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Antagonistic Effects of *Bacillus* Species in Biocontrol of Tomato *Fusarium* Wilt

Caroline Fadeke Ajilogba, Olubukola Oluranti Babalola* and Faheem Ahmad

Department of Biological Sciences, Faculty of Agriculture, Science and Technology North-West University Mafikeng Campus, Private Bag X2046 Mmabatho 2735

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ABSTRACT *Fusarium solani* is the causative organism of *Fusarium* wilt of tomato (*Lycopersicon esculentum* Mill). Four *Bacillus* spp identified as *B. amyloliquefaciens, B. cereus, B. pumilus* and *B. subtilis* were tested for biocontrol activities *in vitro* as zone of inhibition and *in vivo* as percent disease control and disease incidence. The result from *in vitro* analysis showed that *B. amyloliquefaciens* inhibited the growth of *F. solani* the most by 95.2% while *B. cereus* had the highest growth of *F. solani* and inhibition of 55.7%. *B pumilus* and *B. subtilis* showed inhibition of 70.46% and 82.1% respectively and were significantly lower ($p=0.05$) than the control with 100% *F. solani* growth. *In vivo*, *B. cereus* had the least disease incidence and highest percent disease control (18.75% and 81.2%). This was significantly different from the control (100% and 0%), *B. amyloliquefaciens* (25% and 75%), *B. pumilus* (37.5% and 62.5%) and *B. subtilis* (37.5% and 62.5%). Also, growth parameters like shoot and root length from treatment with *B. cereus* (38 mm ±1.47 and 31 mm ±1.22) were significantly longer ($p=0.05$) compared to the control. This result shows that these four *Bacillus* spp are very effective biocontrol agents and should be harnessed for further biocontrol applications.

INTRODUCTION

Tomato is one of the most important vegetables because of its health benefits and phytochemical properties. Because of its low calorie and absence of cholesterol, it is one of the recommendations of diets needing low cholesterol. They are quite rich in many important nutrients and vitamins which include phosphorus and potassium and also vitamins B and C. They are also very important against common cancers like breast and prostate cancer.

As important as tomato is nutritionally and in being an important cash crop for smallholders and medium-scale commercial farmers in Africa, soil-borne pathogens inflicts a lot of diseases and infections on it (Babalola and Glick 2012). Such diseases include Bacterial wilt, root knot nematodes disease, early blight, late blight and Fusarium wilt. Fusarium wilt is a devastating disease of tomato and causes a lot of loss to farmers worldwide. symptoms begins as gradual yellowing and wilting of the lower leaves (Khan and Khan 2002) which is brought about by the growth of the microconidia inter-cellularly in the xylem of the stem and root. As a result of the failure of the infected xylem of the plant to meet the water requirement of the plant, death of the tomato plant is inevitable (Burgess et al. 2008). Spores from the conidia are released into surrounding tissues as the plant dies. They later form chlamydospores that fall back into the soils (Jones 2000). These spores can remain in the soil for as long as 30 years until favourable conditions are available and they can re-infect plants (Thangavelu et al. 2004).

Several microorganisms are being used in the control of tomato pests and diseases. Listed among tomato pest control agents are *F. compactum* and *F. arthrosporioides* (Babalola 2010a,b,c). Included in tomato disease control agents are *Trichoderma, Pseudomonas* and *Bacillus* species. Bacillus-based biocontrol agents are quite important in the management of pests and plant diseases (Jacobsen et al. 2004). Varieties of *Bacillus* and *Paenibacillus* help to promote the health of crops and control diseases by producing antibiotic metabolites, suppressing plant pathogens, others antagonise plant pathogens by competing for nutrients like iron and phosphate, others indirectly fix nitrogen which they make available to the plants and help stimulate plant nutrient uptake (Gardener 2004). This research seeks to elucidate the biocontrol abilities of these four *Bacillus* spp in order to make use of these abilities for further biocontrol interventions.

MATERIAL AND METHODS

Bacillus spp Inoculum Preparation

The following typed cultures and locally isolated organisms (LIO) from the culture collec-

*Address for correspondence:
Telephone (work): +27 183892568
Fax: +27 183892134
E-mail: olubukola.babalola@nwu.ac.za

tion of the Microbial Biotechnology Research Group of the Biological Sciences department of the North-West University Mafikeng Campus, South Africa were used for this study: *B. subtilis* (ATCC 11774), *B. cereus* (ATCC 11778), *B. amyloliquefaciens* (LIOBac179), *B. pumilus* (LIOBac269). The inoculum preparation was carried out according to Cavaglieri et al. (2005). Two loopfuls of each of the bacteria from 3-day old cultures on tryptic soy agar (TSA) were transferred separately to 50 ml tryptic soy broth (TSB) medium and incubated overnight at 28±2°C. Viability was confirmed by standard plate count method using trytone soy broth plus 2% agar (TSBA). These inocula were prepared in order for them to be used *in vitro* for antifungal activities of the *Bacillus* isolates and also their biocontrol activities *in vivo*.

The inocula for use in the greenhouse were prepared from a 24 h shaken culture of each of the *Bacillus* isolate incubated at 28±2°C. Tenfold serial dilution was carried out and a concentration of 5×107 was achieved Five ml of *Bacillus* suspension containing 5×107 cfu/ml was used as inoculant in the greenhouse.

Preparation of Phytopathogenic Fungi

Fusarium solani ATCC 36031 (Davies Diagnostic, South Africa) was used for the research. Fungal strains and inoculum was prepared by culturing it on Potato dextrose agar (PDA) for 10 days in Petridishes. The microconidial suspension of *F. solani* was prepared by pouring 1 ml of sterile water in each petri dish in order to loosen the spores from the medium. The inoculum was then scrapped with the aid of a sterile spatula from the surface of the Petri dishes in other to be sure of the viability of the cells and 1 ml was made up to 20 ml in sterile bottles. The bottles were properly shaken in the rotary shaker to dislodge the spores from the mycelia of the fungi to get a concentration of 10^6 spore concentration. The spore concentration was adjusted to the required concentration of 10^9 spores/ml by taking 1ml of the spore suspension and diluting with 9 ml sterile distilled water and then injected into sterile soils in the greenhouse (Adebayo and Ekpo 2005).

Antifungal Activities of *Bacillus* spp

Based on antifungal activities, the *Bacillus* spp were tested for the following:

Detection of Hydrogen Cyanide (HCN) Production

Production of HCN was detected according to the method of Lorck (1948) freshly grown cells were spread on a tryptone soy agar (30 g) to which 4.5 g/l glycine had been added and a sterilized filter paper saturated with 1% solution of picric acid and 2% sodium carbonate was placed in the upper lid of the Petridish, which was then sealed with parafilm and incubated at 30°C for 4 days, a change in colour of the filter paper from yellow to reddish brown was an index of cyanogenic activity while no colour change represent no cyanogenic activity.

Determination of Indole Acetic Acid Production (IAA)

Eight grammes of nutrient broth (Merck) was suspended in 500 ml of distilled water; freshly grown cultures were inoculated into 10 ml nutrient broth to which tryptophan had been added (1 mg and 3 mg tryptophan) in each test tube and incubated at 30°C for 48 h. A 4 ml culture was removed from each test tube and centrifuged at 10,000 rpm for 15 min. An aliquot of 1 ml of supernatant was transferred into a fresh tube to which 50 µl of 10 mM orthophosphoric acid and a 2 ml of Salkowski reagent comprising of (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. The mixture was incubated at room temperature for 25 minutes. The development of a pink colour indicated the presence of indole acetic acid (Brick et al. 1991). The absorbance of the pink solution from each isolate was measured and recorded at 530 nm using spectrophotometer (Thermo Spectronic, Merck, SA).

Phosphate Solubilisation

Phosphate solubilization is a complex phenomenon for selectively screening the bacteria which have the ability to release inorganic phosphate from tricalcium phosphate. Pikovskaya's medium, is a selective medium for phosphate solubilizing microorganisms (PSM) was used to which tricalcium phosphate (TCP) has been added as it will enhance formation of halo zones. The medium was autoclaved at 121°C for 15 min and poured into Petridishes. Isolates were streaked on the Petridishes and incubated for 3 days at 27°C. Bacillus isolates that were able to

solubilize developed clear zones around colonies (Pikovskaya 1948).

Antagonistic Activity of *Bacillus* spp

The test was carried out to see the effect of the *Bacillus* isolates on the fungi before the growth of the fungi *in vitro*. Fungal inhibition tests were performed by plate assay. A loop of *Bacillus* culture was streaked over the surface of a PDA plate (Biolab) and after 4 days of incubation at 28°C, each Petridish was inoculated with a loopful containing mycelia of F. solani. Petridishes were then incubated at 25°C for 10 days and examined for inhibition of fungus growth by the *Bacillu*s isolates. A zone of inhibition around the *Bacillus* isolate indicated positive response. The width of cleared zones of antagonism (distances between the bacterial and fungal growth) were measured after 10 days. Each experiment was repeated four times. The results were expressed as the mean values with standard error deviation in inhibition distance between the growths of the corresponding *Fusarium* isolate and the presence of the *Bacillus* isolate tested. Percentage inhibition was calculated as follows:

% inhibition = 1-(fungal growth/control growth) × 100

Control experiment with only growth of *Bacillus* isolates and fungi in two separate Petridishes were observed.

Green House Experiment

Two-week old tomato seedlings were transplanted into 24cm-diameter pots. Each pot contained sterile vermiculite, peat, and perlite in the ratio 3:4:1. At 5 weeks, the different treatments with *Bacillus* isolates were applied as outlined in the experimental design using 4 trials (each trial comprised of 120 plants) for each of the *Bacillus* isolates, a negative control with no *F. solani* and a positive control with inoculation of *F. solani*. Plants were watered daily in the green house at 25°C at 60-90% relative humidity for 10 weeks. At the end of 10 weeks, samples were harvested to assess the effect of the various *Bacillus* isolates on the different growth parameters. Significant difference was assessed from the mean of each of the different treatments. The experiment was repeated twice.

The growth parameters assessed include: length of shoot and root length and randomly selected seedlings were used to determine each parameter per treatment.

Disease Scoring and Data Recording

Disease incidence was recorded by counting the number of infected plants and dividing it with the total number of plants assessed in each treatment. The result obtained was converted to percentage using the formula:

Disease incidence = (Number of diseased plants/ number of plants assessed) x 100 (Haruna et al. 2012).

Percent disease control was obtained by the formula below

Number of diseased plants in control = Number of disease plant in treatment ×100 Number of plants in control

RESULTS

In vitro Antagonistic Effects of *Bacillus* spp

In vitro analysis revealed that the four *Bacillus* isolates *viz B. amyloliquefaciens, B. cereus, B. pumilus and B. subtilis* inhibited the growth of *F. solani* significantly. The results obtained are presented in Figures 1, 2.

The results obtained showed that *B. amyloliquefaciens* inhibited the growth of *F. solani* the most by 95.20% with only 2.50 mm growth in diameter of *F. solani* while *B. cereus* had the highest growth of *F. solani* with 23.07 mm growth in diameter and inhibition of 55.70%. The other *Bacillus* isolates also inhibited the growth of *F. solani* significantly as shown on Figure 1.

Mechanism of Biocontrol of *Bacillus* spp

The mechanism of antagonism of the four *Bacillus* isolates was investigated and the mechanism results are presented (Table 1).

Phosphate Solubilization

Bacillus isolates were examined for their ability to solubilize phosphate as an indirect mechanism of biocontrol by modifying the environmental condition. In order to examine the *Bacillus* isolates for their ability to solubilize phosphate, a standard agar medium; (pH 6-7) containing 5 g of tricalcium phosphate (TCP) as a sole source of phosphorus was prepared and used. Only *B.*

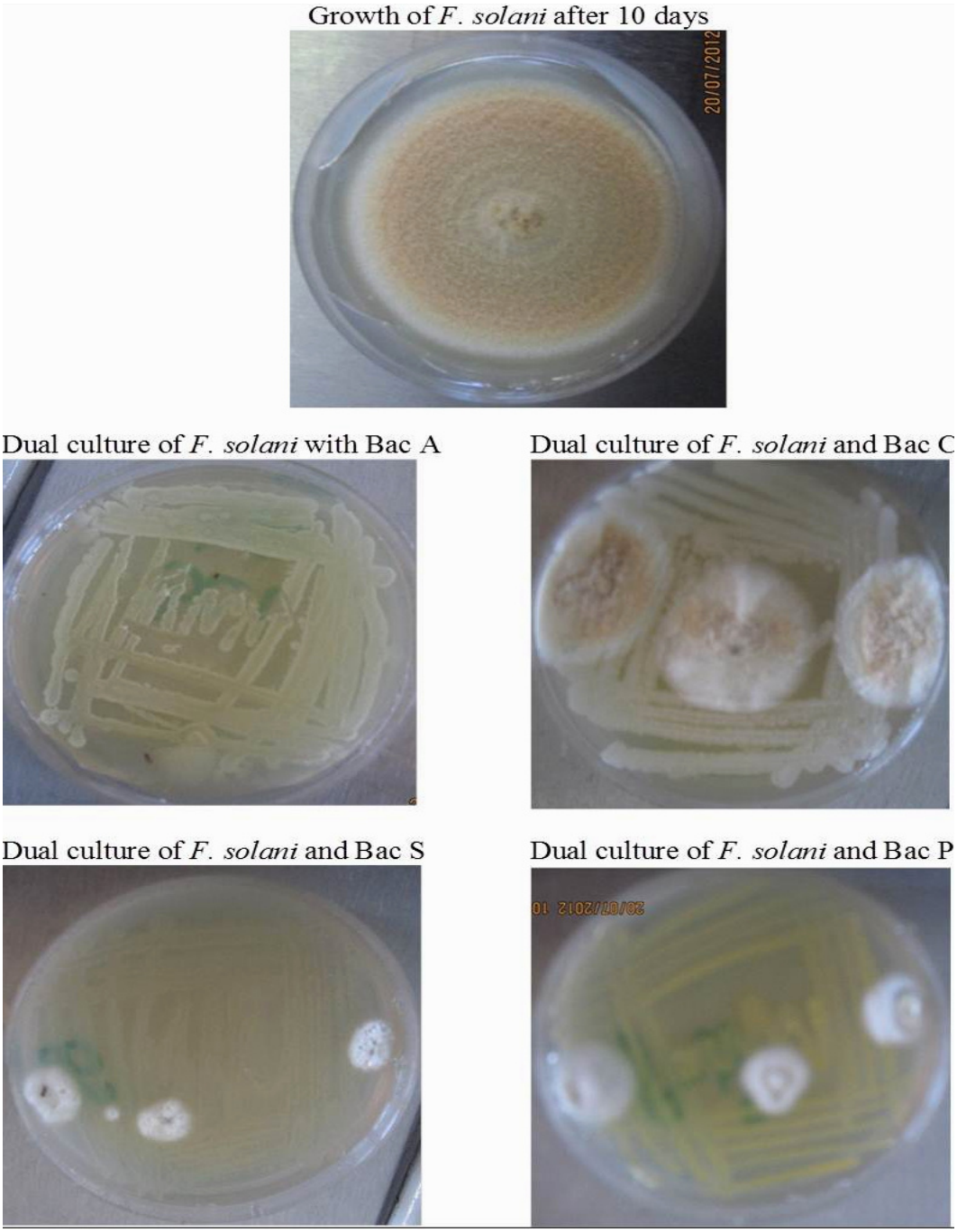

Fig. 1. Inhibitory effects of the four *Bacillus* spp against *F. solani in vitro* Petridishes containing PDA were inoculated with both *F. solani* and the *Bacillus* isolates to measure mycelia growth inhibition by *Bacillus* isolates. (A) show *F. solani* growth diameter of 52 mm (control). (B), (C), (D) and (E) shows 2.5 mm, 23.07 mm, 9.32mm and 15.36mm growth diameter respectively. Values are mean of four replicates. Bac A = *B. amyloliquefaciens,* Bac C = *B. cereus,* Bac P = *B. pumilus,* Bac S = *B. subtilis.*

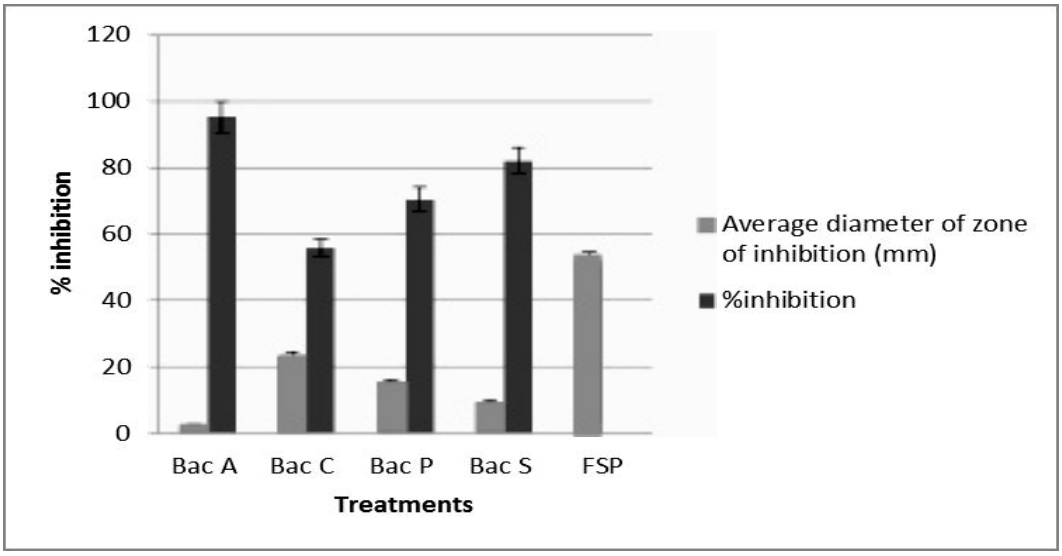

Fig. 2. Inhibitory effect of the four *Bacillus* isolates against *F. solani in vitro* at *P*=0.05. Bac A = *B. amyloliquefaciens,* Bac C = *B. cereus,* Bac P = *B. pumilus,* Bac S = *B. subtilis.* Percent inhibition value for Bac A, Bac C, Bac P and Bac S is 95.20, 55.70, 70.46 and 82.10 respectively while FSP is the growth of *F. solani* alone in the Petri dish. Each value is average of four replicates.

Table 1: Mechanism of inhibition by the four *Bacillus* spp

Antifungal metabolite production					
Treatments	*Phosphate solubilization*	*HCN*	*IAA*		
			No tryptophan	*1mg tryptophan*	*3mg tryptophan*
Bac A	+	-	0.249	0.258	0.294
Bac C	-	-	0.248	0.284	0.294
Bac P	-	-	0.261	0.271	0.284
Bac S	-	-	0.226	0.247	0.263
Control (Sterile distilled water)	-	-	0.064	-	-

All isolates were negative to HCN production and all were positive to the utilization of tryptophan which is a precursor of IAA while only Bac A solubilized phosphate. Bac A = *B. amyloliquefaciens,* Bac C = *B. cereus,* Bac P = *B. pumilus,* Bac S = *B. subtilis.*

amyloliquefaciens showed clear zones around the streaked isolate. All the others were negative without any clear zones (Table 1).

HCN Production

In vitro production of HCN by the four *Bacillus* spp was carried out using the picric acid assay. None of these isolates produced HCN (Table 1).

IAA Production

Production of IAA by all the *Bacillus* isolates was detected by the production of pink colour by all of them. Production of IAA was not dependent on the presence of tryptophan even though highest concentration was read from *Bacillus* isolates to which tryptophan had been added. This also means that there is correlation between the amount of tryptophan and amount of IAA produced. All the *Bacillus* isolates produced indole acetic acid when grown in media containing tryptophan which is obvious by the production of pink colour by all isolates in different concentrations (Table 1). Using spectrophotometer (Thermo Spectronic, Merck, SA), absorbance at 530 nm revealed that *B. amyloliquefaciens* and *B. cereus* had the highest absorbance of 0.29 nm from the test tube having 3

mg tryptophan while *B. subtilis* had the least absorbance of 0.26 nm from the 3 mg test tube. All the *Bacillus* isolates inoculated with or without tryptophan showed different levels of absorbance but the levels of absorbance gradually increased from isolates inoculated without tryptophan to isolates inoculated with 3 mg tryptophan. Increase in the absorbance from zero tryptophan to 1 mg tryptophan was 3% while that of 1 mg tryptophan to 3 mg tryptophan was 13.95% in *B. amyloliquefaciens*. Level of absorbance exhibited by *B. cereus*, increased from zero tryptophan to 1 mg tryptophan with 14.51% while from 1 mg tryptophan to 3 mg tryptophan with 3.52%. In *B. Pumilus*, the increase from zero tryptophan to 1 mg tryptophan was 3.83% while that of 1 mg tryptophan to 3mg tryptophan was 4.79%. Absorbance level in *B. subtilis*, increased from zero tryptophan to 1 mg with 9.29% while from 1 mg to 3mg with 6.47%. This shows that the highest increase in the level of absorbance based on the increase in tryptophan was exhibited by *B. amyloliquefaciens* while *B. cereus* exhibited the lowest.

Biocontrol Potentiality of *Bacillus* spp on *Fusarium* Wilt of Tomato Plants in the Screen House

As a result of the *in vitro* performance of the four *Bacillus* isolates in antagonising the growth of *F. solani*, greenhouse experiments were carried out to analyse their biocontrol activities. Greenhouse experiment was carried by using completely randomized block design, with 4 main blocks:

- Tomato planted without *F. solani* and *Bacillus* isolates.
- Tomato planted with only the different *Bacillus* isolates.
- Tomato planted with both *Bacillus* isolates and *F. solani*.
- Tomato planted with only *F. solani*

The result obtained is presented in Table 2 and Figure 3a, b, c, d.

Degree of Disease Incidence in Tomato Plants After Treatment with *Bacillus* spp

Incidence of disease the screen house is quite different compared to the pattern of inhibition *in vitro*. Disease incidence varied from 18.75% in treatments with Bac C to 25% in treatments with Bac A and 37.5% in treatments with Bac P and Bac S. They were significantly different from the control which had 100% disease incidence though there was no occurrence of wilt on plants that were not infected with F. solani and that were in turn not treated. In summary, all four *Bacillus* spp were effective in reducing disease incidence and thus disease control.

Table 2: Effect of various treatments on plant growth parameters in tomato plants treated with *Bacillus* spp and infected with *Fusarium solani*

Treatments	*Shoot length (mm)*	*Root length (mm)*
Bac AF	38±1.22b	29.5±1.04b
Bac CF	38±1.47b	31±1.22b
Bac PF	27±0.70a	18.5±1.65a
Bac SF	29±2.27a	18.5±1.25a
Control	24±0.85a	16.75±1.10a

Values are mean of 4 replicates ± SE. Each replicate had a total of 120 plants. Values with different letters are significantly different at *P*= 0.05 by Duncan's LSD. Bac A = *B. amyloliquefaciens,* Bac C = *B. cereus,* Bac P = *B. pumilus,* Bac S = *B. subtilis.*

Degree of Disease Control Using *Bacillus* spp as Treatments

Percent disease control using *Bacillus* spp in the screen house followed the pattern of percent disease incidence. The treatment with the highest disease control activity was Bac C with 81.2% disease control. Others are 75% in Bac A and 62.5% in Bac P and Bac S. This is quite significantly different from the control having 0% disease control.

Plant Growth Parameters

Plant growth parameters to be evaluated from the tomato plants treated with the four *Bacillus* spp include; shoot and root length.

Shoot Length of the tomato plants treated with Bacillus isolates

The shoot length of the plants not infected with FSP but treated with Bac C was 42 mm long and the longest. It was significantly different from the control which was 32 mm long and also from Bac P which was 36 mm long and Bac S which was 35 mm long but not significantly different from Bac A which was 38 mm long. While the plants that were infected with FSP and the treated with *Bacillus* spp also had Bac A and Bac C hav-

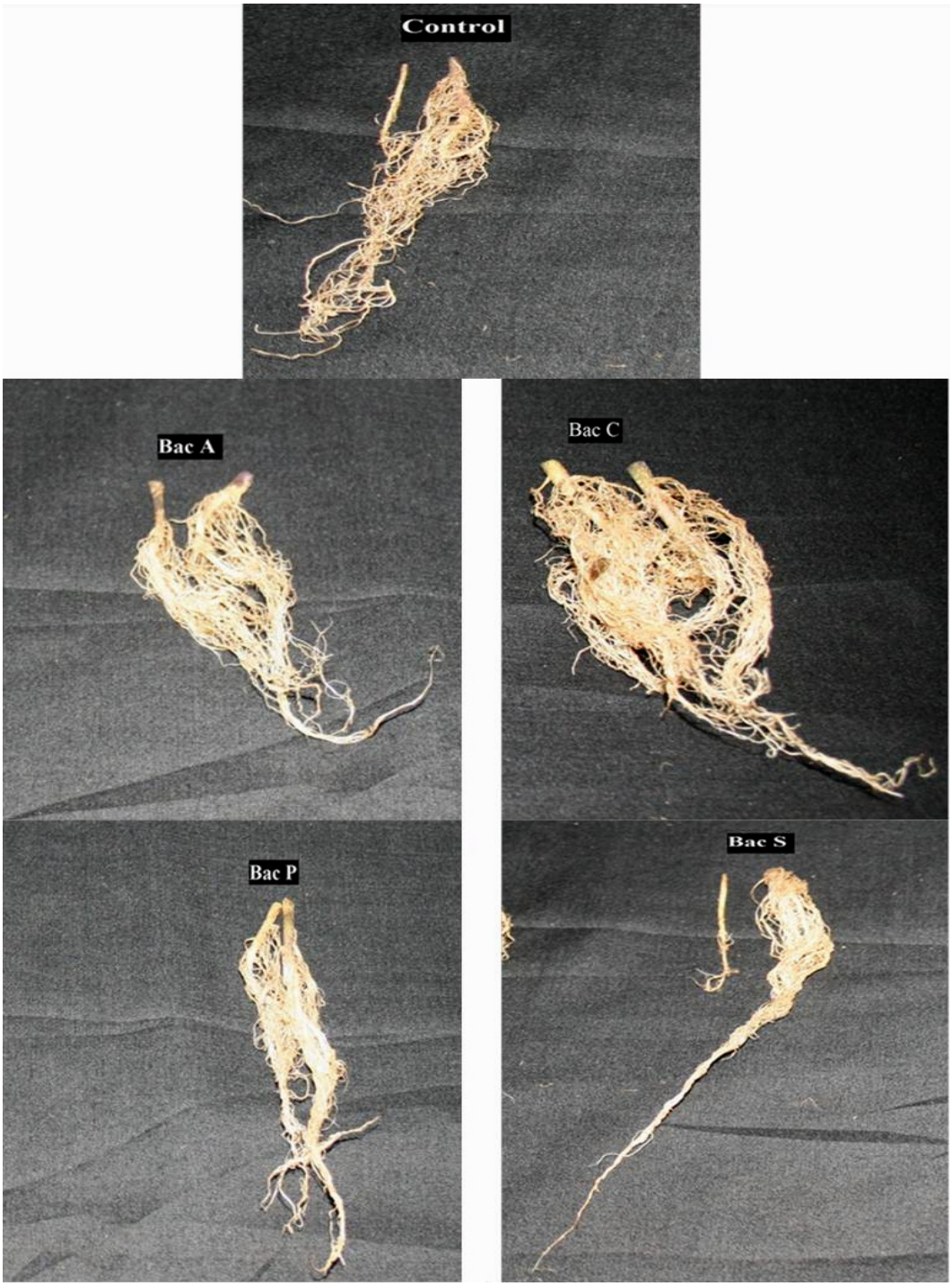

Fig. 3. Dry roots of tomato plants that have been treated with different *Bacillus* isolates. Bac A = *B. amyloliquefaciens*, Bac C = *B. cereus,* Bac P = *B. pumilus,* Bac S = *B. subtilis*

ing the longest shoot length of 38 mm and were significantly different from the control which had shoot length of 24 mm and Bac P and Bac S which had shoot length of 27 mm and 29 mm respectively (Table 2).

Root Length of the Tomato Plants Treated with Bacillus isolates

For plants not infected with FSP but treated with *Bacillus* spp, the root length of plants treated with Bac C was the longest with 34 mm and was significantly different from the Bac A which had root length of 29 mm, Bac P and Bac S which had 23.25 mm and 23.5 mm respectively. Root lengths of plants treated with Bac C and Bac A were significantly different from the other treatments and from the control with root length of 21.5 mm. Plants infected with FSP and treated with Bac C also had the longest root length of 31 mm and was significantly different from the control having 16.75 mm. Bac A having root length of 29.5 mm was not significantly different from Bac C but both of them were significantly different from Bac P and Bac S having root length of 18.5 mm each (Table 2).

DISCUSSION

In vitro Antagonistic Effects of *Bacillus* spp on *Fusarium solani*

Eleven *Bacillus* spp isolated from the rhizosphere were evaluated for their PGPR and biocontrol potential against *F. solani in vitro*. The result revealed that all the *Bacillus* spp suppressed the mycelial growth of *F. solani* in varying degree ranging from 55.7% by *B. cereus* (Bac C) to 95.2% by *B. amyloliquefaciens* (Bac A). This inhibitory activity of Bac A was reported to be as a result of antifungal compounds or metabolites released into the PDA medium (Dihazi et al. 2012). Also it has been reported that *B. amyloliquefaciens* strains have been able to inhibit the growth of a variety of fungal pathogens because of their ability to produce a vast array of antibiotics such as zwittermicin, bacillomycin, fengycin, bacilysin and difficidin (Athukorala et al. 2009; Chen et al. 2009). *B. subtilis* also inhibited the growth of *F. solani* by 82.1% *in vitro*. This result agrees with Adebayo and Ekpo (2005), because *B. subtilis* inhibited fungal growth and also promoted the growth of tomato plant in screen house trial. *B. subtili*s has been shown to have a broad spectrum of antimicrobial activities over diverse fungal and bacteria pathogen (Grover et al. 2009). Over 70 % of mycelial growth of *F. solani in vitro* was inhibited by *B. pumilus* (Bac P). This may be as a result of production of antibiotic, competition with pathogen for nutrients and direct antagonism (Akhtar et al. 2010). *Bacillus* spp are known to reduce wilting index in *F. udum*, increase plant growth and cause rapid colonization of tomato tissue in order to induce systemic resistance against *F. oxysporum* (Kloepper et al. 2004).

Mechanism of Biocontrol of *Bacillus* spp against *Fusarium solani*

Phosphate Solubilization

B. amyloliquefaciens solubilized phosphate out of the four *Bacillus* isolates used in this research. Majority of the strain isolated from potato crop rhizosphere that solubilizes tricalcium phosphate (58%) belonged to *B. amyloliquefaciens* isolates and they also had *in vitro* antagonism against *Rhizoctonia solani* and *Fusarium solani* (Calvo *et al*. 2010). *B. amyloliquefaciens* sks-bnj-1 (AY 932823) possessed multiple plant growth-promoting traits which included production of indole-3-acetic acid (IAA), solubilization of zinc, production of ACC deaminase, solubilization of phosphate, production of phytases, HCN and cellulases. It also improved the growth of soybean by improving nutrient assimilation, rhizosphere properties and yield (nutrient content of soybean) compared to unioculated control (Sharma et al. 2013a). *B. amyloliquefaciens* AM1 and D29 inhibited the growth of *R. solanacearum* T-91 and produced IAA, siderophore and solubilize phosphate (Almoneafy et al. 2012). *B. subtilis* and *B. cereus* isolated from groundnut rhizosphere were able to solubilize phosphate (Maheswar and Sathiyavani 2012). *B. subtilis* inhibited the growth of *F. oxysporum* (25-34%) *in vitro* and *Botryodiphodia theobromae* isolated from post-harvest rotten yam tuber (100%). It was able to solubilise phosphate and promote elongation of root in seedlings (70-74%) of *Cicer arietinum* compared to the control (Swain and Ray 2009). *B. subtilis* strain D16 inhibited the growth of *R. solanacearum,* produced IAA and siderophore but did not solubilize phosphate which is similar to the result of this research (Almoneafy et al. 2012).

Other *Bacillus* isolates that solubilise phosphate include *B. thuringiensis, B. sphaericus* and *B. megaterium* (Akgül and Mirik 2008). Phosphorus is a very important macronutrient needed for plant growth and development. Microorganisms help to convert insoluble phosphorus in the soil to soluble ones that are accessible by plants for growth and increased yield (Saharan and Nehra 2011). This helps to increase the uptake of phosphorus by plants (Chen et al. 2006; Igual et al. 2001), they are therefore quite important to biotechnological aspect of agriculture in other to meet the phosphorus needs of plants

HCN Production

Positive colour change of filter paper to reddish brown indicated the production of HCN. None of the four *Bacillus* isolates produced HCN which is similar to Singh et al. (2008) in whose research the *Bacillus* isolate were all negative for HCN. Cyanide is a toxic and dreaded chemical produced by many rhizobacteria. Some bacteria synthesis it, others excrete it and yet others metabolize it in other to avoid predation and competition (Zeller et al. 2007). Hydrogen cyanide is a gas that affects the metabolism of most root especially of weeds negatively. Production of Hydrogen cyanide in *Bacillus* is about 50% in both rhizospheric soils and nodules compared to *Pseudomonas* that is over 80% (Ahmad et al. 2008; Charest et al. 2005). Plant growth was enhanced invitro by most of the rhizospheric isolated that produced HCN (Wani et al. 2007). HCN produced by rhizospheric bacteria isolated from chickpea rhizosphere also promoted plant growth directly, indirectly and synergistically (Joseph et al. 2006). According to Karuppiah and Rajaram (2011), most of the *Bacillus* isolates (*Bacillus* BA1, BA3, BA4, BA6, BA7, and BA8) from rhizosphere of vegetable plants produced HCN and siderophore and so had antifungal activity against *Penicillium* spp, *F. oxysporum* and *Cercospora* spp. HCN has been reported as been effective in the control of wilt of cucumber caused by *Pythium ultimum*. It chelates metals and upsets perspiration (Keel et al. 1996). *B. amyloliquefaciens* sks-bnj-1 (AY 932823) produced HCN among other Plant growth promoting characteristics; improved plant growth and increased soybean yield (Sharma et al. 2013). Others include *B. megatatrium* JUMB1, JUMB2, JUMB3, JUMB4, JUMB5, JUMB6 and JUMB 7 which all produced HCN, IAA, Ammonia and siderophore but were unable to solubilize phosphate (Shobha and Kumudimi 2012). Reports have shown that HCN influences plant growth indirectly especially isolates from rhizosphere of chickpea, rice and mangrove (Joseph et al. 2007; Samuel and Mathklaruppan 2011; Shobha and Kumidimi 2012).

IAA Production

All the *Bacillus* spp produce indole acetic acid from tryptophan to enhance plant growth as the pink colour was produced by all isolates in different concentrations. This is similar to production of auxin which is the commonest form of IAA by *B. amyloliquefaciens* KPS46 which also supported growth of soybean (Buensanteai et al. 2008). Most of the strains isolated from the rhizosphere of potato crop (81%) were from the *B. amyloliquefaciens* strain and they produced IAA (Calvo et al. 2010). Others are *B. amyloliquefaciens* AM1 and D29 and B. subtilis strain D16 (Almoneafy et al. 2010). *B. cereus* RS87 significantly promoted growth of root length, plant height and seedling emergence over control and produced IAA (Jetiyanon et al. 2008). The ability of *B. amyloliquefaciens* FZB24 to enhance growth and control plant disease could be as a result of production of plant hormones such as indole-3-acetic acid (IAA) (Bottini et al. 2004; Bloemberg and Lugtenberg 2001). *B. subtilis* B1, B6, B28 and B99 significantly promoted growth and biocontrol activity against *F. oxysporum* f.sp ciceris in chickpea compared to untreated control (15.8-44.8 %). They were observed to produce IAA, HCN and antifungal volatiles among others (Karimi et al. 2012). *B. subtilis* WR-W2 and B. amyloliquefaciens MR-A1 produced different concentration of IAA with *B. subtilis* producing more compared to *B. amyloliquefaciens.* Sometimes, auxins are produced when there is a precursor such as L-tryptophan, which helps to increase the production of IAA in *Bacillus amyloliquefaciens* FZB42 (Idris et al. 2007). *B. licheniformis* K11 and *B. subtilis* AH18 both produced antifungal β-glucanase, siderophere and auxins. They were also involved in phosphate solubilization. This led to up to 20% increase in leaf, stem and root growth of red pepper and tomato (Lim and Kim 2009). According to Joseph et al 2007, while working with chickpea, all *Bacillus* isolates produced IAA. Production of IAA

in plants help to increase root dry weight and thereby increase the plants' ability to take up N, P, K compared to non-inoculated control (Etesami et al. 2009). It helps to stimulate plant growth and increased the uptake of N, P, K, Ca and Mg in sweet potato cultivar (Farzana and Radizah 2005). It caused increase in vegetables especially cucumber, pepper and tomato (Kidoglu et al. 2007). It is responsible for early growth promotion in soybean (*Glycine max* L) and corn (Zea mays L) (Cassana et al. 2009). The response of plant to different concentration of auxin (Sarwar et al. 1994) is different and the difference can depend on the type of microorganism (Ahmad et al. 2005). Even though some microorganisms produce high concentration of auxin, that is, IAA and this helps to increase plant growth and yield in wheat crop (Khalid et al. 2004), others producing low concentration of IAA also improve plant growth (Tsavkelova et al. 2007).

Relationship between Growth Parameters and Disease Incidence

In this research the various treatments reduced disease incidence and promoted growth parameters compared to the control in the greenhouse. They were all effective in promoting tomato growth which led to increase in the shoot and root dry weight compared to the control. This is because where *Bacillus* spp or and their by-products are applied to plants, the outcome is disease control (Gardener 2004).

According to (Singh et al. 2008), chir-pine seeds treated with *B. subtilis* BN1 demonstrated early seed emergence, viability and increased biomass. In comparison to uninoculated seeds and seeds infested with *M. phaseolina*, disease severity was significantly reduced. *B sphaericus* and *B. brevis*-2 increased plant length significantly while *B. megaterium, B. polymyxa, B. sphaericus*, *B. brevis* -1 and *B. thuringiensis* increased significantly by 30-54% the number of pods. Pod weight was increased by 25% while seed yield by 35% in plants treated with *B. thuringiensis* (de Freitas et al. 1997).

Shoot and root length were enhanced as well as increase in fresh biomass and total dry matter using rhizospheric *Bacillus* spp for the biocontrol of anthracnose caused by *Colletotrichum acutatum* on pepper. AB05 (*B. amyloliquefaciens*) and AB12 (*B. subtilis*) inhibited the growth of *C. acutatum* by 60% and induced increase in weight of pepper fruit. In the greenhouse disease was more than 30%. These rhizobacteria solubilized phosphate and produced phytohormone IAA which are factors regarded as systemic acquired resistance induced in different and diverse plants making such isolates to be considered as potential biocontrol agents (Lamsal et al. 2012).

Two strains of *B. pumilus* (203-6 and 203-7) and one of *B. mycoides* (Strain Bac J) were able to significantly reduce the severity of *Cercospora* leaf spot of sugar beet which is caused by *Cercospora beticola* Sacc. They were able to do this by eliciting ISR (Bargabus et al. 2002; Bargabus et al. 2004; Kloepper et al. 2004). Growth of banana plantlets increased and *Fusarium* wilt of banana caused by *F. oxysporum cubense* was controlled as a result of treatment with *B. pumilus* ENF24 (Figueiredo et al. 2010). *B. cereus* was effective in suppressing alfalfa diseases, enhancing the emergence of seedling and increasing nodulation in common beans (Camacho et al. 2001; Figueiredo et al. 2007). *B. megaterium* has been found to increase growth parameters in the root which include the length of the root and the dry matter content of the root (Kaymak et al. 2008).

B. subtilis FZB24 and FZB37 inhibited mycelial growth of *F. oxysporum, R. solani* and Sclerotinia *Sclerotiosum in vitro*. Incidence of *F. oxysporum* disease was significantly reduced by up to 50% while plant height, root and shoot fresh weight increased significantly compared to the control. The result of the greenhouse was quite different from the result *in vivo* which means that antifungal activities *in vitro* did not always correlate with disease reduction *in vivo* (Schmledeknecht et al. 2001). This is quite similar to the result from this research. Bacteria that antagonise soil-borne pathogen *in vitro* are not necessary the most effective *in vivo* and vice-versa (Chérif et al. 2002). *Bacillus* spp from the rhizosphere have been reported to be effective against a variety of soil borne pathogens. They are able to do this using diverse mechanisms (Choudhary and Johri 2009; Kloepper et al. 2004). Colonization of root was not inspected in this research but from the morphology of the root samples, those treated with *Bacillus* isolates had more root hairs compared to the uninoculated control.

CONCLUSION

This research shows that *Bacillus* species are quite important and effective as biocontrol agents. Their effectiveness is also observed in their ability to promote growth in plants. Research is continuing to be able to formulate them into microbial agents that will be health and environmentally friendly.

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