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Incidence and Disease Severity of Bacterial Wilt in *Solanum scabrum* and Pathogen Identification in the Buea Municipality, Cameroon

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ABSTRACT

Solanum scabrum Mill., commonly known as African nightshade, is an important indigenous vegetable in sub-Saharan Africa, valued for its nutritional and socio-economic benefits. However, its production in the Buea Municipality, Cameroon, is threatened by bacterial wilt caused by *Ralstonia solanacearum*, alongside other diseases such as Verticillium wilt and Tomato Yellow Leaf Curl Virus (TYLCV). This study aimed to assess the incidence and severity of bacterial wilt and other diseases affecting *S. scabrum* in Buea Municipality, identify the bacterial pathogen using cultural characteristics and evaluate the effectiveness of common management practices employed by farmers. A cross-sectional survey was conducted across five major production sites; Bolifamba, Bwitingi, Koke, Molyko, and Muea between November to December, 2023. Disease incidence and severity were assessed using quadrat sampling. Symptomatic plants were collected for pathogen isolation and characterization through cultural, morphological, and biochemical methods. A pathogenicity test was conducted to confirm the causal organism. The efficacy of selected management strategies, including the application of Mancozeb, ginger extract, black pepper extract, and wood ash, was tested under field conditions. Bacterial wilt recorded the highest incidence (32.48%) and severity (1.68%) across the study area, with Koke and Bwitingi being the most affected sites. Verticillium wilt and Tomato yellow leaf curl virus disease (TYLCV) had lower incidences of 4.68% and 6.14%, respectively. Cultural identification confirmed *Ralstonia solanacearum* as the causal agent of bacterial wilt based on colony morphology, gram-negative reaction, and pathogenicity tests. Field trials indicated that Mancozeb significantly reduced disease incidence (41.3%) and severity (13.6%) compared to other treatments. Wood ash was the least effective, resulting in the highest disease incidence (91.5%) and severity (54.5%). Bacterial wilt, caused by *R. solanacearum*, poses a significant threat to *S. scabrum* production in Buea Municipality. Although some management practices are employed, their efficacy remains limited. Integrated disease management approaches, incorporating resistant varieties, improved cultural practices, and appropriate biocontrol methods, are recommended for sustainable control of bacterial wilt in *S. scabrum*.

Keywords: *Solanum scabrum,* Disease, Incidence, Severity, Bacterial wilt, *Ralstonia solanacearum,* Management

1. INTRODUCTION

African nightshade (*Solanum scabrum* Mill.) is an important indigenous leafy vegetable widely cultivated and consumed in many

parts of sub-Saharan Africa, including Cameroon (Schippers, 2002). It is valued not only for its nutritional benefits, being rich in vitamins, minerals, and antioxidants but also for its socio-economic importance, especially among smallholder farmers and urban dwellers who rely on its production for income generation and household food security (Abukutsa-Onyango, 2007; Akutse et al., 2020). Despite its importance, the production of *S. scabrum* is hampered by several biotic constraints, of which bacterial wilt is among the most destructive (Nono-Womdim et al., 2002).

Bacterial wilt, primarily caused by *Ralstonia solanacearum*, is a soil-borne vascular disease that affects over 200 plant species worldwide, particularly within the Solanaceae (Hayward, 1991; Genin & Denny, 2012). The bacterial has been reported to infect solanacous crop in tropical Africa, including tomato (Bamazi et al., 2022). Infected plants typically exhibit rapid wilting, leaf yellowing, stunted growth, and eventual death (Schell, 2000). The disease has been reported to cause significant yield losses, estimated between 30% and 90% in susceptible crops under favorable conditions (Elphinstone, 2005; Prior et al., 2016). In Cameroon, bacterial wilt poses a serious threat to the cultivation of solanaceous crops, including *Solanum lycopersicum*, *Solanum melongena,* and *African nightshade Solanum scabrum* (Fontem, 2001).

The causal agent, *R. solanacearum*, is a highly heterogeneous species complex with wide host range, diverse pathogenicity, and high genetic variability (Fegan & Prior, 2005). Although molecular techniques such as PCR and sequencing are now widely used to confirm pathogen identity and diversity, (Opina et al., 1997), cultural and morphological methods remain relevant and accessible tools for pathogen isolation and preliminary identification, particularly in resource-limited settings (Schaad et al., 2001; Elphinstone et al., 1996).

In Cameroon, and in Buea municipality in particular, there is limited documentation on the incidence and severity of bacterial wilt in *S. scabrum*, despite anecdotal reports of increasing disease prevalence in major production zones in Buea Municipality.

Understanding the incidence and severity of bacterial wilt in *S. scabrum* production areas is essential for developing effective management strategies. Additionally, documenting the cultural and morphological characteristics of the causal agent in local contexts is necessary for guiding future research and extension efforts. This study therefore sought to assess the incidence and severity of bacterial wilt affecting *S. scabrum* in selected farming areas of Buea Municipality; isolate and culturally identify the bacterial pathogen associated with the disease, using cultural characteristics.

2. MATERIALS & METHODS

2.1 Study Area

The study was conducted in selected farming communities within the Buea Municipality, located in the Fako Division of the Southwest Region of Cameroon. Buea lies between latitudes 4°09' and 4°20'N and longitudes 9°12' and 9°20'E, at an altitude ranging from 870 to 1,200 meters above sea level. The area experiences a humid tropical climate with an annual rainfall of approximately 3,000 mm and average temperatures ranging from 18°C to 27°C. Agriculture, particularly vegetable farming, is a major livelihood activity in the municipality.

2.2 Field Survey Assessment

A field survey was carried out between November and December, 2023 in five major *Solanum scabrum* production sites: Bolifamba, Bwitingi, Koke, Molyko, and Muea. In each site, five farms were randomly selected for evaluation, giving a total of 25 farms. Field inspections were conducted to assess disease incidence and severity and composites samples were collected from each field for culturing.

2.3 Disease Incidence and Severity

Disease incidence was determined using a 10 m x 10 m quadrat method. Ten quadrats were randomly laid per farm. Within each quadrat,

the number of infected plants showing characteristic bacterial wilt symptoms (wilting, leaf yellowing, stunted growth) were counted and this was expressed as a percentage of the total number of plants assessed:

$$\text{Disease incidence (\%)} = \left(\frac{\text{No. of infected plants}}{\text{Total number of plants assessed}}\right) \times 100 \quad (1)$$

Disease severity was rated using a visual disease rating scale modified from Winstead and Kelman (1952) (Table 1). Following this scale, a severity score was assigned to each infected plant. The mean severity index was calculated across quadrats, using the formular on equation (2) (Chiang et al., 2017).

Table 1: Disease severity rating scale

Scale	Symptoms Description
0	No symptoms observed
1	1–10% leaf area affected
2	11–25% leaf area affected
3	26–50% leaf area affected
4	51–75% leaf area affected
5	More than 75% of the plant affected or dead

The disease severity index (DSI) was calculated using the following formula.

$$\text{Disease Severity Index (DSI)(\%)} = \frac{\sum(\text{Rating} \times \text{Number of plants in that rating})}{\text{Total number of plants} \times \text{Maximum rating}} \times 100 \quad (2)$$

2.4 Isolation and cultural identification of the pathogen

2.4.1 Sample collection

Symptomatic plants were uprooted, the lower stem and roots were cleaned, collected in Ziplock bags in a cooler containing ice and transported to the Life Science Laboratory of the Faculty of Science, University of Buea for bacteria isolation. The bacterial pathogen was done following standard bacteriological procedures (Basu et al., 2015).

2.4.2 Isolation of the bacterial pathogen

The isolation was done following the protocol described in streak plate protocol (Katz, 2008), in which Stem sections (1-2 cm) were surface-sterilized in 70% ethanol for 30 seconds, rinsed twice in sterile distilled water, and macerated in sterile water. A loopful of the suspension was streaked onto nutrient glucose agar (NGA) (Basu et al., 2015). Plates were incubated at 28°C for 48–72 hours. Colonies were examined for characteristic features such as smooth, glistening, and whitish colonies.

2.5 Gram Staining and Pathogenicity Test

Bacterial isolates were subjected to Gram staining (Ansar et al., 2023). Microscopic examination was done using a light microscope. Samples were placed on a clean slide, spread over an area of 1cm circumference and a cover slip place on the inoculated area and observed under a light microscope at 40X magnification.

To confirm the pathogenicity of the isolated bacteria, healthy *S. scabrum* seedlings (4–6 weeks old) were inoculated by growing them in soil drenching with a bacterial suspension (~10^8 CFU/mL) (Koch, 2016). Control plants were treated with sterile distilled water. Plants were observed for symptom development for over a two-week period. Re-isolation was done from symptomatic plants and compared to the original isolates (Koch, 2016).

2.6 STATISTICAL ANALYSIS

Quantitative data on disease incidence and severity were subjected to descriptive statistics (means, percentages). Analysis of variance (ANOVA) was used to compare disease incidence and severity across locations using SPSS version 23 software. A significance level of $p < 0.05$ was used for all statistical tests.

3. RESULT

3.1 Incidence and severity of common diseases of *S. scabrum* in the Buea municipality

Various disease symptoms were observed in the field. These included (a) Bacteria wilt disease (b) Tomato yellow leaf curl virus (TYLCV) disease and (c) Verticillium wilt symptoms (Figure 1).

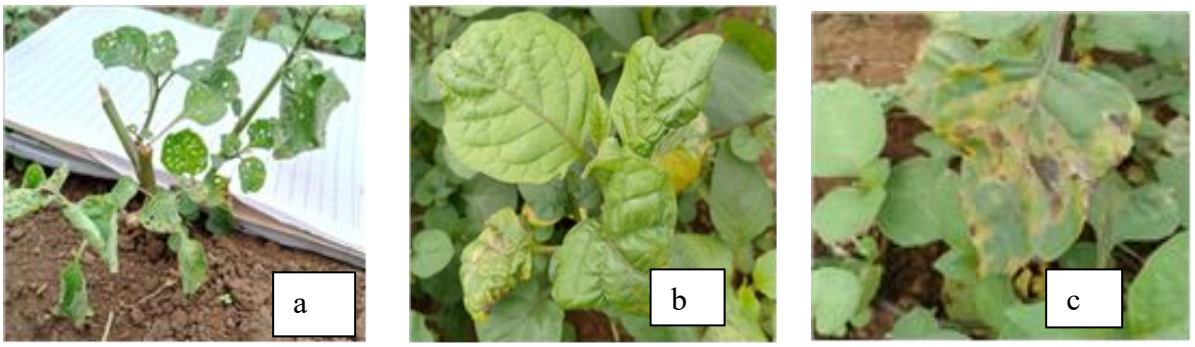

Figure 1: Symptoms of: (a) Bacteria wilt disease (b) Tomato yellow leaf curl virus disease and (c) Verticillium wilt disease as observed in the field.

The incidence and severity of diseases varied across the study sites, with notable differences in fungal (Verticillium wilt), bacterial (bacterial wilt), and viral (Tomato Yellow Leaf Curl Virus (TYLCV) diseases. There was no significant difference (p=0.092) in viral disease incidence across sites, while significant differences were recorded for fungal disease (p=0.042) and bacterial disease (p=0.002) across sites (T able 2). Verticillium wilt had the highest incidence in Muea (8.2%) and the lowest in Molyko (3.2%), while bacterial wilt was most prevalent in Koke (50%) and least in Molyko (17.35%) (Table 2).

Table 2: Variation of disease incidence across study sites

Study site	Disease incidence (%)		
	TYLCV disease	Verticillium wilt disease	Bacterial wilt disease
Bolifamba	6.25 ± 6.01^{a}	5.00 ± 2.83^{ab}	25.00 ± 0.00^{b}
Bwitingi	10.50 ± 0.71^{a}	6.75 ± 0.35^{ab}	24.50 ± 0.71^{b}
Koke	6.75 ± 2.47^{a}	4.95 ± 0.07^{ab}	50.00 ± 0.00^{a}
Molyko	6.35 ± 0.35^{a}	3.20 ± 1.70^{ab}	17.35 ± 12.23^{b}
Muea	7.00 ± 0.00^{a}	8.20 ± 3.11^{a}	26.50 ± 0.71^{b}
P-value	0.092^{ns}	0.042*	0.002**

Disease severity also varied significantly, with the highest severity of TYLCV recorded in Bwitingi (2.1%) and the lowest in Molyko (1.49%). The most severe cases of Verticillium wilt were recorded in Molyko (2.05%), while bacterial wilt severity peaked in Bwitingi (2.25%) and was lowest in Bolifamba (1.49%) (Table 3).

Table 3: Variation of severity across study sites

Study site	Disease severity (%)		
	TYLCV Disesase	Verticillium wilt disease	Bacterial wilt disease
Bolifamba	1.55 ± 0.07^{ab}	1.40 ± 0.14^{a}	1.49 ± 0.30^{b}
Bwitingi	2.10 ± 0.14^{a}	1.55 ± 0.07^{a}	2.25 ± 0.07^{a}
Koke	1.60 ± 0.28^{ab}	1.60 ± 0.28^{a}	1.90 ± 0.14^{ab}
Molyko	1.49 ± 0.12^{ab}	2.05 ± 0.35^{a}	1.70 ± 0.14^{ab}
Muea	1.60 ± 0.14^{ab}	1.60 ± 0.00^{a}	1.95 ± 0.07^{ab}
P-value	<0.001***	<0.001***	<0.001***

Bacterial wilt recorded the highest incidence (32.48%), followed by TYLCV (6.14%) and Verticillium wilt (4.68%). However, the severity of these diseases did not vary significantly across the types (Table 4).

Table 4: Variation of disease incidence and severity across disease type

Disease	Incidence (%)	Severity (%)
Bacterial wilt	32.48 ± 14.34^{a}	1.68 ± 0.50^{a}
Verticulum wilt	4.68 ± 3.05^{b}	1.37 ± 0.69^{a}
TYLCV	6.14 ± 3.80^{b}	1.39 ± 0.69^{a}
P-value	<0.001***	0.4^{ns}

3.2 Colony Characteristics of *Ralstonia solanacearum* on Culture Media

Characteristic colonies of *Ralstonia solanacearum* were observed after 24 hours of inoculation. Colonies grew uniformly across the plates, with some showing a pinkish, creamy white to light yellow colour and a slightly raised smooth surface. Sub-culturing of the colonies resulted in the growth of pure cultures, which were circular, creamy white, slightly opaque, and exhibited a smooth, shiny appearance (Figure 2).

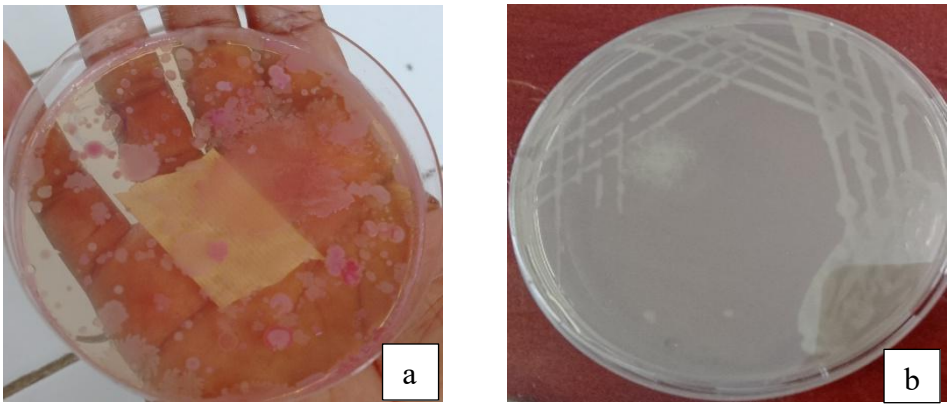

Figure 2: Culture plates showing pinkish to creamy white coloration of bacterial colonies: a) before subculturing and b) pure culture after subculturing

Gram staining test and microscopic observation revealed characteristics consistent with *Ralstonia solanacearum*. Gram staining revealed the bacterium's gram-negative nature, by not retaining the crystal violet stain, but retained the safranin counterstain, indicating a thin peptidoglycan layer in the cell wall (Figure 3). Microscopically, the bacteria appeared as motile, non-sporulating, rod-shaped cells with one or more polar flagella, displaying a distinct pinkish-red pigmentation due to the production of ralsolamycin.

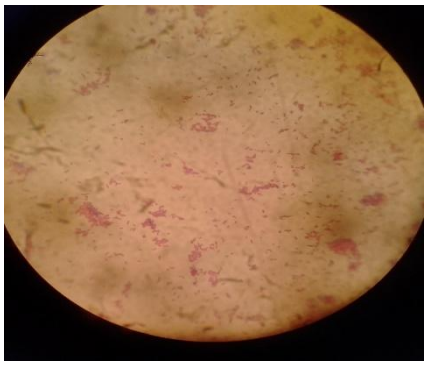

Figure 3: Micrograph of rod-shaped bacterial cells with safranin counterstain.

3.3 Pathogenicity Test

Inoculated healthy *S. scabrum* plants developed wilting symptoms 7–10 days after inoculation. The symptoms included drooping of leaves, yellowing, and eventual wilting of the entire plant, consistent with

bacterial wilt. No symptoms were observed in control plants. Re-isolation from symptomatic plants yielded bacterial colonies similar in morphology and characteristics to the original isolates, thereby fulfilling Koch's postulates (Figure 4).

Figure 4: *Solanum scabrum* plants used for pathogenicity test: a) asymptomatic control plants; b) symptomatic inoculated plants

4. DISCUSSION

4.1 Incidence and Severity of Bacterial Wilt and Other Disease

This study reveals the high prevalence of the disease on *Solanum scabrum*. The disease was observed in all the villages under survey. Bacterial wilt was identified as the most prevalent and severe disease affecting *S. scabrum* in the study area, with an overall incidence of 32.48% and severity of 1.68%. These findings confirm previous reports that bacterial wilt is a major constraint in Solanaceous crops in Cameroon and other tropical regions (Fontem, 2001; Elphinstone, 2005; Bamazi et al., 2022). The incidence of these diseases can be due to contamination of the soils in these study localities. Most of the chemical nutrient added on to the soil do not respect manufactural instructions. This high dose of soil argumentation can be favourable for the pathogen to survive. The disease was most prevalent in Koke and Bwitingi, areas characterized by favorable conditions for *R. solanacearum*, such as high soil moisture and temperatures conducive to pathogen proliferation (Hayward, 1991; Yuliar et al., 2015).

Verticillium wilt and Tomato Yellow Leaf Curl Virus (TYLCV) were also detected, though at significantly lower incidences. The presence of these diseases indicates a complex disease environment that complicates management practices. Notably, TYLCV, previously reported primarily in tomato crops, has increasingly been found to affect other Solanaceous vegetables, posing new threats to production (Czosnek & Ghanim, 2002).

4.2 Identification and Characterization of the Bacterial Pathogen

The isolation and cultural characterization of the bacterial pathogen revealed colonies typical of *Ralstonia solanacearum*. The colonies displayed fluidal, irregular margins with creamy white to pinkish coloration on NGA media, consistent with descriptions by Maji and Chakrabartty (2014) using TZC agar medium with tomato plant. Similar results were reported by Elphinstone (2005). Gram staining confirmed its gram-negative as also reported by Dinesh and Amit (2021), rod-shaped morphology, and pathogenicity tests reproduced typical wilt symptoms, fulfilling Koch's postulates. These results corroborate previous studies identifying *R. solanacearum* as the principal causal agent of bacterial wilt in Solanaceous crops in Cameroon (Fegan & Prior, 2005; Prior et al., 2016).

The absence of effective control measures, particularly integrated disease management (IDM) approaches, likely contributed to the persistent high incidence of bacterial wilt in the region. Similar constraints have been reported in smallholder farming systems where limited access to resistant germplasm

and lack of disease management knowledge prevail (Saddler, 2005).

These results highlight the need for integrated approaches combining cultural, biological, and chemical strategies to manage bacterial wilt effectively. Promoting the use of resistant varieties, improving crop rotation practices, and enhancing farmer knowledge through extension services are critical recommendations for sustainable management (Yuliar et al., 2015; Huet, 2014).

CONCLUSION

This study highlights bacterial wilt, caused by *R. solanacearum*, as a major disease of *S. scabrum* in the Buea Municipality, significantly affecting productivity. Although farmers employ various management practices, their efficacy remains limited. The findings underscore the need for integrated disease management strategies that combine cultural practices, the use of resistant varieties, and possibly biological control methods. Further research, including molecular characterization of pathogen strains and screening of resistant *S. scabrum* varieties, is recommended to inform sustainable disease management in the region.

Declaration by Authors

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Conflict of Interest: There is no conflict of interest among the authors.

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