

Green solutions for bacterial wilt: Harnessing botanicals and soil amendments in tomato production

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Abstract

This study evaluated the efficacy of botanical extracts and soil amendments in managing bacterial wilt caused by *Ralstonia solanacearum* in tomato production. *Ralstonia solanacearum* samples were isolated from infected tomato samples and characterized morphologically and biochemically. Ten invasive weed species were screened for antimicrobial activity against five *Ralstonia solanacearum* isolates. *Chromolaena odorata* and *Cynodon dactylon* extracts exhibited potent antimicrobial properties, with inhibition ranges of 6-14 mm on nutrient agar plates and a Minimum Inhibitory Concentration (MIC) of 250 µl/ml. Field trials with soil amendments enriched with selected botanicals resulted in significant improvements in plant growth parameters, including a 15% increase in plant height and a 20% increase in healthy leaves per plant. Disease severity was reduced by 70% compared to the control group. Soil analysis revealed a 25% increase in organic matter content and improved nutrient availability in the amended soil. Pathogen incidence on tomato plants decreased by 60% in the amended soil. Multiplex PCR identified four *Ralstonia solanacearum* phylotypes, with phylotype I and IV widely distributed, while phylotype II and III were predominant in specific regions. These findings contribute to the development of sustainable management strategies for bacterial wilt, promoting environmentally friendly practices in tomato production, and enhancing global food security.

Key words: Bacterial wilt, Botanical extracts, Cameroon, *Chromolaena odorata*, *Cyanodon dactylon*, *Ralstonia solanacearum*

Résumé

Cette étude a évalué l'efficacité des extraits botaniques et des amendements du sol dans la gestion du flétrissement bactérien causé par *Ralstonia solanacearum* dans la production de tomates. Des échantillons de *Ralstonia solanacearum* ont été isolés à partir d'échantillons de tomates infectées et caractérisés morphologiquement et biochimiquement. Dix espèces de mauvaises herbes envahissantes ont été testées pour leur activité antimicrobienne contre cinq isolats de *Ralstonia solanacearum*. Les extraits de *Chromolaena odorata* et de *Cynodon dactylon* ont montré des propriétés antimicrobiennes puissantes, avec des plages d'inhibition de 6 à 14 mm sur des plaques de gélose nutritive et une concentration minimale inhibitrice (CMI) de 250 µl/ml. Les essais sur le terrain avec des amendements du sol enrichis en extraits botaniques sélectionnés ont conduit à des améliorations significatives des paramètres de croissance des plantes, notamment une augmentation de 15 % de la

hauteur des plantes et de 20 % du nombre de feuilles saines par plante. La gravité de la maladie a été réduite de 70 % par rapport au groupe témoin. L'analyse du sol a révélé une augmentation de 25 % de la teneur en matière organique et une meilleure disponibilité des nutriments dans le sol amendé. L'incidence de l'agent pathogène sur les plants de tomates a diminué de 60 % dans le sol amendé. La PCR multiplex a identifié quatre phylotypes de *Ralstonia solanacearum*, les phylotypes I et IV étant largement distribués, tandis que les phylotypes II et III étaient prédominants dans des régions spécifiques. Ces résultats contribuent au développement de stratégies de gestion durable du flétrissement bactérien, en promouvant des pratiques respectueuses de l'environnement dans la production de tomates et en renforçant la sécurité alimentaire mondiale.

Mots-clés : Flétrissement bactérien, Extraits botaniques, Cameroun, *Chromolaena odorata*, *Cynodon dactylon*, *Ralstonia solanacearum*

Introduction

Tomato production in Buea, Cameroon, encounters numerous challenges that impede its productivity and profitability. These challenges include low input usage, soil degradation, inadequate agricultural research and extension, and market development constraints (Jones, 2019). Furthermore, bacterial diseases, particularly *Ralstonia solanacearum*, pose a significant threat to tomato crops globally (Gomez *et al.*, 2020). This pathogen exhibits aggressive characteristics, spreads rapidly through the soil, and causes severe damage to infected crops (Mansfield *et al.*, 2012). Consequently, the need arises to address these challenges and develop effective strategies for sustainable tomato production. Chemical control measures are unsustainable, and limited information exists on effective management strategies for bacterial wilt in this region (Nweke, 2017). The pathogen's pathogenicity features, including its long survival in soil and ability to infect a wide range of host plants, do not only lead to poor yields and food insecurity in Buea but complicate control measures such as crop rotation and diversification (Tans-Kersten *et al.*, 2001; Elphinstone, 2005). To combat bacterial wilt and other tomato-related diseases effectively, a holistic approach that considers the complex interactions between plants, pathogens, and the environment is necessary (Smith *et al.*, 2018). This approach integrates various strategies, such as disease-resistant cultivars, cultural practices, and biological control agents, to achieve sustainable and resilient tomato production (Jones, 2019). The addition of organic matter and botanicals improves soil fertility, enhances nutrient availability, and suppresses soil-borne pathogens (Liu *et al.*, 2019). These practices contribute to robust plant growth and resilience against bacterial wilt and other diseases. This study addresses the need for sustainable management strategies for bacterial wilt in tomato production by investigating the efficacy of botanical extracts (Sikirou *et al.*, 2018; Garcia *et al.*, 2019) and soil amendments (Sikirou *et al.*, 2018) to mitigate *Ralstonia solanacearum*. The findings will contribute to developing green solutions for bacterial wilt and promoting environmentally friendly practices in agriculture.

Literature Summary

Bacterial wilt, caused by *Ralstonia solanacearum*, is a devastating disease that poses significant economic losses to tomato production globally (Paudel *et al.*, 2020a). Due to concerns about the adverse effects of synthetic pesticides and chemical fertilizers on human health and the environment, alternative management strategies have been sought (OEPP, 2018a; Mamphogoro, 2020; Paudel *et al.*, 2020a, 2020b). The utilization of botanicals, which are plant-derived products containing bioactive compounds with antimicrobial and plant growth-promoting properties is promising approach (Kumar *et al.*, 2017). The antimicrobial, the reaction mechanisms, and plant growth-promoting

properties of botanicals and their suitability for combating bacterial wilt were reviewed. The application of soil amendments, which enhance the physical, chemical, and biological properties of soil were examined (Sakr, 2016). Various types of soil amendments are examined, along with their impact on soil health, tomato plant growth, and yield (Chen *et al.*, 2020; Ji *et al.*, 2020). The effectiveness of specific soil amendments in managing bacterial wilt disease is also evaluated. The biology and epidemiology of *Ralstonia solanacearum*, including its morphology, life cycle, and modes of transmission, with emphasis on the genetic diversity of the pathogen, which contributes to its ability to infect a wide range of plant hosts and adapt to different environmental conditions were reviewed (Genin and Denny, 2012). The taxonomy and advancements in studying the genetic traits of *Ralstonia solanacearum* are also discussed. Over time, the classification of the pathogen has evolved, with milestones such as the proposal of phylotypes, biovars, sequevars, ecotypes, and races (Prior *et al.*, 2016). The literature review concluded by identifying the gaps in the existing knowledge base and highlights on the potential for future research in this area (Liu *et al.*, 2021). The need for further exploration of botanicals and soil amendments as sustainable management strategies for bacterial wilt in tomato production is emphasized and forms the basis of this study.

Research Approach

The identification and characterization of *Ralstonia solanacearum* (RS) phylotypes responsible for Bacterial Wilt (BW) of tomatoes in Buea was a critical step in understanding the disease and developing effective control measures. A synoptic summary of the materials and methods used to achieve this objective, included sample collection, isolation, pathogenicity testing, biochemical tests, DNA extraction with a Fungal/Bacterial DNA extraction KIT, and Conventional Multiplex PCR.

To identify botanicals with sensitivity to the RS-isolates, 10 invasive weed species were screened. The plant materials were collected, pooled together, weighed, washed, dried, ground into fine powder, centrifuged, the supernatant transferred into sterile vials stored under 4 °C, and the residues discarded. The efficacy of the botanicals in mitigating BW of tomatoes in vitro was determined by measuring the isolates sensitivity to the extracts, the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) by the agar well diffusion/cavity-plating method, Muller Hinton Broth (MHB), and Muller Hinton Agar (MHA), respectively. The phytochemical analyses of the plants' extract were conducted using standard methods optimized to determine the presence of major antioxidant secondary metabolite classes, including saponins, alkaloids, flavonoids, tannins, phenolics, oxalates, phlobatamins, and terpenoids (Iqbal *et al.*, 2015) and compared with the amounts accumulated in the tomatoes grown under these botanical amendments. The resulting data were subjected to descriptive statistics, correlations, PCA, and ANOVA using MINITAB version 23.0

The effects of soil amendments with selected botanicals in the mitigation of RS-wilt of cobra fl in situ was achieved by considering several aspects. These included soil analysis to determine the soil nutrient status and identify any nutrient deficiency, screen house preparation to control insects and other pests (vectors of the disease), soil amendment preparation and application to complement any absent nutrients and to reduce the RS-wilt. There was the measurement of growth, yield parameters, and disease severity to evaluate the efficacy of the soil amendments and plant extracts.

Results

Figure 1 shows the bacterial wilt symptom and the morphological characteristics of the pathogen.

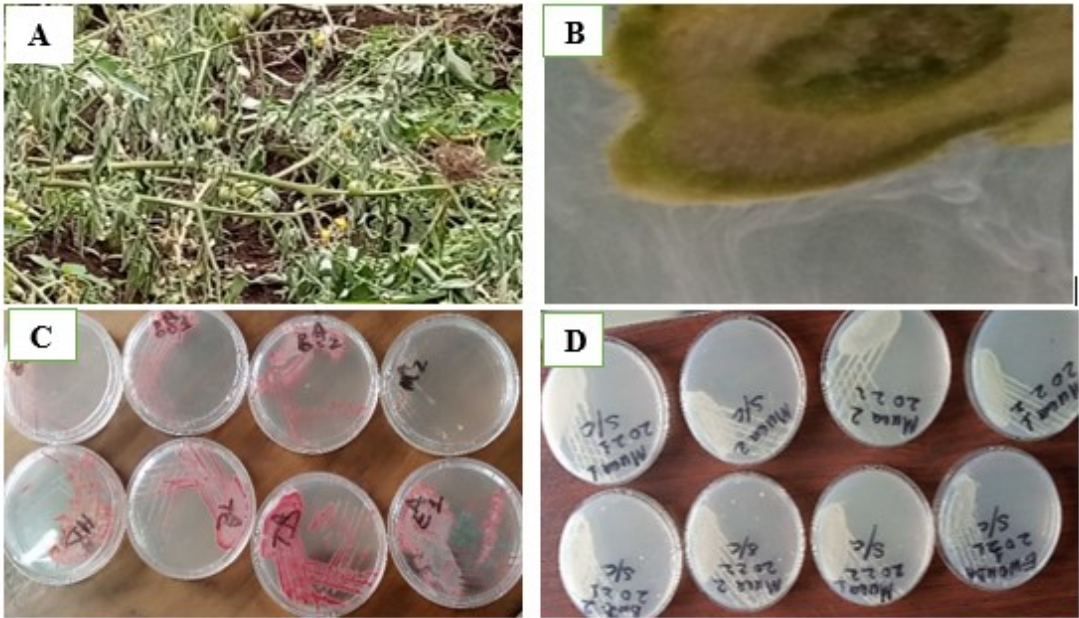


Figure 1. The tomato bacterial wilt syndrome and the morphological characteristics of *Ralstonia solanacearum*. A = wilting tomatoes affected by *R. solanacearum* (RS), B = stem streaming field test, C = RS colonies on Triphenyl tetrazolium chloride (TZC) plates and semi-selective growth medium, D = RS colonies on Nutrient agar plates. The biochemical tests conducted indicated that the symptoms were those of bacterial wilt caused by *Ralstonia solanacearum* (Table 1).

Table 1. The responses of the *Ralstonia solanacearum* isolates to the different tests

S/N	Name of test	Reaction	Appearance	Remarks
1	Stem streaming test	Positive	Milky white ooze	Bacteria involved is presumptively RS.
2	Growth on hydrated culture medium (Nutrient agar)	Positive	pearly cream-white, flat, irregular, and fluidal colonies	Virulent bacteria showed pearly cream-white, flat, irregular, and fluidal colonies.
3	KIA	Positive	Yellow slant/butt	Bacteria yields yellow slants confirming glucose and lactose fermenting.
4	Gram staining	Negative	Small rod-shaped cells pink in colour.	Gram staining showed gram negative bacteria
5	Pathogenicity test	Positive	General wilting starting with young leaves.	Bacteria isolates produced typical symptoms of BW.

After isolation, morphological, and biochemical characterisation Multiplex PCR was used for the molecular detection of different *R. solanacearum* phylotypes using primers targeting the *IpxC* gene and the (Internal Transcribed Spacer) ITS intergenic region between the 16S and 23S rRNA genes (Figure 2).

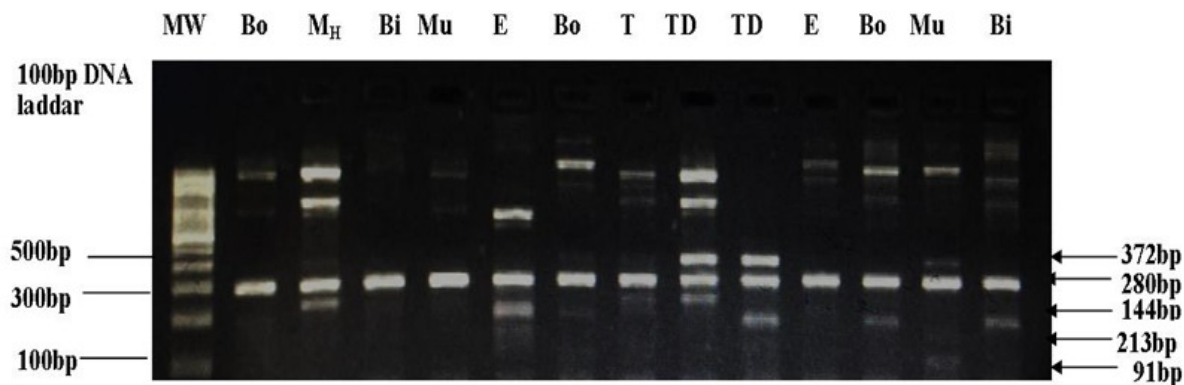


Figure 2. Profile of 2% gel showing different *Rastonia solanacearum* phylotypes. MW = Molecular Weight marker, Bo = Boduma, Mu = Muea, Bi = Bitingi, E= Ewunda, and T or TD = Tiko.

Table 2. Distribution of *Ralstonia Solanacearum* phylotypes and their associated names in different localities

Phylotypes	Names	Localities				
		Boduma	M _{uea}	Ewunda	Tiko	Bitingi
I	<i>R. pseudosolanacearum</i>	+	+	+		+
II	<i>R. solanacearum</i>		+			
III	<i>R. pseudosolanacearum</i>		+			
IV	<i>R. syzygii</i>	+	+	+	+	

All the four known phylotypes of *R. solanacearum* were identified in Buea indicating a high diversity of the pathogen that is related to its virulence and high yield losses in Buea but more studies are needed to identify the specific Biovars, sequevers, and races (Table 2).

The diameters of inhibition of the positive controls (antibiotics: Chloramphenicol, 67 mm and rifampicin, 74 mm) were significantly higher at $P < 0.001$ than that of the plant extracts. For the botanicals, the dried *Chromolaena odorata* extract showed the smallest (6.8 mm) diameter of inhibition on NA plates inoculated with the Tiko isolate while the highest (13 mm) was given by the fresh aqueous extracts of *Cynodon dactylon* on the plates inoculated with the Ewunda isolate. Generally, the isolates were more sensitive to the *Cynodon dactylon* than *Chromolaena odorata* (Figure 3).

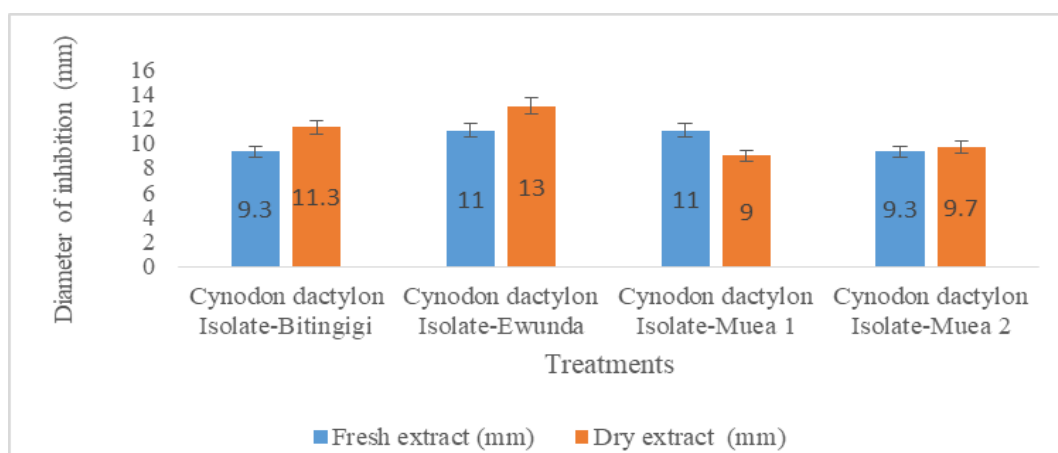


Figure 3. The diameter (mm) of inhibition of cynodont dactylon plant extract on *Ralstonia solonacearum* isolates

The Minimum Inhibitory Concentration (MIC) of both extracts was 250 $\mu\text{g}/\text{ml}$ while the Minimum bactericidal Concentration was 500 $\mu\text{g}/\text{ml}$, respectively. This is significantly lower than those of the positive controls: 0.5 $\mu\text{g}/\text{ml}$ for MIC and 50 $\mu\text{g}/\text{ml}$ for MBC for both Chloramphenicol and Rifampicin.

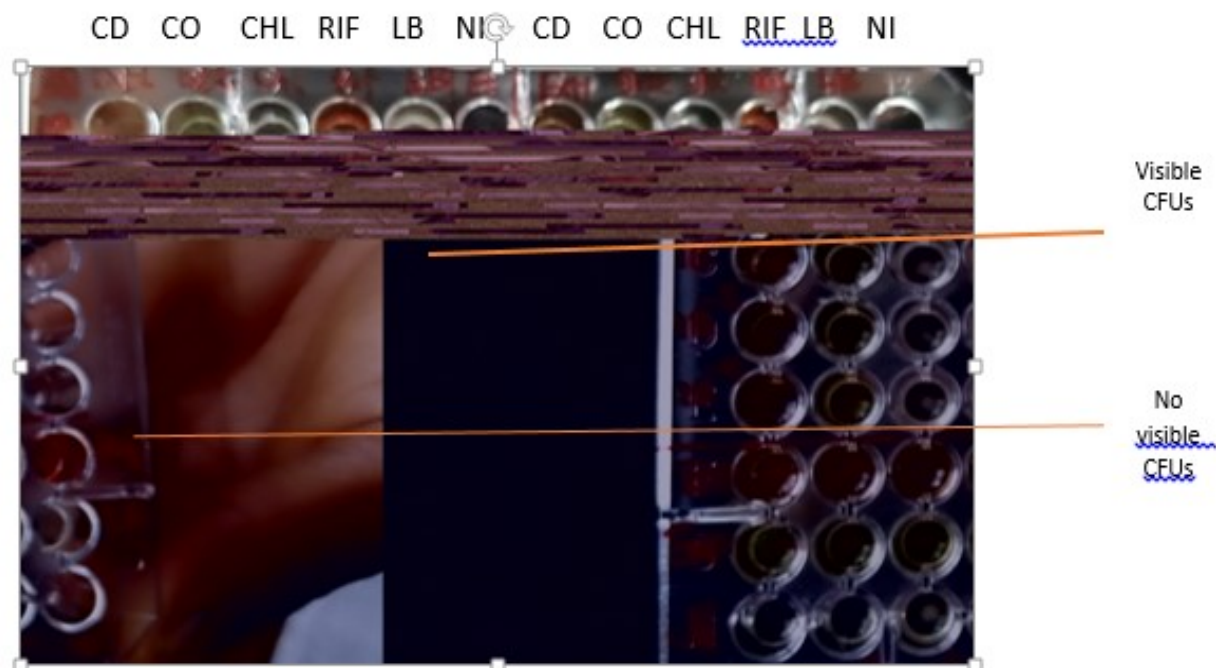


Figure 4. The determination of Minimum Inhibitory Concentration (MIC) by the serial dilutions on MHB. All wells had MHB, 10 μl of bacterial isolates, and different serial dilutions of CD = *Cynodon dactylon*, CO = *Chromolaena odorata*, Chloramphenicol, Rifampicin for the tests. The controls LB had no extract or antibiotic while NI had only Distilled water.

Table 3. The phytochemical analysis and antioxidant activity of the selected plant extracts

Samples	Units	Chromolaena odorata extracts	Cynodon dactylon extracts	Solanum lycopersicum Fruits	Tomatoes/ CD	Tomatoes/ CO	P values
Alkaloids	(mg/Kg)	0.445 $\pm$ 0.01a	0.34 $\pm$ 0.03b	0.23 $\pm$ 0.02c	0.235 $\pm$ 0.01c	0.21 $\pm$ 0.21c	0.001
Saponins	(mg/Kg)	5.06 $\pm$ 3.41a	5.56 $\pm$ 1.53a	0.31 $\pm$ 0.13b	0.20 $\pm$ 0.00b	0.25 $\pm$ 0.21b	0.050
Tannins	(mg/Kg)	619.59 $\pm$ 0.00a	426.09 $\pm$ 1.44b	64.10 $\pm$ 1.41c	64.13 $\pm$ 0.01c	64.12 $\pm$ 1.41c	0.050
DPPH	0	0.05 $\pm$ 0.00b	0.07 $\pm$ 0.00b	0.34 $\pm$ 0.00a	0.33 $\pm$ 0.01a	0.34 $\pm$ 0.00a	0.001
Antioxidants	$\mu\text{g}/\text{ml}$	54.01 $\pm$ 0.45b	57.21 $\pm$ 1.41b	88.38 $\pm$ 0.84a	79.24 $\pm$ 4.89a	77.87 $\pm$ 6.07a	0.001
Total Phenolic Content	mg GAE/Kg	1647.93 $\pm$ 18.20b	1286.57 $\pm$ 20.20c	1814.85 $\pm$ 12.93a	1808.99 $\pm$ 7.07a	1823.99 $\pm$ 0.01a	0.001
Oxalates	mg/Kg	283.31 $\pm$ 8.01c	395.59 $\pm$ 3.86c	2545.23 $\pm$ 21.3a	2465.39 $\pm$ 49.5a	2080.39 $\pm$ 70.70b	0.001
Flavenoids	mg/Kg	44.38 $\pm$ 0.04a	7.35 $\pm$ 0.03b	1.55 $\pm$ 0.04c	1.97 $\pm$ 0.78c	1.32 $\pm$ 0.00c	0.001

Values are reported as $M \pm \text{STD}$. Means (M) are obtained from 3 separate treatments. STD ($\pm$): Standard Deviation. Means that do not share a letter are significantly different. DPPH = 2, 2-Diphenyl-1-picrylhydrazyl, GAE = Gallic Acid Equivalent, Tomato/CD and CO= Tomatoes grown in *Cynodon dactylon* and *Cynodon odorata* amended soils, respectively

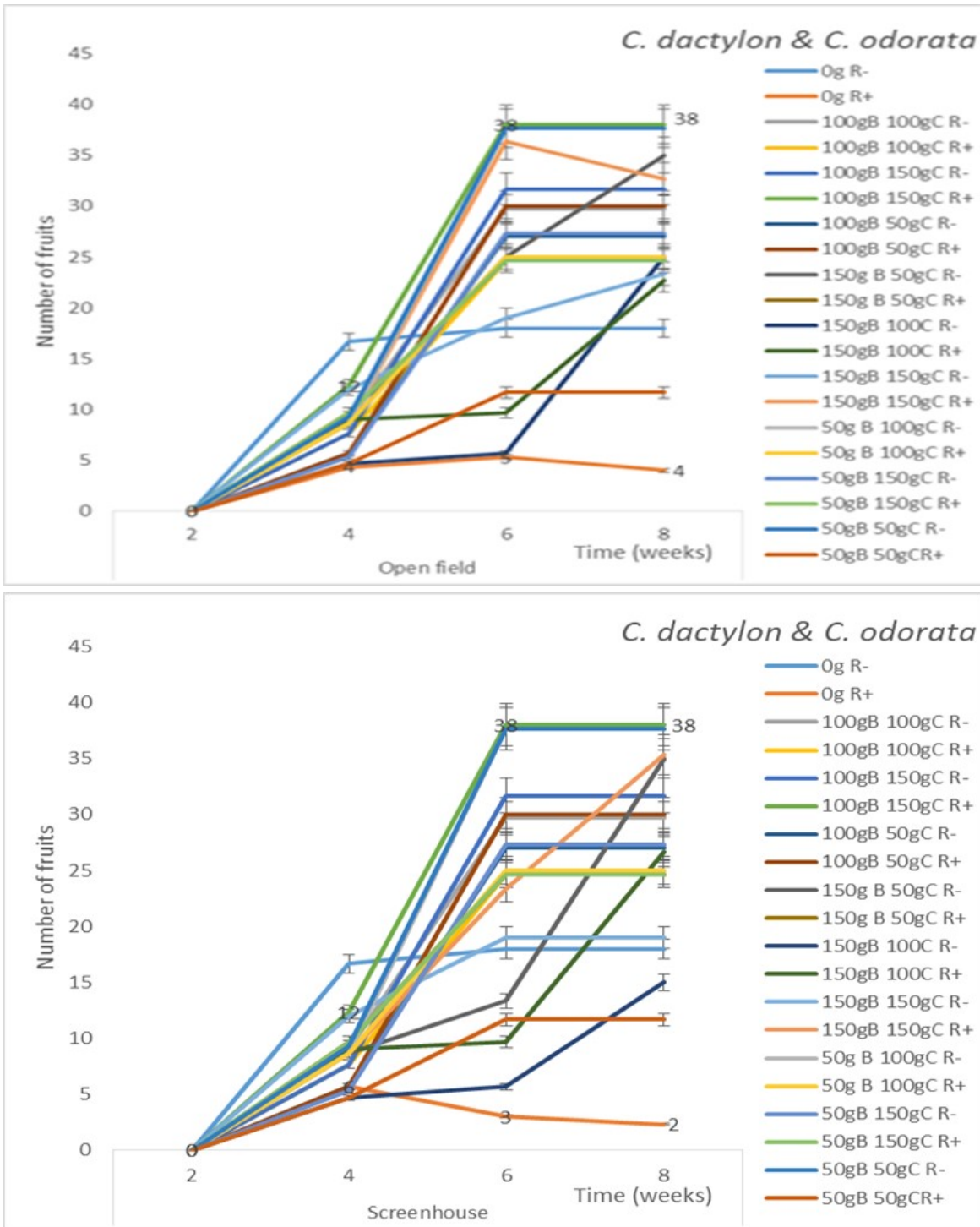
The results revealed significant differences ($P > 0.05$) in the quantities of phytochemicals among the tested compounds (Table 3). The *Cynodon odorata* extracts exhibited the highest levels of alkaloids (0.445 ± 0.01 mg/kg), tannins (619.59 ± 0.00 mg/kg), and flavonoids (44.38 ± 0.04 mg/kg). Surprisingly, these extracts also showed the least DPPH activity (0.05 ± 0.00) and antioxidant content (54.01 ± 0.45 µg/ml), as well as low oxalate levels (283.31 ± 8.01 mg/kg). On the other hand, *Cynodon dactylon* extracts had the highest saponin content (5.56 ± 1.53 mg/kg) but exhibited the least total phenolic content ($1286.57.20.20$ mg GAE/kg). The tomato fruits grown without any plant extracts (control experiments) had the highest DPPH activity (0.40 ± 0.00) and oxalate levels (2545.23 ± 21.3 mg/kg), while showing the lowest tannin content (64.10 ± 1.41 mg/kg). Interestingly, tomato fruits grown with *Cynodon dactylon* extracts accumulated the least saponins (0.20 ± 0.00 mg/kg). Conversely, tomato fruits cultivated with *C. odorata* extracts exhibited the highest total phenolic content (1823.99 ± 0.01 mg GAE/kg) but had the lowest alkaloid (0.21 ± 0.21 mg/kg) and flavonoid (1.32 ± 0.00 mg/kg) contents.

The macronutrient analysis of soil samples showed significant variations across depths and with the application of poultry manure ($P < 0.001$) (Table 4). Nitrogen (N) levels were adequate with poultry manure (545.0 ± 50 kg/ha) and at the 0-15 cm depth (305.0 ± 75 kg/ha), but inadequate at the 15-30 cm depth (167.5 ± 69 kg/ha). Phosphorus (P) levels were insufficient at both depths (0-15 cm: 12.5 ± 2 kg/ha, 15-30 cm: 11.0 ± 5 kg/ha) despite poultry manure application (43.8 ± 15 kg/ha). Potassium (K) levels were below the recommended range at both depths (0-15 cm: 49.3 ± 10 kg/ha, 15-30 cm: 28.7 ± 21 kg/ha) despite poultry manure amendment (542.0 ± 229 kg/ha). Calcium (Ca) and magnesium (Mg) levels were generally adequate, while sodium (Na) levels were positively influenced by poultry manure (65.9 ± 0.02 mg/kg) compared to control samples (1.59 ± 0.74 mg/kg).

Table 4. Macronutrient levels in soils of different depths and soils amended with poultry manure

Soil samples	N (kg/ha)	P (kg/ha)	K (kg/ha)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)
Poultry manure	545.0 ± 50^a	43.8 ± 15^a	542.0 ± 229^a	639.8 ± 3.5^b	152.5 ± 2.6^b	65.9 ± 0.02^a
0 - 15	305.0 ± 75^b	12.5 ± 2^b	49.3 ± 10^b	706.7 ± 3.5^a	205.0 ± 10.3^a	1.59 ± 0.74^b
15 - 30	167.5 ± 69^c	11.0 ± 5^b	28.7 ± 21^b	706.7 ± 3.5^a	204.8 ± 8.9^a	1.59 ± 0.74^b
P	***	***	***	***	***	***

Values are reported as $M \pm \text{STD}$. Means (M) are obtained from 4 separate samples. STD ($\pm$): Standard Deviation. Means that do not share a letter are significantly different.



Conclusion

The study highlights the efficacy of botanical extracts and soil amendments in managing bacterial wilt in tomato production caused by *Ralstonia solanacearum*. The use of botanicals and organic amendments resulted in improved plant growth, reduced disease severity, and enhanced soil fertility. These findings contribute to the development of sustainable and environmentally friendly strategies for bacterial wilt management, promoting global food security and resilient tomato production.

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