Protection of Potted Okra against *Fusarium* wilt using Different Concentrations of *Bacillus thuringiensis*

*Okereke, V. C., Rehua, N. & Godwin-Egein, M.I.

Department of Crop and Soil Science, Faculty of Agriculture,
University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.
* chykeoky@yahoo.com

Abstract: The bio-pesticide ability of commercially produced *Bacillus thuringiensis (*Bt*)* against wilt diseases of Okra was evaluated in a pot experiment. The experiment employed one cultivar of Okra (Lima F1) and four concentrations of Bt applied at three different times. The experiment was laid out in a 4 x 3 factorial experiment in completely randomized design replicated four times. Bt was applied as soil drenches at 3, 4 and 5 weeks after sowing. Data were collected on plant height, leaf wilt and plant wilt at weekly intervals and the percentage mean values were derived from the data. The results showed that *Fusarium* sp had the lower prevalence in pot treated with *Bacillus thuringiensis.* The treatments had no effect on plant height. Application of *Bacillus thuringiensis* at three levels suppressed both leaf wilt and plant wilt when compared with the control. Lower levels of the wilt diseases were obtained especially at Bt 0.5 g and 1.0 g per 10,000 g of potted soils, and this was significant for plant wilt. Although the commercially produced Bt strain used in this study did not show evidence of plant growth stimulation as observed in the plant height, but was effective in the reduction of *Fusarium* an important causal organism of wilt diseases in the humid tropics.

[Okereke, V. C., Rehua, N. & Godwin-Egein, M.I. **Protection of Potted Okra against *Fusarium* wilt using Different Concentrations of *Bacillus thuringiensis*.** *Nat Sci* 2016;14(4):27-32]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 4. doi:10.7537/marsnsj14041604.

Key words: *Bacillus thuringiensis, Okra,* Lima (F1), bio-control, leaf wilt, plant wilt, *Fusarium* sp

Introduction

Several microbial antagonists and bio control agents have shown promising results in the control of soil-borne pathogens (Ghaffar, 1992), replacing the most widely used methods such as the cultural practices and chemical control using synthetic fungicides. However, the use of some of these synthetic chemicals has caused environmental problems, with consequences such as human toxicity, as well as fungicide resistance. The effect of pesticides on increased crop resistance for over four decades, the emerging, re-emerging, and endemic plant pathogens are still challenging crop safety worldwide (Berg, 2009 and Gilbert, 2013). Chemicals leave residues in grains, fruits, vegetables and soil that may be harmful to both the ecosystems and human health. Due to increase in cost of chemical pesticides and environmental hazards involved with their application emphasis is now given on the biological control agents against plant pathogens. An alternative measure is the use of antagonistic microorganisms such as: some species of the genus *Bacillus* which is recognized as one of the most effective biological control agent because of their pathogen growth inhibition properties (Sid *et al.,* 2003 and Schisler *et al*., 2004).

Bacillus spp. are non-pathogenic, easy to cultivate, and also are protein and metabolite secretors. These characteristics make them appropriate for the formulation of stable and viable biological products that could be used for soil-borne disease management (Kloepper, 1997). At present, the most successfully commercially distributed species from this genus is *Bacillus thuringiensis* (Valadares *et al.*, 1998). The secondary metabolites produced by *Bacillus* have been found to show antibacterial and/or antifungal activity against plant pathogens and food borne pathogenic organisms (Shoji 1978; Smirnov *et al*., 1986).

Result of *in vitro* study using *Bacillus* spp as bio-control agent against various plant pathogens has showed positive responses against foliage and soil-borne pathogens such as *Alternaria dauci* and *Rhizoctonia solani*. Asaka and Shoda, (1996) associated the inhibitory effect of *B. thuringiensis* on plant pathogenic fungus with enzyme production that act against the fungal cell wall. Mojica-Marín *et al.*, (2008) showed that *B. thuringiensis* proved to be efficient in the control of *R. solani* of chilli pepper in *in vitro* assays and antibiosis appears to be the principal mode of action (Lee *et al*., 2003 and Ligon *et al*., 2000). Waheed *et al*., (2013) also reported a significant reduction in disease severity of tomato seedlings treated with *B. thuringiensis* strain 199 against *Fusarium* wilt and advocated the need to develop inoculum for commercial application at the field level. It has also been shown that where *Bacillus* was applied at the time of transplanting, plants had an increase in height, flowering and fruiting compared to traditional crop management. Szczech and Shoda,

(2006) also reported that the aapplication of some *Bacillus* strains to seeds or seedlings was found to be effective in the suppression of soil-borne diseases and successfully induced systemic resistance in the treated plants. This study was therefore aimed at determining the antagonistic effect of *Bacillus thuringensis* (Bt) applied at different concentrations on wilt pathogens of potted okra in the humid tropics.

Materials and Methods

This experiment was conducted at the Faculty of Agriculture Research and Teaching Farm, University of Port Harcourt, Rivers State, Nigeria.

Experimental materials

Okra cultivar (Lima F1) was purchased from Agri-Tropic Nig. Ltd., Rivers State; a sub-branch of Technisem company, Lounge-Jumelles (France). Commercially produced (*Bacillus thuringiensis* (Bt) Biothor 120 g) was obtained from Tratamientos Bio-Ecology, S.A san Javier Spain.

Pot preparation and sowing of Okra seeds

10,000 g of top soil collected from the study location was added into a 22.5 cm (diameter) pot perforated at the bottom. The pots were filled with water and left overnight to drain out. Three Okra seeds were sown per pot after been soaked in sterile distilled water over night to enhance seed germination. Three weeks after germination, the pots were transferred to a shade to avoid the damaging effects of rain splashes on the young Okra seedlings and watered appropriately.

Treatment preparation and application

Three different concentrations of *Bacillus thuringiensis* (Bt) were prepared into three different concentrations as shown below;

- Bt_1 = 0.5 gram of Bt /100ml water
- Bt_2 = 1.0 gram of Bt /100ml water
- Bt_3 = 1.5 gram of Bt / 100ml water

The weighing was done using a sensitive weighing balance. These concentrations of Bt were applied as soil drenches on the surface of the potted soils. Control plots (Bt_0) were treated with 100 ml of sterile distilled water only. The application of Bt was done at three different timings three, four and five weeks after sowing.

Isolation of fungal pathogens

Soils were collected from the treated and control pots where one gram of the different soil samples were dispersed in the bottom of sterile Petri dishes (9 cm in diameter), melted but cooled potato Dextrose Agar (PDA) + streptomycin (50mg/L) was poured over them and incubated at 28°C for 7 days during which the fungal organisms growing out were isolated. Three subcultures were made to obtain pure cultures of the organisms.

Data Collection

Data on plant height, number of leaves per plant, number of wilted leaves, number of plants per pot and number of wilted plants per pot were collected weekly from five weeks after sowing till nine weeks after sowing. Percentage wilted leaves and plants were derived from the formula below and the mean value was derived.

$$\%\ \text{leaf wilt} = \frac{\text{Number of wilted leaves}}{\text{Total number of leaves per plant}} \times 100$$

While,
$$\%\ \text{plant wilt} = \frac{\text{Number of wilted plants}}{\text{Total number of plants per pot}} \times 100$$

Data were also collected on fungal count and percentage prevalence recorded.

Experimental Design and Data Analysis

The experiment was a 4 (concentration) by 3 (time of application) factorial in a completely randomized design (CRD) replicated four times. Data were analyzed using analysis of variance (ANOVA) (using GenStat 16th Edition; VSN Industrial Ltd, UK) and means were separated using the standard error of difference (SED) at 5% probability level.

Results

Data on fungal isolation showed that *Fusarium* spp were less prevalent (< 30%) in soils treated with *Bacillus thuringiensis* compared with the control where the prevalence rate was above 30% (Table 1). *Trichoderm* sp and *Rhizoctonia* sp were all prevalent in both control and treated pots, indicating that the Bt had no negative effect on the population of these organisms.

Table 1: Fungal pathogens prevalent in soils treated with *Bacillus thuringiensis* in potted Okra

Treatment	*Fusarium* sp	*Trichoderm* sp	*Rhizoctonia* sp
Control pot	+	+	+
Treated pot	-	+	+

+ Presence (> 30%), - absence (> 30%).

Effect of different concentrations of Bt on mean plant height

The research work was performed to investigate the potential of *Bacillus thuringiensis* to induce systemic resistance against wilt of potted Okra. Bacillus can induce a distinct broad spectrum resistance response in both below and above ground parts of the plants. Results showed no significant (P >0.05) effect of concentration of Bt on the mean height of potted okra (Fig. 1). Although the application of Bt at 0.5 g/ 10,000 g of potted soils supported the highest plant height (8.94 cm).

Effect of different concentrations of Bt on % mean leaf wilt

Mean leaf wilt (%) showed significant (P<0.001) effect of Bt application. The three concentrations of Bt suppressed leaf wilt disease of potted Okra when compared with the control pots (Fig. 2). Mean % leaf wilt ranged from 11.1 – 44.3 % in Bt at 0.5 g/10, 000 g and Bt at 0 g/ 10,000 g (Control), respectively.

Effect of different concentrations of Bt on % mean plant wilt

Mean plant wilt also showed significant (P<0.05) effect of treatment. Application of Bt at 0.5, 1.0 and 1.5 g per 10,000 g of potted soils significantly reduced the disease given plant wilt value of 18.3, 20.0 and 27.8 % respectively, compared to the control which had a disease value of 38.9 % (Fig. 3). Furthermore, a comparing among Bt treatments also showed that application of Bt at 0.5 and 1.0 g per 10,000 g of potted soils was more effective in disease suppression than at 1.5 g per 10,000 g of potted soil.

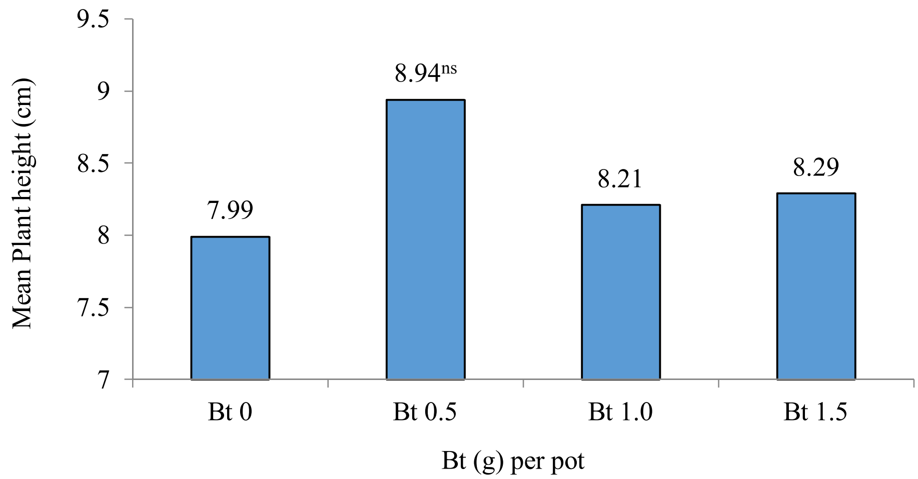

Figure 1: Effect of concentration of *Bacillus thuringiensis* applied as bio agent at 3, 4 and 5 weeks after sowing on mean plant height (cm) of potted Okra plant. SED = 1.14

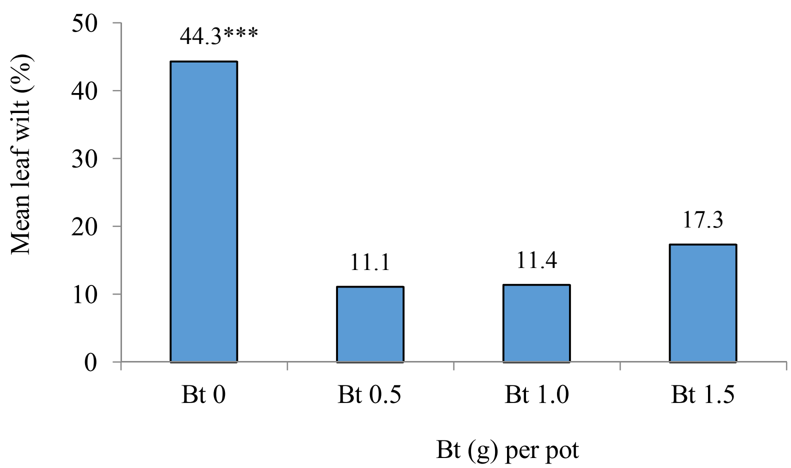

Figure 2: Effect of concentration of *Bacillus thuringiensis* applied as bio agent at 3, 4 and 5 weeks after sowing on mean leaf wilt (%) of potted okra plant. SED = 6.2

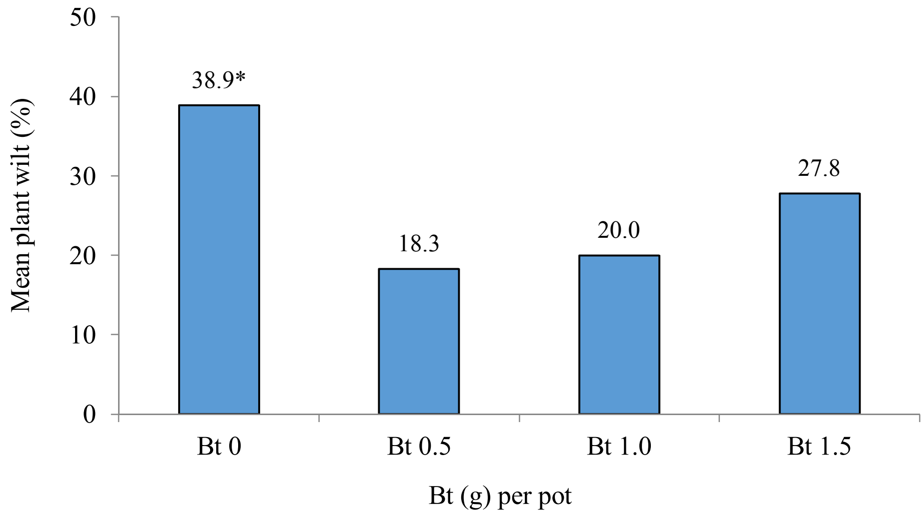

Figure 3: Effect of concentration of *Bacillus thuringiensis* applied as bio agent at 3, 4 and 5 weeks after sowing on mean plant wilt (%) of potted okra plant. SED = 7.34

Discussion

The biological control of soil-borne pathogens is one of the possible alternatives to chemical control in sustainable agriculture. *B. thuringiensis,* used in this study successfully reduced the population of *Fusarium* in the treated soil, as this organism has been implicated as one of the causal agent of wilt disease of cultivated plants (Waheed *et al*., 2013). This result agrees with many workers on the use of antagonistic bacteria to successfully control soil-borne pathogens causing diseases in crops (Krebs *et al*., 1998; Dawar *et al.,* 2008 and Mojica-Marin *et al*., 2008). *Fusarium* spp are soil-borne pathogens which could be spread through irrigation water or rain splashes. According to Waheed *et al*., (2013), this pathogen attack vascular system of a plant and the authors suggested the protection of the entrance point of the pathogen as means of control instead of changing the entire soil mycoflora. However, there was no evidence that the different concentrations Bt had any significant effect on the plant growth of the Okra cultivar (Lima F1) used in the experiment as observed in the plant height.

The data obtained from mean plant height showed no difference among treatments suggesting that the strain of the commercially produced Bt used in the experiment may not have the potential to stimulate plant growth in potted Okra. This contrasted the works of Bai *et al*., (2002) and Kloepper *et al.,* (2004) who showed that *B. thuringiensis* enhanced plant growth in soybean and transplanted vegetables. Other studies; Zaki *et al*., 1998; Heydari and Misaghi, 2003; Bharathi *et al.,* 2004; Heydari *et al*., 2005 and Jayaraj *et al*., 2005, clearly revealed that application of antagonistic bacteria are very important in the modern agriculture both as disease suppressing and plant growth promoting agents.

Furthermore the different concentrations employed in the study were effective in the suppression of the pathogens. The possible explanation could be that the amount of Bt in the soil was enough to colonize the soil to effect the control of the wilt pathogens. *Bacillus* spp has been shown to produce antibiotics (Lee *et al*., 2003 and Ligon *et al*., 2000). Pukall *et al*. (2005) identified four toxin producing strains of *Bacillus* spp namely *B. pumils, B. fusiformis, B. subtilis,* and *B. mojavensis* apart from normal toxin producer, *B. cereus,* hydrolytic enzymes produced by *B. subtilis* are important determinants of antibiosis. The authors also suggested that *B. cereus* produces lytic enzymes and antibiotics while *B. subtilis* possesses a lytic factor in its cell wall. The commercially produced *B. thuringiensis* used in this present study may possess some of these chemical compounds which might have accounted for its effectiveness in wilt disease reduction, thus a promising bio-control agent against *Fusarium* wilt.

Conclusion

From this investigation, it is clear that *B. thuringiensis* could reduce *Fusarium* population and protect crops against *Fusarium* wilt disease. Leaf wilt and plant wilt were significantly lower with the application of the bacterium at all concentration. 0.5 – 1.5 g of Bt in 10,000 g of potted soil was effective in wilt disease reduction and could be recommended in pot experiments. It implies that bacterium which show efficacy in pot experiments may have practical application in the biological control programs and potentially replace the use of chemicals. The use and application of such bio-agents in commercial quantities in the field needs to be properly investigated and their uses can reduce the over dependence on the use of chemical pesticides. These bio-agents could also form an important component of integrated crop management (ICM) program that may help farmers achieve a sustainable agricultural system.

Corresponding Author:
Dr. V.C. Okereke
Department of Crop and Soil Science
Faculty of Agriculture
University of Port Harcourt
PMB 5323 Port Harcourt, Nigeria.
Email: chykeoky@yahoo.com

References

1. Asaka, O., Shoda, M. Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB1 4. Applied Environmental Microbiology 1996; 62:4081- 4085.
2. Bai,Y., D'Aoust, F., Smith, D.L. and Driscoll, B.T. Isolation of plant growth promoting *Bacillus thuringiensis* strains from Soya bean root nodules. Canadian Journal of Microbiology 2002; 48: 230-238.
3. Berg, G. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology 2009; 84: 11–18.
4. Bharathi, R., Vivekananthan, R., Harish, S., Ramanathan, A., Samiyappan, R. Rhizobacteria-based bioformulations for the management of fruit rot infection in chilies. Crop Protection 2004; 23: 835-843.
5. Dawar, S., Hayal, S., Anis, M., Zaki, M.J. Effect of seed coating material in the efficacy of microbial antagonists for the control of root rot on okra and sunflower. Pakistan Journal of Botany 2008; 40: 1269-1278.
6. Ghaffar, A. Use of micro-organisms in the biological control of soil-borne root infecting fungi. NSRDB project. Final research report Department of Botany. University of Karachi, Karachi-75270, 1992; Pakistan, pp. 85.
7. Gilbert, J., Haber, S. Overview of some recent research developments in Fusarium head blight of wheat. Canadian Plant Pathology 2013; 35: 149–174.
8. Heydari, A., Misaghi, I.J. The role of rhizosphere bacteria in herbicide-mediated increase in *Rhizoctonia solani*-induced cotton seedling damping-off. Plant Soil 2003; 257: 391-396.
9. Heydari A, Fattah H, Zamanizadeh HR, Hassanzadeh N, Naraghi L. Investigation on the possibility of using bacterial antagonists for biological control of cotton seedling damping-off in the greenhouse. Applied Entomology and Phytopathology 2005; 72: 51-69.
10. Kloepper, J.W. Current Status and Future Trends in Biocontrol Research and Development in the U.S., In: International Symposium on Clean Agriculture, Sapporo: 1997; OECD, pp. 49-52.
11. Kloepper, J. W., Ryu, C.-M., Zhang, S. Induced systemic resistance and pro - motion of plant growth by *Bacillus* spp. Phytopathology 2004; 94(11): 1259-1266.
12. Krebs, B., Hoding, B., Kubart, S., Workie, M.A., Junge, H., Schmiedeknecht, G., Grosch, R., Bochow, H., Hevesi, M. Use of *Bacillus subtilis* as biocontrol agent. I. Activities and characterization of *Bacillus subtilis* strains. Journal of Plant Disease Protection1998; 105: 181 -197.
13. Lee, J.Y, Moon, S.S, Hwang, B.K. Isolation and *in vitro* and *in vivo* activity against *Phytophthora capsici* and *Colletotrichum orbiculare* of phenazine-1 carboxylic acid from *Pseudomonas aeruginosa* strainGC-B26. Pest Management Science 2003; 59: 872-882.
14. Ligon, J.M., Hill, D.S., Hammer, P.E., Torkewitz, N.R., Hofmann, D., Kempf, H.J., Van Pee, K.H. Natural products with antifungal activity from Pseudomonas biocontrol bacteria. Pest Management Science 2000; 56: 688-695.
15. Mojica, M.V., Luna-olvera, A.H., Sandoval-Coronado, C.F., Pereyra Alferez, B., Morales-Ramos, L.H., Hernandez-Luna, C.E., Alvarado-Gomez, O.G. Antagonistic activity of selected strains of *Bacillus thuringiensis* against *Rhizoctonia solani* of chili pepper. African Journal of. Biotechnology 2008; 7(9): 1271-1276.
16. Pukall, C.R., Schumann, P., Hormazabal, V., Granum, P. Toxin producing ability among Bacillus spp. outside *Bacillus cereus* group. Applied and Environmental Microbiology 2005; 71(3): 1178-1183.
17. Schisler, D. A., Slininger, P. J., Behle, R. W., Jackson, M. A. Formulation of *Bacillus* spp for biological control of plant diseases. Phytopathology 2004; 94(11): 1267-1271.
18. Shoji, J. (1978). Recent chemical studies on peptide antibiotics from the genus *Bacillus*. Advanced Applied Microbiology. 24:187-214.

19. Sid, A.A., Ezziyyani, M., Pérez-Sanchez, C., Candela, M.E. Effect of chitin on biological control activity of *Bacillus* spp. and *Trichoderma harzianum* against root rot disease in pepper (*Capsicum annuum*) plants. European Journal of Plant Pathology 2003; 109 (6): 633-637.
20. Smirnov, V.V., Reznik, S.R., Vasilievskaya, I.A. Aerobe endospore forming bacteria. Budapest Medicina KoÈnyvkiado Â; 1986 (in Hungarian).
21. Szczech, M., Shoda, M. The effect of mode of application of *Bacillus subtilis* RB14-C on its efficacy as a biocontrol agent against *Rhizoctonia solani*. Journal of Phytopathology 2006; 154: 370– 377.
22. Valadares, I.M.C., Souza, M.T., Shiler, W. Engenharia genética demicroorganismos agentes de controle biológico. In: Melo IS de; Azevedo JL de (Ed.) Controle biológico. Jaguariúna: Embrapa CNPMA 1998; V.1, pp 208-216.
23. Waheed, A., Mahboob, A., Javed, A.A. *Bacillus thuringenesis* strain 199 can induce systemic resistance in tomato against Fusarium wilt. European Journal of Microbiology 2013; 3 (4): 275-280.
24. Zaki, K., Misaghi, I.J., Heydari, A., Shatla, M.N. Control of cotton seedling damping-off in the field by *Burkholderia cepacia*. Plant Diseases 1998; 82: 291-293.

3/4/2016