects of combining different *Trichoderma* spp. on the disease as integrated with resistant varieties.

**KEY WORDS:** Biocontrol, root rot, faba bean, *Trichoderma harzianum, Fusarium solani*

(Article chronicle: Received; 19-09-2016; Revised 21-09-2016; Accepted: 23-9-2016

## INTRODUCTION

Among the root rot diseases affecting faba bean, black root rot caused by *Fusarium solani* (Mart) Appel & Wollenw is the most destructive one (Akrami *et al*., 2009). The disease usually appears at pre-emergence and early seedling stages during the growing season inducing early seedling death and necessitating massive replanting to replace dead plants (Negussie *et al*., 2008; El-Mougy and Abdel-kader, 2009; Muktar *et al*., 2011). Black root rot is a major biotic stress in faba bean growing areas of Ethiopia (Tesfaye, 1995, 1999) causing yield loss amounting 45 - 70% on farmers' fields under severe conditions (Stewart and Dagnachew, 1967; Habtamu and Dereje, 1985; PPRC, 1996). The disease is a threat to production of faba bean in the high lands of Ethiopia (Bogale *et al*., 2009). In favorable conditions and during severe infections the disease can cause complete crop loss (Negussie *et al*., 2008) and in susceptible cultivars, yield loss may reach 100% (Mukankusi *et al*., 2011). Water logged black clay soils predispose faba beans to the disease where it occurs frequently (Negussie *et al.* 2008). The disease causes severe rotting which causes black discoloration of the roots and ultimately results in death of the plant ((Dereje and Tesfaye, 1994; PPRC, 1996). Poor soil fertility and intensive bean cultivation has been reported to aggravate root rot caused by *F. solani* in countries like Kenya, Rwanda, Burundi, Zaire and Uganda (CIAT, 1992). The pathogens causing root rot live near the rhizosphere and survive for a long period by forming resistant structures making management of the disease a difficult task (El-Mougy and Abdel-Kader, 2009). The pathogen causes damping off of seedlings, chlorosis, stunted growth and death in severe conditions (Otsyula, 1998). Use of chemical fungicides for control of faba bean root rot is neither efficient nor economical. Although there are no adequate control measures in the field for *Fusarium* rots, some agronomic practices such as crop rotation, good soil drainage and use of disease free or fungicide treated seeds may help reduce losses (Agrios, 2005). Other management options include use of broad bed furrows (BBF) and resistant varieties (ICARDA, 2006).

A promising alternative to chemical control and a good complement to agronomic control options is biological control. Use of antagonistic microorganisms as biological control agents for the management of soil borne pathogens provides ecologically friendly and pathogen specific component of integrated disease management that minimizes the economic impacts caused by root rots (Landa, *et al*., 2004; El-Kassas and Khairy, 2009; Rosa and Herrera, 2009). Among biological control agents used against plant diseases, *Trichoderma* spp. are the most studied. *Trichoderma* spp. are abundant rhizosphere colonizers that suppress plant pathogens and promote plant growth and health with several mechanisms including mycoparasitism, antibiosis, competition, inducing systemic resistance and enhancing root growth (Whipps and Lumsden, 2001; Harman *et al*., 2004; Mohidden *et al*., 2012).

The effective use of microbial biological antagonists of pathogens such as *Trichoderma* spp. particularly, for soil borne pathogens has been demonstrated by many scientists (Zheng and Sinclair, 2000; Lewis and Lundsen, 2001; Lewis *et al*.,1998).

Previously conducted *in-vitro* laboratory experiments and *in-vivo* pot experiments (Belay, 2010; Belay and Anteneh, 2015; Eshetu *et al*., 2015) have shown promising results in the use of *Trichoderma* spp. for biological control of the black root rot pathogen *F. solani.*

Despite the control options mentioned above, the disease still remains difficult to control especially in black clay soils. Moreover, little attempts have been made to demonstrate the field level effects of the antagonistic fungi (*Trichoderma* spp.) on the black root rot pathogen *F. solani*. The objective of this study was therefore to study the effects of managing faba bean black root rot through the combined use of varietal resistance and the antagonistic fungus *Trichoderma harzianum* both as soil treatment and seed dressing in the field.

## MATERIALS AND METHODS

The isolate of *T. harzianum* used for the experiments was originally isolated from soils of coffee forest in Jimma (Ethiopia) and identified at molecular level (Temesgen, 2005). The study was conducted in two consecutive main cropping seasons (2009 and 2010). *T. harzianum* mass produced on coffee husk (2009) or wheat bran (2010) was used with or without combination with three varieties *viz*. a susceptible variety (Kassa), a moderately resistant variety (Wolki) and a resistant variety (Wayu) and dressed to the seed or applied to the soil in rows.

For seed dressing a flask containing 250ml of wheat bran or coffee husk moistened with 150ml of distilled water, mixed with glass rod and covered with aluminum foil was sterilized by autoclaving at 121$^{o}$C and 15 PSI for 1hr and allowed to cool for 2hrs before being inoculated with 5ml of the *T. harzianum* suspension containing spores adjusted to 1x10$^{8}$ spores/ml. the flasks were left to sporulate for three weeks. To treat the seeds, the contents of the flasks on which the spores grew were poured on to a sterilized

**Table 1. Treatment combinations used for the experiments in 2009 and 2010 with the substrate used for mass production of *Trichoderma harzianum***

| Treatment | Year | Remarks |
|---|---|---|
| Kassa with *T. harzianum* | 2009 | *Trichoderma harzianum* mass produced on coffee husk |
| Kassa without *T. harzianum* | 2009 | *Trichoderma harzianum* mass produced on coffee husk |
| Wolki with *T. harzianum* | 2009 | *Trichoderma harzianum* mass produced on coffee husk |
| Wolki without *T. harzianum* | 2009 | *Trichoderma harzianum* mass produced on coffee husk |
| Wayu with *T. harzianum* | 2009 | *Trichoderma harzianum* mass produced on coffee husk |
| Wayu without *T.harzianum* | 2009 | *Trichoderma harzianum* mass produced on coffee husk |
| Kassa with *T. harzianum* | 2010 | *Trichoderma harzianum* mass produced on wheat bran |
| Kassa without *T. harzianum* | 2010 | *Trichoderma harzianum* mass produced on wheat bran |
| Wolki with *T. harzianum* | 2010 | *Trichoderma harzianum* mass produced on wheat bran |
| Wolki without *T. harzianum* | 2010 | *Trichoderma harzianum* mass produced on wheat bran |
| Wayu with *T. harzianum* | 2010 | *Trichoderma harzianum* mass produced on wheat bran |
| Wayu without *T.harzianum* | 2010 | *Trichoderma harzianum* mass produced on wheat bran |

glass bowel and mixed with Tween 20 used as a sticker. Finally, the seeds to be planted were rolled on the bowel until fully covered with the wheat bran or coffee husk containing the spores. In the case of soil treatment the same procedure was used except that 250g of the substrates containing well sporulated *T. harzianum* was applied to the soil in rows before sowing and incorporated into the rhizosphere.

There were a total of six treatments in each of year 2009 and 2010 (Table 1). Each variety was sown with and without *T. harzianum.* The experimental design was randomized complete block design (RCBD) with three replications in a 2 x 3 factorial arrangement. Plot size was 4.8m² and the distance between blocks and plots was 1.5m and 1m respectively. The spacing between rows and plants was 40cm and 5cm respectively. A total of 152 seeds were planted per plot. A well developed sick plot containing high amount of root rot inoculum established at Ambo plant protection research center (APPRC) was used for experiments. Data collection was done at emergence, seedling, podding, maturity and harvesting stages by rouging out dead plants. Data analysis and statistical comparison was conducted using the ANOVA procedure of the SAS software version 9.2. followed by mean separation using LSD test.

## RESULTS AND DISCUSSION

In the 2009 field experiments highly significant variations in the percentage of dead plants at harvesting stage was observed due to varietal differences (among varieties only) for soil treatment ($P$ = 0.0001, F = 35.43, df = 2) and seed dressing ($P$< 0.0001, F = 93.71, df = 2) respectively. In the soil treatments, the lowest percentage of dead plants was observed from the resistant variety Wayu (26.1%), followed by Wolki (74.78%) and Kassa (92.1%) which were not significantly different from each other (Fig. 1). In the seed dressing treatments also, Kassa was highly affected (95.2%) followed by Wolki (83.6%) both of which were significantly different from Wayu (30.8%). Similarly, significant variations ($P$ = <0.0001, F = 25.69, df = 2) were observed among the varieties at maturity stage in the soil treatments as well as for seed dressing treatments ($P$<

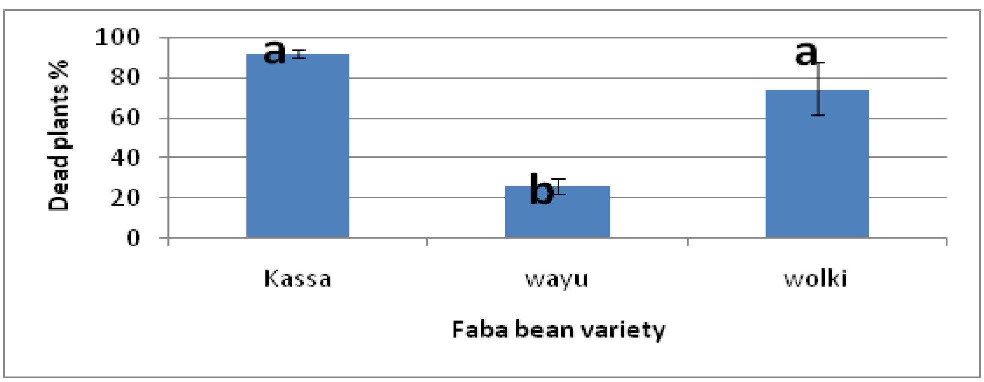


**Fig. 1. Mean percentage (± SE ) of dead faba bean plants due to black root rot of three varieties for soil treatment with *Trichoderma harzianum* at harvest in 2009. Bars with different letters on the top are significantly different. P <0.0001, LSD = 21.69, CV (%) = 21.89, α = 0.05.**

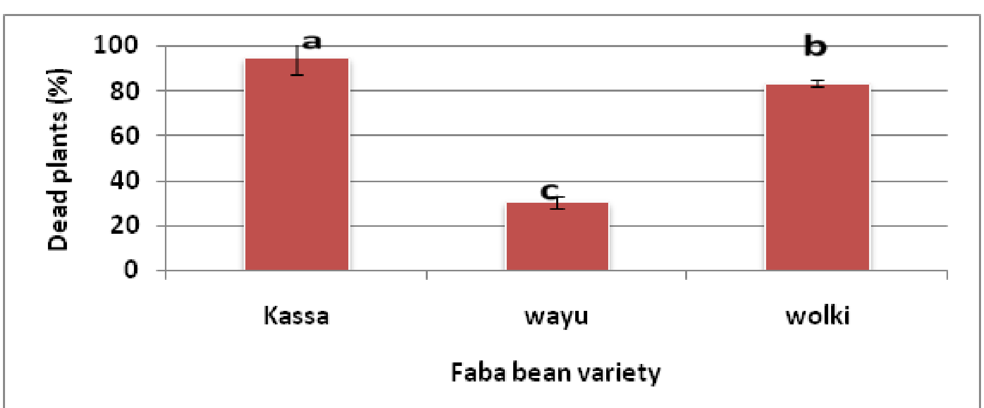


**Fig. 2. Mean percentage (± SE) of dead faba bean plants due to black root rot of three varieties for seed dressing treatment with *Trichoderma harzianum* at harvesting in 2009. Bars with different letters on the top are significantly different P <0.0001, LSD = 10.92, CV (%) = 12.42, α = 0.05.**

0.0001, F = 25.59, df = 2). Significant variations were also observed among the varieties at podding and seedling stages in the seed dressing treatments (*P* = 0.0001and *P* = 0.005 respectively) and soil treatment (*P*= 0.0002 at podding, *P* = 0.0013 at seedling). No significant variations were observed at emergence stage (*P* > 0.05). No significant interaction effects were observed between variety and *Trichoderma* treatments at all the stages (*P*>0.05).

Yield per plot also varied significantly among the three varieties in the seed dressing treatments (*P* = 0.0001, F = 20.57, df = 2) and in soil treatments (*P* = <0.0001, F = 64.85, df=) (Table 2). Variety Wayu yielded highest in the seed dressing (1326.7g/plot) followed by Wolki (118.7g/plot) and Kassa (134.5g/plot). Similarly, Wayu yielded highest in the soil treatments (1108.9g/plot) followed by Wolki (244.80g/plot) and Kassa (63.95g/plot). There were no interaction effects (*P*>0.05).

**Table 2. Yield ± standard error (SE) of three varieties of faba bean treated with *Trichoderma harzianum* as seed dressing and soil treatment in 2009**

| Seed dressing | | | |
|---|---|---|---|
| Variety | Yield (g/plot) | F | P |
| Wayu | 1326.7 ± 328.78a | 20.57 | 0.0001 |
| Wolki | 118.7 ± 115.47b | | |
| Kassa | 134.5 ± 60.14b | | |
| LSD (α=0.05) | 470.77 | | |
| CV (%) | 71.07 | | |
| **Soil treatment** | | | |
| Variety | Yield(g/plot) | F | P |
| Wayu | 1108.9 ± 107.98a | 64.85 | 0.0001 |
| Wolki | 244.8 ± 126.59b | | |
| Kassa | 63.95 ± 21.24b | | |
| LSD (α=0.05) | 213.68 | | |
| CV (%) | 35.95 | | |

Values with similar letters in a column are not significantly different

In the 2010 experiments, slightly different results were observed. The soil treatment in 2010 produced significant variations among varieties (*P* = 0.0012, F = 12.47, df = 2) and *Trichoderma* treated plots (*P* = 0.005, F = 11.16, df = 1) in the percentage of dead plants at harvesting stage. There was also a significant interaction between varieties and *Trichoderma* treatments (*P* = 0.0047, F = 8.66, df = 2) at this stage. Variety Wayu showed the least percentage of dead plants (56.58%) significantly differing from Kassa (93.86%) and Wolki (77.08%) (Table 3). Use of *T. harzianum* as soil treatment showed significantly less percentage of dead plants (65.64%) than the treatments without *T. harzianum* (86.04%) for all the varieties. The seed dressing treatments at harvesting stage were also significant among the varieties (*P*< 0.0001, F = 42.56, df = 2) and between *Trichoderma* treated and untreated plots (*P* = 0.002, F = 15.83, df = 1) (Table 4). The highest percentage of dead plants was observed in variety Wolki (78.51%) followed by Kassa (70.29%) which were significantly different from Wayu (32.02%). Use of *T. harzianum* as a seed dressing resulted in lesser percentage of dead plants (51.54%) than without *T. harzianum* (69%) (Table 4). Percentage of seeds which died before emergence also varied significantly among varieties for seed dressing treatment only (*P* = 0.0013, F = 12.14, df = 1). Significant variations were observed among varieties at podding stage both for soil treatment and seed dressing treatments (*P* = 0.008, F = 7.23, df = 2 and *P* = 0.002, F = 10.59, df = 2 respectively). Significant variations were also observed among the three varieties (*P* <0.0001, F = 42.56, df = 2) at maturity for soil treatments. Similarly, significant variations were observed among the three varieties (*P* <0.0001, F = 35.03, df = 2) and between the *Trichoderma* treated and untreated plants (*P* = 0.011, F = 8.83, df = 1) in the percentage of dead plants at maturity stage for seed dressing treatments. However, there were no significant interaction effects between *Trichoderma* and varieties at this stage. There were no significant differences at seedling stage (*P*>0.05).

**Table 3. Two way (Variety X *Trichoderma*) interaction of Mean percentage of dead plants (± SE) of three varieties due to black root rot at harvesting for soil treatment with *Trichoderma harzianum* in 2010**

| Variety | With *T. harzianum* | Without *T. harzianum* | Variety Means | P | F |
|---|---|---|---|---|---|
| Kassa | 91 ± 6.7 | 96.71 ± 1.4 | 93.86 ± 4.1 A | 0.005 | 8.66 |
| Wayu | 28.51 ± 10.1 | 84.65 ± 7 | 56.58 ± 8.6 C | | |
| Wolki | 77.41 ± 9.7 | 76.75 ± 6.5 | 77.08 ± 8.1 B | | |
| *T. harzianum* treatment Means | 65.64 ± 8.8 b | 86.04 ± 5 a | LSD(α=0.05)16.29<br>CV (%) = 13.3 | | |
| LSD (α=0.05) | 13.3 | | | | |
| CV (%) | 17.07 | | | | |

Values with different letters in a column and across rows are significantly different

**Table 4. Overall mean percentage of dead plants (± SE) of three varieties due to black root rot at harvesting stage for seed dressing with *Trichoderma harzianum* in 2010**

| Vareity | | | | | |
|---|---|---|---|---|---|
| | kassa | Wayu | Wolki | F | P |
| Mean | 70.29 ±2.9 a | 32.02 ±5.01 b | 78.51 ± 4.3 a | 42.56 | <0.0001 |
| LSD (α =0.05) | 11.72 | | | | |
| CV (%) | 15.45 | | | | |
| *T. harzianum* treatment | | | | | |
| | With *T. harzianum* | Without *T.harzianum* | | F | P |
| Mean | 51.54 ± 5.7 b | 69 ± 2.4 a | | 15.83 | 0.002 |
| LSD (α =0.05) | 9.57 | | | | |
| CV | 15.45 | | | | |

Values with similar letters in a row are not significantly different

In the 2010 experiments, the yield varied significantly among the varieties for seed dressing ($P< 0.0001$, F = 23.04, df = 2) with no interaction effects. The highest yield was obtained from variety Wayu (824g/plot) followed by Kassa (256.4g/plot) and Wolki (186.5g/plot) which were not significantly different (Table 5.). There were no significant yield variations whatsoever in the soil treatments ($P>0.05$).

**Table 5. Yield ± standard error (SE) of three faba bean varieties treated with *Trichoderma harzianum* as seed dressing 2010**

| Seed dressing | | F | P |
|---|---|---|---|
| Variety | Yield(g/plot) | | |
| Wayu | 824.7±49.53 a | 23.04 | <0.0001 |
| Wolki | 186.5±38.13 b | | |
| Kassa | 256.4 ±152.44b | | |
| LSD (α= 0.05) | 224.69 | | |
| CV | 42.28 | | |

Values with similar letters in a column are not significantly different

Minimization of chemical pesticides and optimization of alternative management strategies are important components of sustainable management of soil borne pathogens (Harrier and Watson 2004). *Trichoderma* spp. are known to suppress many soil borne pathogens such as *Fusarium solani* and are most commonly used biological control agents (Whipps and Lumsden,2001; Harman *et al*., 2004). In the 2009 experiments, the effects of the *T. harzianum* were not obviously seen as they were probably masked by varietal effects. This may be attributed to the time it may have taken the bio-agent to get established in the rhizosphere.

However, in the 2010 experiments, significant effects of the bio-agent (*T. harzianum*) were clearly shown. Variety Wayu sown with *T. harzianum* applied to the soil resulted in the least percent of dead plants (28.51%) indicating the importance of combining varietal resistance with biological control agent. In agreement with the findings of this study, Abdel-kadir *et al*. (2015) found 35% and 57.3% reduction in root rot incidence on faba bean at pre-emergence and post emergence stages respectively with seed dressing of *T. harzianum* under field conditions. Hamed *et al*. (2012) also found up to 65 and 68 % reduction in root rot incidence at pre-emergence and post emergence stages respectively in field experiments using *T. harzianum* mass produced on rice straw as soil treatment. Similarly, Abdel-Kadir (1997) also reported that application of *T. harzianum* to the soil significantly reduced root rot incidence on faba bean plants.

The mechanisms for suppression of soil borne pathogens due to antagonistic fungi include antibiosis, competition for space and nutrient, mycoparasitism and degradation of the toxins produced by the pathogen (Arras, 1996, Elad 1996). In addition to this, the role of varietal resistance in controlling *Fusarium* root rots cannot be over emphasized and therefore developing and deploying resistant cultivars is the most effective and the cheapest control strategy for the disease (Navarrot *et al*., 2003; Mukankusi, 2011).

In this study also the importance of combining varietal resistance and bio-agent has been demonstrated. Variety Wayu showed significantly lesser percentage of dead plants as compared to the susceptible variety Kassa and the moderately resistant variety Wolki. Thus it can be concluded that the approach can be used as a component of integrated disease management for this notorious disease. Significant increase in yield was also observed in *T. harzianum* treated fields. As *Trichoderma* spp. are known to have plant growth promoter effects (Naseby *et al*., 2000), the increase in yield obtained during this study can be attributed to this fact. The results of this study agree with the findings of Sing and Sing (2004) and Rojo *et al*. (2007) who reported increase in yield and reduction in infection in different crops that were treated with *T. harzianum*. Similarly up to 50% increase in yield was obtained in faba bean treated with *T. harzianum* as a seed coating treatment (Abdel-Kader *et al*., 2011).

In conclusion, this study has demonstrated the effectiveness of *T. harzianum* in combination with resistant variety Wayu to reduce the incidence of faba bean root rot caused by *Fusarium solani*. Future research should focus on studying the effects of combining different *Trichoderma* spp. on the disease as integrated with resistant varieties.

**ACKNOWLEDGEMENT**

The authors would like to thank Dr. Getaneh Woldeab and Ato Birhanu Bekele who handled the early stages of the experiments. Compliments also go to Ato Tesfaye Hailu and Ato Beddasso Jebessa for technical assistance during the experiments. The research was funded by the Ethiopian Institute of Agricultural Research (EIAR) regular budget.

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