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ANTAGONISTIC EFFECT OF NATIVE *BACILLUS* ISOLATES AGAINST BLACK ROOT ROT OF FABA BEAN

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ABSTRACT

Faba bean (*Vicia faba* L.) is one of the most important pulse crops grown in eastern Africa. Black root rot (*Fusarium solani*) is known to cause great yield losses in faba bean, especially in the highlands of Ethiopia. The objective of this study was to evaluate the biological control ability of native *Bacillus* species on the basis of their antagonistic effects against *F. solani.* The study was conducted *in vitro* and in a greenhouse. All tested *Bacillus* isolates significantly ($P \leq 0.05$) reduced radial mycelial growth of the pathogen. Seven bacterial isolates restricted growth of the pathogen to <14 mm diameter, and showed 39-44% efficacy over the positive control. Isolate BP048 was the most effective, with 43.6% efficacy. The lowest inhibitory effects, 15.5 and 27.8%, were recorded from isolates BS083 and BS0102, respectively. The culture filtrate of the bacterial isolates also inhibited *F. solani* spore germination. *In vivo*, the isolates significantly reduced severity of black root rot on artificially inoculated faba bean seedlings. The antagonist *Bacillus* isolates kept black root rot severity low with more than 50% disease suppression, compared to the untreated control.

Key Words: Ethiopia, *Fusarium solani, Vicia fabae*

RÉSUMÉ

La féverole (*Vicia faba* L.) est l'un des plus importants légumes grains cultivé en Afrique de l'Est. La maladie de pourriture racinaire noire (*Fusarium solani*) cause des pertes de rendement en grain chez la féverole, surtout dans les terres émergées de l'Ethiopie. L'objectif de cette étude était d'évaluer les potentialités de lutte biologique au travers de l'effet antagoniste des espèces native de *Bacillus* contre *F. solani.* L'étude a consisté en des expérimentations *in vitro* et en serre. Toutes les souches bacteriennes testées réduisent de façon significative ($P \leq 0.05$) la croissance des colonies mycéliennes de l'agent pathogène. Sept souches bactériennes ont réduit la croissance du pathogène à moins de 14 mm de diamètre, et ont montré 39 à 44% d'efficacité par rapport à l'expérience témoin. La souche BP048 était le plus efficace (43.6%). Les moindres effets inhibiteurs, 15.5 et 27.8%, ont été enregistré respectivement chez les souches BS083 et BS0102. Le filtrat du milieu de culture des souches bactériennes ont aussi inhibé la germination des spores de *F. solani*. En culture *In vivo*, les souches bactériennes ont réduit de façon significative la sévérité de la maladie de pourriture racinaire noire sur des plants de féverole inoculés artificiellement. Les souches antagonistes de bacille maintiennent faible la sévérité de la maladie de pourriture racinaire noire avec plus de 50% d'éradication, par rapport au témoin non traité.

Mots Clés: Ethiopie, *Fusarium solani, Vicia fabae*

INTRODUCTION

Faba bean (*Vicia fabae* L.) is one of the most important pulse crops grown in eastern Africa. The production of the crop is, however, threatened by black root rot caused by *Fusarium solani* (Mart.) Sacc. (Hasanzade *et al.*, 2008; Bogale *et al*., 2009). In the highlands of Ethiopia, faba bean black root rot is among the most important disease in black clay soils, where water-logging is a big problem (Birhanu *et al*., 2003; Eshetu *et al.,* 2013). It causes up to complete crop yield losses in epidemic seasons (PPRC, 1996).

Many strategies have been used to minimise the effect of root rot on faba bean production; examples include use of resistant varieties, and cultural practices such as crop rotation and furrow planting to drain out excess water, and seed treatments (Tesfaye, 1996; SARC, 2005). However, results from cultural practices and chemicals are ineffective since the pathogen survives in the soil in the absence hosts (Avilés *et al*., 2003; Abeysinghe, 2007). Biological control, through the use of natural antagonistic microorganisms, provides an alternative method for managing soilborne pathogens (Rosa and Herrera, 2009). Biological control is ecologically friendly, safe and specific for controlling pathogens (El-Kassas and Khairy, 2009). Therefore, it can be used as a component of integrated disease management option to minimise economic impacts of root rot (Landa *et al.,* 2004).

A number of fungi and bacteria are known to be very effective antagonists against soil-borne pathogens (Shoda, 2000). *Bacillus*-based biological control agents have great potential in integrated disease management (IDM) options, together with cultural control, resistant cultivars, fungicides or others biological control agents (Jacobsen *et al*., 2004). This genus comprises of a heterogeneous group of Gram-positive, aerobic or facultative anaerobic, endospore-forming bacteria. *Bacilli* survive in many diverse environments, often with extreme variations in temperature, nutrient, and other stresses (Driks, 2004). These properties are associated with the ability to produce peptide antibiotics and contribute to the utilisation of *Bacillus* spp. to manage several root and foliar diseases (Kloepper *et al*., 1999; Driks, 2004.).

Bacillus spp. were reported as effective in controlling a wide range of pathogens, such as *F. oxysporum* f.sp *ciceris,* on chickpea (Karimi *et al.,* 2012; Moradi *et al*., 2012), *F. oxysporum* f. sp. *lycopersici* on tomato (Kumar *et al*., 2012), chocolate spot (*Botrytis fabae)* on faba bean (Sahile *et al.,* 2009), and root-knot nematodes (*Meloidogyne javanica*) on bean (Dawar *et al.,* 2008). The objective of this study was to evaluate native *Bacillus* spp. for controlling *F*. solani causing root rot on faba bean in Ethiopia.

MATERIALS AND METHODS

Sample collection and isolation. Fifty-eight faba bean roots and 103 soil samples were collected from three major faba bean growing districts (Delanta, Jamma, and Woreillu) of the northeast highlands of Ethiopia. The districts are located between 39°07′ to 39°26′E and 10°19′ to 11°35′N, with altitude ranging from 2551 to 3017 metres above sea level (m.a.s.l.). Vertisols dominate the soil types in the area and are characterised by water-logging (Abebe, 2012). The rainfall is bimodal, with a short period in April or May, and the main rain season during June to September. The annual rainfall average is 839.9 mm. The monthly mean minimum and maximum temperatures are 7.3 and 19.6 °C, respectively.

Healthy faba bean plants were up-rooted and the roots were collected in a plant sampling paper bag. At the same time, approximately 100 g of rhizosphere soil was carefully transferred into sterile plastic bags, and refrigerated at 4 °C, at the Plant Pathology Laboratory, Haramaya University.

Isolation of *Bacillus* spp. from healthy faba bean roots and rhizosphere soil was done using the serial dilution technique (Watesman, 1922). Each composite soil sample was thoroughly mixed and pulverised using a porcelain mortar and pestle; and sieved through a 0.5 mm soil screen mesh, before 1 g was suspended in 9 ml sterile distilled water. The suspensions were made homogeneous by agitation using Vortex Mixer, before serial dilutions of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared. Isolations from plant samples were made from root segments, by washing with sterile water and removing adhering soil; before cutting the roots into pieces (2-3 cm). The pieces were

surface-sterilised in 1% sodium hypochlorite, for 3 minutes, and rinsed twice in sterilised distilled water. Sterilised root sections of approximately 5 g were washed in 50 ml sterile distilled water on a wrist action shaker for 30 minutes and serial dilutions were made as described above.

One millilitre of serially diluted suspension from each soil, and root dilutions, was pipetted into sterile Petri plate, containing autoclaved King' B medium and Nutrient Agar (NA) medium, cooled to about 50 ËºC was poured into the plate. To uniformly spread the suspension, the Petri plate media were mixed by gently swirling in clockwise and anti-clockwise directions. Isolates of bacteria were picked for antagonism studies after incubation of the plates at 28±1 °C for 48 hr. Representative colonies were selected from the countable plates and re-streaked on a new plate, but of the same media to obtain pure colonies.

All bacterial isolates were Gram-stained, and Gram-positive and motile bacterial isolates were tested for catalase reaction and growth at 45 °C (Schaad *et al.,* 2001). All catalase- positive and growth at 45 °C, rod-shaped and endospore forming bacteria were identified as *Bacillus* isolates and were maintained at 4 °C on slants of NA for further studies. *Bacillus* isolates showing potential antagonistic activity were selected and their cultural characteristics evaluated on PDA (Table 1).

***In vitro* antagonistic test.** Twelve isolates of *Bacillus* spp. were tested for antagonistic effects against *F. solani,* using the dual culture method (Abdel-Kader *et al*., 2002; Kamil *et al*., 2007). Cultures of *Bacillus* isolates were picked aseptically and streaked in the centre of Petri plate containing Potato Dextrose Agar (PDA) medium. *Fusarium solani* culture, consisting of five millimetre diameter agar discs, were taken with a sterilised cork-borer from the growing margin of a five-day-old culture and placed on either side of *Bacillus* inoculated plates. A culture plate without biological control agent treatment was used as control or check.

The treatments were replicated three times and arranged in a completely randomised design (CRD), and incubated at 25±2 °C for 5-7 days. Cultures were observed daily and data on growth inhibition zone and colony diameter were recorded for each plate. The radii of the fungal colony towards and away from the antagonistic colony were measured and the percent growth inhibition was calculated using the following formula (Abeysinghe, 2007).

$$\% \text{ Inhibition} = \frac{(R - r)}{R} \text{ X } 100$$

..... Equation 1

TABLE 1. Cultural characteristics of *Bacillus* spp. on Potato Dextrose Agar (PDA) medium

Isolate code	Colony shape	Colony elevationfrom side	Colony margin	Colony surface texture	Colony growth after 48 hrs
BP018	Irregular and spreading	Hilly	Lobate	Smooth shiny	Fast
BP037	Round	Convex	Smooth	Smooth shiny	Slow
BP048	Irregular	Flat	Lobate	Smooth shiny	Fast
BP079	Irregular and spreading	Flat	Lobate	Irregular and spreading	Fast
BP101	Round	Convex	Smooth	Wrinkled	Slow
BS024	Irregular and spreading	Flat	Wavy	Dry	Slow
BS052	Rounded with wrinkled margin	Hilly	Lobate	Dry	Slow
BS069	Round and raised margin	Convex	Wavy	Smooth shiny	Slow
BS071	Irregular	Flat	Lobate	Smooth shiny	Intermediate
BS082	Round	Flat	Smooth	Dry	Intermediate
BS083	Rounded with wrinkled margin	Flat	Wavy	Smooth shiny	Slow
BS102	Round	Convex	Smooth	Smooth shiny	Slow

Where, *R* is the maximum radius of the fungal colony away from the bacterial colony; *r* is the radius of the fungal colony opposite the bacterial colony.

The effect of *Bacillus* isolates on conidial germination of *F. solani* was evaluated by taking a loopful of *Bacillus* isolate culture multiplied in 250 ml nutrient broth, in 500 ml Erlenmeyer flasks and incubated in a shaker-incubator at 120 rpm at 25 °C (Yonas and Amare, 2007) for three days. Culture filtrates were obtained by sedimenting the cells through centrifugation at 6000 rpm for 10 minutes, and passing the supernatant through sterile Whatman-1 filter paper. The culture filtrate was diluted to 0, 25, 50 and 75% with sterile distilled water, and 0.5 ml from each concentration was pipetted into three replicated single-well depression slides. Sterile distilled water, without culture filtrate, was used as a control.

Conidia from 10 day old *F. solani* culture were transferred with a sterile loop to 10 ml sterile distilled water containing 0.05% Tween-80, before determining the concentration to 10^6 conidia ml^{-1}. Then, 0.5 ml of conidial suspension was pipetted into each depression slide and incubated in a humid chamber at 25 °C for 48 hr. Data on spore germination were recorded 12 hourly under a compound microscope fitted into 10x eye pieces with 40x objective magnification.

Greenhouse experiment. Twelve *Bacillus* isolates were tested for their ability to control *Fusarium* black root rot, on faba bean seedlings. The antagonistic isolates' spore concentration, grown on Nutrient Agar (NA), was adjusted to 10^8 CFU ml^{-1}, using spectrophotometer. Surface disinfected seeds of susceptible faba bean cultivar, CS-20DK, were dipped in the spore suspension of each *Bacillus* isolate for five hr. Seeds soaked in sterile water (without antagonists) for the same period of time were used as control.

Five inoculated seeds per 15 cm diameter pot were planted into sterile black soil (70:16:14 clay: silt: sand), and kept in the greenhouse at 24-28 °C day, and 15-20 °C night, temperature regimes. One week after planting (at 2 - 4 leaf stages) of the seedlings, each pot was artificially drenched (10^6 conidia ml^{-1}) with the most aggressive *F. solani* isolate. The experiment was conducted in a randomised complete block design (RCBD) with three replications, and repeated two times.

One month after planting, plants were removed from the soil and roots were washed with tap water. Root rot severity was scored (necrotic lesions on roots and hypocotyls) using 0-4 rating scale, where: 0 = hypocotyls and roots white and firm, no root pruning; 1 = slightly brown or discolored hypocotyls and roots, slight root pruning; 2 = moderately discolored hypocotyls and roots, extensive root pruning; 3 = darkly discolored hypocotyls and roots, hypocotyls completely collapse or, severe root pruning; and 4 = dead or dying plant (Ondrej *et al.,* 2008), Based on root rot severity, percent disease suppression was calculated using the following formula (Villajuan-Abgona *et al.*, 1996).

$$\% \text{ Suppression} = \frac{A - B}{A} \text{ X } 100$$

..... Equation 2

Where: A is the disease severity exhibited in the root region due to *F. solani* alone; and B is the disease severity exhibited in the root region after inoculation with both the pathogen and bacterial antagonists.

Data on biomass and shoot length were also recorded at this stage.

Data analyses. Statistical analyses were performed using General Linear Modeling (GLM) procedure of SAS® System for Windows Version 9.1 software (SAS, 2004). Severity ratings were normalised before analysis using square root transformation with the formula:

$(X + 0.5)^{1/2}$ ……..…….. Equation 3

Where: X = the severity rating of black root rot (Gomez and Gomez, 1984).

The Least significant difference (LSD test at 5 %) level was used to compare treatment means.

RESULTS

***In vitro* antagonistic test.** Potential *Bacillus* isolates occured on healthy faba bean roots and soil rhizospher in the study areas (Table 2).

However, the distribution of these isolates varied among the districts. All 12 isolates of the antagonists reduced mycelial growth of the pathogen (*F. solani*). Significant differences (Pd"0.05) were obtained among *Bacillus* isolates in inhibiting the mycelial growth and their lytic effects on the pathogen (Table 3). The effect of *Bacillus* spp. on the pathogen, five days after incubation, showed that antagonism had no physical contact with the mycelia of the pathogenic fungal pathogen (Fig. 1), where the mean diameter of inhibition was 14.2 mm compared to 22.4 mm in the control. This trend was maintained throughout the incubation period. Mycelial growth inhibition ranged from 18.5 to 43.6%, where maximum (43.6%) reduction in mycelial growth rate was obtained from isolate BP048, and the lowest (18.5%) inhibition zone was recorded from isolate BS083. Seven *Bacillus* isolates restricted the mycelia growth to less than 14 mm diameter, and showed 39-44% protection efficacy (Table 3).

In most cases, the antagonist *Bacillus* isolates were able to inhibit mycelial growth and clear lysis was formed between antagonists and the pathogen isolates (Fig. 1). Lysis effects were evidenced by limited growth or complete absence of fungal mycelium in the inhibition zones surrounding the streak of the isolates tested. The lysis formed by the different antagonistic isolates ranged from 3.8 to 9.2 mm. Bacterial isolates BP0101 and BS024 caused the highest (9.2 mm); lysis area, while isolates BP018, BP037 and BP048 showed lysis areas of 8.3, 8.8 and 8.5 mm, respectively. The lowest (3.8 mm) lysis was recorded from isolate BS083. The other isolates showed less than 8.3 mm lysis area (Table 3).

Culture filtrates of bacterial isolates significantly ($P \leq 0.05$) reduced *F. solani* spore germination (Fig. 2), whereas the germination of

TABLE 2. Occurrence of *Bacillus* isolates on the rhizospher of faba bean fields in the highlands of northeastern Ethiopia

Districts	Altitude	Grid reference	Potential antagonistic *Bacillus* isolates
Delanta	2818-2935	50-51^{0}E, 12^{0}N	BS083, BP0101, BS102
Jamma	2562-2562	52-53^{0}E, 11^{0}N	BP018, BP037, BP048, BP079, BS024
Woreillu	2631-3090	54-56^{0}E, 11-12^{0}N	BP048, BS052, BS069, BS071, BS082

TABLE 3. *In vitro* effect of *Bacillus* isolates towards *Fusarium solani* grown on Potato Dextrose Agar (PDA)

Isolate code	Lysis (mm)	Inhibition (mm)	Inhibition(%)	Radial growth of *F. solani* (mm)
BP018	8.3±0.3	13.3±1.2	40.1±4.7	22.3
BP037	8.8±0.7	13.3±0.3	39.6±0.8	22.0
BP048	8.5±0.5	12.2±0.8	43.6±1.5	26.9
BP079	8.0±0.5	14.5±1.3	35.9±5.4	22.7
BP0101	9.2±0.3	13.0±0.8	42.6±4.5	22.7
BS024	9.2±0.3	13.7±0.6	39.1±9.8	24.4
BS052	7.7±0.7	13.5±0.8	39.4±4.4	20.9
BS069	6.3±0.3	14.3±1.0	36.3±4.5	22.6
BS071	7.5±0.5	13.3±0.3	39.6±1.0	22.4
BS082	6.8±0.6	14.3±0.3	34.6±3.9	22.3
BS083	3.8±0.6	18.3±0.7	18.5±1.8	22.5
BS102	8.0±0.5	16.0±0.5	27.8±2.3	22.2
Mean	7.8	14.15	36.4	22.4
C V %	6.1	5.8	12.3	8.7
LSD (5%)	0.81	1.39	7.56	3.29

untreated spores was about 83.4%; while *Bacillus* isolate BP0101 at 100% culture filtrate concentration recorded the lowest (9.9%) level of spore germination. This was 88% reduction over the control. As the culture filtrate concentration increased, the level of germinated spores reduced (Fig. 2). At 50, 75 and 100% concentrations, mean spore germination level of 41.1, 31.3 and 20.1%, with 50.7, 62.5 and 75.2% reduction, respectively, over the control were recorded.

Greenhouse test. Application of *Bacillus* isolates as seed treatments significantly ($P \leq 0.05$) reduced black root rot infection, and enhanced plant heights and biomass (Table 4). *Bacillus*-treated faba bean seedlings showed up to 78.4% reduction in root rot severity over the control.

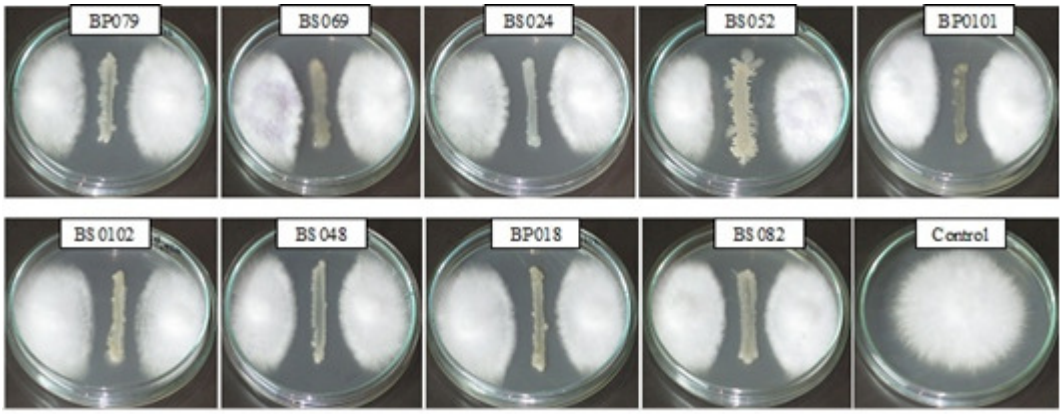

Figure 1. *In vitro* antagonistic effect of selected *Bacillus* isolates against *Fusarium solani* using in dual culture method. Each Petri plate has bacterial antagonists at the center and the pathogen *F. solani* at opposite side of antagonist. Antibiotic effect of bacterial antagonist inhibits the growth of the pathogen.

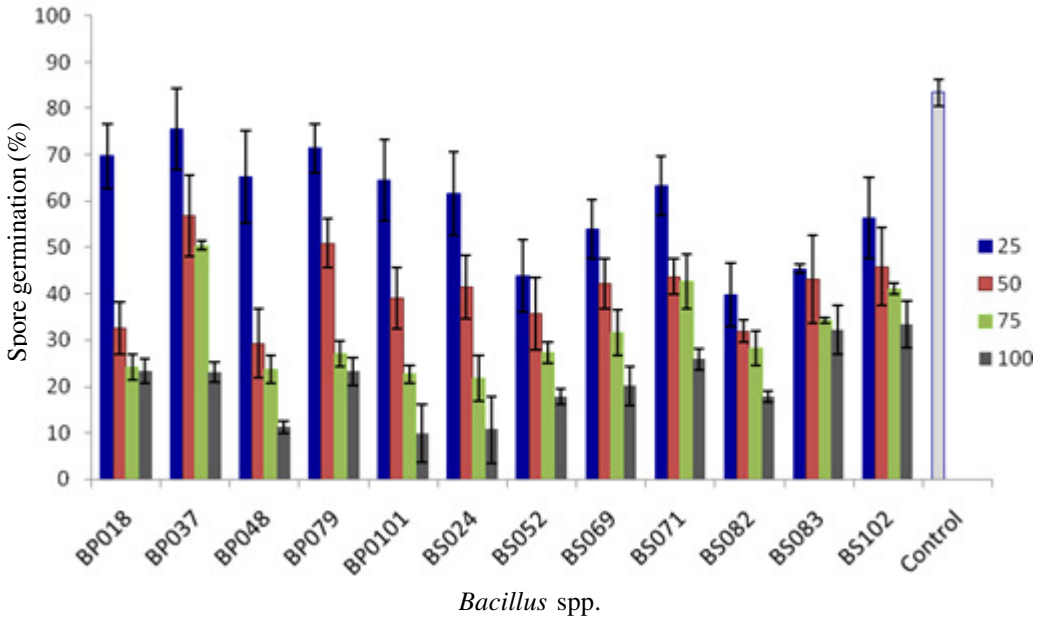

Figure 2. Effect of *Bacillus* isolates and their culture filtrate concentrations on germination of *Fusarium solani* spores.

TABLE 4. Effect of *Bacillus* isolates on root rot severity and growth parameters of faba bean under green house condition[1,2]

Isolate code	Disease severity (0-4 scale)	Disease suppression (%)	Plant height (cm)	Plant biomass	
				Fresh weight (g)	Dry weight (g)
BP018	1.17(1.27)	62.3	33.0±1.1	50.0±2.4	7.3±0.5
BP037	1.3(1.33)	58.1	28.6±0.5	40.7±1.5	6.1±0.5
BP048	0.73(1.23)	76.5	32.2±1.0	48.4±1.7	6.9±0.2
BP079	1.0(1.2)	67.7	32.5±4.4	45.1±1.7	6.8±0.2
BP0101	0.67(1.2)	78.4	33.3±1.4	49.9±3.2	7.5±0.5
BS024	1.0(1.2)	67.7	32.5±1.0	47.2±2.8	6.9±0.4
BS052	1.0(1.2)	67.7	30.6±1.7	46.3±3.6	6.9±0.4
BS069	1.0(1.2)	67.7	31.2±1.8	46.8±1.8	7.1±0.4
BS071	0.67(1.2)	78.4	34.1±3.7	50.2±0.8	7.4±0.5
BS082	1.0(1.2)	67.7	28.7±1.1	50.9±4.6	7.6±0.8
BS083	1.2(1.3)	61.3	29.3±2.7	44.6±1.6	6.5±0.3
BS0102	1.2(1.27)	61.3	34.4±2.7	44.8±1.3	6.8±0.1
Control	3.1(1.87)	0	23.3±1.2	21.7±4.0	3.2±0.8
Mean			31	45.1	6.7
CV (%)	5.3		7.3	5.9	7.4
LSD (5%)	0.11		3.83	4.48	0.82

[1]Values in parentheses are square root transformed (x + 0.5)1/2 values

Isolates BP048, BP0101 and BS071 were the most effective antagonists and reduced root rot severity by 76.5, 78.4 and 78.4%, respectively, over the control. Isolates BP079, BS024, BS052, BS069 and BS082 resulted in more than 65% suppression of black root rot; while isolates BP018, BP037, BS083 and BS0102 resulted in disease reductions of more than 50% (Table 4).

Bacillus-treated seedlings increased fresh biomass by 46.6 to 57.4% over the control (Table 4). Isolates BP018, BP0101, BS071 and BS082 resulted in 56.6, 56.5, 56.8 and 57.4%, respectively, increased the fresh biomass over control. *Bacillus* isolates also significantly increased plant height by 18.5 to 32.3% over the control (Table 4).

DISCUSSION

Native *Bacillus* isolates obtained from healthy faba bean roots and rhizosphere soil from northeastern highlands of Ethiopia, showed high inhibition activity on mycelial growth of *F. solani* in dual culture as well as in the greenhouse (Tables 3 and 4). Isolation of bacteria from within the rhizosphere of the target crop is essential for successful identification of potential biological control agents (Williams and Asher, 1996). All the antagonistic *Bacillus* isolates reduced mycelial growth of *F. solani* in dual culture. *In vitro* mycelial growth inhibition by the bacterial isolates against *F. solani* varied (Table 3). Some bacterial isolates highly inhibitory to *F. solani* growth, whereas others showed only limited activity. This suggests that the type of antifungal metabolite produced by the isolates may vary (Ahmed-Idris *et al*., 2007)

The observed mycelial growth inhibition and lysis formation among the colonies of pathogen (Table 3; Fig. 1) might be due to the effect of the bacterial diffusible inhibitory antibiosis substances, which could have suppressed and restricted the growth of the pathogen. *Bacillus* spp. are known to produce different kinds of antibiotics that are used in the biological control of plant pathogens (Asaka and Shoda, 1996; Arguelles-Arias *et al*., 2009). Thus, the production of antibiotics by the *Bacillus* spp. and their uses in the biological control of plant pathogens has been reported in many reviews (Pukall *et al.*, 2005; Killani *et al.,* 2011). In an earlier report, Young *et al.* (1974) stated that *B.*

subtilis produces at least five different antibiotics, namely subtillin, bacitracin, bacillin, subtenolin, and bacillomycin. Other antibiotics produced by *Bacillus* spp. are fengycin A and fengycin B. mycobacillin, iturin A, sufactin, mycosubtilin, fungistatin, subsporin, bacilysin, chlorotetetain (Arguelles-Arias *et al.,* 2009).

Formation of colourless lysis by biological control agents, suggest the production of colourless metabolites by the *Bacillus* isolates, which diffused into the culture medium and possibly inhibited the radial mycelial growth of the fungal pathogen. This mechanism could include any of the compounds reported by Fravel (1988). *Bacillus* species have a number of characteristics useful for biocontrol of plant diseases. They form endospores, which can withstand ecological stresses like high temperature and moisture stress for long periods (Driks, 2004). The varying level of growth inhibition of *F. solani* by the antagonist might indicate the existence of the different mechanisms of antibiosis exhibited by different antagonistic agents against different pathogens.

Moreover, the *Bacillus* antagonists kept the germination of *F. solani* spore below 50%, in the culture filtrate concentration test (Fig. 2). Similar results were reported on direct relationships between the population density of *Bacillus* and yeast antagonists on the spore germination and hyphal growth of anthracnose (*Colletotrichum gloeosporioides*) on mango (Yonas and Amare, 2007). Most bacterial isolates were able to reduce *F. solani* spore germination at lower culture filtrate concentrations. Effectiveness at low concentration indicates typical characteristics of ideal antagonists (Yonas and Amare, 2007). Therefore, the antibiosis compounds produced by *Bacillus* isolates that were evaluated in this study possessed promising properties, which drastically reduce the mycelial growth of the test pathogen. Such property is helpful in disease reduction by checking or curbing the survival and spread of the pathogen.

In most biological control studies, a large number of antagonists have been commonly isolated and screened *in vitro* for antagonistic activity. However, tests based on *in vitro* mycelial inhibition and root colonisation do not always correlate with biological control efficacy under natural field conditions (Williams and Asher, 1996). All promising isolates from the *in vitro* study were, therefore, evaluated under greenhouse conditions. The *Bacillus* isolates which showed high efficacy *in vitro* also significantly reduced the black root rot severity in the greenhouse.

The effective colonisation of faba bean roots by antagonists might have contributed to their capability to inhibit infection by *F. solani*. Among the potential biological control agents active in the rhizosphere, several members of the Genus *Bacillus* are reported to be effective in controlling a variety of fungal plant diseases (Sahile *et al.,* 2009; Karimi *et al.,* 2012; Moradi *et al*., 2012; Kumar *et al*., 2012). Most of these agents were able to inhibit the mycelial growth of the pathogen effectively *in vitro*. Similarly, in the current study, the majority of *Bacillus* isolates significantly inhibited *F. solani in vitro* and in the greenhouse (Table 3, Table 4) and have potential as biocontrol agents in faba bean rhizosphere. Isolates BP048, BP0101 and BS071 showed higher antagonistic activity in the rhizosphere of faba bean seedlings.

Moreover, the effect of antagonists on the faba bean plant growth under pot condition increased plant height and biomass, compared with *F. solani* inoculated faba bean plants alone. Better overall growth of seedlings indicates the efficiency of *Bacillus* antagonists in controlling faba bean black root rot. This is in accordance with earlier results of Weller (1988), who showed some fungal and bacterial strains were capable of promoting plant growth. In addition to production of secondary metabolites, the antagonistic effects of the bacterial isolates may attribute to the competition, which occurs between the two organisms requiring the same nutrients and the predominant use of these nutrients by antagonists reduces the amount available to the other counterpart.

CONCLUSION

Indigenous *Bacillus* spp. as a biopesticide for the control of *F. solani* of faba bean is quite promising under the conditions of the highlands of northern-eastern Ethiopia. All the tested bacterial isolates inhibit *F. solani* both in the dual culture assay and in the greenhouse experiments.

Further research should be directed towards ascertaining in details the mode of action of these effective isolates. Field studies should be undertaken to confirm the effectiveness of the isolates under natural field conditions as component of integrated disease management.

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