ANTIBACTERIAL POTENCY OF *Pelargonium zonale* and *Psidium guajava* AGAINST BACTERIAL WILT OF POTATO UNDER GREENHOUSE CONDITION

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Abstract

Pelargonium zonale and *Psidium guajava* plant extracts have demonstrated in-vitro antibacterial activity against *Ralstonia pseudosolanacearum* sp. nov. Nevertheless, their antibacterial effectiveness against this disease has not been examined under greenhouse condition. This study explored the antibacterial efficacy of ethanolic leave extracts of *P. zonale* and *P. guajava* in management of *R. pseudosolanacearum* sp. nov. of potatoes under greenhouse condition. The experiment was set in a completely randomized design (CRD) of 2*6 factorial arrangements [2 varieties (Shangi (Highly susceptible) and Sherekea (Moderately resistant) and 6 treatments] with 3 replicates. The treatments comprised; 2 ethanolic leave extracts (*P. guajava* and *P. zonale*) at 50 mg/mL, 2 positive controls [ENRICH BM (Bronopol 27%w/w) and KOBE 1.2 SL (Chrysophanol 12g/l)] at commercial rates and 2 negative controls (Untreated control and 1% DMSO). Data was collected on disease incidence, disease severity, plant heights, number of stems, yield (Kgs) and number of bacterial wilt colony counts. The study results revealed that all the treated plants exhibited significantly low disease incidences, low area under disease progress curves (AUDPCs), high number of stems, high stem heights, low bacterial wilt colony counts and high yield (Kgs) compared to negative controls. However, the efficacy results were dependent on resistant levels of each variety against *R. pseudosolanacearum* sp. nov. Potato variety Sherekea exhibited the highest efficacy from the two plant extracts demonstrating the synergistic effect of host plant resistance/tolerance and botanicals in management of *R. pseudosolanacearum* sp. nov. The study outcome was comparable to those of Enrich BM (Bronopol 27%w/w) and KOBE SL (Chrysophanol 12g/l). Further research is required to determine the effectiveness and stability of the two extracts against the target pathogen in the field.

Keywords: Antibacterial; *Pelargonium zonale*; *Psidium guajava*; In-vitro; In-vivo

1. Background

Potato (*Solanum tuberosum* L.) is ranked fourth after wheat, rice, and maize among the most important food crop globally, providing nourishment to over one billion people (Taiy *et al.,* 2017; Harahagazwe *et al.,* 2018; Gautam *et al.,* 2021). Regarding production volume, the crop is ranked second after maize in Kenya, contributing to food security and poverty alleviation. Its tubers are the major source of carbohydrates and other key dietary nutrients such as potassium, vitamin C, and fiber (Beals, 2019). Besides its nutritional values, the crop provides employment and income generation for various stakeholders along its value chain (Taiy *et al.,* 2017; Mwakidoshi *et al.,* 2021). However, field infestation by bacterial wilt pathogens has affected its optimal production worldwide. Approximately 50 – 100% yield losses have been reported in bacterial wilt infested potato fields (Kromann *et al.,* 2014; Muthoni *et al.,* 2014).

Ralstonia pseudosolanacearum sp., a damaging pathogen found primarily in tropical, subtropical, and temperate climates, is the causal agent for potato bacterial wilt (Shimelis *et al.,* 2014; Boschi *et al.,* 2017). The pathogen is soil-borne, and overwinters in the soil, plant debris, and alternate hosts such as solanaceous weeds (Kromann *et al.,* 2014; Boschi *et al.,* 2017). Bacterial wilt pathogen is disseminated through latently infected tubers, infested water (irrigation water and or run-offs), infested soil adhering to tools, farm handlers' shoes, and through mechanical activities in the farm, which causes wounds to potato crop (Kwambai *et al.,* 2011; Shimelis *et al.,* 2014). Complete eradication of bacterial wilt pathogen has proved difficult due to a lack of satisfactory management options. Thus, farmers have been advised to adopt an ecofriendly management option [integrated disease management (IDM)] against the disease (Sharma *et al.,* 2017). Nevertheless, the site-specific nature and variety of bacterial wilt strains have constantly affected the effectiveness of IDM as a management option (Priou *et al.,* 1999; Karim & Hossain, 2018).

There is limited adoption of IDM in bacterial wilt management in potato fields which has resulted to widespread use of conventional bactericides (Sarkar & Chaudhuri, 2016; Biswal & Dhal, 2018). The overreliance on conventional bactericides as a management option against bacterial wilt has posed health risks to humans and the environment. When these bactericides leak into water bodies, they cause aquatic health problems whereas those that infiltrates into the soil cause terrestrial health hazards (Rahman *et al.,* 2012; Mulugeta *et al.,* 2020). Developing countries are the most affected since farmers in these countries lack quality personal protective equipment (PPEs) coupled with their sluggish adoption of good agricultural practices (GAPs) (Mulugeta *et al.,* 2020). The negative environmental impacts from the excessive use of conventional bactericides have consequently shifted various scientists' focus to research eco-friendly bacterial management options (Rahman *et al.,* 2012).

Studies by different researchers have confirmed various plant extracts' antibacterial activity against bacterial wilt pathogens (Abo-Elyousr *et al.,* 2009). Apart from commercialized botanicals (formulated plant extracts), plant extracts have shown efficacy against bacterial wilt pathogen when used in various forms; aqueous form, dried powder form, and or green manure form (Din *et* *al.,* 2016; Chen *et al.,* 2020; Abd-Elrahim *et al.,* 2021). The use of plant extracts in dried powder form and/or as green manure is advantageous since, in addition to their antibacterial activity, they also improve soil properties (biological, physical, and chemical properties) and plant growth parameters (Chen *et al.,* 2020). When used as dried powder (organic amendment), the powder mixes with soil water to release water-soluble secondary metabolites with antibacterial activity against bacterial wilt pathogen. The secondary metabolites' method of action against the bacterial wilt pathogen is compound/metabolite dependent (Din *et al.,* 2016).

Before moving on to in-vivo studies, the efficacy screening of plant extracts' antibacterial potency against plant pathogenic bacteria typically starts with in-vitro experiments to determine their viability (Abd-Elrahim *et al.,* 2021). The antibacterial activity of *P. zonale* and *P. guajava* against *Ralstonia pseudosolanacearum* sp. nov. was confirmed by in-vitro experiments by Okeyo *et al.,* (2022a). Therefore, the purpose of this study was to determine if *P. zonale* and *P. guajava* ethanolic leave extracts were effective at controlling *R. pseudosolanacearum* sp. nov. of potatoes grown in a greenhouse.

2.0 Materials and methods

2.1 Bacterial isolation, identification, and inoculum preparation

Bacterial inoculum was isolated from infected potato plant tissues as described by Kelman (1954). Biovar identification of the isolated bacteria through the utilization of disaccharide sugars (lactose, maltose, and cellobiose) and oxidization of hexose alcohols (Mannitol, Sorbital, dulcitol, trehalose dextrose) was conducted according to Okeyo *et al.,* (2022a). The virulent colonies of the isolated bacteria were then multiplied in several plates containing Casamino Acid-Peptone-Glucose (CPG) medium at 28±10C for 48 hours. 48 hours old bacterial cells were harvested in sterilized distilled water, and the suspension was adjusted to attain the optical density (OD) of 600 nm (approximately

107 to 108 cfu / mL) using a spectrophotometer and hemocytometer, according to Mihovilovich *et al.* (2017) and Chen *et al.,* (2020).

2.2 Preparation of plant extracts

Leave samples from *Pelargonium zonale* and *Psidium guajava* were cleaned, dried, and crushed into fine powder and 1Kg of each powder was soaked in 10L of ethanol at a 1:10 (w/v) ratio for 48 hours with frequent stirring as described by Okeyo *et al.* (2022a). After 48 hours, the solutions were filtered to get rid of the solid particles using two layers of muslin cloths and Whatman filter papers. The filtrates were subsequently evaporated into pastes using a water bath at temperatures just below the boiling point of ethanol (60°C). The pastes were left to air dry overnight before being weighed and kept at 4°C.

2.3 Experimental design

A completely randomized design (CRD) of 2*6 factorial arrangement (two varieties and six treatments) with three replicates was used to set up the greenhouse experiment. The first level of treatments comprised two potato varieties (Shangi and Sherekea) which, according to Okeyo *et al.* (2023), were highly susceptible and moderately resistant, respectively. The second level of treatments consisted of ethanolic leave extracts (*Psidium guajava* and *Pelargonium zonale*) tested at a dosage rate of 50mg/mL, two commercial bactericides as positive controls [ENRICH BM (Bronopol 27%w/w), a conventional bactericide and KOBE 1.2 SL (Chrysophanol 12g/l) a botanical bactericide] applied at their commercial rates and two negative controls (1% DMSO as and untreated control).

2.4 Greenhouse experiment

The greenhouse experiment was carried out at the University of Nairobi field station, upper Kabete campus. Forest soil was collected from a tree forest (*Eucalyptus spp.)*, which has been out of cultivation for over ten years, and autoclaved for 1 hour at 121°C as described by Mahmood *et al.* (2014). Upon cooling, 4 kg sterile plastic pots with a surface area of $0.03M^2$ were half-filled with the cooled sterile soil. Five pots were used per treatment translating to 60 pots per replicate. Uniform wounds/physical injuries were created on each well-sprouted certified seed potato tuber using a sterile knife. The pots were inoculated with 10 mL of bacterial suspension adjusted to 600 nm OD (approximately 10^7 to 10^8 cfu / mL) using a syringe, as described by Mihovilovich *et al.* (2017). The injured tubers were planted at the center of each half-filled plastic pot (one tuber per pot). The tubers were then covered with sterile soil to about ¾ full.

Each treatment (ethanolic leave extracts (*P. guajava* and *P. zonale*), ENRICH BM (Bronopol 27%w/w), KOBE 1.2 SL (Chrysophanol 12g/l), and 1% DMSO) was mixed with sterile distilled water in different containers at recommended rates and the mixed solutions applied as a soil drench to their target treatment pots at the rate of 90ml per pot translating to (3L/M2) as described by (Kumar, 2021). Two weeks after plant emergence, 2 foliar applications of the above treatments were applied on the emerged plants at 2 weeks intervals. Each treatment was mixed with sterile distilled water at recommended rates, and the solution was applied to respective test plants as a foliar spray using hand sprayers. Maximum plant coverage was observed during foliar treatment applications. All potato agronomic practices (watering, fertilization, insect pest, and disease control) except the application of additional bactericides were conducted according to potato optimal production requirements. The experiment was repeated once, and in each experimental period, the experiment was terminated once the test plants exhibited senescence symptoms.

2.5 Determination of *Ralstonia solanacearum* population in the soil at harvest

During the experiment termination phase, soil samples were taken from the rhizosphere of potato plants in respective treatments and shipped to the laboratory to assess *R. solanacearum* population.

A sterile trowel was used to collect soil samples per pot from all five pots per treatment, and the samples were mixed thoroughly to form a composite sample from which 10 g of soil sample was sub-sampled and placed in plastic bags. The bags were labeled and sent to the lab for microbial scrutiny. Using Kelma's TZC agar media, bacterial isolation and quantification from soil samples were performed in the lab as described by Okeyo *et al.* (2023). The number of bacterial colonies was counted, and the bacterial population was determined using the following formula;

$$CFU/mL = \frac{Total\ number\ of\ colonies}{Plated\ volume\ (mL)} * dilution\ factor$$

2.6 Data collection

Data was collected on the number of stems, plant heights, disease incidence, disease severity, yield (Kgs), and bacterial colony counts. Disease indices (incidence and severity) data were recorded after plant emergence at two weeks intervals until the untreated control attained ≥80% wilt incidence (Mihovilovich *et al.,* 2017).

Disease severity scale of 1-3 based on the affected plant's degree of wilting, where 1 = healthy plant, 2 = ≤50% of the plant foliage wilted, and 3 = ≥50% of plant foliage wilted was used. The scored severity scales were converted to percent disease severity (%) using the following formula;

$$S = 100\left(\frac{\sum n}{N * Max.\ score\ in\ the\ scale}\right)$$

Where S = percent disease severity, Σn = summation of wilt scores, and N = an overall number of plants evaluated per treatment (Mihovilovich *et al.,* 2017).

Disease incidence was recorded by assessing the number of symptomatic plants per treatment at two weeks intervals. The scored values were converted to percent bacterial wilt incidence [BWI (%)] using the following formula;

$$BWI(\%) = \frac{X}{Y} * 100$$

Where BWI = percent bacterial wilt incidence, X = the number of symptomatic plants, and Y = the overall number of assessed plants per treatment (Okeyo *et al.,* 2018).

The harvested tubers were graded as symptomatic tubers (either externally visible and/or internal bacterial wilt symptoms) and asymptomatic tuber (for tubers without external and internal bacterial wilt symptoms). The harvested tubers were weighed and weights recorded in kilograms (Kgs).

2.7 Data analysis

The data from the two experiments, except disease severity, was first subjected to Levene tests to test for equal variance (Levene, 1960) before testing for mean differences using the Mann-Whitney U test at a 5% probability level (Wilcoxon, 1945; Mann & Whitney, 1947). Since there were no significant differences between the treatments, the data were subsequently pooled together and then subjected to normality testing using Shapiro-Wilk tests (Shapiro & Wilk, 1965). Where the data lacked equal variance as well as a normal distribution, the data were transformed using the formula;

$$\text{Log}\ (X + 1)$$

The pooled data were then subjected to a two-way analysis of variance (ANOVA) using R software, version 4.2.2 (R Studio Team 2020). Tukey's Honest significant difference (HSD) at $p \leq 0.05$ in agricolae package was used to separate the treatment means.

The percent disease severity data per treatment was used to compute the area under disease progress curves (AUDPCs) using the following formula;

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where yi= percent severity score at the ith observation, ti = time in days at the ith observation, and n= the total number of observations.

3.0 Results

3.1 Biovar identification through carbohydrate fermentation tests

All of the disaccharide sugars (lactose, maltose, and cellobiose) and hexose alcohols (mannitol, sorbital, dulcitol, trehalose, and dextrose) were oxidized by the bacterial isolate within five days of icubation. The universal bottles that had been inoculated with the isolated bacteria changed color from red to yellow to show the oxidation reaction, whilst the untreated controls (non-inoculated bottles) retained the red color.

3.2 Efficacy of ethanolic leave extracts of *P. zonale* and *P. guajava* against *Ralstonia pseudosolanacearum* sp. nov. of potatoes under greenhouse condition

The analysis of variance (ANOVA) results revealed highly significant interactions between treatments and varieties (Treatment x variety) among all the tested parameters except for the number of stems (NS), stem heights (SH), and Final wilt incidence (FWI) (Table 1.0). ANOVA results also revealed highly significant effects among treatments and varieties across all the response values.

Table 1.0: F-test statistics of two-way ANOVA of percent plant emergence, number of stems, stem heights, colony counts, final wilt incidence, symptomatic tubers, number of tubers and weight (Kgs)

Source of variation	Df	NS	SH	CC	FWI	NAT	Weight (t/ha)
Treatment	5	5.61***	3.95**	10447.81***	4.77***	155.88***	66.20***
Variety	1	42.20***	50.48***	2773.66***	140.48***	962.17***	469.04***
Treatment * variety	5	0.63	2.02	10.50***	1.63	49.67***	12.46***
Residuals	60						

Significance codes: 0 ‘***’, 0.001 ‘**’, 0.01 ‘*’ 0.05 ‘s’ 0.1 ‘’ 1. Df = Degree of freedom, NS= Number of stems, SH = Stem heights, CC = colony counts, FWI = Final wilt incidence and NAT = Number of asymptomatic tubers.

3.3 Effect of ethanolic leave extracts of *P. zonale* and *P. guajava* treatment application on mean number of stems and stem heights

The mean number of stems significantly differed at $p \leq 0.05$ across treatments and varieties. However, the interaction effect was insignificant at $p \leq 0.05$ among treatments and varieties (Treatment x variety) (Table 1.0). KOBE 1.2 SL recorded the highest number of stems in Shangi (0.93) and Sherekea (0.88), untreated controls, and 1% DMSO recorded the least for both varieties, respectively. Shangi had the highest overall mean number of stems (0.89), while Sherekea had the least (0.81). The transformed mean stem heights differed significantly at $p \leq 0.05$ across varieties. With the exception of the untreated control, all the transformed mean stem heights of Shangi were insignificantly different at $p \leq 0.05$ across all treatments. At the same time, those of Sherekea exhibited insignificant differences across all the treatments. Sherekea recorded the highest average mean stem heights, while Shangi recorded the least (Table 2.0).

Table 2.0: Effect off treatment application on transformed mean number of stems and stem heights for the two potato varieties

Treatments	Transformed mean number of stems and stem heights			
	Number of stems		Stem heights	
	Shangi	Sherekea	Shangi	Sherekea
Untreated control	0.86 ± 0.02 abcde	0.77 ± 0.04 e	1.69 ± 0.09 c	1.82 ± 0.03 a
Psidium guajava	0.91 ± 0.04 ab	0.80 ± 0.04 cde	1.78 ± 0.03 ab	1.85 ± 0.02 a
Pelargonium zonale	0.88 ± 0.03 abc	0.80 ± 0.07 cde	1.78 ± 0.04 ab	1.83 ± 0.03 a
ENRICH BM	0.87 ± 0.07 abcd	0.83 ± 0.07 bcde	1.78 ± 0.06 ab	1.83 ± 0.04 a
KOBE 1.2 SL	0.93 ± 0.05 a	0.88 ± 0.04 abc	1.78 ± 0.01 ab	1.83 ± 0.04 a
1% DMSO	0.86 ± 0.03 abcde	0.78 ± 0.03 de	1.72 ± 0.03 bc	1.81 ± 0.03 a
Grand mean	0.89	0.81	1.76	1.83
MSD	0.09	0.09	0.08	0.08

The values are average number of stems ± standard deviations. Means within the same column having same letter(s) do not differ significantly at p ≤0.05, MSD = mean square displacement.

3.4 Effect of ethanolic leave extracts of *P. zonale* and *P. guajava* treatment application on potato bacterial wilt incidence and area under disease progress curve

Bacterial wilt incidences (BWIs) varied across treatments and varieties and were significantly different at $p \leq 0.05$ at 38 days after planting (DAP), 52 DAP, and 66 DAP. For Shangi, BWI commenced at 38 DAP and increased rapidly to 66 DAP. A part from *Pelargonium zonale* leaf extracts which exhibited BWI at 38 DAP, most treatments expressed BWIs at 52 DAP for Sherekea, and the BWI progressed slowly to 66 DAP (Table 3.0). In general, Shangi exhibited high final bacterial wilt incidences (FBWIs) at all treatment levels compared to Sherekea. The area under disease progress curves (AUDPCs) varied across treatments and varieties. For both varieties, untreated control and 1% DMSO recorded the highest AUDPCs of 2155.68 (untreated control) and 2154.28 (1% DMSO) for Shangi and 1236.55 (untreated control) and 1237.04 (1% DMSO) for Sherekea respectively. In general, Shangi exhibited high AUDPCs at all treatment levels compared to Sherekea (Figure 1.0).

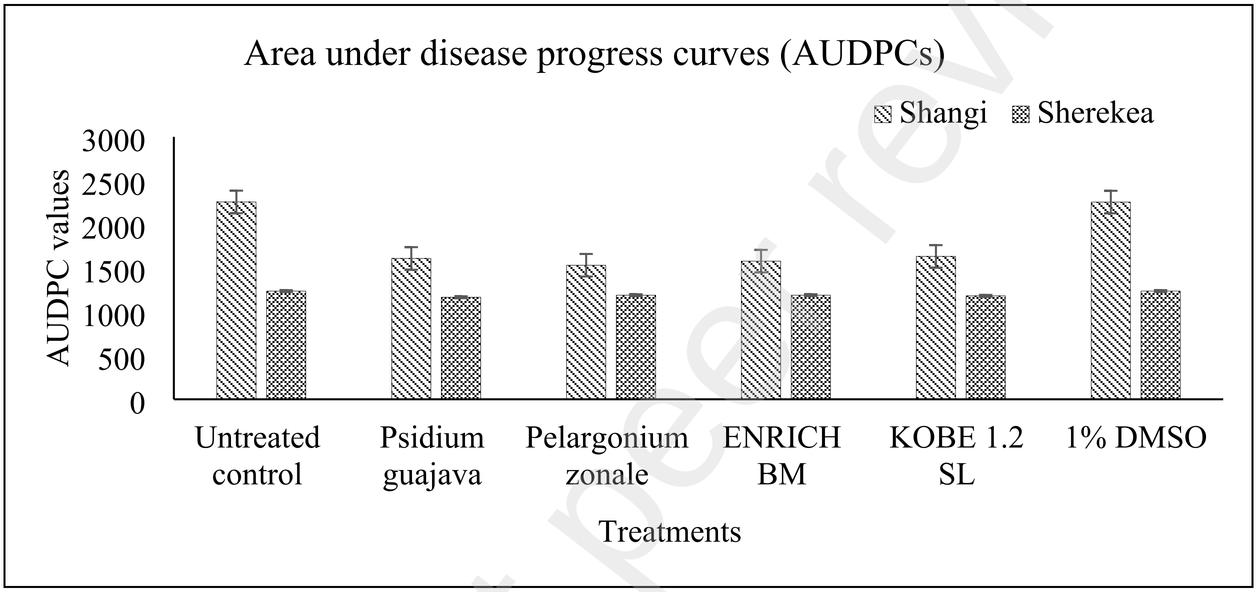

Figure 1. Effect off treatment application on Area under disease progress curve (AUDPC) of the Shangi and Sherekea potato varieties. Bars represent standard error.

Table 3.0: Effect off treatment application on mean bacterial wilt incidence for the two potato varieties

Treatments	38 DAP		52 DAP		66 DAP	
	Shangi	Sherekea	Shangi	Sherekea	Shangi	Sherekea
Untreated control	1.30 ± 0.65 a	0.00 ± 0.00 c	1.91 ± 0.00 ab	0.44 ± 0.68 cd	2.00 ± 0.00 a	0.93 ± 0.73 bcd
Psidium guajava	0.66 ± 0.72 abc	0.00 ± 0.00 c	1.23 ± 0.63 abc	0.00 ± 0.00 d	1.79 ± 0.12 ab	0.00 ± 0.00 e
Pelargonium zonale	0.71 ± 0.78 abc	0.22 ± 0.54 bc	1.52 ± 0.15 ab	0.22 ± 0.54 d	1.67 ± 0.09 ab	0.27 ± 0.66 de
ENRICH BM	0.71 ± 0.78 abc	0.00 ± 0.00 c	1.45 ± 0.20 ab	0.00 ± 0.00 d	1.67 ± 0.09 ab	0.49 ± 0.76 cde
KOBE 1.2 SL	0.54 ± 0.83 abc	0.00 ± 0.00 c	1.39 ± 0.72 ab	0.00 ± 0.00 d	1.74 ± 0.16 ab	0.44 ± 0.68 cde
1% DMSO	1.20 ± 0.60 ab	0.00 ± 0.00 c	1.92 ± 0.08 a	1.10 ± 0.54 bc	2.00 ± 0.00 a	1.20 ± 0.60 abc
Grand mean	0.85	0.04	1.57	0.29	1.57	0.56
MSD	1.06	1.06	0.81	0.81	0.88	0.88

The values are average bacterial wilt incidence ± standard deviations. Means within the same column having same letter(s) do not differ significantly at p ≤0.05, MSD = Mean square displacement, DAP = Days after planting.

3.5 Effect of ethanolic leave extracts of *P. zonale* and *P. guajava* treatment application on potato yield parameters [number of asymptomatic tubers and tuber weights (Kgs)]

The mean number of asymptomatic tubers by visual assessment significantly differed at $p \leq 0.05$ across treatments and varieties. KOBE 1.2 SL (Chrysophanol 12g/l) of Sherekea recorded the highest number of asymptomatic tubers (1.87), while untreated control of Shangi recorded the least (1.41) (Table 4.0). Sherekea had the highest overall mean number of asymptomatic tubers (1.76), while Shangi had the least (1.59). However, dissection of all the sampled asymptomatic tubers per treatment per variety revealed brown discoloration of the vascular ring and the adjacent tissues extending to the pith and/or tuber cortex. Mean tuber wights (Kgs) differed significantly at $p \leq 0.05$ across treatments and varieties. KOBE 1.2 SL (Chrysophanol 12g/l) of Sherekea recorded the highest mean transformed tuber weights (0.31), while untreated control and 1% DMSO of Shangi recorded the least (0.17). Sherekea had the highest overall transformed mean tuber weights (0.29), while Shangi had the least (0.22).

Table 4.0: Effect off treatment application on transformed proportion/percent mean number of asymptomatic tubers and tuber weights (Kgs) for the two potato varieties

Treatments	Percent mean No. of asymptomatic tubers and tuber weights (Kgs)			
	Number of asymptomatic tubers		Tuber weighs (Kgs)	
	Shangi	Sherekea	Shangi	Sherekea
Untreated control	1.41 ± 0.04 g	1.71 ± 0.02 cd	0.17 ± 0.01 g	0.26 ± 0.02 def
Psidium guajava	1.68 ± 0.04 def	1.78 ± 0.01 b	0.24 ± 0.01 f	0.29 ± 0.01 abc
Pelargonium zonale	1.67 ± 0.03 def	1.75 ± 0.01 bc	0.25 ± 0.01 ef	0.28 ± 0.01 bcd
ENRICH BM	1.67 ± 0.01 ef	1.75 ± 0.01 bc	0.25 ± 0.01 ef	0.30 ± 0.02 ab
KOBE 1.2 SL	1.65 ± 0.03 f	1.87 ± 0.02 a	0.25 ± 0.01 ef	0.31 ± 0.01 a
1% DMSO	1.43 ± 0.02 g	1.70 ± 0.02 de	0.17 ± 0.01 g	0.27 ± 0.01 cde
Grand mean	1.59	1.76	0.22	0.29
MSD	0.05	0.05	0.02	0.02

The values are average proportion of symptomatic tubers ± standard deviations. Means within the same column having same letter(s) do not differ significantly at p ≤0.05, MSD = mean square displacement.

3.6 Effect of ethanolic leave extracts of *P. zonale* and *P. guajava* treatment application against *Ralstonia pseudosolanacearum sp. nov.* colony counts

The transformed mean bacterial wilt colony counts from soils sampled around the rhizosphere of the two potato varieties differed significantly at p≤0.05 across treatments and varieties. Untreated control and 1% DMSO recorded the highest mean transformed colony counts of 3.15 and 3.16 for Shangi and 3.06 and 3.05 for Sherekea, respectively. *Pelargonium zonale* recorded the least mean transformed colony counts of 2.70 for Shangi and 2.63 for Sherekea (Table 5.0).

Table 5.0: Transformed mean bacterial wilt colony counts per treatment recorded from the soil samples sampled around the rhizosphere of two potato varieties

Treatments	Transformed mean bacterial wilt colony counts	
	Shangi	Sherekea
Untreated control	3.15 ± 0.01 a	3.06 ± 0.00 b
Psidium guajava	2.78 ± 0.01 d	2.69 ± 0.00 f
Pelargonium zonale	2.70 ± 0.00 f	2.63 ± 0.01 g
ENRICH BM	2.80 ± 0.01 c	2.73 ± 0.00 e
KOBE 1.2 SL	2.78 ± 0.01 d	2.70 ± 0.01 f
1% DMSO	3.16 ± 0.00 a	3.05 ± 0.00 b
Grand mean	2.9	2.81
MSD	0.01	0.01

The values are average transformed mean bacterial wilt colony counts ± standard deviations. Means within the same column having same letter(s) do not differ significantly at $p \leq 0.05$, MSD = mean square displacement.

4.0 Discussion

The bacterial isolate completely oxidized all of the disaccharide sugars and hexose alcohols within 5 days of incubation. These results confirmed the findings of Rahman *et al.,* (2010), Popoola *et al.,* (2015), Boschi *et al.,* (2017), Khasabulli *et al.,* (2017), and Okeyo *et al.,* (2022a) who classified *Ralstonia pseudosolanacearum* sp. nov. bacterial isolate with similar traits as biovar III race 1 *(Ralstonia solanacearum* (phylotype I)). The mean number of stems varied across treatments and varieties. All the treated pots exhibited a higher mean number of stems per variety than untreated controls. The soil drench of various treatments (plant extracts and conventional bactericide) used in this study might have suppressed apical dominance on the planted tubers resulting in an increased number of lateral buds, sprouts and hence increased number of stems (Biruk-Masrie *et al.,* 2015). Similarly, Biruk-Masrie *et al.,* (2015) also reported many stems from potato tubers treated with extracts from essential oils. KOBE 1.2 SL (Chrysophanol 12g/l), recorded the highest mean number of stems for both varieties, and this could be an indication that KOBE 1.2 SL had high phytochemical composition compared to other treatments. In general, Shangi recorded the highest number of stems per treatment compared to Sherekea, which can be attributed to differences in their genetic traits and a high number of tuber eyes recorded on the Shangi variety (Nielson *et al.,* 1989).

The average stem heights varied significantly between varieties at $p \leq 0.05$. In contrast to Sherekea, the mean stem heights of Shangi did not differ significantly across any of the treatments except for untreated control. Both varieties did not exhibit significantly different stem heights from pots with various treatment applications compared to negative control pots. The high stem heights from treated pots can be attributed to low disease indices scored (Priou *et al.,* 1999; Chen *et al.,* 2020). Additionally, studies by Okeyo *et al.,* (2022b) reported the presence of Succinic acid (one of the major components used in the manufacture of bio-stimulants) in low concentrations from the ethanolic leave extracts of *Psidium guajava* and *Pelargonium zonale*, and this could have resulted to slight increase in stem heights in plants treated with the two extracts compared to untreated plants (Zeikus *et al.,* 1999; Levchyk *et al.,* 2017). Liu *et al.,* (2016) also reported the bio-stimulant effect of Chrysophanol on potato plants, which can explain the high stem height in the plants treated with Chrysophanol. Sherekea exhibited the highest average stem heights compared to Shangi, and this can be attributed to the difference in the expression of genetic traits for plant height from the two varieties.

The final bacterial wilt incidences (FBWIs) and area under disease progress curves (AUDPCs) varied across treatments and varieties. All the treated plants recorded low FBWIs and AUDPCs compared to negative controls. This was consistent with the study's findings of Oboo *et al.* (2014), Chen *et al.* (2020), and Abd-Elrahim *et al.* (2021), who reported in-vivo efficacy of plant extracts (essential oils) against bacterial wilt of potatoes with regards to disease incidence. It has been demonstrated that the bacterial wilt pathogen can be controlled by induced host plant resistance through the use of KOBE 1.2 SL (Chrysophanol 12g/l) and ENRICH BM (Bronopol 27%w/w) (https://agroduka.com/enrich-bm; Liu *et al.,* 2016), while the mode of action of both *P. guajava* and *P. zonale* against this pathogen is still unknown. In general, Shangi exhibited high FBWIs and AUDPCs at all treatment levels compared to Sherekea, and this can be attributed to varied degrees of resistance levels of the two varieties to bacterial wilt pathogen (Patil *et al.,* 2012; Muthoni *et al.,* 2014; Okeyo *et al.,* 2023).

The average proportion of asymptomatic tubers and tuber weight (kgs) significantly differed across treatments and varieties. All the treated plants recorded a significantly higher number of asymptomatic tubers and tuber weights (Kgs) than negative controls. These results concurred with that of Abd-Elrahim *et al.,* (2021), who revealed increased yield parameters from bacterial wilt-inoculated potato plants treated with plant extracts in-vivo. Sherekea variety recorded the highest yield parameters per treatment compared to Shangi, and this can be attributed high bacterial wilt resistance level of Sherekea compared to Shangi (Okeyo *et al.,* 2023). KOBE 1.2 SL (Chrysophanol 12g/l) recorded the highest yield parameters per variety, which can be attributed to its high concentration of antibacterial compound compared to other treatments. The overall proportion of antibacterial compounds per plant extract was less than 10% for both *P. zonale* and *P. guajava* (Okeyo *et al.,* 2022b). Most of the harvested tubers lacked externally visible symptoms, which can be attributed to late bacterial symptom establishment and certified tubers used in this study. However, dissection of all the sampled asymptomatic tubers per treatment revealed 100% bacterial wilt incidence (brown discoloration of the vascular ring and adjacent tissues extending to the pith and/or tuber cortex). The 100% wilt incidence on dissected tubers indicates that potato tubers harvested from all the treated pots had latent infection symptoms and, therefore, cannot be used as seeds (Patil *et al.,* 2012). A hundred percent wilt incidence on the sampled tubers can be attributed to wounds created on the tubers at planting to avoid disease escape.

Bacterial wilt colony counts from soil samples around the rhizosphere revealed significantly low bacterial wilt populations from all treated pots compared to the negative controls. These results confirmed the study outcomes of Chen *et al.* (2020) and Abd-Elrahim *et al.* (2021), who reported a reduced bacterial wilts population from soils sampled from pots treated with various plant extracts in-vivo. *P. zonale* recorded the least mean bacterial wilt population among all the treated pots for both varieties (Shangi and Sherekea). These results confirmed the in-vitro study results of Okeyo *et al.* (2022a), who reported *P. zonale* as the most effective plant extract against bacterial wilt of potatoes. In general, Shangi had the highest bacterial wilt population per treatment, which can be attributed to the high disease indices scored per treatment for Shangi compared to Sherekea.

5.0 Conclusion

The present study revealed in-vivo antibacterial efficacy of *P. zonzle* and *P. guajava* leave extracts against soil inoculated *R. pseudosolanacearum* sp. nov. However, the efficacy results were dependent on resistant levels of varieties used in the study. Sherekea (moderately resistant variety) demonstrated the highest efficacy of the two plant extracts, illustrating the synergistic effect of host plant tolerance/resistance and botanicals in the management of bacterial wilt of potatoes. These results were comparable to those of Enrich BM (Bronopol 27%w/w) a conventional bactericide and KOBE SL (Chrysophanol 12g/l) a botanical bactericide. Additional research should be conducted to determine the effectiveness and stability of *P. zonale* and *P. guajava* against the target pathogen under field condition.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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