NIGERIAN JOURNAL OF BIOTECHNOLOGY

Nig. J. Biotech. Vol. 36 (1): 94-102 (June 2019)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v36i1.13

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Antagonistic effect of *Bacillus thuringiensis* for the control of bacterial wilt of tomato (*Lycopersicon esculentum* Mill)

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Abstract

Tomato is affected by a lot of diseases including bacterial wilt caused by *Ralstonia solanacearum* (Rs), resulting in fruit yield loss. Chemical control of this disease is not advisable because of hazardous effects on human and environment, hence, the need to use biocontrol agents such as *Bacillus thuringiensis* (Bt). This study investigated the potential of Bt in the control of Rs under screenhouse condition. Six treatments with three replications in completely randomized design were carried out. Agronomic parameters were measured and data collected were analyzed using one - way analysis of variance. Means were then separated using Duncan's Multiple Range Test at $p \leq 0.05$. Inoculation of Bt and Rs simultaneously significantly suppressed the incidence of bacterial wilt and promoted plant growth. Higher plant heights (72.70 - 111.00 cm), stem girth (1.63 - 1.90 cm) and number of leaves (103.33 - 125.33) were recorded for plants inoculated with Bt at 9th week after planting compared to plants inoculated with Rs alone. Highest disease incidence was recorded in plants inoculated with Rs alone (100 %), while plants inoculated with Bt had the highest disease reduction (67%). This study revealed that Bt may be used in the control of bacterial wilt of tomato.

Key words: *Bacillus thuringiensis*, screenhouse, Bacterial wilt, *Ralstonia solanacearum*, Tomato.

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Introduction

Tomato (*Lycopersicon esculentum* Mill) belongs to the Solanaceae family, it is one of the most important vegetables consumed in the world (Olaniyi et al., 2010). The fruits are eaten raw as salads or ground into a paste and are an important source of antioxidants, minerals and vitamins (Frusciante et al., 2007. Soil-borne pathogens inflict a lot of diseases and infections on tomato (Babalola and Glick, 2012). Such diseases include Bacterial wilt, root-knot nematodes disease, early blight, late blight and *Fusarium* wilt (Ajilogba et al., 2013).

Bacterial wilt of tomato is a systemic vascular disease caused by *Ralstonia solanacearum* (Abeer and Hend, 2013). A soil-borne plant bacterial pathogen notorious for its lethality, persistence, complex subspecies, wide host range, and broad geographic distribution (Elphinstone, 2005). Bacterial wilt is a common disease in tropical, subtropical and some temperate regions of the world (Fegan and Prior, 2005), it is endemic in most tomato-growing areas of Nigeria, causing 60 to 100% loss in yield (Popoola et al., 2015).

Soil fumigation, suppressive soil, short rotation, and resistant cultivars have been suggested as integrated control strategies for bacterial wilt (French, 1994). However, while chemical soil disinfection can temporarily eradicate most microbial flora, pesticide-resistant pathogens may rebound, causing even more damage than those originally targeted for control (Gamliel et al., 2000). Therefore, environment - friendly biopesticide and beneficial microorganisms are alternatives to chemical pesticides (Bailey and Lazarovits, 2003; Céline et al., 2007; Weller, 2007; Ongena and Jacques, 2008). Biological control of *R. solanacearum* has been achieved using several Beneficial Microorganisms which include *Bacillus* spp. (Ji et al., 2008), *Pseudomonas* spp. (Ramesh et al., 2009; Vanitha et al., 2009), avirulent mutants of *R. solanacearum* (McLaughlin and Sequeira, 1988; Frey et al., 1994, *Acinetobacter, Enterobacter* spp. (Xue et al., 2009), *Stenotrophomonas maltophilia* (Messiha et al., 2007) and *Actinomycetes* (Tan et al., 2006) under laboratory and/or greenhouse conditions. *In vitro* analysis revealed that four *Bacillus* isolates viz *B. amyloliquefaciens, B. cereus, B. pumilus* and *B. subtilis* inhibited the growth of *Fusarium solani* significantly (Ajilogba et al., 2013).

Bacillus thuringiensis (BT), a spore-forming bacterium is well known for its insecticidal properties associated with its ability to produce crystal inclusions during sporulation. These inclusions are proteins encoded by cry genes and have shown to be toxic to a variety of insects and other organisms like nematodes and protozoa (Konecka et al., 2007). Recently, in Japan, *Bacillus thuringiensis* attracted considerable attention as a potential biological control agent for the suppression of *R. solanacearum* growth and the development of wilt symptoms in tomato plants (Hyakumachi et al., 2013). Hence the need to investigate the antagonistic activity of native *Bacillus thuringiensis* in the control of bacterial wilt affecting tomato plants in Nigeria.

Materials and methods

The study was conducted in the Screenhouse of College of Plant Science, Federal University of Agriculture, Abeokuta, Ogun State. The study location lies within the savanna agroecological zone of South-west Nigeria (Latitude 7°N, Longitude 3.5°E in Odeda Local Government Area of Ogun State).

Preparation of seedlings and inoculum

Beske tomato seeds (susceptible to bacterial wilt disease of tomato) were raised in the nursery and transplanted after two weeks into 5kg soil (sterilized in a hot air oven at 120°C for 2 hours) in plastic buckets in the screenhouse. *Ralstonia solanacearum* (pathogen) was isolated from diseased tomato plant showing symptoms of bacterial wilt as described by Zubeda and Hamid (2011) and *Bacillus thuringiensis* (antagonist) was isolated from cultivated soil sample obtained from the Directorate of University Farms, Federal University of Agriculture, Abeokuta, according to Palma (2015). Pathogenicity test was carried out on *R. solanacearum* according to the methods described by Abeer and Hend, 2013; Hyakumachi et al., 2013 and Elsharkawy et al., 2015.

The two isolates were confirmed using biochemical and molecular methods. The pathogen and antagonist were grown separately on nutrient agar plates for two days at 28±2°C after which growth was scrapped into sterile distilled water in microcentrifuge tubes. The cells of the pathogen were removed by centrifugation at 7,000 rpm for 10 minutes; pellets were resuspended in sterile distilled water and adjusted to a final density of 1×10^7 CFU / ml (Elsharkawy et al., 2015). Antagonist suspension was adjusted to a final density of 1.8×10^8 CFU/ml. The suspensions were applied to 3-week old tomato seedlings. The experimental design used was Completely Randomized Design (CRD), replicated three times. Experimental treatments

Inoculation of *Bacillus thuringiensis* and *Ralstonia solanacearum* simultaneously (B+R)

Inoculation of *Bacillus thuringiensis* first and *Ralstonia solanacearum* a week after (Bf)

Inoculation of *Ralstonia solanacearum* first and Bacillus thuringiensis a week after. (Rf)

Inoculation of *Ralstonia solanacearum* only (Ro)

Inoculation of *Bacillus thuringiensis* only (Bo)

Inoculation with sterile water. (control)

The following agronomic data were collected at 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 weeks after planting: plant height (cm), stem girth (cm), fresh and dry root weights (g), number of flowers and number of leaves. Disease incidence and reduction were determined at the end of the experiment.

$$\%DI = (NDP \times 100)/NPA$$

Where % DI is percentage disease incidence, NDP is the number of diseased plants and NPA is the number of plants assessed.

$$\%DR = (C-T)/C$$

Where %DI is percentage disease reduction, C is percentage disease reduction in untreated plants, T is percentage disease incidence in treated plants

Data analysis

Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 for windows (SPSS, Chicago IL, USA). Descriptive statistics (mean and standard deviation) and analysis of variance (one-way) with means separated using Duncan multiple range test and level of significance was considered as $p \leq 0.05$.

Results

Effect of Bacillus thuringiensis on Tomato Plant height

Plant height increased with weeks after planting in all the treatments with highest plant height recorded in treatments B+ R (*Bacillus thuringiensis* and *Ralstonia solanacearum* applied at the same time) and Bo (*Bacillus thuringiensis* only) having 111.00 cm. The least plant height was recorded in treatment Ro that had only the pathogen with 63.67 cm (Figure 1).

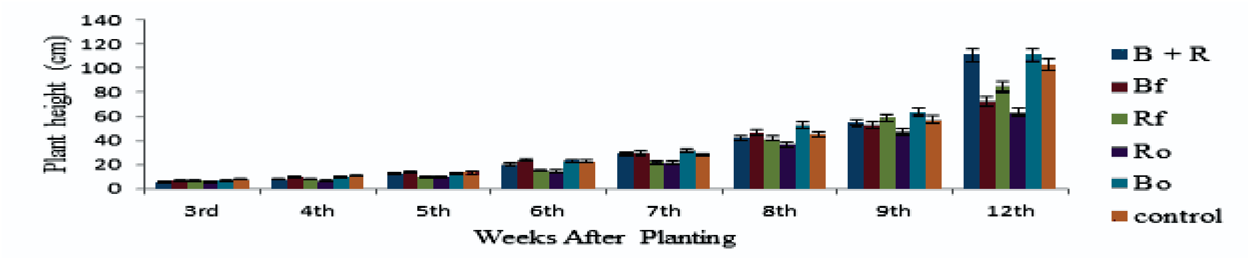

Figure 1: Effect of treatments on tomato plants height at different weeks after planting

KEY
B + R – Antagonist (Bt) and pathogen (Rs) applied simultaneously
Bf – Antagonist applied first, pathogen applied a week after
Rf – Pathogen applied first, antagonist applied a week after
Ro – Pathogen only
Bo – Antagonist only
Control - Sterile water

Effect of Bacillus thuringiensis on Tomato Plant stem girth

Stem girth also increased with weeks after planting (WAP) in all the treatments until when the experiment was terminated. Treatment Bo where the antagonist was applied alone, had the highest stem girth of 1.90 cm and the least was recorded in treatment Ro where only the pathogen was inoculated having 1.20 cm (Figure 2)

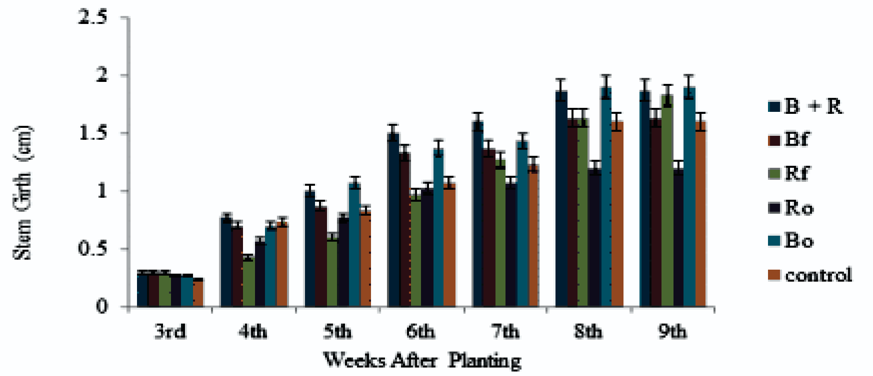

Figure 2: Effect of treatments on stem girth of tomato plants at different weeks after planting

KEY
B + R – Antagonist (Bt) and pathogen (Rs) applied simultaneously
Bf – Antagonist applied first, pathogen applied a week after
Rf – Pathogen applied first, antagonist applied a week after
Ro – Pathogen only
Bo – Antagonist only
Control - Sterile water

Effect of Bacillus thuringiensis on number of leaves of Tomato Plant

The number of leaves increased with weeks after planting across treatments. The highest number of leaves was recorded in control (130) whereas the least was recorded in Ro having only pathogen (102) (Figure 3).

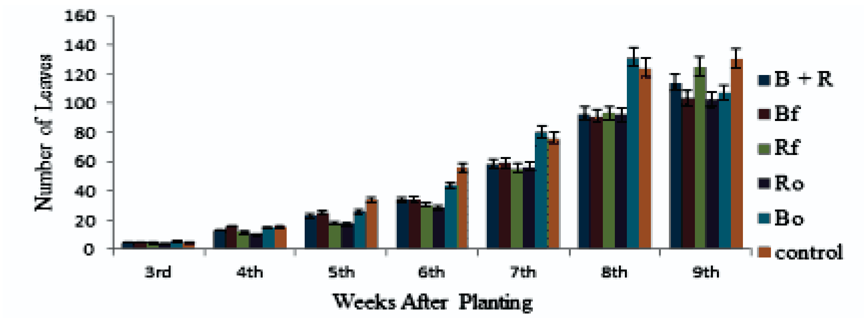

Figure 3: Effect of treatments on the number of leaves of tomato plants at different weeks after planting
KEY
B + R – Antagonist (Bt) and pathogen (Rs) applied simultaneously
Bf – Antagonist applied first, pathogen applied a week after
Rf – Pathogen applied first, antagonist applied a week after
Ro – Pathogen only
Bo – Antagonist only
Control - Sterile water

Effect of Bacillus thuringiensis on number of flowers of tomato plants

At the end of the experiment, treatment B + R (*Bacillus thuringiensis* and *Ralstonia solanacerum* applied at the same time) had the highest number of flowers (17) as shown in figure 4

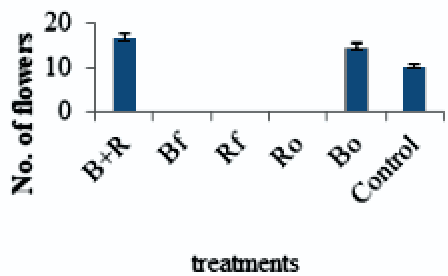

Figure 4: Effect of treatments on the number of flowers
KEY
B + R – Antagonist (Bt) and pathogen (Rs) applied simultaneously
Bf – Antagonist applied first, pathogen applied a week after
Rf – Pathogen applied first, antagonist applied a week after
Ro – Pathogen only
Bo – Antagonist only
Control - Sterile water

Effect of Bacillus thuringiensis on Fresh and Dry Shoot Weight of tomato plant

Highest fresh and dry shoot weights were observed in treatment Bo having only *Bacillus thuringiensis* while the least was observed in treatment Bf, where *Bacillus thuringiensis* was applied first and then *Ralstonia solanaearum* applied a week after (figure 5).

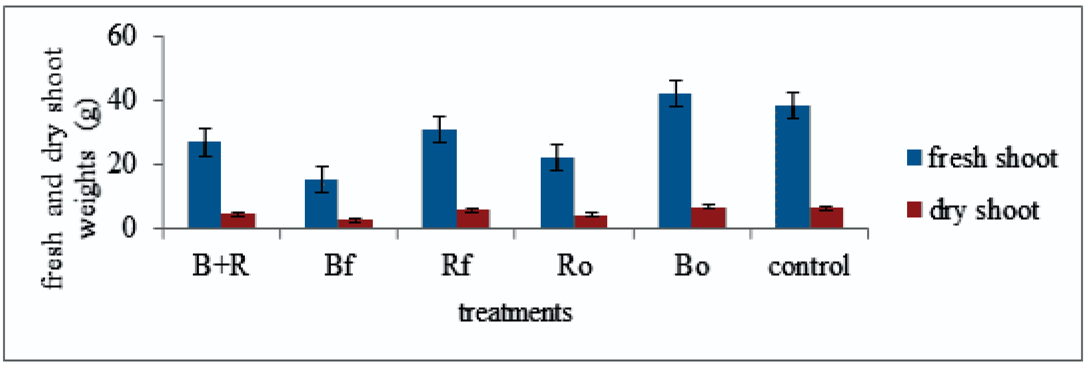

Figure 5: Effect of treatments on fresh and dry shoot weights
KEY
B + R – Antagonist (Bt) and pathogen (Rs) applied simultaneously
Bf – Antagonist applied first, pathogen applied a week after
Rf – Pathogen applied first, antagonist applied a week after
Ro – Pathogen only
Bo – Antagonist only
Control - Sterile water

Effect of Bacillus thuringiensis on Fresh and Dry root Weight of tomato plant

Treatments B+R (*Bacillus thuringiensis* and *Ralstonia solanacearum* applied at the same time) and Ro with pathogen only had the highest fresh root weight while the least fresh root weight was observed in treatments Bo (*Bacillus thuringiensis* only) and control. The highest dry root weight was observed in treatment B+R (*Bacillus thuringiensis* and *Ralstonia solanacearum* applied at the same time) while the least was observed in control (Figure 6).

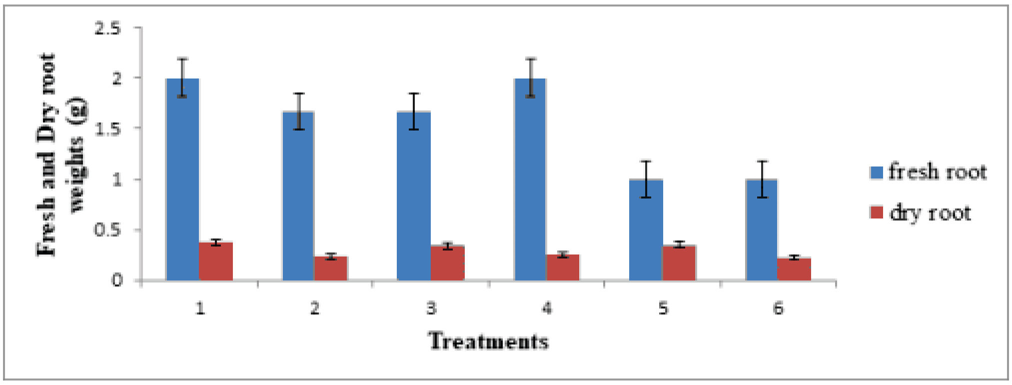

Figure 6: Effect of treatments on fresh and dry root weights
KEY
B + R – Antagonist (Bt) and pathogen (Rs) applied simultaneously
Bf – Antagonist applied first, pathogen applied a week after
Rf – Pathogen applied first, antagonist applied a week after
Ro – Pathogen only
Bo – Antagonist only
Control - Sterile water

Disease Incidence and Reduction

Disease incidence was highest in treatment Ro (100 %) while the least was recorded in treatments Bo and control (0%). Percentage disease reduction was highest in treatments having *Bacillus thuringiensis* (B+R, Bf and Rf) with 67% while the least was recorded in treatment having only *Ralstonia solanacearum* (Ro) with 0% (Figure 7).

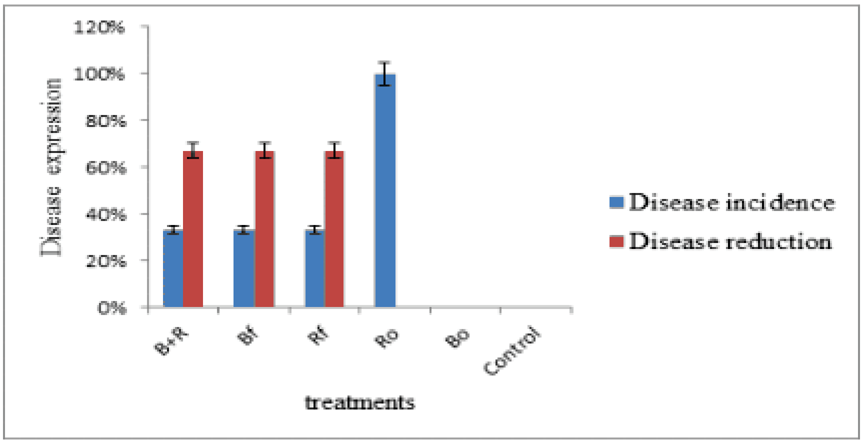

Figure 7: Disease Incidence and Reduction
KEY
B + R – Antagonist (Bt) and pathogen (Rs) applied simultaneously
Bf – Antagonist applied first, pathogen applied a week after

Discussion

Bacterial wilt caused by *Ralstonia solanacearum* is a dangerous disease in tropical, subtropical and some temperate regions of the World (Elsharkawy et al., 2015). A species complex with a wide host range infecting more than 450 crop species belonging to more than 50 families (Swanson et al., 2005; Aliye et al., 2008). Control methods against various diseases caused by this phytopathogen include the use of resistant varieties, crop rotation, biological control e.t.c. (Elsharkawy et al., 2015).

Bacillus thuringiensis a Gram-positive spore-forming bacterium is commonly known as an important biocontrol agent for the control of many agricultural insect pests and vectors of human diseases (Chattopadhyay et al., 2004; Noura et al., 2007). This is owing to its ability to produce during sporulation characteristic proteinaceous crystalline toxins (delta-endotoxins) (Schnepf et al., 1998). It has also attracted considerable attention as a potential biocontrol agent for the suppression of plant diseases (Reyes-Ramirez et al., 2004; Zhou et al., 2008).

Results from this study revealed that inoculation of *Bacillus thuringiensis* and *Ralstonia solanacearum* into apparently healthy *Beske* tomato seedlings under screenhouse condition significantly reduced the manifestation of bacterial wilt disease. Higher growth rates were recorded for plants height, number of leaves, number of flowers and shoot weights across treatments treated with *Bacillus thuringiensis*. This is in agreement with the previous studies of Armada et al. (2015) and Kassogue et al. (2016) who found Bt to improve plant growth significantly. Disease suppression was also higher in treatments B+R (*Bacillus thuringiensis* applied first and *Ralstonia solanacerum* applied a week after), Bf (*Bacillus thuringiensis* applied first and *Ralstonia solanacearum* applied a week after), Rf (*Ralstonia solanacearum* applies first and *Bacillus thuringiensis* applied a week after) and Bo (only *Bacillus thuringiensis* applied). This is in agreement with Mitsuro et al. (2012) who found *Bacillus thuringiensis* to significantly suppress the growth of *Ralstonia solanacearum* and the development of wilt symptoms in tomato plants. Suppressive ability of *Bacillus thuringiensis* could be due to the production of several compounds, including b-exotoxins, antibiotics, degrading enzymes, bacteriocins, and a signal molecule in the bacterial quorum-sensing system as opined by Dong et al. (2002), Cherif et al. (2003), Murphy et al. (2003), Cherif et al.(2008), Zhou et al.(2008) and Raddadi et al.(2009).

Conclusion

From this study, it can be concluded that native *Bacillus thuringiensis* isolated from cultivated soil can be used in the biological control of the soil-borne plant bacterial pathogen, *Ralstonia solanacearum*, the causal agent of bacterial wilt of tomato and also to improve the overall health of tomato plants under screenhouse condition. Further study should be carried out on field trials under different local environmental conditions. After successful field trials, farmers should be encouraged to embrace the use of *Bacillus thuringiensis* in place of chemicals.

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