

## Effect of *Bacillus thuringiensis* applied as Soil drenching on *Fusarium* wilt of four Okra Cultivars

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**Abstract:** A pot experiment was conducted to evaluate the effect of *Bacillus thuringiensis* (Bt) applied as soil drenching against wilt disease of potted Okra. The experiment employed four Okra cultivars namely; Clemson spineless, Sahari, Lima and Yodana and three concentrations of *Bacillus thuringiensis* (0 g, 1.0 g and 1.5 g per 10,000 g of potted soil). The experiment was laid out in a 4 x 3 factorial in Completely Randomized Design (CRD) replicated four times. The application of Bt was done at four weeks after sowing and data were collected on plant height, % leaf wilt and % plant wilt. Results showed *Fusarium* sp was implicated as the main causal agent of the wilt disease. Pots treated with *Bacillus thuringiensis* tend to have increased plant height when compared with the control pots. On average, plant height of Clemson spineless was significantly higher than all the other tested cultivars (17.35 cm), and Yodana had the least (7.51 cm) plant height. The different concentrations of *Bacillus thuringiensis* also reduced the severity of the *Fusarium* wilt disease. There was cultivar sensitivity to Bt application, both the % leaf wilt and plant wilt were significantly ( $P < 0.05$ ) reduced in Lima and Sahari when compared with the other cultivars. The commercially produced strain of *B. thuringiensis* used in this present study is a promising natural bio-agent against *Fusarium* and could be considered as an alternative to chemical pesticides in Okra disease management strategies. The bio-agent could be studied further and tested for control of other plant pathogenic fungi causing diseases and reduction of yield in susceptible Okra cultivars.

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**Key words:** *Bacillus thuringiensis*, *Fusarium*, Okra, cultivar, bio-control, leaf wilt, plant wilt

### Introduction

Several factors have led to an increased interest in the application of biological control for plant disease management (Robert and Lohrke 2003; Spadaro and Gullino, 2005). Such factors include the desire for a more sustainable approach to agriculture in general, concerns about the impact of synthetic agrochemicals on human health and the environment, the high level of pathogens developing resistant to commonly used fungicides and the increasing cost of these chemicals. Both *in vitro* and *in vivo* experiments have shown biological control to be effective against soil borne pathogens. These biological control agents reduce plant diseases by mechanisms such as antibiosis, competition, stimulation of resistance and the production of extracellular cell wall-degrading enzymes (Zhang *et al.*, 2008; Susi *et al.*, 2011).

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 ability to inhibit the growth of other pathogens (Schisler *et al.*, 2004). Application of some *Bacillus* strains to seeds or seedlings has been found effective in

the suppression of soil borne diseases and has successfully induced systemic resistance in the treated plants (Kloepper, *et al.*, 2004; Szczech and Shoda, 2006). Paul *et al.* (1998) showed a successful *in vitro* suppression of grey mould on grapevine caused by the pathogen *Botrytis cinerea* using a strain of *Bacillus* which induced the production of phytoalexins by the crop plant. Many species of *Bacillus* including *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, *B. cereus*, *B. mycoides* and *B. thuringiensis*, are known to suppress growth of several fungal pathogens such as *Rhizoctonia*, *Fusarium*, *Sclerotinia*, *Sclerotium*, *Gaeummanomyces*, *Nectria*, *Pythium*, *Phytophthora* and *Verticillium* (Zhang *et al.*, 2009; Haleem *et al.*, 2011; Basurto-Cadena *et al.*, 2012). The main property of antagonist bacterial strains as suggested by Bottone *et al.* (2003); Hernández *et al.* (2006) is production of antifungal antibiotics, which seem to play a major role in biological control of plant disease (Schisler *et al.*, 2004; Hernández *et al.*, 2006; Hernández *et al.*, 2008). Many of these antifungal substances have been characterized and identified as peptide antibiotics (Katz and Demain, 1977).

*B. thuringiensis* is a plant growth promoting bacterium which produces bacteriocin compounds (Gray *et al.*, 2006) that inhibits the growth of other pathogens. *B. thuringiensis* had been recorded for its fungicidal activity against *F. Oxysporum* (Knaak *et al.*, 2007). According to Akram *et al.* (2013), *B. thuringiensis* has been used to induce systemic resistance in plants to ward off the devastating plant pathogenic microorganisms. Kloepper *et al.* (2004) and Szczech and Shoda, (2006) reported that the application of *B. thuringiensis* to the seedlings stage of crops was found to be effective for suppressing soil borne diseases and has successfully induced systemic resistance in the treated plants. This study was therefore aimed at determining the antagonistic effect of *Bacillus thuringiensis* (Bt) applied as soil drenches at different concentrations on *Fusarium* wilt of potted Okra in the humid tropics.

## Materials and Methods

### Research site and Experimental Materials

The research was conducted at the Teaching and Research Farm, University of Port Harcourt. The study employed four Okra cultivars namely; Clemson spineless, Lima, Sahari and Yodana, purchased from Agri-Tropic Nig. Ltd., Rivers State, Nigeria; a sub-branch of Technisem company, Lounge-Jumelles (France), and a commercially produced *Bacillus thuringiensis* "Biothor" procured from Tratamientos Bio-Ecology, S.A, San Javier Spain.

### Pot preparation and sowing

10, 000g of top soil collected from the study site was put into a 22.5 cm (diameter) pot. Three Okra seeds from the four different cultivars were sown per pot after been soaked in sterile distilled water over night to enhance germination. Three weeks after sowing, the pots were transferred under shade to avoid the damaging effects of rain splashes on the young Okra plants and were watered regularly when needed.

### Treatment preparation

*B. thuringiensis* (Biothor) was prepared into two concentrations. 1.0 g of Bt to 10,000 g of potted soil (Bt<sub>1.0</sub>) and 1.5 g of Bt to 10,000 g of potted soil (Bt<sub>1.5</sub>).

### Treatment application

The Bt were dissolved in 100 ml of water and applied as soil drenches. Control pots were treated with 100 ml of sterile distilled water (Bt<sub>0</sub>). Application of treatment was done at four weeks after sowing. The treatment was carefully applied to cover the surface of the pot diameter and ensure proper colonization of the soil by the control agent.

### 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 mm in diameter), melted but cooled Potato Dextrose Agar

(PDA) + streptomycin (50 mg/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 (cm), number of leaves, number of wilted leaves, and number of dead plants were taken from 5 weeks after sowing through to the termination of the experiment at 10 weeks after sowing. Percentage wilted leaves and wilted plants were derived mathematically. Data were also collected on fungal count and percentage prevalence calculated.

### Experimental design and data analysis

The experimental design was a 4 x 3 Completely Randomized Design (CRD) replicated four times. Analysis of variance (ANOVA) was obtained using GenStat (GenStat ® 16<sup>th</sup> Edition; VSN Industrial Ltd, UK) and means separated using the standard error of difference (SED) at 5 % level of probability.

## Results

Data on fungal isolation showed that *Fusarium* sp was implicated as the causal agent of the wilt with prevalence rate of below 30% in soils treated with *Bacillus thuringiensis* compared with the control where the prevalence rate was above 30% (Table 1). *Trichoderma* sp and *Rhizoctonia* sp were all widespread in both control and treated pots, indicating that the Bt had no major effect on the population of these organisms.

### Effect of cultivar

Tables 2 - 4 below showed the effect of cultivar on plant height, % leaf wilt and % plant wilt respectively, after the application of *Bacillus thuringiensis*. The result showed a significant effect of cultivar at 5 WAS (P = 0.014); 6 WAS (P = 0.007); 7 WAS (P = 0.002); 8 WAS (P < 0.001); 9 WAS (P = 0.006) and 10 WAS (P < 0.001) on plant height (Table 1) after the application of *Bacillus thuringiensis*. Clemson spineless had the tallest plants (17.35 cm), followed by Sahari (13.00 cm), Lima (10.34 cm) while Yodana had the shortest plant height of (7.51 cm) at 10 WAS. At 6 WAS (P = 0.01) and 7 WAS (P = 0.006), Clemson spineless and Lima had significantly lower % leaf wilt contrasting the effect of Sahari and Yodana (Table 2). Although, at 10 WAS Clemson spineless and Sahari had the highest % leaf wilt but their effect was not statistically significant. There was a significant effect of cultivar on the % plant wilt of potted okra at 7 WAS (P < 0.001); 8 WAS (P = 0.002) and 9 WAS (P = 0.04) (Table 3), where Yodana and Clemson spineless had significantly higher % plant wilt compared to Sahari and Lima which had significantly the lowest

incidence of plant wilt. At 10 WAS the highest % plant wilt incidence was recorded for Yodana (61.1 %) followed by Clemson spineless (41.7 %) and their

effect was significantly ( $P = 0.03$ ) different from Lima and Sahari which had % plant wilt of 30.6%.

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

Treatment	<i>Fusarium</i> sp	<i>Trichoderm</i> sp	<i>Rhizoctonia</i> sp
Control pot	+	+	+
Treated pot	-	+	+

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

**Table 2: Plant height of Okra cultivars after the application of *Bacillus thuringiensis***

Cultivar	Weeks after sowing					
	5	6	7	8	9	10
Sahari	6.56	7.44	8.77	10.18	12.10	13.00
Clemson	8.12	9.75	11.39	13.05	14.76	17.35
Lima	6.58	7.17	8.01	8.80	9.88	10.34
Yodana	5.12	5.47	5.57	6.06	7.02	7.51
S.E.D	0.851	1.11	1.33	1.51	2.03	2.11
<i>P. value</i>	0.014 *	0.007 *	0.002 *	0.001 ***	0.006 *	0.001 ***

\*\*\* Highly significant; \* Significant; ns = not significant.

**Table 3: Percentage leaf wilt of potted okra after the application *Bacillus thuringiensis***

Cultivar	Weeks after sowing					
	5	6	7	8	9	10
Clemson	0.00	2.80	4.40	21.50	26.70	38.10
Lima	0.00	9.90	12.50	15.30	25.70	28.50
Sahari	14.60	27.50	39.90	41.30	42.00	50.40
Yodana	13.9	20.80	18.70	18.70	19.40	22.20
SED	8.58	7.56	9.69	10.34	12.79	14.48
<i>P. value</i>	0.16 <sup>ns</sup>	0.01*	0.006*	0.07 <sup>ns</sup>	0.35 <sup>ns</sup>	0.25 <sup>ns</sup>

\* Significant; ns = not significant

**Table 4: Percentage plant wilt of potted Okra after the application of *Bacillus thuringiensis***

Cultivar	Weeks after sowing					
	5	6	7	8	9	10
Clemson	12.5	12.5	12.5	12.5	33.3	41.7
Lima	18.1	18.1	18.1	26.4	30.6	30.6
Sahari	8.3	22.2	22.2	22.2	30.6	30.6
Yodana	26.4	44.4	58.3	61.1	61.1	61.1
SED	11.00	12.62	10.91	11.91	12.19	13.17
<i>P. value</i>	0.40 <sup>ns</sup>	0.08 <sup>ns</sup>	0.001***	0.002*	0.04*	0.03*

\*\*\* Highly significant ( $P. value < 0.001$ ); \* Significant; ns = not significant

### Effect of concentration

Tables 5 – 7 below showed the effect of different concentrations of *B. thuringiensis* applied at 4 weeks after sowing on plant height, % leaf and % plant wilt of potted Okra. There was a significant effect of concentration of *B. thuringiensis* on plant height at 7 WAS ( $P = 0.04$ ), 8 WAS ( $P = 0.04$ ), and 9 WAS ( $P = 0.04$ ), but the difference were not significant ( $P > 0.05$ ) at 5, 6 and 10 WAS. The tallest Okra plant was recorded

where pots were treated with Bt at 1.5 g per 10,000 g of soil (13.33 cm) followed by 1.0 g per 10,000 g of soil (13.28 cm), when compared with the control pots (9.55 cm) at 10 WAS (Table 4). Percentage leaf wilt was significant at 6 WAS ( $P = 0.002$ ) and 7 WAS ( $P = 0.007$ ) (Table 5) with the control pots having the highest incidence of leaf wilt of 29.8% and 34.3% at 6 and 7 WAS, respectively. The result also showed that at 10 WAS Bt<sub>1.5</sub> had a better control of the leaf wilt symptom

of the tested potted okra cultivars with leaf wilt percentage of occurrence of 27.7% as compared to the control pots (45.8%). The least occurrence of plant wilt was observed in pots treated with Bt<sub>1.0</sub> (19.8%) while the highest was observed in the control pot (63.5%). There was significant effect of concentration of *B.*

*thuringiensis* on the % plant wilt of the potted okra at 7 WAS ( $P = 0.02$ ), 8 WAS ( $P = 0.005$ ), 9 WAS ( $P = 0.005$ ) and 10 WAS ( $P = 0.002$ ). Data at 5 WAS ( $P = 0.38$ ) and 6 WAS ( $P = 0.24$ ) showed no significant effect of concentration of *Bacillus thuringiensis*.

**Table 5: Effect of concentration of *Bacillus thuringiensis* applied at 4 weeks after sowing on height of potted Okra plant.**

Concentration	Weeks after sowing					
	5	6	7	8	9	10
Control	6.32	7.00	7.31	8.03	8.29	9.55
Bt <sub>1.0</sub>	7.20	8.48	10.10	11.43	12.23	13.28
Bt <sub>1.5</sub>	6.26	6.90	7.89	9.11	12.29	13.33
SED	0.74	0.96	1.15	1.31	1.76	1.83
<i>P. value</i>	0.37 <sup>ns</sup>	0.20 <sup>ns</sup>	0.04*	0.04*	0.04*	0.04*

\* Significant; ns = not significant

**Table 6: Effect of concentration of *Bacillus thuringiensis* applied at 4 weeks after sowing on the % leaf wilt of potted Okra.**

Concentration	Weeks after sowing					
	5	6	7	8	9	10
Control	16.7	29.8	34.3	36.9	43.2	45.8
Bt <sub>1.0</sub>	1.6	6.5	6.4	20.3	24.9	30.8
Bt <sub>1.5</sub>	3.1	9.5	16.0	15.5	17.2	27.7
SED	7.43	6.54	8.39	8.96	11.07	12.54
<i>P. value</i>	0.097 <sup>ns</sup>	0.002*	0.007*	0.056 <sup>ns</sup>	0.069 <sup>ns</sup>	0.32 <sup>ns</sup>

\* Significant; ns = not significant

**Table 7: Effect of concentration of *Bacillus thuringiensis* applied at 4 weeks after sowing on the % plant wilt of potted Okra.**

Concentration	Weeks after sowing					
	5	6	7	8	9	10
Control	24.0	31.3	41.7	50.0	57.3	63.5
Bt <sub>1.0</sub>	13.5	13.5	13.5	13.5	19.8	19.8
Bt <sub>1.5</sub>	11.5	28.1	28.1	28.1	39.6	39.6
SED	9.52	10.93	9.45	10.32	10.55	11.40
<i>P. value</i>	0.38 <sup>ns</sup>	0.24 <sup>ns</sup>	0.02*	0.005*	0.005*	0.002*

\* Significant; ns = not significant.

## Discussion

There has been a growing interest in non-pathogenic bacteria due to their efficacy as bio-control agents (Kloepper *et al.*, 2004; Akram *et al.*, 2013; Zaher *et al.*, 2013 and Abada and Eid, 2014). In this study, *Bacillus thuringiensis* was evaluated for the management of wilt disease of Okra in a pot experiment and also to evaluate cultivar sensitivity to Bt application. It is more realistic to protect the entrance point of these disease organisms in the plant than changing the entire soil micro flora. Akram *et al.* (2013) suggested the use of microorganisms that could induce systemic resistance in plants. Kloepper *et al.* (2004) also reported that the colonization of plant roots by

strains of non-pathogenic bacteria, such as various species of the genus *Bacillus* can induce different resistance response in both below and above ground parts of cultivated plants. Different plants and different cultivars of a particular plant species could possess varying degrees of resistance to pathogen attack (Jarosz and Burdon, 1991). It was evident in this study that there was cultivar sensitivity to *B. thuringiensis* in application. The different concentrations of Bt used in this study tend to have progressively increased the height of the potted okra plants from 7 WAS to 10 WAS. This is in agreement with other workers (Mogica-Marin *et al.*, 2008; Zongzheng *et al.* 2009; Girish *et al.*, 2010; Nihorimbere *et al.* 2010; Érica,

2015) who observed that *Bacillus* spp stimulates plant growth in addition to suppression of plant diseases. Thus, an application of as low as 0.5 – 1.0 g of *B. thuringiensis* per 10,000 g of soil can effectively suppress *Fusarium* wilt pathogens associated with Okra. The higher percentage of plant wilt as observed at 10 WAS for the pots treated with Bt<sub>1,5</sub> could suggest that the synergy between *B. thuringiensis* and soil-borne pathogens at later stages of the plant growth may have had effect on the different cultivars. Furthermore, crop productivity depends largely on the photosynthetic efficiency of the leaves, invariably; excessive leaf wilt would result to poor production of some crops. The commercially produced *B. thuringiensis* used in this study revealed high potency in suppressing both leaf and plant wilt pathogens of potted Okra plant compare to the control pots. This result agrees with the findings of Kloepper *et al.* (2004) and Szczech and Shoda, (2006), who reported that the application of some *Bacillus* strains at the seedling stage of crops was effective in suppressing soil borne diseases.

It is also in agreement with other researchers who had reported that *Bacillus* spp have significant inhibitory activity against many plant pathogens including *Fusarium moniliforme* (Agarry *et al.*, 2005) and *F. oxysporum* (Nikam *et al.*, 2011).

### Conclusion

The antagonistic bacterium, *Bacillus thuringiensis* against *Fusarium* wilt pathogen was pathogenic, and resulted in the suppression of Okra wilt diseases. Leaf wilt and plant wilt were significantly lower with the application of the treatments. On the basis of overall performance, the timing of *B. thuringiensis* and method of application was very effective against the fungal pathogen. Bacteria which show efficacy in pot experiments may have practical application in the biological control programs which can potentially replace the use of chemicals. The use and application of such bio-agents in the field can result in the reduction of the application of harmful chemicals, protect the environment and biological resources and can also be an important component of integrated crop management (ICM) program that may help the farmers to achieve a sustainable agricultural system.

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