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Full Length Research Paper

In vitro evaluation of some fungicides and bioagents against common bean anthracnose (*Colletotrichum lindemuthianum* Sacc. & Magnus*)* Briosi & Cavara

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Bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara is one of the most devastating seed-borne diseases of common bean (*Phaseolus vulgaris* L.) in Ethiopia. The aim of the present investigation was to evaluate the antifungal activities of bioagents and fungicides which can be used to control bean anthracnose. Three fungicides viz., Mancozeb, Folpan and Mancolaxyl, and three bioagents viz., *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers. Fr. and *Pseudomonas fluorescens* Migula, were screened *in vitro* for their antifungal activities against common bean anthracnose, *C. lindemuthianum* using the dual culture and microtitre double-dilution techniques. Antagonistic effects of the three bioagents tested by the dual culture method showed highly significant (P<0.01) percentage of inhibition of the mycelia germination of *C. lindemuthianum.* The highest percentage of inhibition of the mycelia germination (80.39%) was obtained from *T. viride,* followed by 75.49% from *T. harzianum* and 40.2% from *P. fluorescens*. Similarly, highly significant (P<0.01) differences were observed in the radial growth of mycelia of *C. lindemuthianum.* The highest growth of mycelia (3.4 cm) was measured from the control (*C. lindemuthianum*), whereas the least (0.67 cm) was obtained from the dual culture containing *T. viride.* The *in vitro* assays revealed that all the antagonistic bioagents produced siderophores which were capable of inhibiting mycelia growth of the pathogen. The mancozeb fungicide was found to be fatal to *C. lindemuthianum* at four different concentrations poisoned on potato dextrose agar medium.

Key words: Bio agents, *Colletotrichum lindemuthianum*, dual culture, fungicides, *in vitro, Phaseolus vulgaris.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important food grain legume crop, cultivated in almost every part of the world (Ibarra-Perez et al., 1997). For many households in the tropics, the crop is a good source of cash and food nutrients (Popelka et al., 2004). Globally, common bean is cultivated in nearly 28 million hectares of land, producing about 20 million tons annually (FAOSTAT, 2008). The highest production and area

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under common beans in Africa is in the east and central regions, where beans are mainly grown by resource poor farmers (Wortmann et al., 1998). In Ethiopia, common bean is mainly cultivated in the eastern, southern, south-western and rift valley regions of the country (Habtu et al., 1996). Annual area coverage is estimated to be around 200,000 ha (CSA, 2010). The crop is increasingly becoming important to the national economy and to the farmers as food and cash income. Despite its economic significance and wide area of production, the national annual average yield of common bean in Ethiopia is low, ranging from 0.615-1.487 tons/ha between the years 2004 and 2010 (CSA, 2010). Such a low figure is far below the corresponding yield recorded at research sites (2.5-3 tons/ha) using improved varieties (EEPA, 2004). The low national yield could be attributed to various constraints as low adoption of improved agricultural technologies, drought, diseases and pests, lack of improved seed varieties, poor cultural practices, shortage of land and environmental degradation (Legesse et al., 2006, Kutangi et al., 2010). Due to extreme differences in agro-climatic conditions and cropping practices in Ethiopia, these production constraints could vary from one region to the other.

Common bean production is influenced by both biotic and abiotic stresses; biotic factors are responsible for major losses. Six major diseases (anthracnose, rust, angular leaf spot, common bacterial blight, Bean Golden Mosaic Virus and Bean Common Mosaic Virus) are known to hamper common bean production. However, the most important among these is anthracnose which is found in almost every bean growing region of the world (Kelly et al., 1994). It is considered as one of the most destructive disease (Pastor-Corrales and Tu, 1989). Anthracnose of the common bean is caused by *Colletotrichum lindemuthianum,* a hemibiotrophic fungus. The disease generally occurs by contaminated seeds or infected plant debris (Dillard and Cobb, 1993). This disease may lead to major or total crop loss, particularly in a case where a susceptible variety is grown (Fernandez et al., 2000; Sharma et al., 2005).

Chemical and biological methods are very useful alternatives among the different strategies for plant growth promotion and disease suppression.

Bioagents (living antagonistic organisms) however, can be safer, more biodegradable and less expensive to develop as compared to synthetic fungicides (Amin et al., 2014). The use of *Pseudomonas fluorescens* and *Trichoderma* species are becoming increasingly common as an effective, economic and environment friendly approach and also effectively controlling many seed and soil borne pathogens including *Colletotrichum.* Padder et al. (2010) reported that seed dressing or soil application of *Trichoderma viride*, *Trichoderma harzianum* and *Gliocladium virens* caused significant inhibition of mycelial growth of *C. lindemuthianum*, effectively controlling the seed borne infection and increasing the seed germination; extracellular metabolites like siderophores, antibiotics, lytic enzymes and volatile compounds produced by rhizobacteria (*Pseudomonas fluorescens* and *Bacillus capacia*) effectively reduced lesions and damages caused by *C. lindemuthianum* on bean plants.

Recent innovations showed that biological control of crop diseases is getting increased attention as an environ-mentally sound approach. But in Ethiopia, the method has received comparatively little attention. Apparently, the management of bean anthracnose through biocontrol agents, particularly*, P. fluorescens, T. viride* and *T. harzianum* has not been studied so far in Ethiopia. Therefore, the present work was carried out with the objective of the evaluation of some fungicides and bioagents for the management of common bean anthracnose disease under *in vitro* conditions.

MATERIALS AND METHODS

Project location

In vitro evaluation of the antagonistic activities of three bio agents: *T. harzianum*, *T. viride* and *P. fluorescens* and the efficacy of three fungicides; Mancozeb (Unizeb 80% WP), Folpan 80% WDG and Mancolaxyl 72 WP (Mancozeb + Metalaxyl) against common bean anthracnose disease was carried out in Plant Science Research Laboratory, Ambo University, Ethiopia. All the three bioagents and fungicides were obtained from the Department of Plant Science of the university. Ambo is located 120 km west of Addis Ababa at 8°98' South latitude and 37°83' North longitude. It has a total geographical area of 83,598.69 km^2, with elevation ranging from 1380-3300 m above sea level.

Sample collection and isolation of common bean anthracnose pathogen

Anthracnose infected common bean variety Mexican-142 pods were collected from the University farm and washed in running tap water. About 1 g of the diseased sample was surface sterilized in a solution of 1% hypochlorite and macerated three times in sterile distilled water and the filtrate was further diluted using sterile distilled water. From the appropriate dilutions, 0.1 ml of an aliquot was spread-plated in duplicates on pre-dried surfaces of Potato Dextrose Agar (PDA) medium and incubated at 26°C for 10 days. The colonies of *C. lindemuthianum* was picked up from the culture plates and further sub-culturing and purified through repeated spread-plated on the PDA medium (Das et al*.,* 2003).

Antifungal assay (dual culture method)

The dual culture method was used to evaluate the antagonistic effects of the bioagents. A 5 mm diameter agar disc of each of the three bio-agents (*T. harzianum, T. viride* and *P. fluorescens*) were taken from five day old cultures and placed, separately, at the periphery of Petri plates (90 mm, diameter) containing solid PDA. A similar size of agar disc of *C. lindemuthianum* was placed at the periphery of each Petri plate with the bioagents, but on the opposing end. *C. lindemuthianum* agar disc was placed in a similar manner on a fresh PDA plate, as control. All pairings were carried out on three replicates and incubated at 25°C. Antagonistic activity was assessed after five days of incubation by measuring the radius

Figure 1. Bean crops with anthracnose infected pods.

Figure 2. *C. lindemuthianum* growth on agar plates.

$$\text{PIMG (\%)} = \frac{R1 - R2}{R1} \times 100$$

of the mycelia growth (R2) of *C. lindemuthianum* in the direction of the antagonistic colony and the radius of *C. lindemuthianum* in the control plate (R1). The two readings, R1 and R2 were transformed into percent inhibition of mycelium growth (PIMG) according to Skidmore and Dickinson (1976). The number of days taken for the antagonists to overgrow the whole colony of *C. lindemuthianum* was also recorded.

Siderophore production assay

The procedure of Schupp et al. (1988) was used for detection of siderophore production by the three bioagents. Whatman No.1 filter paper soaked in an indicator solution containing 1% ammonium ferric sulfate [$FeNH_4(SO_4)_2.12H_2O$] in sulfuric acid was placed on 4-day old single colonies of each of the three bioagents grown on Des-4 medium containing; 2% Dextrin, 2% Manitol, 1.2% L-asparagine, 0.025% L-lysine, 0.01% L-methionine, 0.01% L-theronine, 0.5% $CaCO_3$, 0.025% $MgSO_4$, 0.05% K_2HPO_4, 0.005% $ZnSO_4$ and 2% agar. Brown halo formation around the colonies of each bio agent was recorded and used as detection for siderophore production.

Efficacy of fungicidal assay

The efficacies of each of the three fungicides, Mancozeb, Folpan and Mancolaxyl against *C. lindemuthianum* were evaluated using the Food Poisoned Technique, as described by Nene and Thapliyal (1979). Potato dextrose agar (PDA) liquid medium was mixed with different concentrations (100, 250, 500 and 1000 ppm) of each of the three fungicides, poured into sterilized Petri plates and allowed to solidify. A disc of 7 mm diameter of *C. lindemuthianum* grown on a solid PDA medium was cut with the help of a sterilized cork borer and placed aseptically in the center of each of the Petri plates containing the test fungicides and incubated at room temperature for 7 days. Culture discs grown under the same conditions on PDA without the test fungicides were used as controls. The radius of the mycelia growth in each fungicidal concentration was measured.

Statistical analysis

Analysis of variance (ANOVA) was performed for the antifungal antagonistic assay using Statistical Analysis System (SAS) version 9.1.3 software (SAS Institute, 2002). Least significance difference (LSD) was used to separate treatment means ($P<0.05$).

RESULTS AND DISCUSSION

Isolation of the pathogen

The target anthracnose pathogen was isolated from freshly collected common bean pod samples (Figure 1) at the University farm. All the isolates produced characteristic central blackish surrounding whitish on PDA medium (Figure 2) and this observation was confirmed with the observation of Ainsworth et al. (1973).

Antifungal assay

Antagonistic effects of the three bio agents tested showed highly significant ($P<0.01$) percentage of inhibition of the mycelia growth (PIMG) of *C. lindemuthianum.* The highest PIMG (80.39%) was obtained from *T. viride,* followed by 75.49% from *T. harzianum* and 40.2% from *P. fluorescens*. Similarly, highly significant ($P<0.01$) differences were observed in the mycelia growth of *C. lindemuthianum.* The highest growth of mycelia (3.4 cm) was measured from the control, whereas the least (0.67 cm) was from the dual culture containing *T. viride* (Table 1; Figure 3). This is similar to the works of Padder et al. (2010) who, in India, recorded mycelia growth inhibition of 69.21 and 64.2% with *T. viride* and *T. harzianum*, respectively, against a local strain of *C. lindemuthianum*.

T. viride was overgrown on the whole colony in dual culture plates and started sporulation in 10 days and *T. harzianum* in 12 days after inoculation of the dual culture. Contrarily, the bacterial antagonistic, *P. fluorescens* did

Table 1. Effect of dual cultures of bioagents on mycelial growth, PIMG and the number of days taken for the antagonists to overgrow the whole colony of *C. lindemuthianum* on PDA.

Bioagents used	Mycelial growth (cm)	PIMG (%)	Overgrowth of colony (days)
T. viride	0.67^{c}	80.39^{a}	10
T. harzianum	0.83^{c}	75.49^{a}	12
P. fluorescens	2.03^{b}	40.2^{b}	-
Control	3.4^{a}	-	-
C.V	13.5	11.12	-
LSD (0.05)	0.47	16.48	-

PIMG= Percentage inhibition of mycelial growth, PDA = potato dextrose agar.

Figure 3. Mycelial inhibition in dual cultures; A = *T. viride* vs. *C. lindemuthianum*, B = *T. harzianum* vs. *C. lindemuthianum* and C = *P. fluorescens* vs. *C. lindemuthianum*.

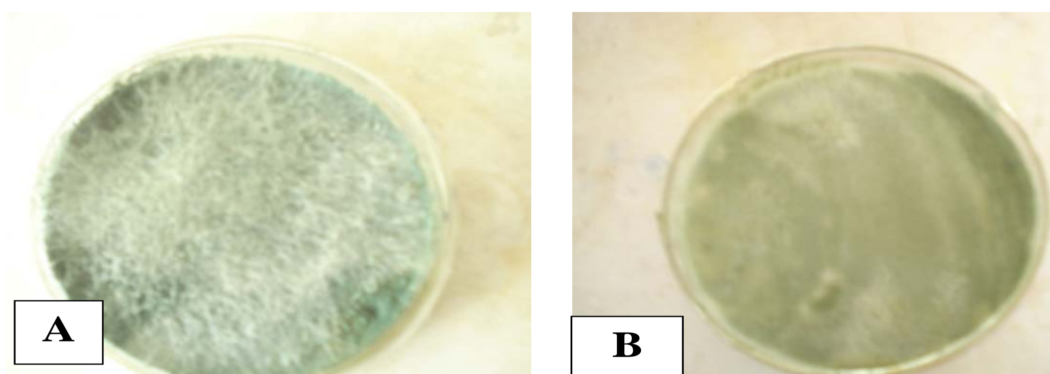

Figure 4. Overgrowth of colony of *C. lindemuthianum* and sporulation; A = *T.* viride, 10 DAI and B = *T. harzianum*12 DAI, Where: DAI = days after inoculation.

not show any signs of colony overgrowth (Table 1; Figure 4).

Siderophore production

The *in vitro* detection of siderophore production by the three bioagents viz., *T. viride*, *T. harzianum* and *P. fluorescens* revealed that all produced the extracellulariron chelating metabolites. Cultures of the three bioagents formed brown halo around their colonies immediately after addition of the indicator, soaked filter papers. Cultures of *T. viride*, formed dark brown halo and *P. fluorescens* formed light brown halo, whereas cultures of *T. harzianum* formed an intermediate brown halo around their colonies (Figure 5).

O'Sullivan and O'Gara (1992) reported that *P. fluorescens* inhibit the plant pathogens through the production of antibiotics, chitinolytic enzymes and

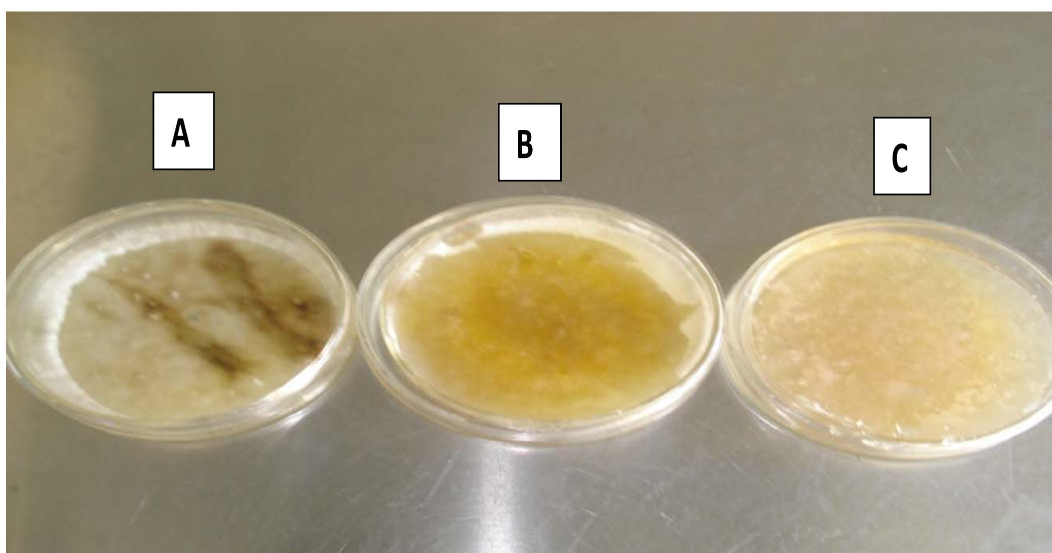

Figure 5. Brown halo formation around colonies of bioagents; A = *T. viride*, B = *T. harzianum* and C = *P. fluorescens*.

Table 2. Effect of different concentrations of test fungicides on the radial growth of mycelia of *C. lindemuthianum* using the food poison technique.

Test fungicide	Mycelial growth of *C. lindemuthianum* in different fungicidal concentrations (cm)			
	100 ppm	250 ppm	500 ppm	1000 ppm
Mancolaxyl	3.0	2.63	0.67	0
Folpan	3.2	2.97	2.4	0
Mancozeb	2.4	2.23	0	0
Control	3.5	3.5	3.5	3.5

ppm= parts per million.

siderophores; extracellular metabolites like siderophores, antibiotics, lytic enzymes and volatile compounds produced by rhizobacteria (*P. fluorescens* and *Bacillus cepacia*) effectively reduced lesions and damages caused by *C. lindemuthianum* on bean plants. Siderophore production by different *Trichoderma* strains is documented (Heidrun et al., 1991). Padder et al. (2010) suggested a hyphal interaction mechanism in which hyper parasitism along with the production of antibiotics and secondary metabolites like siderophores to have contributed to the inhibition of mycelial growth, and control of seed borne infection.

Efficacy of fungicides

Four different concentrations of the three synthetic fungicides evaluated via the food poison technique showed inhibition of mycelial growth to a varying extent. No growth of *C. lindemuthianum* was observed in any of the three fungicides at a concentration of 1000 ppm. Mancozeb showed the least mycelia growth with 2.4 cm at 100 ppm and 2.2 cm at 250 ppm concentrations, whereas the growth of mycelia was not allowed at both 500 and 1000 ppm. Mancolaxyl showed comparatively better inhibition at 100, 250 and 500 ppm fungicidal concentrations than folpan (Table 2).

Conclusions

Anthracnose has been reported as a serious threat to bean production in the major common bean growing regions of Ethiopia, especially in areas like Ambo. Frequent rainfall and moderate temperature that prevail during the main cropping season predispose the crop to attack by various pathogens including *C. lindemuthianum*. *In vitro* evaluation of fungicides and bioagents provide useful preliminary information regarding the efficacy against a particular pathogen within a short period of time

and therefore serve as a guide for further field testing in the future. The production of siderophores and mycelial inhibition by either overgrowing or exhibiting inhibition zones that was detected *in vitro* by all the bioagents are considered to be one of the mechanisms that controlled the development of anthracnose in the future experiment-tal field study. Among the tested fungicides, Mancozeb was the most effective, showing a strong inhibition even at the lowest dosage. The bioagents evaluated in this study were found to be economically important options that need to be further investigated.

Conflict of interest

The authors declare that they have no conflict of interest.

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