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***Research Article***

# *In Vitro* Control of Bacterial Pathogens Affecting Tomatoes from Irrigated Tomato Farms in Watari, Dawakin Tofa, Kano State Using Plant Extracts

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**ABSTRACT**
A study was conducted to control bacterial pathogens affecting tomatoes from irrigated tomato farms in Watari, Dawakin Tofa, Kano State, using plant extracts. Infested plant parts and soils were collected for isolation and identification of pathogenic bacteria using morphological and biochemical characterization. Based on traditional knowledge, three plants were collected and taken to the laboratory for herbarium identification using voucher numbers and binomial naming. The phytochemicals associated with the three plants were determined by ethanolic extraction and screening. The effect of the plant extracts on the culture of bacterial colonies was also determined. The study found that the effectiveness of plant extracts against bacterial isolates compared favorably with synthetic bactericides. Using different concentrations (1 g/5 mL, 1 g/10 mL, and 1 g/15 mL), the results showed that plant extract from *P. biglobosa, T. indica*, and *E. globulus* can be incorporated into integrated pest and disease management in tomatoes and help reduce the overuse of synthetic chemicals. 1 g/5 mL of the ethanolic extracts of *E. globulus* had the highest antibacterial activity, with a 15 mm zone of inhibition against *E. faecalis*, 18 mm zone of inhibition against *P. syringae*, and a 12 mm zone of inhibition against *B. subtilis* when compared with 20 mm and 15 mm of synthetic bactericides.

**Keywords:** Bacteria; *Eucalyptus globulus; Parkia biglobosa; Tamarindus indica;* Watari

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## INTRODUCTION

Agricultural activities will continue to be the primary source of income for Nigeria's rapidly growing population (Ugonna *et al.,* 2016). Tomato (*Solanum esculentum)* is one of the major vegetables grown in the country and is consumed in various forms (Ugonna *et al.,* 2016) Bacterial wilt of tomato caused by the *Ralstonia solanacearum* is endemic in most Tomato growing areas of Kano and Nigeria at large causing 60 to 100% loss in yield (Popoola *et al.,* 2015). Tomato being a cash crop in many parts of the world, control measures to safeguard the crop from nematodes and bacterial pathogens are usually taken seriously (Luka, 2017). Several methods have been used for the control but the most spectacular of all this is the use of chemical nematicides and anti-bacteria (Luka, 2017). However, chemical bactericides are not much used by peasant farmers because they are expensive, toxic persistent, and require skills for their application. (Umar, Muhammad and Okusanya, 2010; Luka, 2017).

Besides causing significant yield reduction, the bacteria interact synergistically with other plant pathogens, most especially nematodes, resulting in the disease complexes, that impact more heavily on crop yield (Ayomide, 2020) Continuous cropping on the same piece of land further aggravates the nematodes and bacterial menace as the population increases above the economic threshold level, thereby reducing yield (Feng *et al.,* 2013).

## MATERIALS AND METHODS

### Description of the Study Area

The study was carried out at Watari irrigation Centre, Dawakin Tofa LGA of Kano State from February to April 2023. The irrigation centre lies between latitude 12.1$^0$N longitude 8.32$^0$E elevations of 482 meters respectively (Figure 1).

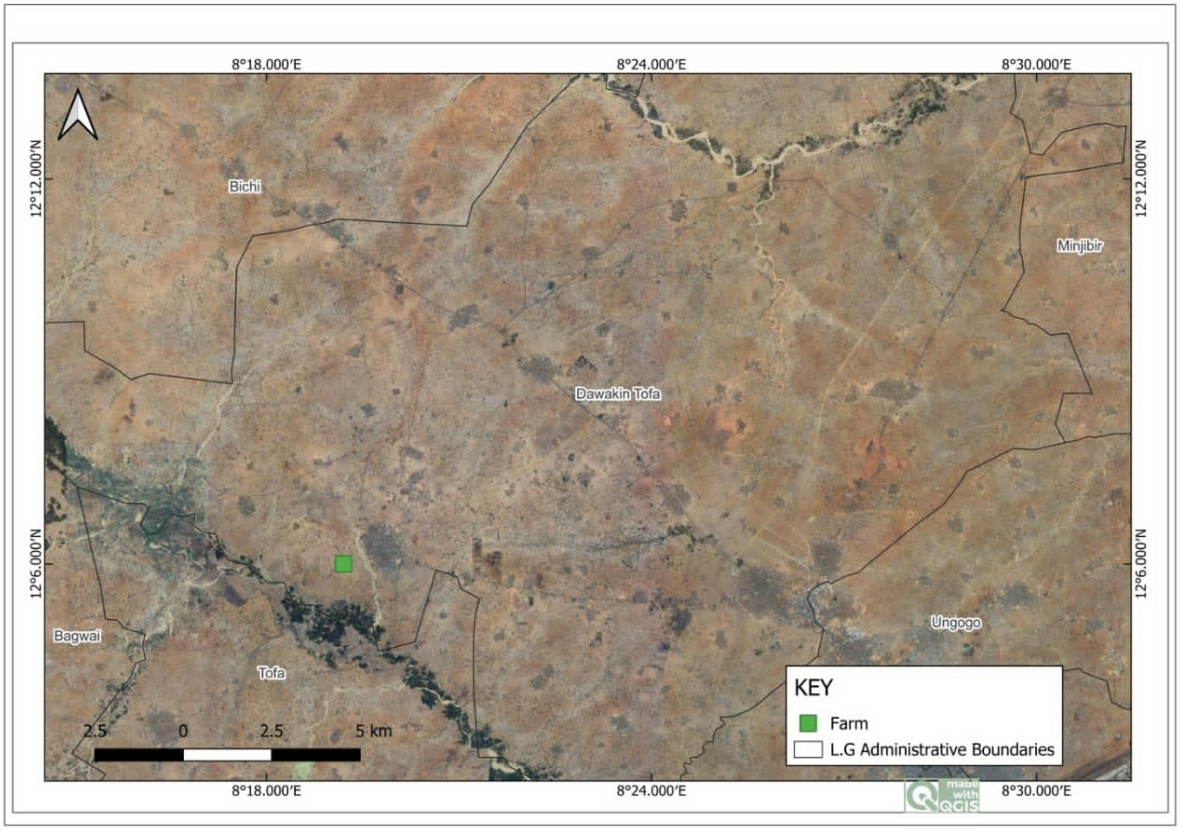


**Figure 1: Satellite image of the study location (Source: GIS Lab, Bayero University Kano)**

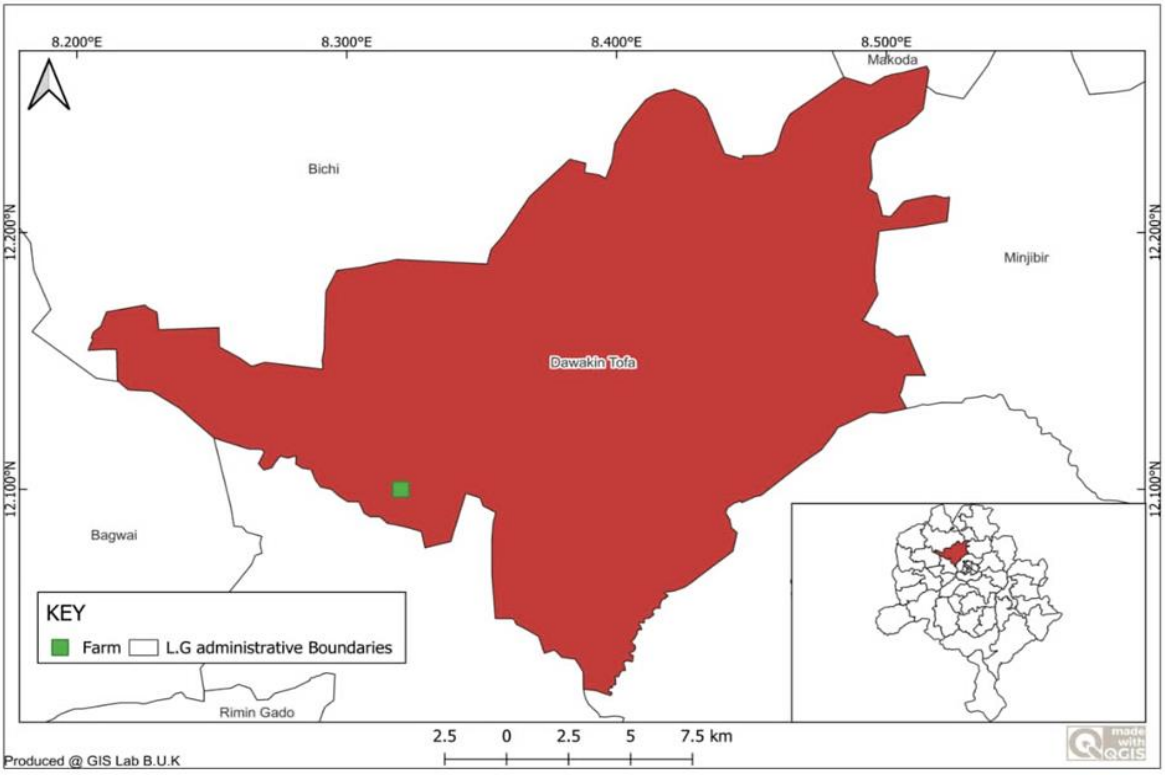


**Figure 2: The map of Dawakin Tofa Showing the study location (Souce: GIS Lab B. U. K June 6, 2023)**

**Field Sample/Data Collection**

A trowel was used to gently removed soil adhering to the root system of the diseased plants selected in a zigzag pattern. The soil was then removed and collected, the plants also expected to be infested with bacteria was scored based on severity of the incidence the diseased plants and the soil samples were stored in a clearly labelled polyethene bags and transported to the laboratory for further analysis Missinga *et al.* (2018).

**Isolation of Bacteria**

The procedures described by Feng *et al.,* (1994), Saxer *et al.* (2013) and Padder *et al.* (2017) were slightly modified in order to isolate the bacteria from diseased tomato plants and soil. The collected tomato plant samples were separated into root, shoot and leaf portions. The sampled portions were washed three times in flowing tap water and cut into pieces measuring between 2 and 4 mm using a sterile scalpel. The cut plant tissues were carefully placed in a well labelled beaker containing 5% v/v sodium hypochlorite solution and allowed to stand for a minute to ensure surface sterilization and rinsed thoroughly in three changes of sterilized distilled water. Thin slices of the tissue were plated on solidified nutrient agar with the aid of sterile forceps and incubated at $37^0$C for 48 hours. Discrete colonies were counted and recorded. Repeated sub culturing was carried out to obtain pure cultures.

Soil samples from the top 5cm layer of the rhizosphere of infected plants were collected and diluted with distilled water, 1g/10mL of it was collected with sterile pippete and gently mixed by swirling with molten nutrient agar at $45^0$C (pour plate technique), allowed to set and incubated at $37^0$C for 48hrs in a repeated pattern till pure cultures were obtained (Padder *et al.,* 2017).

**Identification of Bacteria**

The bacteria were identified using procedure outlined by Saxer *et al.,* (2010) where the isolates characterized morphologically and biochemically using (Catalase, Citrate, starch hydrolysis and gelatin liquefaction tests).

**Collection and Identification of Plants for Extraction**

Based on folklores or traditional use of plant extracts, three (3) plants reported to possess anti-bacterial properties were sampled and collected at random from the study area for identification. The plants were collected in the month of February 2023, and brought to the herbarium of Federal University Dutsin-ma, Katsina State Nigeria for identification.

**Preparation of Crude and Ethanol Extracts of the Plants**

In the laboratory, 500 mL of ethanol was mixed with 60 g of each plant after the plant materials (leaves) were dried to a constant weight and ground into powder using a clean pestle and mortar and sieved with an aperture of 0.5 mm. The test plants' dried leaves were thoroughly extracted using ethanol (Analytical Grade, BDH laboratory supplies) in a Soxhlet apparatus for about 12 hours. The resulting ethanol extract was then filtered through Whatman paper No. 1 and concentrated using the Buchi Rotvapor R-200 at $45^0$C under reduce pressure to produce a crude mix of 23.5% (Eloff, 1998; Rios, 2005).

**Preparation of Plant Extracts for Preliminary Phytochemical Screening**

The plants were dried for two to four weeks on a laboratory bench at 25 to 27 $^0$C (Valencia and Oyabanji, 1997). After drying to a constant weight for 12 hours at 35 $^0$C in the oven, the plants were ground for extraction and phytochemical analysis. Alkaloids, Terpenes, Saponins, Flavonoids, Glycosides, Sterols, and Tannins were examined using standard methods for the phytochemical analysis of the test plants, *Parkia biglobosa, Tamarinddus indica,* and *Eucalyptus globulus.*

**Preparation of Extract Concentrations for the Studies**

To test the bactericidal properties of the extracts, 1.0 g of each extract was dissolved in 5 mL of distilled water. Additional concentrations of the extracts were also prepared by dissolving 1.0 g in 10 mL and 1.0 g in 15 mL of distilled water, and their efficacy was determined using agar well diffusion method.

**Experimental Design**

Completely randomized design was adopted for the experiments, with three replicates. Data obtained was subjected to analysis of variance at 5% level of probability using Minitab statistical tool and the means were separated using turkey post-hoc.

## RESULTS

Twenty-five isolates (25) of bacteria were isolated from tomato roots and leaves portion and also the soil sample collected from the study area on Nutrient agar medium, colonies with different morphological characters on nutrient agar were further characterized (Table 1). Out of the four isolates, 1 was gram positive cocci, 1 gram negative rod, and 2 gram positive rods, Biochemical characterization of the bacterial isolates using citrate, catalase, gelatin liquefaction, starch hydrolysis and motility tests revealed the presence of *Bacillus subtilis, Enterococcus faecalis, and Pseudomonas syringae* as shown in Table 2.

The preliminary phytochemical screening of ethanolic extracts of *T. indica, P. biglobosa* and *E globulus* revealed the presence of phytochemicals

including, alkaloids, flavonoids, glycosides, phenols, terpenes, saponins, sterols and tannins (Table 3).

**Table 1: Colony Characteristics of Different Isolates of Bacteria**

| Isolates | Shape | Pigmentation | Appearance | Gram Staining Reaction | Shape Under microscope |
|---|---|---|---|---|---|
| LBI | Circular | Milky | Shiny | Gram-Positive | Cocci |
| LBI | Irregular | White | Shiny | Gram-Negative | Rod |
| RBI | Circular | Milky | Shiny | Gram-Positive | Rod |
| SBI | Circular | Milky | Shiny | Gram-Positive | Rod |

Key: LBI= Leaf bacterial isolates, RBI= Root bacterial isolates, SBI= Soil bacterial isolates

**Table 2: Biochemical Characterization of the most predominant Bacterial Isolates**

| Tests | *B. Subtilis* | *E. Faecalis* | *P. Syringae* |
|---|---|---|---|
| Citrate | + | - | + |
| Catalase | + | - | + |
| Starch hydrolysis | + | - | - |
| Gelatin liquefaction | + | - | + |
| Motility | + | - | + |

Key: + = Positive, - = Negative

**Table 3: Phytochemical Constituents of the Investigated Plant Species**

| Test plant<br>Phytochemicals | *P. biglobosa* | *T. indica* | *E. globulus* |
|---|---|---|---|
| Alkaloids | + | + | + |
| Flavonoids | + | - | + |
| Glycosides | + | - | + |
| Phenols | + | - | + |
| Terpenes | + | + | - |
| Saponins | + | + | + |
| Sterols | + | + | + |
| Tannins | + | + | + |

Key = Present (+), Absent (-)

The antibacterial properties show increase in inhibitory effect with increase in concentrations of the different ethanol extracts as shown on table 4, 5 and 6.

The inhibitory activity of *T. indica* on bacteria isolated from the study area by varying concentrations was weak when compared with that of treated control, though the inhibitory activity increased with increase in concentration. For *Enterococcus faecalis,* the ethanolic leaf extracts at l.0g/15 mL exhibited no inhibitory effect, l.0g/10 mL had 7 mm zone of inhibition (i.e., very weak inhibitory effect), 1.0g/5 mL had 8.0 mm zone of inhibition (i.e., weak inhibitory effect), while the control had 17 mm zone of inhibition. For *Pseudomonas syringae* 1.0g/15 mL had 7.0mm (A very weak inhibitory effect), 1.0g/10 mL had 8.0 mm (weak inhibitory effect), and 1.0g/5 mL had 10 mm (partially strong inhibitory effect) while the treated control had 17 mm zone of inhibition also. For *B. subtilis* 1.0g/15 mL had no inhibitory effect... 1.0g/10 mL had 7.0mm (a very week inhibitory effect), 1.0g/5 mL had 10mm (partially strong), while the treated control had 19 mm zone of inhibition (Table 4).

**Table 4: Inhibitory Effect of *T.indica* on Bacterial Isolates from Tomato farmers' Fields of Dawakin Tofa, Kano State**

| | ----------Zone of inhibition of each concentration---------- (mm) | | | |
|---|---|---|---|---|
| **BacterialIsolates** | **(1g/5mlL)** | **(1g/10mL)** | **(1g/15 mL)** | **(Control)** |
| *E. faecalis* | 8.0 | 7.0 | 6.0 | 17.0 |
| *P. syringae* | 10.0 | 8.0 | 7.0 | 17.0 |
| *B. subtilis* | 10.0 | 7.0 | 6.0 | 19.0 |
| LSD P < 0.05 | 0.01 | 0.10 | 0.56 | 0.001 |

The inhibitory activity of different concentrations of *P. biglobosa* against the test organisms were concentration dependent. For *E. faecalis* 1.0g/15ml had 6mm indicating no any inhibitory effect 1.0g/10ml had 8.0mm inhibitory effect (i.e., week), 1.0g/5ml had 9.0mm a week inhibitory effect, while the treated control (i.e., chemical control) had 22mm. For *P. syringae* 1.0g/15ml had no inhibitory effect, 1.0g/10 mL had 9mm (a week inhibitory effect), l.0g/5ml had 10mm (A partially strong inhibitory effect). The treated control had 16mm, while 1.0g/15ml on *B. subtilis* exhibited no inhibitory effect. 1.0g/10ml had 8mm exhibited weak inhibitory effect, l.0g/5ml had 10mm zone of inhibition, and while the treated control had 17mm (Table 5).

**Table 5: Inhibitory Effect of *P. biglobosa* on Bacterial Isolates from Tomato Farmers Field of Dawakin Tofa, Kano State**

| | ----------Zone of inhibition of each concentration---------- (mm) | | | |
|---|---|---|---|---|
| **Bacterial Isolates** | **(1g/5mlL)** | **(1g/10mL)** | **(1g/15 mL)** | **(Control)** |
| *E. faecalis* | 9.0 | 8.0 | 6.0 | 22.0 |
| *P. syringae* | 10.0 | 9.0 | 6.0 | 16.0 |
| *B. subtilis* | 10.0 | 8.0 | 6.0 | 17.0 |
| LSD P < 0.05 | 0.10 | 0.37 | 0.76 | 0.01 |

The inhibitory activity/effect of different concentrations of *E. globulus* varied also by dissolving l.0g of the ethanolic extract of *E. globulus* in different volumes of Distilled water. The activity of the extract on the bacterial isolates increases with the increase in concentration. For *E. faecalis* l.0g/15 mL had 11 mm (A strong inhibitory effect) l.Og/10mL had 12mm (strong inhibitory effect) l.0g/5mL had 15mm (very strong inhibitory effect) while the treated control had 20mm respectively. *P. syringae* l.0g/15mLhad 13mm (A very strong inhibitory effect) l.0g/10mL had 15mm (A very strong inhibitory effect) l.0g/5mL had 18mm zone of inhibition (A very strong inhibitory effect), while the treated control had 20 mm zone of inhibition. And for *B. subtilis* l.0g/15mL had 6mm zone of inhibition (exhibited no any inhibitory effect) l.0g/10mL had 8mm (A weak inhibitory activity l.0g/15mL had 12mm a strong inhibitory effect while the treated control had 15mm zone of inhibition as indicated clearly on Table 6.

**Table 6: Inhibitory Effect of *E. globulus* on Bacterial Isolates from Tomato Farmers field of Dawakin Tofa, Kano State**

| | ----------Zone of inhibition of each concentration---------- (mm) | | | |
|---|---|---|---|---|
| **Bacterial Isolates** | **(1g/5mlL)** | **(1g/10mL)** | **(1g/15 mL)** | **(Control)** |
| *E. faecalis* | 15.0 | 12.0 | 11.0 | 20.0 |
| *P. syringae* | 18.0 | 15.0 | 13.0 | 20.0 |
| *B. subtilis* | 12.0 | 8.0 | 6.0 | 15.0 |
| LSD P < 0.05 | 0.30 | 0.62 | 0.92 | 0.05 |

## DISCUSSION

Analysis of the diseased tomato plants and the soil samples collected from the study area revealed the presence of three bacterial species including *Pseudomonas syringae, Bacillus subtilis,* and *Enterococcus faecalis*. It is clear that bacteria cause enormous problems in the plant production industry and that in adequate control can lead to serious problem in food production. Therefore, continued research including using plant based – products are required to provide effective biological products that are cheap, less toxic, persistent, and effective (Kumar *et al.,* 2021). Control by using plant-based products may offer relief in the fight against plant bacterial diseases (Tripathi *et al.,* 2020a). This approach has the advantage that there may be reduced development of resistance in the different anti-bacterial compounds in an extract target different receptor. There is, however, an advantage compared to using a single chemical product in ensuring good quality control and variation of activity based on genetic or environmental factors.

Therefore, thorough methods have to be used to find new anti-bacterial product that may be of potential use in agricultural production. We also have to investigate how plants which may be of no use in agricultural and horticultural compliment production for take themselves against pathogens. They may produce novel compounds to overcome pathogens invasion. Yiidz *et al.* (2018) reported *Pseudomonas syringae* as the major problem for a greenhouse production of tomato in the Mediterranean region of Turkey causing bacterial

speck. This result also agrees with that of Kawo *et al.* (2021), where *B. subtilis* was isolated from same Watari irrigation scheme. Yoana *et al.* (2022) also reported *Enterococcus faecalis, Leclercia adecarboxylate, Enterobacter cloacae* and other 16 cross over pathogenic bacteria in tomatoes and pepper fields in Bulgaria.

The phytochemical screening of the ethanolic extracts of *P. biglobosa, T. indica and E. globulus* leaves revealed the presence of Some phytochemicals include alkaloids, terpenes, saponins, flavonoids, glycosides, phenols, sterols, and tannins. Their uses as bactericides and bacterial growth inhibitory activities might be due to the antibacterial properties contained in the extracts, and these properties might have penetrated the body of bacteria and inhibited metabolic activities such as those of respiratory enzymes and hydrolysis of acetylcholine by acetylcholinesterase that function in various metabolic systems.

Many researchers reported that plants that produce alkaloids, phenols, and flavonoids could potentially be toxic to bacteria, *Parkia biglobosa* and *Eucalyptus globulus* containing alkaloids, phenols, and flavonoids phytochemicals in which the bacterial growth inhibition might be attributed to, thereby making it agree with the work of Castillo *et al.,* 2010. Who reported that the use of plants with a high content of polyphenols inhibits bacterial growth, and it also agrees with the work of Soylu *et al.,* 2006?

The results of this research revealed that the ethanolic extracts of the three test plants had antagonistic activity on bacteria, this anti- bacterial activity could be a result of the unique phytochemical components of the P. biglobosa, T. Indica, and E.globulus, like alkaloids, flavonoids, glycosides, phenols, terpenes, saponins, sterols, and tannins this findings are in line with the works of Kutawa *et al.,* (2025) & Luka (2017). It is clear that existing control measures are not enough to deal with emergence of the outbreak of plant bacterial pathogens therefore, continued research including using plant - based products, is required to provide effective biological products that are cheap, less toxic persistent, and effective (Kumar *et al.,* 2021).

## CONCLUSION

The study showed that plant extract is highly effective in reducing bacterial diseases levels and increase crop yield. Therefore, they can be incorporated in integrating pest and disease management programs, thereby reducing heavy application of synthetic bactericides which have negative effect on environment and leaving harmful residues on produces. This will help meet the increasingly stringent quality requirements and hence improved access to prime markets resulting in increased incomes for small scale tomatoes growths and practical application of the extracts is suggested

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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