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NEEM LEAF POWDER REDUCES *RALSTONIA SOLANACEARUM* POPULATION DENSITY, BACTERIAL WILT SEVERITY AND ENHANCES TOMATO FRUIT YIELD

Short communication

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ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* is a major threat to global tomato production. Two field trials were conducted in 2023 (wet season) and 2024 (dry season) at the Federal University of Kashere, Gombe State, to evaluate the use of neem leaf powder in the management of bacterial wilt of tomato. The experiment was conducted using a randomized complete block design with three replications. Neem leaf powder (*Azadirachta indica*) was applied to two tomato cultivars, 'Tandino' and 'Dan Syria', at 15, 30, and 45 g, while untreated (0 g) and streptomycin-treated plots served as negative and positive controls, respectively. Application of streptomycin and 45 g of neem leaf powder 9 weeks after transplanting showed population densities of 1.5 and 1.4 total cfu·g^{-1} soil (2023) and 1.4 and 1.0 cfu·g^{-1} soil (2024), which were significantly lower than the population densities of 8.6 (2023) and 8.0 (2024) cfu·g^{-1} soil recorded in untreated plots. After application of streptomycin and 45 g of neem leaf powder, wilt severity scores of 0.7 and 1.3 (wet season) and 0.7 and 1.0 (dry season) were observed, which was significantly lower than 5.0 in each season in untreated plots. Application of 45 g neem leaf powder resulted in a yield of 432.4 and 437.4 g of fruit per plant, which was significantly higher than the 2.8 and 2.4 g on untreated plots in 2023 and 2024, respectively. It was found that application of 45 g of neem leaf powder can reduce the severity of bacterial wilt in tomatoes and thus increase fruit yield.

Key words: *Azadirachta indica*, *Solanum lycopersicum*, dry season, wet season

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are among the most important vegetable crops in most regions of the world. According to FAOSTAT data (FAO 2018), total production in 2017 was 182 million metric tons from 4.8 million hectares. Growing this highly valued crop in Nigeria supports smallholder farmers who have transitioned from subsistence to commercial agriculture (Hanson et al. 2016).

Pests and diseases in nurseries and in the field are the main factors limiting tomato yields (Prasannath et al. 2014). Among the various tomato diseases, *Ralstonia solanacearum* causes bacterial wilt and is a serious problem in warm regions of the world (Daunay et al. 2010; Huerta et al. 2015). The primary means of transmission of the pathogen are contaminated soil, surface or irrigation water, and tools used by workers, as well as infected seedlings (Champoiseau et al. 2010; Kinyua et al. 2014).

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Due to its extreme persistence in soil, broad host range, and extensive geographic distribution, this pathogen is considered one of the most important phytopathogenic bacteria worldwide (Ray et al. 2024), affecting over 200 plant species from 50 families listed in 80 countries (Mepharishvili et al. 2012; Champoiseau et al. 2010). In tropical and subtropical regions, bacterial wilt ranks second only to late blight in its importance as a limitation to solanaceous crops (Priou 1999; Champoiseau et al. 2010). A combination of preventive, phytosanitary, cultural, chemical, and biological strategies has shown some degree of protection against the pathogen, although no single method is 100% effective in controlling this disease (Champoiseau et al. 2010; EPPO 2014; Kinyua et al. 2014).

To protect the production environment and produce, efforts are being made to use less harmful methods of protection against this pathogen, including plant products known in folk medicine. *Rhazya stricta* is a medicinal shrub found in desert regions. Its dried powder has been investigated for its potential in controlling the disease (Gilani et al. 2007). In addition to increasing host resistance (Pradhanang et al. 2005) and reducing chemicals use (Santos et al. 2006), finely ground *R. stricta* powder, alone or in combination with dried powder from other medicinal weeds (Din et al. 2016; Najeeb et al. 2019), can also serve as an efficient and inexpensive tool for controlling bacterial wilt in tomatoes and potentially other crops. Dried powder from *Conyza canadensis* has been studied *in vitro* and on *ex vitro* grown tomato plants for the control of bacterial wilt. It was found to be effective as a soil amendment by reducing the *R. solanacearum* population in the soil and disease severity, and improving plant growth parameters (Chen et al. 2020). Bacterial wilt of tomato was significantly reduced after soil enrichment with dried medicinal herbs, weed powders, or green manures such as *Withania coagulans*, *W. somnifera*, and *Xanthium strumarium* (Najeeb et al. 2019; Khan et al. 2019, 2020). The invasive, mass-producing weed *Xanthium strumarium* was found to effectively control *R. solanacearum* through its well-known antibacterial activity. Plant growth was significantly improved, bacterial wilt was reduced, and the area under disease progress curve (AUDPC) values were reduced after applying dried *X. strumarium* powder to the soil at different times and rates (Khan et al. 2020). In this study, the effect of neem leaf powder application on *R. solanacearum* population density, bacterial wilt severity, growth and yield of two tomato cultivars was investigated.

MATERIALS AND METHODS

Experimental site

The study was conducted at the Teaching and Research Farm, Crop Production Unit, Faculty of Agriculture, Federal University of Kashere (FUK), Gombe State, during the wet (June to September, 2023) and dry (November 2023 to February 2024) seasons. Kashere is located at an altitude of 431 meters above sea level in the Sudan Savannah Agroecological Zone of Nigeria, and its coordinates are 9°46′0″ N, 10°57′0″ E (Ibrahim et al. 2024). The physicochemical properties of the site were analyzed in the laboratory of the Department of Soil Science, FUK. The soil had a sandy-loam texture, with clay, silt, and sand contents of 14.9%, 36.4%, and 48.7%, respectively. The soil pH was slightly acidic (6.4). The organic carbon content was 0.3%, total nitrogen – 0.2%, available phosphorus – 0.2 $cmol \cdot kg^{-1}$, and the cation exchange capacity (CEC) – 5.8 $cmol \cdot kg^{-1}$. The content of exchangeable bases, such as calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), and sodium (Na^{+}), were 4.3, 0.6, 0.6, and 2.6 $cmol \cdot kg^{-1}$, respectively.

Experimental design

The experiment was conducted using two tomato cultivars: ‘Tandino’ and ‘Dan Syria’, obtained from Kwadon, Yamaltu Deba Local Government, Gombe State. Dried neem leaf powder at 15, 30, and 45 g was applied to the base of each tomato plant (5 cm from the stem), streptomycin was applied at 30 g per plant as a positive control, and an untreated plot (0 g) served as a negative control. The 2 × 5 factorial experiments were conducted in a split-plot design with three replications. Each plot was 3 m × 2 m, with inter- and intra-row spacing of 60 cm and 50 cm, respectively. Each plot consisted of 30 plants, for a total of 90 plants per treatment, with a 0.5 m wide path between plots and replicates.

Seedlings were grown in nursery trays using steam-sterilized sandy loam soil. Three-week-old tomato seedlings were planted in the experimental field on July 15, 2023 (first trial) and December 2, 2023 (second trial). During the dry season, the plots were irrigated daily using a water pump. The pests were controlled foliarly using Cyperforce (cypermethrin 10% EC) at 2-week intervals.

Preparation and application of neem leaf powder

Fresh and healthy leaves of *Azadirachta indica* were obtained from the University grounds. The collected leaves were washed under running water and air-dried at 28±2 °C for 2 weeks. The air-dried plant leaves were ground into a powder using an electric blender and stored in an air-tight paper bag until use. Dried neem leaf powder was applied to the 12 middle plants of the plot at 0, 15, 30, and 45 g in a ring-shaped pattern, 5 cm from each tomato stem, 2 weeks after planting (2 WAP). Subsequent applications were made at 4, 6, and 8 WAP. Similarly, streptomycin, as a positive control, was applied in the same manner as the neem leaf powder.

***Ralstonia solanacearum* population dynamics**

Using a 10 mm diameter cork borer, three soil samples were collected from the rhizosphere of each plant at a depth of 15 cm in each study unit. One week before planting (WBP) of the seedlings, soil samples were collected from the experimental field to monitor initial population density. Subsequent population dynamics were examined at 3, 5, 7, and 9 weeks after planting (WAP). Soil samples from all replicates of the same treatment were mixed to obtain a composite sample (Schönfeld et al. 2003; Grüter et al. 2006; Chen et al. 2020), from which 10 g of soil was collected for 10-fold serial dilution in the laboratory. After dilution, 1 ml of suspension from each tube was inoculated into a Petri dish with tetrazolium chloride and incubated for 48 h, at 28±2 °C (Goszczynska 2000; Popoola et al. 2015). After incubation, bacterial colonies with typical red centers were counted and the number of colony-forming units (cfu) per gram of soil was calculated.

Data collection and analysis

Plant assessment included height (cm), number of leaves, number of flowers, number of branches, and fruit yield (g per plant) at 3, 5, 7, and 9 WAP. Disease incidence was calculated as the percentage of plants showing bacterial wilt symptoms among five sample plants per unit. Disease severity was assessed using a 0–5 rating scale according to Ganiyu et al. (2018), where 0 – 0% plants wilted, 1 – 1–10%, 2 – 10.01–25%, 3 – 25.01–50%, 4 – 50.01–75%, and 5 – 75.01–100%. *R. solanacearum* population dynamics were assessed, and the number of bacteria per g of soil was shown. Data were subjected to analysis of variance (ANOVA) using GenStat Discovery Edition 4, and means separated using the least significant difference at $p \leq 0.05$.

RESULTS AND DISCUSSION

Bacterial population

No significant differences in initial population density were observed between seasons 1 WBP (Fig. 1). At all counting times, bacterial populations did not differ between wet and dry seasons, nor between 15 and 30 g of neem leaf powder. They were halved after application of 45 g of neem leaf powder or streptomycin (7.9 $cfu \cdot g^{-1}$ compared to 3.5 at 3 WAP). Population density in plots treated with 45 g of neem leaf powder and streptomycin gradually decreased over time, reaching 1.0 and 1.4 total $cfu \cdot g^{-1}$ at 9 WAP.

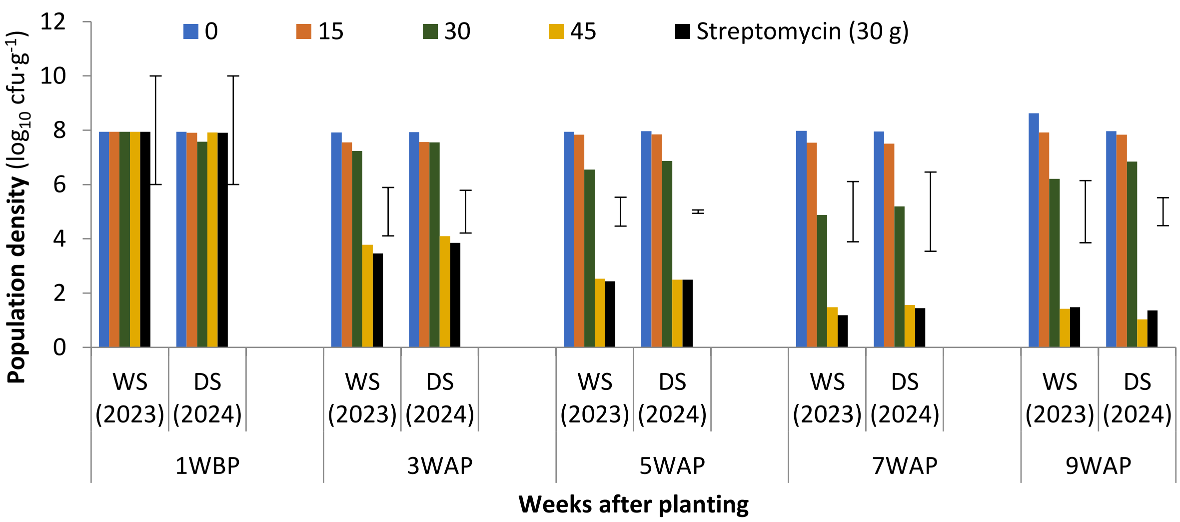

Figure 1. Effect of neem leaf powder application (g per plant) on *R. solanacearum* population dynamics in wet (2023) and dry (2024) seasons

WS – wet season, DS – dry season, WBP – week before planting, WAP – weeks after planting

Plant growth parameters

Application of neem leaf powder or streptomycin did not affect tomato plant height during the wet season, whereas streptomycin reduced growth during the dry season (Table 1). In the dry season, 'Tandino' plants were significantly shorter than 'Dan Syria'. Leaf number and branch number were not affected by neem leaf powder and streptomycin addition, nor by cultivar. Flower number varied between cultivars, being 3 flowers per plant higher for 'Dan Syria' in both seasons. Application of neem leaf powder and streptomycin resulted in a gradual increase in flower number, with neem leaf powder doses being approximately 5 times higher (up to 40 flowers per plant) with 45 g of neem leaf powder or streptomycin.

Disease incidence and fruit yield

The incidence of bacterial wilt between 'Tandino' and 'Dan Syria' did not differ between the wet and dry seasons (Table 2). Application of neem leaf powder resulted in a gradual decrease in bacterial wilt incidence from 100% in the control to 13.3% and 8.3% in the wet and dry seasons, respectively. Diseased plants in the streptomycin-treated group were 6.7% and 7.5% in the wet and dry seasons, respectively. The cultivars did not differ in wilt severity between the wet and dry seasons. Application of neem leaf powder gradually reduced the wilt severity from 5 in the control to 1.3 and 1.0 at 45 g, while application of streptomycin reduced the wilt severity to 0.7. Both cultivars responded similarly in the control group and neem leaf powder-treated groups. During the wet season, fruit yields of 432.4 and 412.2 g per plant recorded in plots treated with 45 g of neem leaf powder or streptomycin, respectively, were significantly higher than the 2.8, 22.0, and 62.6 g per plant recorded in plots treated with 0, 15, and 30 g of neem leaf powder, respectively. Similarly, during the dry season, fruit yield of 2.4, 22.4, and 21.6 g per plant documented in plots with 0, 15, and 30 g neem leaf powder application, were significantly lower than 427.2 and 437.4 g per plant in plots treated with streptomycin or 45 g neem leaf powder, respectively.

The plant powder was not phytotoxic and did not affect tomato growth or development. Application of 45 g of neem leaf powder significantly reduced the incidence and severity of the disease, with an effect similar to streptomycin. According to Najeeb et al. (2019) and Khan et al. (2019), bacterial wilt disease of tomato plants was significantly reduced when the soil was amended with dried powders of various medicinal plants. Researchers investigated dried powder from the medicinal shrub *Rhazya stricta* in arid regions to control bacterial wilt of tomato and found that the plant contains a unique set of secondary metabolites with a wide range of biological activities against microorganisms (Ali et al. 2000; Gilani et al. 2007; Albeshri et al. 2021). Tannins and terpenoids in the plant powder could kill bacteria by directly destroying their cell membranes, as confirmed by Mainasara et al. (2012). Similarly, the neem leaf powder used in this study effectively inhibited bacterial wilt of tomato, suggesting that the inhibition was due to secondary metabolites. Tomato fruit yield was noticeably higher after application of 45 g of neem leaf powder. This result is consistent with Paris et al. (2012), who found that microbial properties are attributed to the synergy of active compounds present in tree biomass, which naturally provide better pathogen control, promoting root and vegetative growth and increasing plant yield.

Table 1. Effect of cultivar and neem leaf powder (g) on tomato growth parameters

	Plant height (cm)		Number of leaves per plant		Number of branches per plant		Number of flowers per plant	
Cultivar	WS 2023	DS 2024	WS 2023	DS 2024	WS 2023	DS 2024	WS 2023	DS 2024
'Tandino'	50.0	48.4	45.2	54.8	6.5	7.1	18.8	21.4
'Dan Syria'	55.5	51.1	56.0	57.3	7.1	8.0	24.2	25.9
LSD(0.05)	ns	2.3	ns	ns	ns	ns	4.8	3.0
Neem leaf powder (g per plant)								
0	55.4	50.0	55.6	55.9	7.8	6.8	8.9	8.0
15	54.9	52.2	56.9	60.3	7.4	7.4	14.0	12.8
30	45.8	46.4	40.6	53.8	5.3	7.8	17.9	19.8
45	56.4	50.9	53.6	57.3	7.0	8.3	35.2	38.1
Streptomycin (30 g per plant)	51.5	49.4	46.3	53.1	6.3	7.7	40.4	39.4
LSD(0.05)	ns	3.6	ns	ns	ns	ns	7.6	4.8
Interaction C × D	ns	ns	ns	ns	ns	ns	ns	ns

WS – wet season; DS – dry season; ns – not significant

Table 2. Effect of cultivar and neem leaf powder (g) on the incidence of bacterial wilt of tomato during the wet and dry seasons of 2023 and 2024

	Incidence of bacterial wilt (%)		Severity of bacterial wilt*		Fruit yield (g per plant)	
Cultivar	WS 2023	DS 2024	WS 2023	DS 2024	WS 2023	DS 2024
'Tandino'	49.7	46.7	2.6	2.5	173.6	180.2
'Dan Syria'	50.7	44.7	2.0	2.1	199.2	200.2
$LSD_{(0.05)}$	ns	ns	ns	0.4	10.9	10.4
Neem leaf powder (g per plant)						
0	100.0	100.0	5.0	5.0	2.8	2.4
15	77.5	65.0	3.3	3.2	22.0	22.4
30	53.3	47.5	1.7	1.5	62.6	21.6
45	13.3	8.3	1.3	1.0	432.4	437.4
Streptomycin (30 g per plant)	6.7	7.5	0.7	0.7	412.2	427.2
$LSD_{(0.05)}$	11.1	9.5	0.8	0.6	30.1	20.3
Interaction C × D	ns	ns	*	*	ns	ns

*Severity scale (0–5): 0 – 0%, 1 – 1–10%, 2 – 10.01–25%, 3 – 25.01–50%, 4 – 50.01–75%, and 5 – 75.01–100% of wilted plants; WS – wet season; DS – dry season; ns – not significant

CONCLUSION

Neem leaf powder, applied at a dose of 45 g after transplanting tomato seedlings into the field, has been shown to accelerate tomato growth and fruit yield. Neem leaf powder reduced the *Ralstonia solanacearum* population in the soil and the incidence of tomato disease. Therefore, this natural leaf powder can prevent bacterial wilt of tomato, which means it is environmentally beneficial and can be used as a cost-effective disease control method. It can even replace synthetic bactericides, which are harmful to both humans and the environment. Before using it on a production scale, experimentation with different tomato cultivars is necessary, as well as ruling out any changes in flavor or deterioration of the fruit. Furthermore, the stability of the powder in the soil and the ease of use and application of the product formula should also be assessed.

Author contribution statement

SG – A – Research concept and design, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article
BY – F – Final approval of the article
SA – F – Final approval of the article
EA – B – Collection and/or assembly of data, D – Writing the article
IA – B – Collection and/or assembly of data, D – Writing the article
MD – F – Final approval of the article
MA – F – Final approval of the article
AP – F – Final approval of the article

Ethical statement

No human or animal was used in this study.

Conflicts of interest

The authors declare that there is no conflict of interest in this study.

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