# CURRENT OCCURRENCE AND MANAGEMENT OPTIONS FOR BACTERIAL WILT CAUSED BY RALSTONIA SOLANACEARUM IN AFRICAN NIGHTSHADE KENYA

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## ABSTRACT

*Ralstonia solanacearum is responsible for bacterial wilt epidemics in a wide range of cultivated crops including solanaceae, bananas, and geraniums among others. The pathogen has the ability to thrive in wide temperature ranges enabling a wide geographical distribution but tends to be aggressive in warm and humid regions. Climatic conditions in Sub-saharan Africa are optimal for the pathogen multiplication. R. solanacearum is capable of surviving for long periods in soil, crop residues and irrigation water owing to its great metabolic adaptability. Indigenous vegetables form an important component in people's diets. African nightshades are solanaceous crops and are among the most popular indigenous vegetables. Their production is threatened by the devastating bacterial wilt pathogen R. solanacearum. Nightshades are important in food security and are a rich source of vitamins, micronutrients and roughage. Initially the vegetable was consumed by the rural poor but its popularity has risen due to its multiple health benefits. They also possess phytochemicals such as antioxidants, one of the body's defense compound against diseases. African nightshades are vital in curbing hidden hunger especially among vulnerable communities. A survey was done in selected areas in Kenya to determine the current status of the pathogen in African Nightshade farms. R. solanacearum affecting African nightshades was confirmed in various parts Western, Central and Rift valley regions. Several management options have been used including use of chemicals as fumigants and amendments, solarization, biological control with the aim of reducing the population. However, no single control method has been completely successful. The aim of this paper is to give insights to the current status of bacterial wilt and the current management options against bacterial wilt in African Nightshades in Kenya.*

**Keywords:** Ralstonia solanacearum, African Nightshade, Management

## INTRODUCTION

### *R. solanacearum* occurrence and host range

Bacterial wilt caused by *R. solanacearum* has a wide host range occurring in tropical and subtropical environments (Agrios, 2005). The first occurrence of *R. solanacearum* in potato was reported in Japan. *R. solanacearum* is classified into races, biovars in reference to the geographical distribution (Champoiseau et al., 2010). Biovar 1 occuring in USA, 3 in Asia, 2 and 5 occur in Australia and China while 4 occurs in India. In Africa, (Figure 1) bacterial wilt disease was reported in many countries (EPPO, 2014). It is distributed globally and in the absence of a susceptible host, alternative hosts or non-host plants enable survival of the pathogen (Granada and Sequeira, 1983; Hayward, 1991; Genin and Denny, 2012). The bacteria has by far potentially posed a threat to food security globally especially where epidemics are reported due to the heavy crop losses (Ravelomanantsoa et al., 2018). The



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pathogen is known to affect food crops causing lethal wilting in more than 200 plant species (Denny, 2006) with more than 450 plant species reported to be host plants (Hayward, 1991).

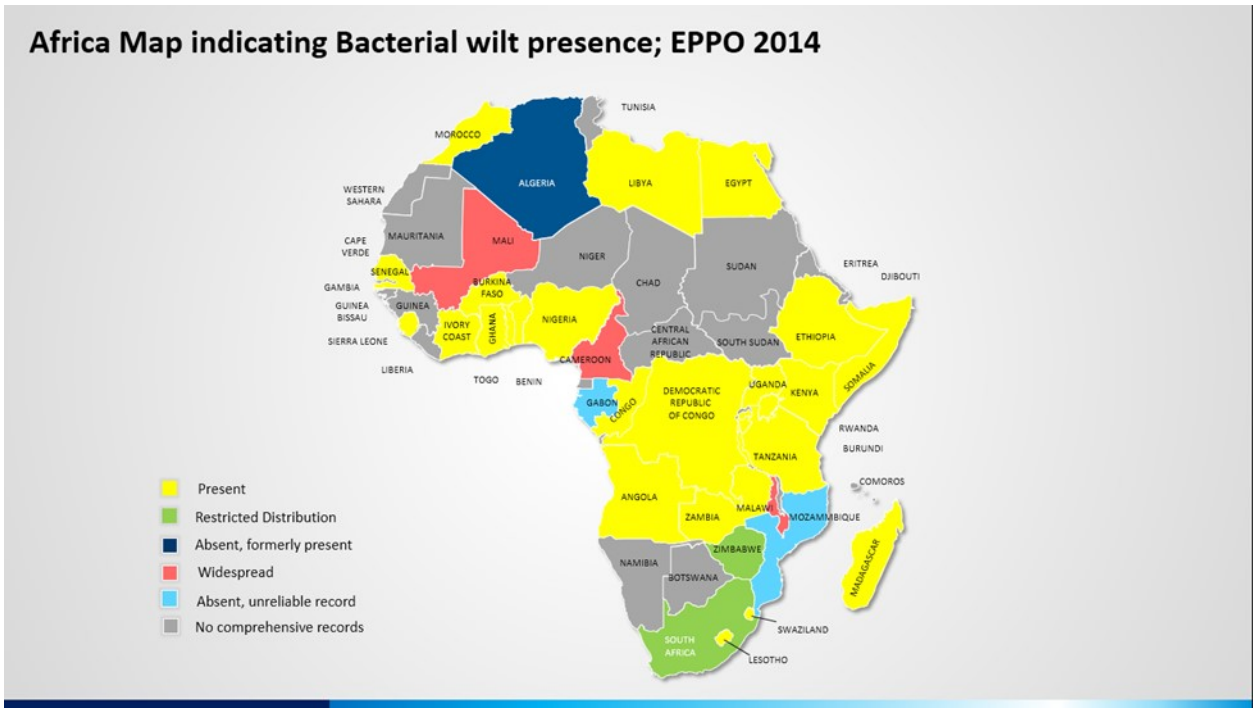


Fig 1: Map showing distribution of Bacteria wilt in Africa

### *R. solanacearum* Classification, Pathogenicity and virulence

Phenotypically, *R. solanacearum* has been historically divided into five races with regard to the particular species of plant they infect. It is further classified to six biovars depending on the pathogens ability to hydrolyze three sugar alcohols and three disaccharides (Fegan & Prior, 2005; Wicker et al., 2012). *R. solanacearum* being a soil borne, enters the plant through wounds or natural openings in the root elongation zone or at the location of developing lateral roots (Kurabachew & Wydra, 2013). The pathogen is highly tissue specific and multiplies rapidly in the xylem vessels thus clogging them resulting to disruption of smooth flow of water and eventual wilting and dying of the plant.

Expression of pathogenicity in *R. solanacearum* is controlled by a complex regulatory network that is dependent upon environmental conditions, the presence of host cells, and bacterial cell population (Schell, 2000; Genin and Denny, 2012). High *R. solanacearum* density in the rhizosphere is one of the important factors triggering bacterial wilt disease epidemics coupled with suitable environmental conditions (Wei et al., 2011). Research has indicated positive correlation of amount of inoculum and disease prevalence (He et al., 2014). Moreover, the pathogen has been found to multiply severely in warm and humid environs (Li et al., 2016) with capacity to survive in the soil for lengthy periods even in absence of a susceptible pathogen. Further, biovar (bv) 2, has been reported to survive in low temperatures (Stevens, 2010). Pathogenicity tests can be done drenching the planting media with inoculum or injecting the test plants with a needle dipped in *R. solanacearum* bacterial culture. Another method is by injuring roots and soaking them in *R. solanacearum* inoculum then planting in sterile media. Controls can be drenched, soaked or injected with sterile water. Test plants should be kept at 28°C to observe the witling symptoms.

The disease process complex involving several phases that are dependent on environmental conditions and physiological state of both the pathogen, and the host (Hayward, 1991).The success of the pathogen upon entry in the plant cells is dependent on the ability to overcome host defense mechanisms. Disease resistance comprises defense responses initiated to counter the pathogen avirulence factors. Detection of bacterial flagellin chitin, lipopolysaccharides, and peptidoglycans triggers plants defenses through recognition of Microbe/Pathogen-associated molecular patterns (Sun et al., 2017; Jones and Dangl, 2006; Zipfel, 2009). *R. solanacearum* must overcome the host defense mechanisms to cause disease. The pathogen employs an intricate network that directs the expression of the various pathogenicity factors (Schell, 2000). Type-III secretion system (T3SS) of *R. solanacearum* has a key function in virulence by enabling the pathogen invade the host. Some strains are reported to have more than 70 T3SS now designated Rips (Ralstonia-injected proteins), (Peeters et al., 2013). Extracellular polysaccharide (EPS) is another key virulence factor of *R. solanacearum*. It is hypothesized that the role of EPS is physical obstruction of the flow of water in the xylem of infected plants or that EPS covers the bacterium thus preventing host plant recognition thus defense mechanisms are not initiated (Milling et al., 2011).

**Detection of *R. solanacearum***

Detection of *R. solanacearum* is important for disease diagnosis and research. Several methods have been employed ranging from physical, biochemical and molecular. Isolation of the bacteria can be from soil, plant tissues and irrigation water. Initial detection can be done visually through looking out for symptoms of wilt in crop fields (Figure 2b). Vascular discoloration is also observed when a longitudinal cross section is done in infected plant stems (Figure 2c). Further tests are required to confirm presence of the pathogen. Bacterial streaming test (Fig 2a) is usually one of the initial tests carried out on suspected tissue samples (IPDN, 2014). The result is usually positive for plants having heavy infestation indicating a dirty cream ooze once a cross section of the stem is suspended in a beaker with clean water.

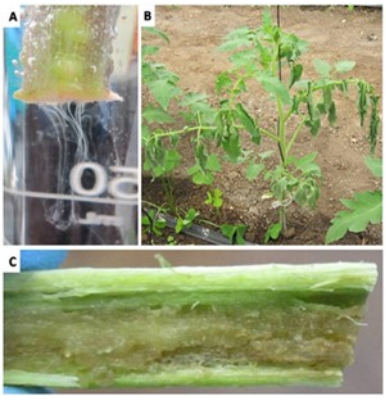


Figure 2: (a) Streaming test, ( b) Wilted tomato plant, (c) Damaged vascular tissues

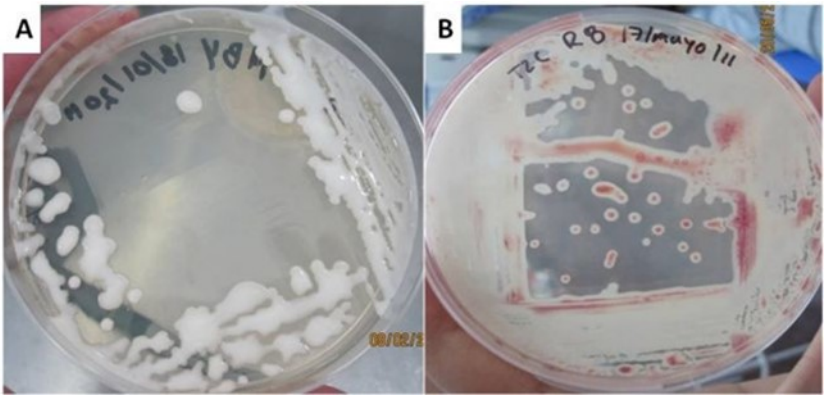


Figure 3: Plates showing avirulent (a) and virulent (b) strains of *Ralstonia Solanacearum*

Further confirmatory tests need to be done on plant tissues. The samples are prepared by washing under running water and sterilizing them with 70% ethanol or 0.5% sodium hypochlorite for approximately 2 minutes. The tissues are then rinsed with sterile distilled water, isolated aseptically and cultured on selective media. Soil samples are suspended in water and then a sample drawn for serial dilution. The isolated suspensions are then inoculated in selective or semi-selective media. This media works by suppressing or



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preventing growth undesirable microorganisms while allowing multiplication of the target microorganism. These media include Kelman's tetrazolium chloride medium (TZC), Casamino acid Peptone Glucose medium (Kelman, 1998) and Semi selective Media South Africa (Englebrecht, 1994). The results after incubating for 48 to 72 hours at 28-30 degrees Celsius are fluidal cream colonies with pink centers (Figure 3).

Biovar determination is based on ability of the *Ralstonia solanacearum* strains to hydrolyze disaccharides (maltose, lactose, and cellobiose) and hexose alcohols (mannitol, sorbitol, and dulcitol). The protocol was described by Hayward 1991. The basal medium ($NH_4H_2PO_4$ 1.0 g, KCl 0.2 g, $MgSO_4.7H_2O$ 0.2 g, Difco bacto peptone 1.0 g, Agar 3.0 g, and Bromothymol blue 0.03 g per liter) containing 1% of each type of sugar. About 200 µl of the melted medium is dispensed into the wells of a microtitre plate. Bacteria inoculum suspension of Optical Density + 0.05 at 600nm is prepared in sterile distilled water from 2-day-old cultures. For the determination of biovars 20 µl of bacterial suspension is added in the microtiter plate and incubated at 28-32$^0$C. The microtitre wells are examined 3-6days after inoculation for color change (Schaad et al., 2001). Oxidation results in production of an acid which changes the medium from green to yellow. Huang et al., (2011) enhanced the biovar test by using phenol red as a pH indicator that changes color at a higher pH when a carbohydrate is utilized. The colour changes from red to yellow upon oxidation. This method significantly reduces cost and time while improving efficiency.

Serological methods such as enzyme linked immunosorbent assays (ELISA) and immunofluorescent (IF) antibody staining are utilized for large numbers of samples and are not very expensive. However, these techniques are only effective with reasonably high bacterial population exceeds $10^4$ cfu/g soil(Pradhanang et al., 2000; Arslan et al., 2014).

Molecular techniques are credited for good understanding of *R. solanacearum* (She et al., 2017). DNA-based means including PCR or real-time PCR using specific primers (Fegan et al., 1998) have been very important. Polymerase chain reaction (PCR) due to being highly sensitive and specific has been used in quantification of *R. solanacearum* in soil (Inoue & Nakaho, 2014). Sequence analysis has been employed in classification of the pathogen into Division I and Division II through Restriction fragment length polymorphism (RFLPs) on the *hrp* gene region and 16S rRNA (Cook et al., 1994; Poussier and Luisetti, 2000). Further, Two more groups of strains were identified by fingerprinting analyses using amplified fragment length polymorphism (AFLP) and polymerase chain reaction RFLP (PCR-RFLP) techniques on targeting the HRP cluster gene and 16S rRNA gene (Poussier et al., 2000a) intergenic spacer region (ITS) between the 16S and 23S rRNA genes, and endoglucanase and *hrpB* genes (Fegan et al., 1998; Poussier et al., 2000b).

**African Nightshade**

African Indigenous Vegetables offer the most affordable source of macro and micronutrients in Kenya and are a rich source of important vitamins such as A, B, and C, and minerals including calcium, iron, and potassium (Uusiku et al., 2010). Initially, they were considered food for the rural poor but their benefits have led to promotion of their utilization (Kebede and Bokelmann, 2017).They contribute to reducing micronutrient deficiencies, responsible for “hidden hunger”, a major hindrance to the current food security status (FAO, 2010). A healthy population is necessary for contribution to economic growth. However, according to Keatinge et al., (2011) increasing lack of vegetable consumption worldwide has been the cause of serious deterioration of human health and resulting to hampering attainment of the Sustainable Development Goals (SDGs). This is most importantly felt in Sub Saharan Africa where malnutrition due to minimal or no consumption of fruits and vegetables is rampant.

African Indigenous vegetables offer vital supplements to diets which are mostly heavy in staples.

African Nightshades have increasingly become important for commercial purposes in Kenya over the recent past with many markets and groceries across the country having them as a major vegetable for sale (Mwaura et al., 2013). It was estimated that in central Kenya, about 9000 tonnes of nightshades and other indigenous vegetables were sold in formal and informal markets between 2008 and 2010 (AVRDC, 2010). This gives an indication of their importance with capacity to support rural, peri-urban and urban populations for income generation. Nightshade cultivation attracts many farmers because they do not require heavy capital investments and can be intercropped with other crops (DFID and R4D, 2010). Furthermore, nightshades are one of the significant African Indigenous Vegetables (AIV) that are important for gender empowerment. Women are majorly involved in all aspects of the many farm produce supply chains (Weinberger et al., 2011).

African nightshades despite their importance have a major challenge, the bacterial wilt caused by *Ralstonia solanacearum*. Nightshades belong to the Solanaceae family together with a wide host range of many other economically important crops also affected by the pathogen such as potato, tomato, eggplants, pepper and tobacco (Elphinstone, 2005). The aggressiveness of the disease is dependent on various biotic and abiotic factors including susceptibility of the host, temperature, moisture and root wounding (Ishihara et al., 2012; Jacobs et al., 2012; Wei et al., 2015).

**Bacterial wilt affecting African Nightshade in Kenya**

**Methodology**

A survey was done during the short rains season September to December 2016 in Central (Gitaru, Kerugoya and sabasaba), Western (Kanduyi and Mayanja) and Rift valley (Kipkaren) regions of Kenya. The aim was to determine the importance as well as major constraints in production of African Nightshade. A structured questionnaire used to get information on African nightshade production practices, the production challenges, presence of bacterial wilt and management practices carried out. Farm Assessment was done to determine of disease incidence in the farms. (Scale: 1-BW present in farm, 0-BW absent in farm). Initial diagnosis was done using the bacterial streaming test. Soil and tissue samples were collected in bags sealed and labelled for laboratory isolations to confirm pathogen presence.

**RESULTS**

The survey indicated that germplasm of African nightshades cultivated in the surveyed areas were classified as either the broad-leafed or narrow-leafed. The broad leafed was more preferred by many farmers though they said the narrow leafed fetch better prices in the market. African nightshades ranked high in importance among other local and exotic vegetables. Among the constraints affecting African Nightshade production pests and diseases ranked the highest (Graph 1).



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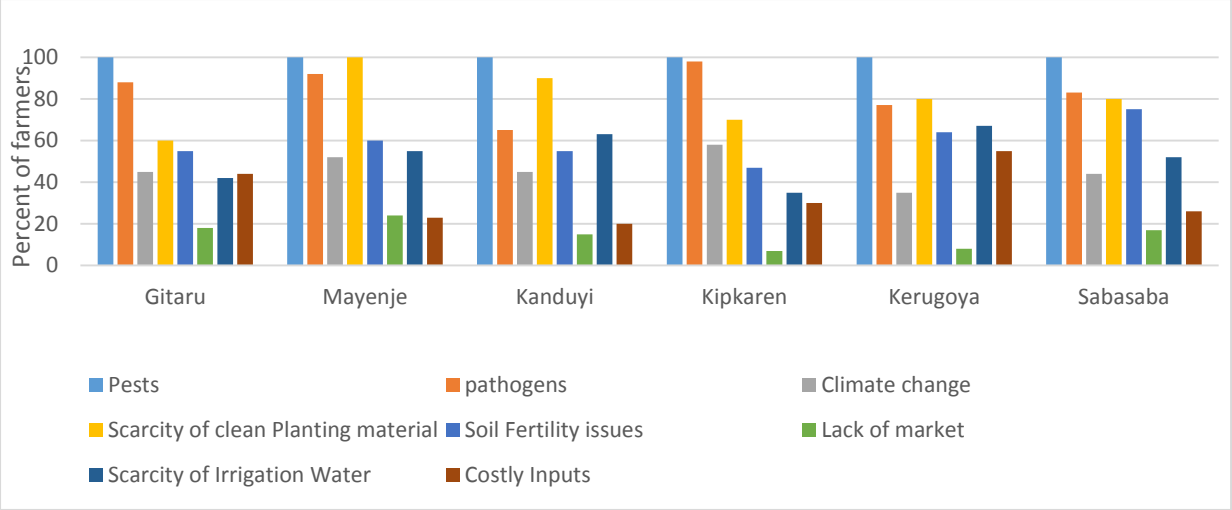


**Graph 1: Constraints affecting production of African nightshade in selected parts of Kenya**

**Figure 4: Wilted African nightshade plants.**

Bacterial wilt was observed in the survey areas (Figure 4). Among the various study areas, farms Kipkaren in Rift valley region indicated the highest disease incidences (Table 1).

**Table 1. Bacterial wilt disease incidence in study areas**

| Survey Area | Nightshade | Potato | Tomato | Other |
|---|---|---|---|---|
| Gitaru | $0.10\pm0.19^{a}$ | $0.70\pm0.19^{b}$ | $0.30\pm0.19^{a}$ | $0.20\pm0.19^{a}$ |
| Mayenje | $0.40\pm0.19^{b}$ | - | $0.20\pm0.19^{a}$ | - |
| Kanduyi | $0.30\pm0.19^{ab}$ | - | $0.10\pm0.19^{a}$ | - |
| Kipkaren | $0.50\pm0.19^{b}$ | - | $0.20\pm0.19^{a}$ | - |
| Kerugoya | $0.10\pm0.19^{a}$ | $0.50\pm0.19^{ab}$ | $0.80\pm0.19^{b}$ | $0.30\pm0.19^{a}$ |
| Sabasaba | $0.10\pm0.19^{a}$ | $0.40\pm0.19^{a}$ | $0.20\pm0.19^{a}$ | - |
| **P** | **0.144** | **0.703** | **0.006** | **0.409** |

Data are the mean ± standard error (SE). Means separated using LSD test, means within the column followed by the same letter are not significantly different at P<0.05. (-) indicates the crop is not grown in the farms surveyed. Other includes crops susceptible to *R. solanacearum*

## Control Methods

Managing *R. solanacearum* has been difficult due to the diversity and complexity of the pathogen. This is especially because of its capacity to grow latently, survive in deep soil layers for long periods, mobility in water ways, and having weeds as alternate hosts (Wang and Lin, 2005). No single management practice thus far has shown satisfactory result in locations where the disease is endemic. However, most researchers have shown the option of reducing the populations of *R. solanacearum* being most viable. This includes controlling bacterial wilt using various methods, such as the use of biocontrol agents with strong inhibition against *R. solanacearum* (Tan et al., 2013; Yuan et al., 2014), the application of compost (Schonfeld et al., 2003), and changing the soil pH (Niwa et al., 2007; Wu et al., 2014).

## Chemical Control

Globally, disease control has been based on commercial Pesticides. Use of chemical bactericides is one of the traditional methods of disease control. However, this method is not popular due to association with environmental pollution and adverse effects that they have on the environment (Fujiwara et al., 2011; Tan et al., 2015). In management of bacterial wilt, pesticides such as algicide (3-[3-indolyl] butanoic acid), fumigants (metam sodium, 1,3-dichloropropene, and chloropicrin) have been used. Enfinger et al., (1979) found that Chloropicrin was effective in reducing populations of *R. solanacearum*. However, use of chloropicrin is regulated due to its toxic and carcinogenic properties. In the past, Methyl bromide was commonly used as a fumigant (Champoiseau et al., 2010) but has been banned in many countries. Antibiotics have been used to manage crops against pathogenic bacteria such as Xanthomonas, Pseudomonas and Erwinia (McManus et al., 2001). Action of Streptomycin and tetracycline antibiotics is through inhibiting protein synthesis. Streptomycin causes irreversible binding to bacterial ribosomes whereas Tetracyclines bind reversibly to bacterial ribosomes (McManus et al., 2001). Antibiotic sprays were used extensively over the years but many pathogen populations have developed resistance resulting to decline in their use (Sundin et al., 2016).

## Cultural practices

These include practices like crop rotation, polyculture, sanitation, manipulation of planting dates among others. These play a key role in disease management (Palti, 1981). Crop rotation helps in managing crop diseases through reducing pathogen population especially of soil borne pathogens (Janvier, 2007). Rouging of diseased plants also help in managing disease epidemics. Burning of crop residues is also eradicates pathogens in the host crop. Sanitation is also important in managing crop diseases through eliminating undesirable crops that can act as alternate hosts. The measures are usually economically feasible. However, control achieved via cultural practices is often inadequate and need to be supplemented with other methods.

## Soil amendment

Application of organic and inorganic fertilizers has been known to effectively suppress activity of the bacterial wilt pathogen. Fine biochar has been found to significantly decrease bacterial wilt incidence. NPK fertilizer was found to reduce bacterial wilt and increase yield (Lemaga et al., 2005). This was successful due to the ability of biochar ability to adsorb to the



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pathogen directly and indirectly via adsorption of root exudates thus interfering with pathogen chemotaxis (Gu et al., 2017). Calcium (Ca) fertilizer has been shown to effectively reduce bacterial wilt incidence and severity. Increased concentration of Ca in the stems of tomato plants significantly reduced the population of *R. solanacearum* (Yamazaki et al., 2000). Further, increase in concentration of Ca ions significantly decreased pectinase activity of *R. solanacearum* (He et al., 2014) thus making it difficult for the pathogen to degrade the cell wall and gain entry into the plant. A combined amendment of rock dust and commercial organic fertilizer significantly reduced the incidence of bacterial wilt in the tomato (Li and Dong 2013). High soil pH and Ca were important factors in management of bacterial wilt by the rock dust amendment.

**Biological control**

Use of Biological control agents (BCAs) and organic matter is on the increase due to the issues associated with use of chemicals. The modes of action of BCAs are characterized by various interactions, such as the competition for nutrients and space, antibiosis, parasitism, and induced systemic resistance (Yuliar et al., 2015). However, the performance of BCAs is hindered by some difficulties, which are associated with the production, storage, and subsequent application of BCAs (Singh et al., 2015; Yuliar et al., 2015). Ability to effectively colonize and survive in the rhizosphere is a condition for strains of antagonistic microorganisms to suppress soilborne diseases (Yuan et al., 2014). Organic fertilizers have been found to supply adequate energy and nutrients for antagonists to improve the suppressive capacity towards pathogens (Sullivan, 2001). Bioorganic fertilizers alter the soils physicochemical and biological properties (Liu et al., 2015) thus improving suppressive capacity of the soil towards bacterial wilt. Many previous studies reported that bacterial wilt was suppressed by organic matter. To suppress bacterial wilt, plant residues (80%) such as fresh plant materials, plant extracts, isolated compounds, and essential oils, have been most commonly used, followed by animal wastes (10%), and simple organic compounds (10%) (Yuliar et al., 2015). Plants contain abundant bioactive materials, such as secondary metabolites, volatile oils, and essential oils that can be exploited to develop new biopesticides (Bhagat et al., 2014). In the development of novel pesticides, secondary metabolites could be used as lead compounds as they have novel modes of action (Bourgaud et al., 2001; Dubey et al., 2011). The possible mechanism of action of plant residues primarily includes antimicrobial activities. The plant residues also suppress pathogens indirectly by improving the physical, chemical, and biological soil properties. Presently, only a few isolated compounds have been used to control tomato bacterial wilt *in planta* or in field conditions (Li et al., 2016).

**Resistance**

Host resistance is considered a useful option for managing bacterial wilt since it is the most environment friendly and effective method to control bacterial wilt. Resistance in many solanaceous plants is quantitative due to many genes contributing to minimal resistance (Thoquet et al., 1996). This results to resistance being strongly influenced by environmental conditions such as soil temperature, pH, and moisture. In Arabidopsis, resistance is governed by a major gene RRS1-R. Several QTLs for resistance to *R. solanacearum* have been mapped, *Bwr-12*, effective against phylotype I (Asian) strains is located in a 2.8-cM interval of chromosome 12, was found to be responsible for 18–56 % of the total resistance (Wang et al., 2013).

Breeding for resistance is a complex process dictated by factors such as the availability and diversity of resistance sources, agronomic qualities, variability of the pathogenic strains and

plant-pathogen interactions (Elphinstone, 2005; Boshou, 2005). Additionally resistance being quantitative, it is typically strain specific, and the diversity of pathogenic strains of *R.solanacearum* has led to the development of resistant lines that are not durable over diverse geographic regions. Another issue that has been problematic for tomato breeders is that small fruit size is linked to resistance to bacterial wilt. Resistant plants according to Prior *et al.* (1996) showed heavy infestation by *R. solanacearum* but without visible symptoms. Additionally, Nakaho *et al.* (2004) indicated suppressed multiplication of bacteria as a result of restricted pathogen movement within the xylem tissues. However more effort in breeding is necessary because resistance to bacterial wilt in many crops has generally been negatively correlated with yield and quality.

Induced resistance focuses on increasing capacity of the cell wall to impart resistance against pathogens. Silicon is considered to be a beneficial element for plants and higher animals (Epstein, 1999). Kiirika *et al.* (2013) reported that the combined application of silicon and chitosan reduced the incidence of bacterial wilt in the tomato by inducing resistance. Strains of specific plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* have been shown to differentially suppress diseases by induced systemic resistance (Ran et al., 2005). Additionally, plant activators including validamycin A and validoxylamine have been known to induce systemic resistance on tomatoes (Pradhanang et al., 2005; Yuliar et al., 2015). Recently, Silicon has been determined to enhance resistance of crops thus aid in managing several pests and diseases in various plant species. Additionally, silicon assists in various abiotic stresses including salt stress, nutrient imbalance, high temperature, freezing among others (Ma J.F., 2004). Silicon acts as a modulator influencing plant defense responses and interacting with key components of plant stress signaling systems leading to induced resistance. Silicon and chitosan have been confirmed to induce resistance to tomato against bacterial wilt (Kiirika et al., 2013).

### Genetic engineering

This provides an opportunity to salvage crops that are important in food security from virulent disease epidemics. Further, it can reduce crop producers' dependence on chemicals. Successful research has been done for example, the *Arabidopsis* NPR1 (non-expresser of *PR* genes) gene was introduced into a tomato cultivar. The research found that wilt incidence was reduced by 70% (Lin et al., 2004). Strategies adopted in Genetic engineering include; Improving host recognition mechanisms as a result of infection, mining R genes, advancing host defense pathways, disarming hosts susceptibility genes, silencing pathogen virulent genes and gene editing (Vincelli, 2016) among others.

## RECOMMENDATIONS

The pathogen *R. solanacearum* on African nightshade needs to be studied in depth due to its capacity to cause disease epidemics. Molecular studies are important in assessing interactions of the pathogen in African nightshades. Induced resistance is a promising method in management of bacterial wilt however further research is needed to determine effectiveness in varying weather conditions. This will form a basis for improving management of bacterial wilt epidemics.

### Disclosure of conflict of interest

The authors declare there is no conflict of interest.

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