

Field Evaluation of Biological Control Measures against Bacterial Soft Rot (*Pectobacterium carotovorum*) on Tomato in Taraba State, Nigeria

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Abstract

Bacterial soft rot caused by *Pectobacterium carotovorum* is one of the most destructive diseases affecting tomato production worldwide, creating a need for sustainable and effective management strategies under field conditions. This study evaluated the effectiveness of selected biological control agents and botanical extracts in managing bacterial soft rot of tomato in Wukari, Taraba State, Nigeria. The experiment was conducted using a randomized complete block design with three replications. The treatments consisted of neem extract, garlic extract, *Bacillus subtilis*, *Trichoderma harzianum*, Mancozeb, and an untreated control. Data were collected on disease incidence, disease severity, plant growth parameters, including plant height and number of leaves, and yield parameters, including number of fruits per plant, fruit weight, and total yield. The data were analyzed using analysis of variance, and treatment means were separated using Duncan's multiple range test at the 5% probability level. The results showed that all treatments significantly reduced disease incidence and severity compared with the untreated control. The lowest disease incidence and severity were recorded in plots treated with *B. subtilis* and *T. harzianum*, which

also significantly improved plant growth and yield parameters. The highest fruit yield, 19.8 t ha⁻¹, was obtained from plots treated with *B. subtilis*, whereas the untreated control recorded the lowest yield, 12.4 t ha⁻¹. The study concludes that biological control agents, particularly *B. subtilis* and *T. harzianum*, are effective in suppressing bacterial soft rot and improving tomato productivity. These findings contribute to sustainable tomato disease management by highlighting the potential of biological control agents as environmentally compatible alternatives for reducing disease pressure and enhancing yield under field conditions.

Keywords: *Pectobacterium carotovorum*, *Bacillus subtilis*, *Trichoderma harzianum*, Bacterial Soft Rot; Tomato Disease Management

Introduction

Tomato (*Solanumlycopersicum* L.) is one of the most important vegetable crops cultivated worldwide and constitutes a major component of human diets due to its high nutritional value. The fruit is rich in vitamins A, C, and E, minerals, and antioxidants such as lycopene which have significant health benefits including reduction of cardiovascular diseases and certain cancers (Arah et al., 2015). Globally, tomato production exceeds 180 million tonnes annually and plays a critical role in food security and income generation for farmers (FAO, 2022). In Nigeria, tomato is widely cultivated across several agro-ecological zones and serves as a major raw material for food processing industries and household consumption (Adenuga et al., 2013).

Despite its economic importance, tomato production in tropical and subtropical regions is constrained by several biotic factors, particularly plant diseases caused by fungi, bacteria and viruses. Among bacterial diseases, bacterial soft rot caused by *Pectobacteriumcarotovorum* (formerly *Erwiniacarotovora*) is one of the most destructive diseases affecting tomato both in the field and during post-harvest storage (Charkowski, 2018). The pathogen infects plant tissues through wounds or natural openings and produces pectolytic enzymes that degrade the middle lamella of plant cells, leading to tissue maceration and soft watery decay (Pérombelon, 2002). The disease is characterized by water-soaked lesions, soft rot of stems and fruits, foul odor and eventual collapse of infected tissues. Under favorable environmental conditions such as high temperature and humidity, the disease spreads rapidly and can cause substantial yield and post-harvest losses.

Bacterial soft rot pathogens have a wide host range including potato, carrot, cabbage, cucumber, and tomato, making their management particularly difficult (Toth et al., 2011). In many tomato-producing regions, yield losses due to soft rot diseases may reach 30–50 % depending on environmental conditions and management practices (Agrios, 2005). In Nigeria, poor storage conditions, mechanical injuries during harvesting and transportation, and high humidity often predispose tomato fruits to infection by soft rot bacteria, leading to significant economic losses for farmers and marketers (Arah et al., 2015).

Management of bacterial soft rot has traditionally relied on the use of chemical bactericides and antibiotics such as streptomycin. However, the effectiveness of these chemicals is often limited due to the development of pathogen resistance, high cost, environmental pollution and potential risks to human health (McManus et al., 2002). Furthermore, increasing public concern over chemical residues in food has encouraged the search for alternative disease management strategies that are safe, environmentally friendly and sustainable.

Biological control and plant-derived botanicals have emerged as promising alternatives for the management of plant diseases. Beneficial microorganisms such as *Bacillus subtilis* and *Trichoderma* species have been widely studied for their antagonistic activity against plant pathogens. These organisms suppress pathogens through mechanisms such as antibiosis, competition for nutrients and space, production of lytic enzymes and induction of systemic resistance in host plants (Harman et al., 2004; Ongena & Jacques, 2008). Several studies have demonstrated the effectiveness of these biological agents in controlling bacterial and fungal diseases in various crops.

Similarly, plant extracts derived from medicinal plants have shown considerable potential in suppressing plant pathogens due to the presence of bioactive compounds with antimicrobial properties. Neem (*Azadirachta indica*) and turmeric (*Curcuma longa*) are among the most widely studied botanicals for plant disease management. Neem contains biologically active compounds such as azadirachtin, nimbin and salannin which exhibit antibacterial and antifungal activities (Isman, 2006). Turmeric contains curcumin and other phenolic compounds that possess strong antimicrobial and antioxidant properties capable of inhibiting the growth of several plant pathogens (Prasad & Aggarwal, 2011).

In addition to biological control measures, the use of resistant or tolerant crop varieties represents an important component of integrated disease management. Tomato varieties differ in their susceptibility to bacterial diseases due to variations in genetic makeup and defense mechanisms. Therefore, evaluating the interaction between tomato varieties and biological control treatments under field conditions is essential for identifying effective disease management strategies.

Although several laboratory studies have reported the antimicrobial activity of biological agents and plant extracts against plant pathogens, there is limited information on their field performance in managing bacterial soft rot of tomato under the agro-ecological conditions of Taraba State, Nigeria. Field evaluation is necessary to determine the practical effectiveness of these control measures under natural environmental conditions.

Therefore, this study was conducted to evaluate the effectiveness of selected biological control agents and botanical extracts in managing bacterial soft rot caused by *Pectobacterium carotovorum* on different tomato varieties under field conditions in Taraba State, Nigeria. The findings of this research will contribute to the development of sustainable and environmentally friendly strategies for managing bacterial soft rot in tomato production systems.

MATERIALS AND METHODS

Study Area

The experiment was conducted during the 2021 and 2022 cropping seasons at the Teaching and Research Farm of the Department of Crop Production and Protection, Federal University Wukari, Taraba State, Nigeria. Wukari lies between latitude 7°52'N and longitude 9°47'E in the southern Guinea savannah agro-ecological zone of Nigeria. The area experiences a tropical climate characterized by a distinct wet and dry season, with annual rainfall ranging from 1000–1200 mm and mean temperatures between 26 and 34 °C. The soil in the experimental site is predominantly sandy loam, which supports the cultivation of vegetable crops including tomato (Adebayo, 1999; FAO, 2019).

Source of Planting Materials

Seeds of improved tomato varieties used for the study were obtained from a certified agro-input dealer in Wukari, Taraba State. The seeds were raised in a nursery bed

for four weeks before transplanting to the field. Healthy and uniform seedlings were selected for transplanting to ensure uniform plant establishment.

Isolation and Identification of the Pathogen

Infected tomato fruits showing typical symptoms of bacterial soft rot were collected from farmers' fields in Wukari and surrounding areas. The infected tissues were surface sterilized using 70% ethanol and rinsed with sterile distilled water. Small portions of diseased tissue were macerated in sterile water and streaked on nutrient agar plates. The plates were incubated at 28 ± 2 °C for 24–48 hours.

Emerging bacterial colonies were purified through repeated streaking and characterized using cultural, morphological, and biochemical tests. Identification of the pathogen as *Pectobacterium carotovorum* was based on colony morphology, Gram staining, and standard biochemical tests as described by Schaadt *et al.* (2001) and Agrios (2005).

Preparation of Bacterial Inoculum

Pure cultures of *Pectobacterium carotovorum* were grown on nutrient broth and incubated for 24 hours at 28 °C. The bacterial suspension was adjusted to approximately 10^8 CFU mL⁻¹ using sterile distilled water following the procedure described by Klement *et al.* (1990). This standardized inoculum was used for artificial inoculation of tomato plants in the field.

Experimental Design and Field Layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each treatment plot measured 3 m × 2 m with plant spacing of 75 cm × 50 cm. Standard agronomic practices such as watering, weeding, and fertilizer application were carried out uniformly across all plots.

Application of Biological Control Treatments

Biological control agents evaluated in the study were prepared according to standard procedures. The treatments were applied as foliar sprays at specified intervals after transplanting. Application was done using a knapsack sprayer in the early morning to minimize evaporation and maximize effectiveness.

Data Collection

Disease Parameters

Disease Incidence (%)

Disease incidence was recorded as the proportion of infected plants in each plot relative to the total number of plants. Observations were taken weekly after symptom appearance.

$$\text{Disease Incidence (\%)} = \frac{\text{number of leaves/fruit infection}}{\text{Total number of leaves/ fruit count}} \times 100$$

Total number of leaves/ fruit count

This parameter indicates the spread of the disease within the experimental population (Agrios, 2005).

Disease Severity

Disease severity was assessed using a rating scale based on the extent of tissue maceration and rotting on the fruits. A 0–5 scale was adopted:

0 = No visible symptoms

1 = Slight softening on fruit surface

2 = Small water-soaked lesions

3 = Moderate rotting covering <50% of fruit

4 = Extensive rotting covering >50% of fruit

5 = Complete fruit maceration

Disease severity index was calculated using the formula described by Wheeler (1969):

$$\text{Disease \ Severity (\%)} = \frac{\text{Sum of all disease rating}}{\text{Total number of observation} \times \text{maximum rating value}} \times 100$$

Total number of observation **X** maximum rating value

Disease severity is widely used to measure the intensity of infection and host damage (Campbell & Madden, 1990).

Growth Parameters

Growth parameters were assessed at regular intervals to determine the effect of treatments on plant development.

Plant Height (cm)

Plant height was measured from the soil surface to the apical meristem using a measuring tape. Measurements were taken from five randomly selected plants in each plot and averaged. Plant height is an important indicator of vegetative growth and plant vigor (Gardner et al., 1985).

Number of Leaves per Plant

The total number of fully expanded leaves was counted on five tagged plants in each plot. Leaves play a major role in photosynthesis and biomass accumulation (Taiz et al., 2015).

Stem Diameter (mm)

Stem girth was measured using a digital caliper at approximately 5 cm above the soil surface. Stem diameter reflects plant structural strength and nutrient uptake efficiency (Gardner et al., 1985).

Yield Parameters

Yield components were evaluated at harvest to determine the effect of treatments on tomato productivity.

Number of Fruits per Plant

The total number of fruits produced by each plant was counted throughout the harvesting period. Data were obtained from five randomly selected plants per plot and averaged.

Fruit Weight (g)

Individual fruit weight was determined using a digital weighing balance. The average fruit weight was calculated by dividing the total fruit weight by the number of fruits harvested.

Total Yield per Plot (kg)

Total yield per plot was determined by weighing all harvested fruits from each plot using a digital scale. Yield data were later converted to tonnes per hectare using standard conversion methods (FAO, 2019).

Marketable Yield

Marketable yield consisted of healthy fruits free from disease symptoms, mechanical damage, or deformities. Diseased or rotten fruits were excluded from this category (Agrios, 2005).

Data Analysis

All data collected were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS). Treatment means were separated using Duncan's Multiple Range Test (DMRT) at a 5% level of probability to determine significant differences among treatments (Gomez & Gomez, 1984)

RESULTS AND DISCUSSION

Effect of Biological Control Treatments on Disease Incidence of Tomato Soft Rot

The results showed that biological control treatments significantly ($P \leq 0.05$) influenced the incidence of bacterial soft rot in tomato plants. Disease incidence increased progressively with time across all treatments, but the magnitude differed significantly among treatments.

The untreated control recorded the highest disease incidence throughout the assessment period, reaching 49.6% at 8 weeks after transplanting (WAT). In contrast, plots treated with *Bacillus subtilis*, *Trichoderma harzianum*, and Mancozeb recorded significantly lower disease incidence compared with the control. Among the biological treatments, *Bacillus subtilis* was the most effective, reducing disease incidence to 20.8% at 8 WAT (table 1 and Appendix 1).

The reduction in disease incidence observed in plots treated with microbial biocontrol agents may be attributed to antagonistic mechanisms such as antibiosis, competition for nutrients, and induced systemic resistance in host plants. Similar findings were reported by Larkin and Fravel (1998), who observed that *Trichoderma* species significantly suppressed soil-borne pathogens through competition and mycoparasitism. Likewise, *Bacillus subtilis* has been widely reported to produce antibiotics and enzymes that inhibit the growth of plant pathogenic bacteria (Kloepper et al., 2004).

Botanical treatments such as neem and garlic extracts also reduced disease incidence compared to the untreated control, although their effects were less pronounced

than microbial biocontrol agents and Mancozeb. The antimicrobial activity of neem and garlic extracts has been attributed to bioactive compounds such as azadirachtin and allicin, which possess strong antibacterial properties (Isman, 2006).

Table 1: Effect of treatments on disease incidence (%) of tomato soft rot at Wukari

Treatment	4 WAT	6 WAT	8 WAT	Mean
Neem extract	12.3 c	18.6 c	24.4 c	18.4
Garlic extract	14.5 c	21.3 c	27.1 c	21.0
Bacillus subtilis	9.2 d	15.7 d	20.8 d	15.2
Trichoderma harzianum	10.4 d	16.2 d	21.9 d	16.2
Mancozeb	7.6 d	13.1 d	18.7 d	13.1
Control	26.8 a	38.5 a	49.6 a	38.3

SE $\pm$ = 1.72, CV (%) = 11.4

Means followed by the same letter within a column are **not significantly different** according to DMRT at P = 0.05.

Appendix 1: ANOVA Summary (Disease Incidence)

Source of Variation	DF	SS	MS	F value
Treatments	5	1420.6	284.1	18.52*
Replication	2	41.3	20.6	1.34
Error	10	153.4	15.3	
Total	17	1615.3		

Significant at $P \leq 0.05$

Effect of Treatments on Disease Severity

The severity of bacterial soft rot symptoms followed a similar pattern to disease incidence. Significant differences ($P \leq 0.05$) were observed among treatments. Disease severity increased with time but remained significantly lower in treated plots compared with the untreated control.

At 8 WAT, the highest disease severity (44.9%) was recorded in the control plots, while the lowest severity (17.4%) was observed in plots treated with Mancozeb. Among the biological control agents, *Bacillus subtilis* and *Trichoderma harzianum* significantly reduced disease severity to 19.8% and 20.6%, respectively (table 2 and Appendix 2).

The reduction in disease severity observed in microbial treatments may be due to the ability of these microorganisms to colonize plant roots and suppress pathogen populations in the rhizosphere. According to Harman et al. (2004), *Trichoderma* species

enhance plant defense mechanisms and reduce disease severity by producing hydrolytic enzymes and antifungal metabolites.

Botanical treatments also contributed to disease suppression, though their effect was moderate compared with microbial treatments. The antimicrobial properties of neem and garlic extracts have been reported to inhibit bacterial pathogens responsible for soft rot diseases (Bowers and Locke, 2000).

Table 2: Effect of treatments on severity (%) of tomato bacterial soft rot

Treatment	4 WAT	6 WAT	8 WAT	Mean
Neem extract	10.5 c	16.8 c	23.6 c	17.0
Garlic extract	12.1 c	18.5 c	25.4 c	18.7
Bacillus subtilis	8.7 d	13.2 d	19.8 d	13.9
Trichoderma harzianum	9.4 d	14.5 d	20.6 d	14.8
Mancozeb	6.9 d	11.6 d	17.4 d	12.0
Control	24.7 a	35.8 a	44.9 a	35.1

SE $\pm$ = 1.51, CV (%) = 10.6

Appendix 2: ANOVA Summary (Disease Severity)

Source	DF	SS	MS	F
Treatments	5	1216.4	243.3	16.74*
Replication	2	34.5	17.2	1.18
Error	10	145.3	14.5	
Total	17	1396.2		

Effect of Treatments on Growth Parameters of Tomato

The growth parameters measured in this study, including plant height and number of leaves per plant, were significantly affected by the treatments. Plants treated with biological control agents exhibited better vegetative growth compared to untreated plants.

The highest plant height (76.2 cm) was recorded in plants treated with *Bacillus subtilis*, followed closely by *Trichoderma harzianum* (75.1 cm), while the lowest plant height (60.8 cm) was recorded in the untreated control. A similar trend was observed in the number of leaves per plant, where *Bacillus subtilis* and *Trichoderma harzianum* produced the highest leaf numbers (tables 3 and 4).

The improved growth performance observed in treated plants may be attributed to the plant growth-promoting activities of beneficial microorganisms. Plant growth-promoting rhizobacteria such as *Bacillus* species enhance plant growth through mechanisms such as nitrogen fixation, phytohormone production, and improved nutrient uptake (Kloepper et al., 2004).

In addition, the reduction of disease pressure in treated plants may have allowed the plants to allocate more resources to vegetative growth rather than defense responses. This observation agrees with findings reported by Harman (2006), who noted that biocontrol agents can enhance plant growth by improving root health and nutrient acquisition.

Table 3: Effect of treatments on plant height (cm)

Treatment	4 WAT	6 WAT	8 WAT	Mean
Neem extract	38.2 b	54.6 b	71.4 b	54.7
Garlic extract	36.9 b	52.1 b	69.3 b	52.8
Bacillus subtilis	41.5 a	58.3 a	76.2 a	58.7
Trichodermaharzianum	40.8 a	57.4 a	75.1 a	57.8
Mancozeb	39.6 ab	55.7 ab	73.8 ab	56.4
Control	30.7 c	45.3 c	60.8 c	45.6

SE $\pm$ = 2.21, CV (%) = 9.8

Table 4: Effect of treatments on number of leaves per plant

Treatment	4 WAT	6 WAT	8 WAT	Mean
Neem extract	18.4 b	26.7 b	33.2 b	26.1
Garlic extract	17.9 b	25.3 b	31.7 b	25.0
Bacillus subtilis	21.3 a	29.6 a	37.5 a	29.5
Trichodermaharzianum	20.8 a	28.9 a	36.2 a	28.6
Mancozeb	19.6 ab	27.5 ab	34.4 ab	27.2
Control	14.7 c	20.4 c	26.1 c	20.4

SE $\pm$ = 1.64, CV (%) = 10.1

Effect of Treatments on Yield Parameters

The treatments significantly influenced tomato yield and yield components (tables 5 and 6). The highest number of fruits per plant (26.4 fruits) was recorded in plots treated with *Bacillus subtilis*, followed by *Trichodermaharzianum* (25.7 fruits). The untreated control produced the lowest number of fruits per plant (16.2 fruits).

Similarly, fruit weight and overall yield were significantly higher in treated plots compared to the control. The highest yield (19.8 t ha^{-1}) was recorded in plots treated with *Bacillus subtilis*, followed by *Trichoderma harzianum* (19.2 t ha^{-1}), while the control recorded the lowest yield (12.4 t ha^{-1}).

The higher yield obtained from treated plots may be attributed to the combined effects of disease suppression and improved plant growth. Reduced disease incidence and severity allowed plants to maintain healthy tissues, leading to increased photosynthetic efficiency and fruit production.

These findings are consistent with previous studies that reported significant yield improvement in tomato following the application of biological control agents. Kloepper et al. (2004) reported that plant growth-promoting rhizobacteria enhance crop productivity by improving plant health and suppressing pathogens. Similarly, Harman et al. (2004) observed increased yield in crops treated with *Trichoderma* species due to improved plant vigor and disease resistance.

Table 5: Effect of treatments on number of fruits per plant

Treatment	Fruits/plant
Neem extract	22.3 b
Garlic extract	21.1 b
Bacillus subtilis	26.4 a
Trichoderma harzianum	25.7 a
Mancozeb	24.5 ab
Control	16.2 c

$SE \pm = 1.32, CV (\%) = 8.7$

Fruit Weight and Yield

Table 6: Effect of treatments on fruit weight and yield

Treatment	Fruit weight (g)	Yield (t/ha)	Mean
Neem extract	82.4 b	16.3 b	
Garlic extract	80.6 b	15.8 b	
Bacillus subtilis	94.2 a	19.8 a	
Trichoderma harzianum	92.6 a	19.2 a	
Mancozeb	88.7 ab	18.1 ab	
Control	67.3 c	12.4 c	

$SE \pm = 2.08, CV (\%) = 9.2$

Overall Implications of the Study

The results of this study demonstrate that biological control agents, particularly *Bacillus subtilis* and *Trichoderma harzianum*, are effective in reducing bacterial soft rot incidence and severity while improving tomato growth and yield. Botanical extracts such as neem and garlic also contributed to disease suppression, although their effects were less pronounced than microbial agents.

The integration of biological control agents in tomato production systems could therefore serve as an environmentally friendly alternative to synthetic chemical pesticides. This approach supports sustainable crop production by reducing reliance on chemical control measures while maintaining crop productivity.

Summary

This study evaluated the effectiveness of selected biological control agents and botanical extracts in managing bacterial soft rot of tomato caused by *Pectobacterium carotovorum* under field conditions in Wukari, Taraba State, Nigeria. The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications.

Treatments included neem extract, garlic extract, *Bacillus subtilis*, *Trichoderma harzianum*, Mancozeb, and an untreated control. Data were collected on disease incidence, disease severity, plant growth parameters (plant height and number of leaves), and yield parameters (number of fruits per plant, fruit weight, and total yield).

The results showed that all treatments significantly reduced disease incidence and severity compared with the untreated control. The highest disease incidence and severity were consistently recorded in the control plots throughout the assessment period.

Among the treatments, *Bacillus subtilis* and *Trichoderma harzianum* demonstrated the greatest ability to suppress bacterial soft rot. These treatments significantly reduced disease incidence and severity while also improving plant growth and yield. Botanical treatments such as neem and garlic extracts also reduced disease levels compared to the control but were less effective than microbial treatments.

Growth parameters were significantly enhanced in treated plots. Plants treated with *Bacillus subtilis* recorded the highest plant height and number of leaves per plant, indicating improved plant vigor. Similarly, yield parameters were significantly higher in

treated plots. The highest number of fruits per plant, fruit weight, and total yield were obtained in plots treated with *Bacillus subtilis*, followed by *Trichoderma harzianum*.

Overall, the results demonstrate that biological control agents can effectively reduce bacterial soft rot incidence and improve tomato productivity under field conditions.

CONCLUSION

The study demonstrated that biological control agents and botanical extracts can play an important role in the management of bacterial soft rot disease of tomato caused by *Pectobacterium carotovorum*. The treatments significantly reduced disease incidence and severity compared with the untreated control.

Among the treatments evaluated, *Bacillus subtilis* and *Trichoderma harzianum* proved to be the most effective in suppressing the disease while enhancing plant growth and yield. These treatments also contributed to improved vegetative growth parameters such as plant height and number of leaves, which are important indicators of plant vigor.

The findings suggest that biological control agents offer a sustainable and environmentally friendly alternative to synthetic chemical pesticides. Their ability to suppress pathogens while promoting plant growth makes them suitable components of integrated disease management strategies in tomato production.

Recommendations

Based on the findings of this study, the following recommendations are made:

1. Application of biological control agents, particularly *Bacillus subtilis* and *Trichoderma harzianum*, should be encouraged for the management of bacterial soft rot in tomato production.
2. Farmers should adopt integrated disease management strategies combining biological control agents with good agronomic practices to effectively reduce disease incidence.
3. Botanical extracts such as neem and garlic may be used as supplementary control measures where microbial biocontrol agents are not readily available.
4. Extension services should promote awareness and training among farmers on the use of biological control agents as environmentally safe alternatives to chemical pesticides.
5. Further research should be conducted to:

- Evaluate the long-term effectiveness of biological control agents under different environmental conditions.
- Investigate combinations of biological agents and botanicals for improved disease management.
- Assess the economic benefits of biological control in commercial tomato production.

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