

Effect of commonly used host and non-host plants for rotation in control of *Ralstonia solanacearum*

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ABSTRACT

Ralstonia solanacearum is a soil-borne bacterial pathogen that causes bacterial wilt disease, a major threat to many plants, particularly those in the Solanaceae family, including potatoes, a key food crop in Kenya. Controlling bacterial wilt is highly challenging, as there are no effective curative chemicals (pesticides). Additionally, the pathogen can survive as a latent infection in the soil and the rhizosphere of certain non-host plants, serving as a reservoir for persistent infections and complicating control strategies. For this reason, an investigation was conducted to evaluate the effect of host and non-host plants on the control of *R. solanacearum* by determining the natural (initial) pathogen population density in the soil using a tetrazolium-based semi-selective medium (SMSA). Potatoes, tomatoes, eggplants, and peppers were used as host plants, while maize, sorghum, cabbage, and beans served as non-host plants. The study assessed *R. solanacearum* population in the soil, disease incidence, root pathogen attachment, and root invasion using a randomized complete block design (RCBD) with four replications (blocks) and eight treatments. Statistical analysis was performed using GenStat 12.1 (PC/Windows XP), and means were compared using Fisher's least significant difference (FLSD) test. A significant difference was observed between the wilt incidence of host and non-host plants ($P < 0.05$). Cabbage was identified as the only safe crop for rotation, as it had a significantly lower *R. solanacearum* population attached to its roots, in the rhizosphere, or within its vascular bundles ($P < 0.05$). Maize asymptotically promoted the persistence of the pathogen within its vascular bundles and accessory tissues. Similarly, beans and sorghum were found to encourage the survival of *R. solanacearum* in the rhizosphere and as root attachments.

Keywords: *Ralstonia solanacearum*, control, rotation crops, non-host plants, rhizosphere.

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INTRODUCTION

One of the most challenging diseases affecting crops in the Solanaceae family is bacterial wilt (BW), caused by *Ralstonia solanacearum* (Yabuuchi et al., 1995), formerly known as *Pseudomonas solanacearum*. This pathogen infects several plant species across more than 50 families and has long been a scourge of tropical agriculture due to its widespread distribution and broad host range. Bacterial wilt is particularly difficult to control, making it a major threat to the production of solanaceous crops. *R. solanacearum* is both a soil- and seed-borne pathogen with limited host resistance (Schaad et al., 2001).

The primary virulence factor of this bacterial pathogen

is exopolysaccharide 1 (EPS 1), which causes wilting by vascular occlusion or rupture of vessel elements due to excessive hydrostatic pressure (Schell, 2000). *R. solanacearum* is classified into three races: race 1 has a broad host range, race 2 primarily affects bananas, and race 3 mainly infects solanaceous crops. The disease is commonly referred to as "bacterial wilt," but in potatoes, it is also known as "brown rot." Bacterial wilt poses a significant economic challenge, affecting approximately three million farming families in 80 countries and causing annual losses exceeding USD 950 million (Lopez and Biosca, 2004). In Kenya, the pathogen has been reported at both low and high elevations (Nyangeri et al., 1984).

Yield losses due to bacterial wilt in traditional potato-growing regions of Kenya are estimated at 50-100% (Ajanga, 1993).

Managing bacterial wilt in regions favorable to its development is highly challenging, as no effective curative chemicals or pesticides are currently available. While resistance breeding has proven effective against race 1 in tropical crops such as eggplant, tomato, peanut, and pepper, it has had limited success in potatoes in South America. Additionally, cultural practices such as using healthy seeds, implementing extensive crop rotation, utilizing specific rotation crops like corn or rice, removing and burning infected plants, and carefully managing water have yielded only marginal results (Lopez and Biosca, 2004). Therefore, soil suppressiveness to bacterial wilt may be influenced by factors beyond soil type alone, including pH, organic matter content, and microbial communities (van Elsas et al., 2005).

MATERIALS AND METHODS

Determination of initial *R. solanacearum* population

The natural (initial) pathogen population density in the soil was determined using a tetrazolium-based semi-selective medium (SMSA) (Schell, 2000). This was done by randomly collecting 20 g of soil from the entire plot up to a depth of 10 cm, three days after inoculation. Three soil samples were collected from each plot in a zigzag pattern. These samples were thoroughly mixed to form a composite sample, from which 10 g of soil was taken and placed in a conical flask. Subsequently, 90 ml of sterile distilled water was added. The mixture was then shaken

using a horizontal shaker at 200 revolutions per minute for 30 minutes.

After shaking, the mixture was allowed to settle for 10 minutes. Then, 1 ml of the suspension was pipetted using a micropipette, placed in an Eppendorf tube, and used as the stock solution. A 0.1 ml (100 μ l) aliquot of the suspension was transferred into 0.9 ml of sterile distilled water in an Eppendorf tube and then serially diluted up to 10^{-5} , using 0.1 ml at each step. The dilutions were gently mixed, and 0.1 ml was pipetted from the 10^{-1} , 10^{-3} , and 10^{-5} dilutions and spread onto SMSA medium using a sterilized glass rod. The plates were then incubated at 28°C for 48 hours.

R. solanacearum colonies were identified by their typical fluidal, irregularly shaped pink appearance with clear margins on SMSA medium. The number of *R. solanacearum* colonies was determined by counting the dilutions that yielded between 30 and 300 colonies. The population of *R. solanacearum* in the soil was then computed based on the number of colonies obtained. Since the field was naturally infested with *R. solanacearum* (race 3, biovar 2), the initial soil population density was artificially increased to approximately 1×10^7 colony-forming units per gram of dried soil (CFU/g) by mixing the soil with a known volume of an *R. solanacearum* suspension containing 1×10^9 CFU/ml.

Trial setup

The crop varieties (Table 1) were purchased from a reputable commercial vendor. Each unit plot measured 4.0 m $\times$ 2.0 m, with a 1.0 m path between plots in all directions. The entire experimental field measured 25.0 m $\times$ 21.0 m.

Table 1. Host and non-host plants used in the study.

Common name	Variety name	Scientific name
Host crops		
Potato	Tigoni	<i>Solanum tuberosum</i>
Tomato	Cal J	<i>Solanum lycopersicum</i>
Eggplant	Black beauty	<i>Solanum melongena</i>
Capsicum	California wanders	<i>Capsicum annuum</i>
Non-host crops		
Maize	Hybrid 624	<i>Zea mays</i>
Sorghum	Katumani	<i>Sorghum bicolor</i>
Beans	Mwitmania	<i>Phaseolus vulgaris</i>
Cabbage	Copenhagen	<i>Brassica oleracea</i>

Potatoes, tomatoes, eggplants, and peppers served as host plants, while maize, sorghum, cabbage, and beans were used as non-host plants (Table 1). The experiment followed a randomized complete block design (RCBD) with four blocks and eight subplots.

The crops were planted using the following spacing:

- **Maize and sorghum:** 75 cm between rows and 30 cm between plants (42 plants per plot).
- **Beans and capsicum:** 50 cm between rows and 20 cm

between plants (56 plants per plot).

- **Potato:** 80 cm × 30 cm (37 plants per plot).
- **Tomato:** 45 cm × 45 cm (37 plants per plot).

- **Cabbage:** 25 cm × 25 cm (46 plants per plot).

- **Eggplant:** 90 cm × 30 cm (46 plants per plot) (Figure 1).



Figure 1. Two months old host and non-host crops in the field.

After planting, the crops were watered only three times, as the experiment was conducted during the rainy season. Some of the host and non-host plants are shown in Figure 1.

Data collection

Data on potential crop rotation for bacterial wilt management was collected in different parts of the study, including the assessment of *R. solanacearum* population, disease incidence, root pathogen attachment, and root invasion. Wilt symptoms were observed in all crops within each plot and recorded for wilt incidence calculations.

The pathogen population in the soil was determined before planting and after harvesting. Root pathogen attachment and root invasion were also analyzed. Wilt incidence for all crop treatments was expressed as a percentage of wilted plants relative to the total number of plants in the plot, as shown in the equation below:

$$\text{Disease incidence (\%)} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

To determine the survival of test bacteria in soil and rhizosphere infestation, four sample plants were used per plot one month after planting and then every month for three months. The number of viable bacteria in the

rhizosphere was assessed by removing "bulk soil" (i.e., soil that is easily dislodged) from the roots by shaking the plants for a few seconds after uprooting them, leaving the "rhizosphere soil" still attached. The roots, along with the rhizosphere soil, were then carefully shaken for one minute into sterile conical flasks. The roots were removed, and the rhizosphere soil was used for population analysis (Saile et al., 1997).

Each 10 g soil sample collected from the rhizosphere was suspended in 90 ml of sterilized water, and the suspension was shaken in a rotary shaker (120 rpm) for 30 minutes. The suspension was then serially diluted in sterile distilled water, and aliquots (100 µl) from 10-fold dilutions were spread onto the surface of each plate of SMSA using a glass rod. The seeded plates were incubated at 28°C for 72 hours, after which colony-forming units (CFU) were counted, and the results were expressed as the total number of bacteria per gram of soil (Yue-Gyu Kang, 2004).

For the evaluation of root attachments, the roots from which rhizosphere soil had been removed were carefully washed for one minute in 100 ml of sterile water in flasks. The roots were then removed, and the flasks containing the suspensions were shaken on a wrist-action shaker for one hour. Dilutions from 1 ml of this suspension were spread on SMSA to determine the number of viable cells, as described above.

***R. solanacearum* counts for primary roots**

To obtain *R. solanacearum* counts for the primary roots, the entire root system was first thoroughly washed under running tap water. The stems were then cut off 1.0 cm above the former soil line. The root systems were immersed in 1.0% sodium hypochlorite (20% v/v household bleach) for two minutes, washed under running tap water again, and the lateral roots were removed. The remaining primary roots were rinsed with 95% ethanol and air-dried. Each segment was placed in a sterile bag with 5 ml of phosphate-buffered saline (0.1 M potassium phosphate buffer, pH 7.3, 0.15 M NaCl, and 3.0 M KCl) and crushed in a stomacher lab blender (Seward Medical, London) for two minutes. Dilutions of the homogenates were spread on a semi-selective medium, and the plates were incubated at 28°C for 48 hours. Viable cell counts were performed as described earlier.

A similar experiment was conducted with other host and non-host plants. The presence or absence of *R. solanacearum* in all host and non-host plant samples was investigated using the method developed by CIP for detecting latent infection in tubers via post-enrichment NCM-ELISA (enzyme-linked immunosorbent assay on a nitrocellulose membrane), as described by Priou et al. (1999).

Data analysis

The effect of host and non-host plants on disease development by *R. solanacearum* was evaluated using a randomized complete block design (RCBD) with four replications (blocks) and eight treatments. Disease incidence was calculated as the percentage of plants that showed wilt symptoms. Statistical analysis was performed using GenStat 12.1 (PC/Windows XP). Means

were compared using Fisher's least significant difference (FLSD).

RESULTS

Population of *R. solanacearum* in the soil

The mean population per plot for each crop was calculated, and the results are shown in Table 2. There was a significant difference between the type of treatment and the mean *R. solanacearum* population in the soil two months after planting ($P < 0.05$), as shown in Appendix 4.

Rhizosphere population

Fisher's protected least significant difference test was used to compare the mean rhizosphere *R. solanacearum* population three months after planting. Eggplant and potato plots had the highest *R. solanacearum* population, but there was no significant difference between them.

This was followed by sorghum, tomato, beans, cabbage, and maize, which also showed no significant difference among them. Capsicum had the lowest mean population, though there was no significant difference when compared to maize and cabbage (Table 3).

Root invasion

Potato had the highest population of *R. solanacearum* in its roots, followed by eggplant, capsicum, tomato, and maize, with no significant difference among them (Table 4). Sorghum and beans had significantly higher *R. solanacearum* populations in their roots compared to cabbage, which had a very low population.

Table 2. Mean *R. solanacearum* population in the soil.

Crop	Mean ($\pm$ SD) of soil <i>R. solanacearum</i> population (cfu $\times 10^3$)	
	Before planting/treatment	After two months
Beans	155 $\pm$ 9.129	300 $\pm$ 8.048
Cabbage	161 $\pm$ 8.602	311 $\pm$ 9.557
Capsicum	192 $\pm$ 6.683	381 $\pm$ 2.582
Eggplant	166 $\pm$ 4.243	306 $\pm$ 2.163
Maize	160 $\pm$ 5.944	312 $\pm$ 1.633
Potato	131 $\pm$ 9.967	246 $\pm$ 5.164
Sorghum	142 $\pm$ 3.367	268 $\pm$ 5.099
Tomato	227 $\pm$ 9.950	439 $\pm$ 7.394

Wilt incidence

Wilt incidence for each crop (treatment) was determined

by calculating the average of the replicate blocks for all crops. The results are shown in Figure 2 and Table 5. According to these results, potato and tomato had the

Table 3. Mean values recorded for *R. solanacearum* population in the rhizosphere.

Crop	Mean population (cfu $\times 10^3$)	Mean difference
Eggplant	25.25	a
Potato	16.50	ab
Sorghum	16.00	b
Tomato	15.75	b
Beans	13.00	b
Cabbage	10.75	bc
Maize	7.50	bc
Capsicum	3.50	c

Means followed by different letters are statistically different at $P < 0.05$ by Fisher's protected least significant difference test.

Table 4. Mean observed values for *R. solanacearum* population inside the root.

Crop	Mean population (cfu $\times 10^3$)	Mean difference
Potato	82.50	a
Eggplant	72.50	ab
Capsicum	42.50	abc
Tomato	40.00	abc
Maize	37.50	abc
Sorghum	32.50	bc
Beans	27.50	bc
Cabbage	5.00	c

Means followed by different letters are statistically different at $P < 0.05$ by Fisher's protected least significant difference test.

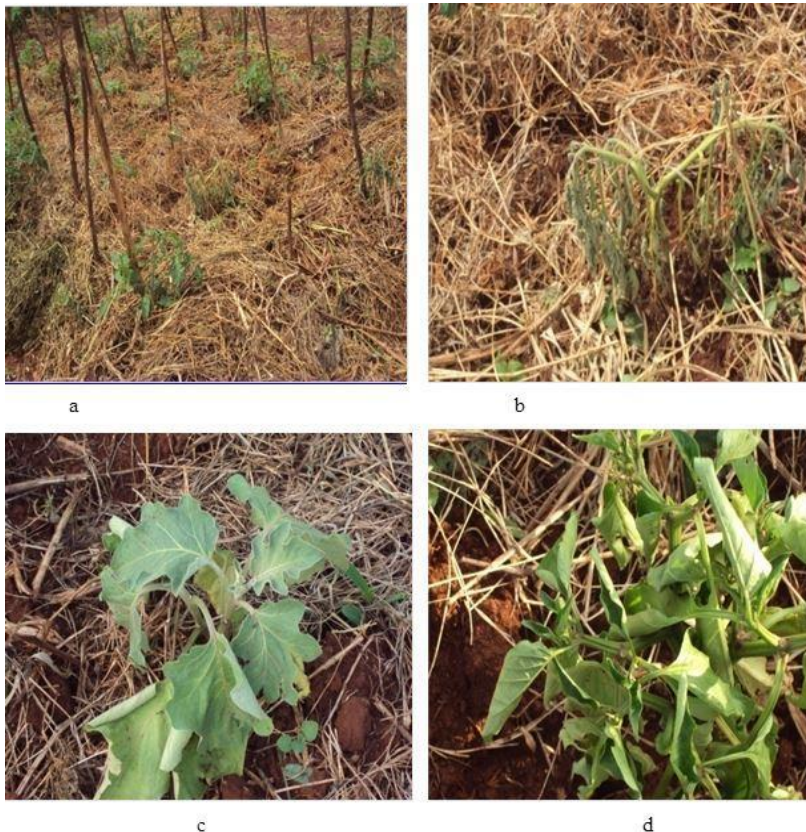
**Figure 2.** Wilted crops in the field: (a) wilted tomato plot, (b) wilted tomato plant, (c) wilted eggplant (d) wilted capsicum.

Table 5. Wilt incidence on crops planted in the field inoculated with *R. solanacearum*.

Crop	Incidence (%)	Mean difference
Potato	100	a
Tomato	100	a
Capsicum	37.9	b
Egg plant	29.3	b
Sorghum	0	c
Cabbage	0	c
Beans	0	c
Maize	0	c

Means followed by different letters are statistically different at $P < 0.05$ by Fisher's protected least significant difference test.

highest wilt incidence, leading to total plant death in all plots containing these crops. Capsicum and eggplant had wilt incidences of 40% and 30%, respectively, while beans, cabbage, maize, and sorghum exhibited no observable wilt symptoms.

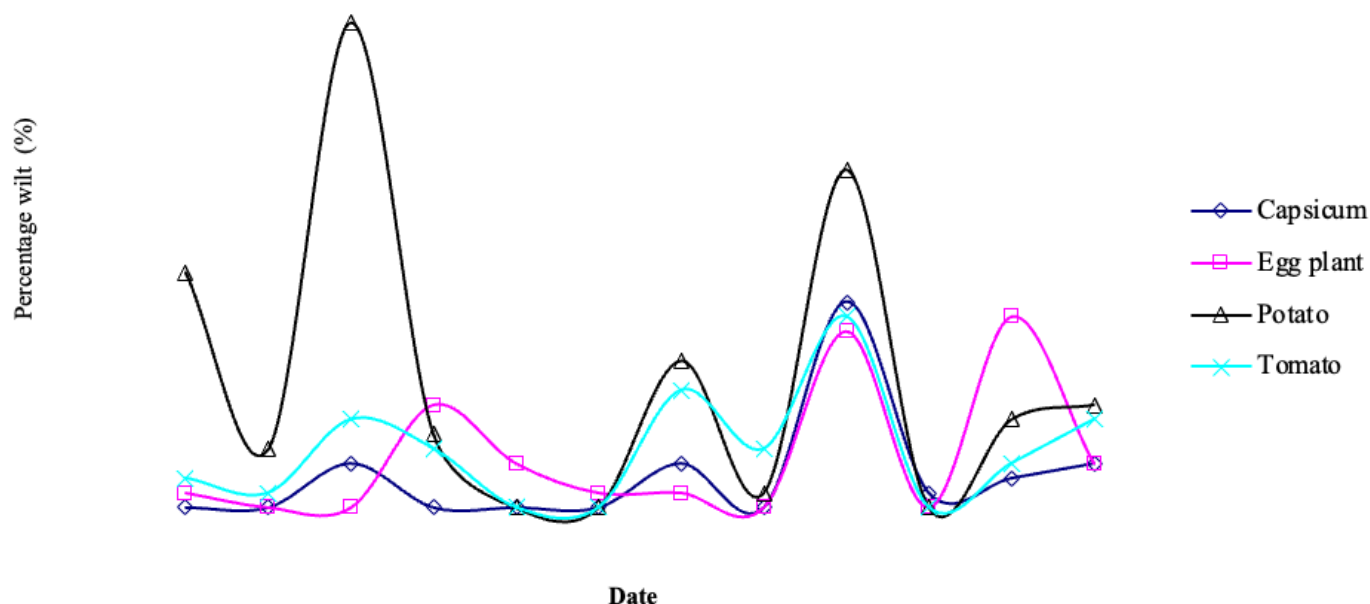
Fisher's protected least significant difference test was performed on the mean wilt incidence per plot to separate the means. The results showed no statistical difference between capsicum and eggplant in wilt incidence, although wilt symptoms were observed in both crops.

Disease progress in the field

Observable wilt symptoms were first noticed one month after planting or transplanting, with potato, eggplant, and tomato showing the earliest symptoms. An increase in the number of wilted crops was observed ten days after

the first symptoms appeared (Figure 3). When soil moisture levels increased drastically following intense rains, a general rise in the number of wilted plants was observed in plots treated and planted with potato, tomato, and capsicum, while eggplants wilted five days later.

By this time, over half of the potato plants in all plots had shown wilt symptoms. There was a slight decrease in the number of wilted crops for two weeks, after which a further increase in moisture levels following heavy rainfall caused a slight rise in the number of wilted crops in the third month. A further increase in the number of wilted plants was observed after five weeks of reduced wilting. This trend declined as soil moisture levels decreased, but it increased again toward the end of the second month, by which time all potatoes and tomatoes had died. At this point, eggplant and capsicum showed an increase in the number of wilting plants, though not all had died.

**Figure 3.** Disease progress in the field.

Latent infection

All samples taken from potato, tomato, capsicum, and eggplant comprising 50% of the tested host plants yielded positive results, showing a dark purple color similar to the dark purple expressed by the *R. solanacearum* citrate buffer positive control.

Sorghum, cabbage and beans comprising 38% of the tested host plants, exhibited green coloration indicating negative results. Half of the maize sample replicates (6%) showed negative results with green coloration, while the other half had positive results with a light purple color, compared to the dark purple color expressed by the *R. solanacearum* buffer positive control (Table 6).

Table 6. Latent infection.

Response of different host and non-host plants in ELISA test	
Crop	Result (+ positive or -negative)
Beans	-
Cabbage	-
Capsicum	+
Egg plant	+
Maize	-50% +50%
Potato	+
Sorghum	-
Tomato	0

DISCUSSION

Potential rotation crops in bacterial wilt management

This study confirms that potato and tomato crops, which are well-known hosts of *R. solanacearum* (USDA, 2003), had the highest number of wilting cases, with no significant difference between them ($P < 0.05$). Both crops experienced 100% loss to *R. solanacearum* by the end of the second month, demonstrating their high susceptibility to infection, as indicated in a study by Schaad et al. (2001). Other known host plants, such as capsicum and eggplant (USDA, 2003), exhibited less than 50% wilt incidence. This finding supports the recommendation that rotating potato and tomato crops should be avoided, as both are highly susceptible to bacterial wilt caused by either race 1 or race 3 strains of *R. solanacearum* (Janse, 1996).

Potato, tomato, eggplant, and capsicum tested positive for the presence of *R. solanacearum* in the NCM-ELISA test performed on them. This result is consistent with findings from the root invasion experiment mentioned above. The reason for this could be that these crops serve as hosts of *R. solanacearum* and are, therefore, highly susceptible to the pathogen (USDA, 2003). Beans have been reported as a host of *R. solanacearum* (USDA, 2003), although Priou et al. (1999) found that beans can be used together with maize in a rotation program to reduce *R. solanacearum* populations in the soil. However, this study did not yield similar results, possibly due to the type of ELISA test used, which is more suitable for detecting the pathogen in potato tubers (CIP, 2001).

Cabbage and sorghum, on the other hand, tested

negative for *R. solanacearum* and can, therefore, be considered the best choices for crop rotation as a control mechanism for the pathogen. This aligns with the recommendation by Priou et al. (1999), which states that the best rotation crops for reducing soil populations of *R. solanacearum* belong to the Gramineae and Brassicaceae families.

This study also confirmed that non-host plants, such as beans, maize, and sorghum, had no wilt incidences. However, they may not be effective rotational crops for controlling bacterial wilt in potatoes. Eggplant and potato had the highest *R. solanacearum* populations in their rhizospheres, with no significant difference between them ($P < 0.05$). French (1994) also recommended that eggplant, pepper, and tobacco should never be planted after a bacterial wilt-infected potato crop. This observation could be due to their high susceptibility to *R. solanacearum*, leading to increased pathogen release from infected plant parts into the soil. Alternatively, these plants may secrete substances that attract the bacteria to their roots or support the pathogen's survival by providing essential nutrients (Priou et al., 1999).

Tomato had a significantly lower rhizosphere population of *R. solanacearum* compared to potato and eggplant, despite being a known host. This could indicate that most of the pathogen population is concentrated within the vascular bundles rather than the rhizosphere.

Sorghum and beans had a lower population of bacteria in their rhizosphere compared to the first three crops, an observation that could be attributed to their non-host nature. This finding aligns with the observation that legumes, such as fabaceous crops (e.g., peas and beans), can be used as rotation crops for potatoes. Moreover, they provide the additional benefit of

increasing soil fertility by fixing atmospheric nitrogen. Additionally, bean and maize rotation has been reported to reduce soil inoculum potential (Priou et al., 1999). This study demonstrated that maize, beans, and sorghum could reduce the population of *Ralstonia solanacearum* but could not completely eradicate the pathogen from the soil, as some *R. solanacearum* cells were still detected in their rhizosphere. Other studies have shown that rotation with cereals and pastures from the *Gramineae* family can also decrease soil inoculum potential, although the time required for eradication varies by location (French, 1994). This study also found that cabbage had a significantly lower *R. solanacearum* population in its rhizosphere, likely due to its non-host nature.

When the roots of the crops under study were analyzed for the presence of *R. solanacearum* cells, a significantly high bacterial population was found in potato tubers, likely due to the crop's susceptibility to the pathogen. It has been documented that *R. solanacearum* affects more than 30 plant species, with the most susceptible crops being potatoes, tomatoes, eggplants, peppers, bananas, and groundnuts (Priou et al., 1999). Similarly, high bacterial populations were also observed in eggplant, capsicum, and tomato. *R. solanacearum* typically enters plants through natural wounds (e.g., excision of flowers, formation of lateral roots) as well as artificial wounds caused by agricultural practices, nematodes, or xylem-feeding insects. Wilting occurs due to vascular dysfunction, which prevents water from reaching the leaves (Stevenson et al., 2001). Additionally, *Ralstonia*'s systemic toxin causes stomatal dysfunction, though there is no evidence that it results in excessive transpiration. Instead, the primary factor contributing to wilting is likely the blockage of pit membranes in the petioles and leaves by the high-molecular-mass EPS1 (Elphinstone, 2005).

This study found that some non-host plants, such as maize and beans, did not exhibit wilt symptoms but still harbored the pathogen within their vascular bundles. This was particularly evident in maize, which exhibited significantly high root infestation despite being a non-host. A similar trend was observed in sorghum and beans, indicating that these crops can serve as reservoirs for the pathogen. Conversely, cabbage exhibited a negligible amount of the pathogen in its roots, making it a very safe choice for rotation with host plants to reduce *R. solanacearum* populations. This finding is particularly relevant considering that *R. solanacearum* has poor survival ability in soil. However, it can persist on the roots of alternate hosts, in undecomposed infected plant tissues, in volunteer tubers from previous crops, or in deeper soil layers where it avoids antagonistic interactions with other soil microorganisms (Wenneker et al., 1999). Eggplant, tomato, potato, and sorghum had the highest numbers of *R. solanacearum* attached to their roots compared to other crops, with no significant difference among them.

Analysis of *R. solanacearum* attachment on the roots

showed that beans and maize had very low attachment, which could be attributed to their non-host nature. However, this does not imply that these crops can be used to eradicate the pathogen from the soil, as the bacterium was still detected in their roots. These findings indicate that, despite being monocots—plants generally not known to suffer from bacterial wilt (Denny, 2006)—maize and sorghum can still encourage the survival of the pathogen by providing attachment sites on their root systems. Based on this study, cabbage remains the safest crop for rotation with host plants to eliminate the pathogen from the soil. This conclusion is supported by the insignificant amount of *R. solanacearum* detected in its root system and rhizosphere soil. Other studies have also found that more dicot species suffer from bacterial wilt than monocots, though the reasons for this differential susceptibility remain unclear (Denny, 2006).

CONCLUSION

Among all the host and non-host plants used in this study, cabbage is the only safe crop for eradicating *R. solanacearum* from the soil through crop rotation. Maize, which has been considered a safe non-host for crop rotation, was found to be less reliable, as it asymptotically supports the survival of the pathogen in its vascular bundles and accessory tissues. Beans and sorghum were also found to promote the survival of *R. solanacearum* in the rhizosphere and as attachments on their roots. The remaining plants used in the study were known host crops of *R. solanacearum* and were not effective in controlling the pathogen due to their proven susceptibility.

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