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Evaluation of Bioagents Seed Treatment Against *Colletotrichum Lindemuthianum*, in Haricot Bean Anthracnose under Field Condition

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Abstract The effect of Pseudomonas fluorescence, Trichoderma viride and Trichoderma harzianum on anthracnose was studied at Ambo, Ethiopia during the 2013 cropping season. There were highly significant differences between disease levels and seed yield among bio agent seed treated plots. Anthracnose on beans from untreated seed-planted was more in diseases incidence and severity than on beans from seed treated plots with Pseudomonas fluorescence, Trichoderma harzianum, and Trichoderma viride. Bean seed yield and yield components aspect were also influenced by the bioagents. Lower hundred seed weight, infected pods per plant and seed yield were obtained from seed untreated plots. There were no significant differences in pods per plant and seeds per pod. Highly significant AUDPC of 1529.8%-day occurred in the control plot and 672.2%-day in the seed treated with Pseudomonas fluorescence plot. Management options that highly reduce anthracnose primary inoculum should be promoted indicating that the purpose of seed treatment was to reduce the primary inoculum.

Keywords: *common bean, Pseudomonas fluorescence, seed treatment, Trichoderma harzianum*

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1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important legume crop in the daily diet of more than 300 million of the world’s population [1]. It has been rated as the second most important source of human food dietary protein and the third most important source of calories of all agricultural commodities produced in Eastern Africa [2]. The Shoa highlands, western Ethiopia is one of the major bean production centers in East Africa [3]. Bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cavi., is the most serious disease attacking bean in cool weathers in Ethiopia. The infected seeds are the most important means of dissemination of this pathogen, which explains its worldwide distribution [4]. For an effective and sustainable management of anthracnose disease, a detailed knowledge of its seed-borne nature including infection sites in the seed, transmission and factors of disease epidemics on the field are required [5].

Due to the risk of anthracnose, the farmers are not able to cultivate beans during the main cropping season in West Showa, Ethiopia. Moreover for sustainable production, pathogen still needs to be controlled in order to ensure healthy plants establishment and growth. In addition to assessment of various management methods like chemical and use of different bio agents are urgently needed in order to provide an alternative to other control methods. The management of anthracnose disease in common bean has been carried out by using some fungicides in some parts of the country and the integrated disease management of bean anthracnose was investigated by using the effect of soil solarization and techniques of timing of fungicide applications as main component for the integrated disease management of anthracnose in eastern Ethiopia [6].

As stated by Padder *et al.* [7], farmers use integrated crop production strategies involving various inputs, practices and means of managing biotic and abiotic stresses. However, uses of chemicals dominate all other inputs, thus leads to degradation of the environment, development of fungicidal resistance along with their harmful effect on human beings and beneficial organisms. Fungicides being highly effective against the anthracnose are mostly used both for seed treatment and foliar sprays in high rainfall conditions which could create problem of fungicidal resistance in the pathogen in due course of time, it is important to find out an alternative to avoid this risk. So, use of non chemical eco-friendly means of control has emerged as a viable alternative under such conditions [8]. The presence of naturally occurring microorganisms with antifungal property has been well recognized and documented, but very few of them have been studied extensively in case of bean anthracnose. These bioagents have been tested against an array of *Colletotrichum* species infecting many commercially important crop plants [9,10].

However, the management of bean anthracnose through bio control agents has not been studied so far in Ethiopia. Much work still needs to be done particularly in West Showa, especially with the bean anthracnose through bio agents, since the disease is still causing much devastation on the crop. Therefore, the present work will be carried out with the main objectives of to evaluate *in vivo* antagonistic activities of bio agents against common bean anthracnose disease, to determine the effect of bioagents applications on yield and yield components of common bean.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted at Ambo University (AU), Research Station during the summer cropping season of 2013, the area is hotspot for common bean anthracnose. The altitude of the study areas was between 1900 and 3100 m. a. s. l, geographical positions of N 08° 43.423-N 10° 12.082 and E 037° 28.902-040° 62.590. Monthly total rainfall, mean maximum and minimum temperature, and relative humidity at the site during the cropping seasons are given in Table 1. The soil of the experimental site is light red in color, clay loam in texture and pH value of 6.8. At the trial site, weather conditions favored the occurrence of the anthracnose disease at different vegetative and reproductive growth stages of common bean.

Table 1. Monthly total rain fall (mm), relative humidity (%), means monthly maximum (max) and minimum (min) temperature (°C) at Ambo, Ethiopia during growing period, 2013

Weather Factors	Months				
	June	July	August	September	October
Maximum temperature	25.7	23	22.7	23.5	25.7
Minimum temperature	15	13.9	14.1	12.9	6.8
Rainfall (mm)	24.6	144.9	179.6	170.4	5.6
Relative humidity (%)	58.7	73	72	65	32

2.2. Bean Genotype

Naturally infected seed used for the field trials, Mexican-142 was obtained from Melkasa Agricultural Research Center, Pulse Improvement Department, was used. The genotype was high susceptibility to anthracnose (*Colletotrichum lindmuthianum*) Sacc. & Magn.) Briosi & Cav. The seed is small in size and white-colored.

2.3. Application of Bioagents

Talc based formulations (28 x 10^{-6} cfu/g product) of *T. viride* and *T. harzianum* [11] were used as seed treatments at 40 g/Kg of seeds soaked in 1 L of water for 24 hrs. Similarly, the talc based formulation of *P. fluorescence* by the method of Kloepper and Schroth, [12] was used as a seed treatment @ 10 g/Kg of seeds soaked in 1 L of water for 24 hrs. Naturally infected seeds of the variety Mexican 142 were treated with each bioagent separately and dried overnight before sowing. Seeds without the application of bioagents were serving as control. The bioagents were obtained from Plant Pathology Laboratory, Department of Plant Science, College of Agriculture and Veterinary Science.

2.4. Treatments and Field Trial

Four treatments were used in a randomized complete block design in three replications. The treatments were: (1) untreated seeds, which used as control (2) treated seeds with *Pseudomonas fluorescence*, (3) *Trichoderma harzianum*, and (4) *Trichoderma viride*. The experiment was conducted on plots size of 1.0 x 1.6 m. The plots were on a potato rotated field on which beans had not been grown for more than 6 years. Bean seeds were sown at a rate of two seeds per hill in four rows on 20 July and thinned to one plant 15 days after sowing (DAP). The rows were 40 cm apart and spacing between plants was 10 cm. The spacing between plots was 1 m. Hand weeding was done twice and no fertilizers were applied.

2.5. Disease Assessment

Bean anthracnose incidence assessment, expressed as proportion of plants diseased, was started at flowering stage. Disease incidence and severity were assessed under natural field infections, every week. Disease severity, area of plant tissue disease, was rated on 10 randomly selected and tagged diseased plants using standard scales of 1 ± 9 [13] where 1 is no visible disease symptom and 9 is disease covering more than 25% of the foliar tissue. Number of infected pods per plant was scored on 10 randomly selected and pre tagged plants. The severity grades were converted into percentage severity index (PSI) [14].

2.6. Crop and Yield Assessment

To obtain a balanced view of the effects of seed treatment bioagents on bean yield and yield components, number of pods per plant and number of seeds per pod were recorded from 10 randomly selected plants and pods at harvest. In addition, bean seed yields at 10% seed moisture content and 100 seeds weight were recorded.

2.7. Data Analysis

Data on bean anthracnose incidence and severity from each assessment date, infected pods per plant, yield and yield components, and AUDPC data were subjected to analysis of variance by using SAS software, version 9.1 computer software. Least significant difference (LSD) values were used to separate differences among treatment means.

3. Results

3.1. Bean Anthracnose Incidence and Severity

Diseases assessment was started on 65 days after planting (DAP) at time of disease onset. The plots showed significantly ($p < 0.05$) different levels of bean anthracnose incidence and severity starting from the all dates of disease assessment (65, 72 and 81 DAP). During each disease assessment, the lowest incidence and severity level of bean anthracnose was recorded on treated seeds with *Pseudomonas fluorescence*, *Trichoderma harzianum*, and *Trichoderma viride* plots. While, the untreated plots had the maximum disease incidence and severity levels consistently for the last three assessment dates.

Table 2. Effect of Bioagents seed treatment on anthracnose severity and incidence at different days after planting

	% Disease Severity			% Disease Incidence			
Treatment	65 DAP	73 DAP	81 DAP	65 DAP	73 DAP	81 DAP	Infected Pods/plant
P. fluorescence	22.8[b]	25.6[b]	45.7[c]	50[a]	63.3[ab]	76.7[ab]	12.8b
Trichoderma harzianum	43.2[a]	48.3[a]	65.9[b]	43.3[a]	56.7[b]	70[b]	17.7ab
Trichoderma viride	50.8[a]	53.8[a]	57.9b[c]	50[a]	60[ab]	66.7[b]	15.9b
Control	53.2[a]	63.2[a]	86.3[a]	66.8[a]	80[a]	93.3[a]	23.5a
LSD (0.05)	14.56	16.17	12.38	NS	22.4	18.8	6.85

DAP = Days after planting; P = *pseudomonas;* Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$)

On the final date of disease assessment i.e. on the 81 th DAP; the incidence and severity level of the disease on untreated plot were 93.3 and 86.3%, respectively. Whereas, disease incidence on plots treated seeds with *Pseudomonas fluorescence*, *Trichoderma harzianum*, and *Trichoderma viride* were 76.7, 70 and 66.7%, respectively; Severity on plots treated seeds with *Pseudomonas fluorescence*, *Trichoderma harzianum*, and *Trichoderma viride* were 45.7, 65.9 and 57.9%, respectively (Table 2).

Infected pods per plant was significantly ($P < 0.05$) different between the levels of treated seeds with *Pseudomonas fluorescence*, *Trichoderma harzianum*, and *Trichoderma viride* (Table 2). Lower infected pods per plant were recorded on plots treated seeds with *Pseudomonas fluorescence* (12.8) followed by *Trichoderma viride* (15.9). While, the maximum number of infected pods per plant was recorded on control plot (23.5).

3.2. Pod Infection Due to Anthracnose

Table 3. Effect of seed treatments (Bioagents) on yield components and grain yield

Treatment	Hundred SW (gm)	Yield (tons/ha)	Seeds/pod	pods/plant
P. fluorescence	23.3[a]	2.26[a]	4.7[a]	26.67[a]
Trichoderma harzianum	21a[b]	1.45[b]	4.8[a]	20.33[a]
Trichoderma viride	18[b]	1.53[b]	4.7[a]	26.2[a]
Control	17.7[b]	0.78[c]	4.2[a]	17.47[a]
LSD (0.05)	3.35	3.35	Ns	Ns

P = *pseudomonas*; Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$); SW = Seed weight; Ns = non significant

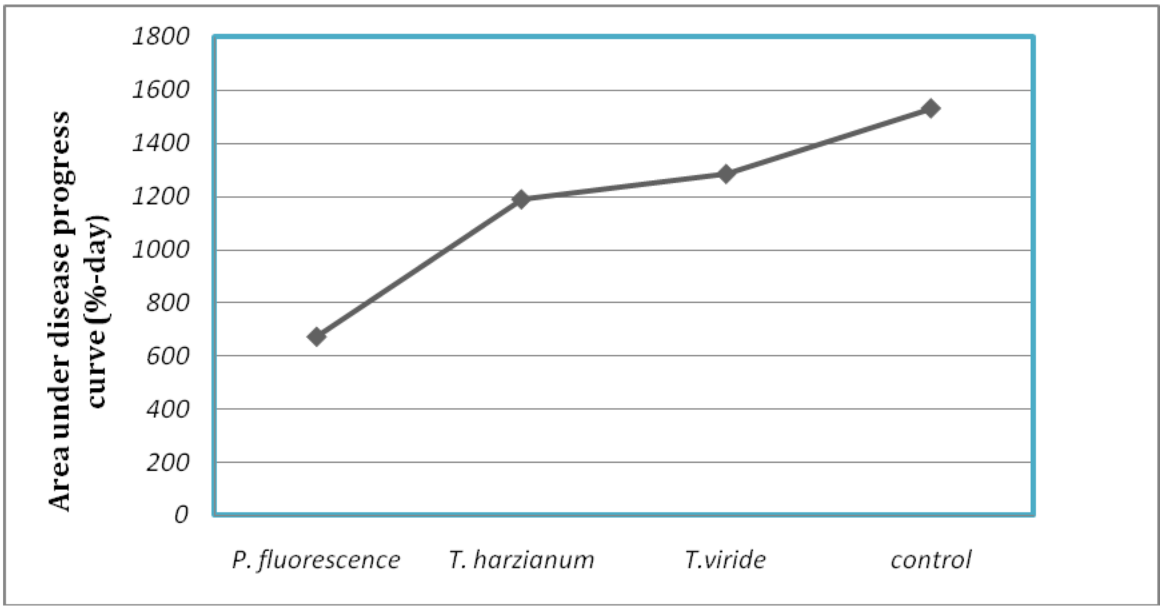

Figure 1. Area under disease progress curve values as affected by treated seeds with *Pseudomonas fluorescence*, *Trichoderma harzianum*, and *Trichoderma viride* at Ambo in 2013 main cropping season

3.3. Yield and Yield Components

The data from this experiment showed that, similar to the disease incidence and severity, there were significant (P < 0.05) hundred seed weight and total yield difference between the treatments. But, there were no significant (P < 0.05) seeds/pod and pods/plant difference (Table 3). Maximum hundred seed weight was observed on treated seed with *Pseudomonas fluorescence* (23.3 gm) followed by *Trichoderma harzianum* (21 gm). Whereas, the minimum was recorded on control plot (17.7 gm). Likewise, the highest yield was obtained from plot treated seed with *Pseudomonas fluorescence* (2.26 tons/ha) and followed by *Trichoderma viride* (1.53 tons/ha). However, the lowest was recorded on control plot (0.78 tons/ha). The variation in yield losses was observed among different treatments. In comparison, in untreated control plots, seed yield losses was notably higher than protected plots.

3.4. Area under Disease Progress Curve

Area under disease progress curve was significantly different at (P < 0.05) among the treatments. Optimum diseases progress was observed on control plot. While, the minimum progress, AUDPC were recorded on treated seeds with *Pseudomonas fluorescence*, *Trichoderma harzianum* and *Trichoderma viride* (Figure 1).

4. Discussion

Excessive use of chemicals in plant disease management has resulted in number of problems related to fungicide resistance, damage to non target flora and fauna and other useful organisms along with hazardous effects of residue on environment have become the main concern of scientists at present [16]. Seed treatment to manage crop pests is an alternative approach to minimize pesticide hazards. It has advantages such as, easy application, low cost, less pollution, selectivity and least interference in the natural equilibrium over soil or foliar application.

Bean anthracnose a cosmopolitan seed borne disease can effectively be controlled if the healthy or treated seed is used for planting [7]. Hence, in the present investigation biocontrol approaches was evaluated to evolve eco-friendly module for its management. In the present investigation all the three biocontrol agents viz., *T. harzianum*, *T. viride* and *P. pseudomonas* significantly reduced diseases epidemics and maximized seed yield. Antagonism with *Trichodermsea* species against an array of phytopathogens has been reported by many workers [17]. Most of them have been reported to produce volatile and non-volatile compounds that inhibit the growth of fungal phytopathogens. Besides, production of various antibiotics along with large number of volatile secondary metabolites plays an important key role in bio-control [18]. The capacity shown by *Trichodermsea* species bioagents to overgrow colonies of *C. lindemuthianum* and coil around its hyphae suggests hyphal interaction mechanism [7]. Moreover, application of *Trichoderma* fungi in agriculture has four beneficial effects for plants. First, it can colonize plant root and its rhizosphere, second, *Trichoderam* fungi control plant pathogens thought parasitism, and antibiosis production, and promote systemic resistance. Third, it improves plant health through increasing plant growth. Ultimately, *Trichoderam* fungi stimulate root growth and improve plant growth [19]. Studies showed that *trichoderma* treatment in tobacco increased fresh weight of root and leaf area index (300%), lateral roots (300%) and the leaf (140%) [20]. various strains of *Trichoderma* have been founded to be effective in plant growth characteristics and enhance biomass production [21]. These fungi inhabit plant root and plant growth characteristics by increasing evolution and production of plants [22] improved root growth and abiotic stress [23] will enhance systemic resistance to diseases. Hyperparasitism along with the production of secondary metabolites and antibiotics might have attributed in inhibition of mycelial growth and seed borne infection, as the *Trichoderma* species either added to the soil or applied as seed treatment, grow rapidly along with the developing root system of the treated plant [23,24,25]. The mechanism of plant disease control by *P. fluorescens like*, production of antibiotics, siderophores, volatile compounds and ammonia, induction of systemic resistance and competition for nutrients [26].

In the present investigation an attempt was made to reduce primary inoculum. However, for development of such a module large number of *Trichoderma* species are to be isolated from the rhizoshere of beans and their screening along with the exploitation of phylloplane microorganisms.

5. Conclusions

Colletotrichum lindemuthianum result in significant yield losses of haricot beans at Ambo, Ethiopia. Farmers, in general still rely on the use of farmers' saved seed system, which is highly infested with anthracnose. The misuse of synthetic fungicides may cause serious environmental and health problems. Bioagents seed treatment antagonists are potential that can be explored to provide effective and safe means to seed borne fungal pathogens. Our recent study showed that *Pseudomonas fluorescence*, *Trichoderma harzianum* and *Trichoderma viride* apparently suppressed the growth of bean anthracnose disease.

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