Original Research Article

Effect of selected soil amendments on the incidences of *Ralstonia solanacearum* isolates in greenhouse on selected solaneceous crops

Comment [Anon1]: Control of *Ralstonia solanacearum* on selected solaneceous crops in greenhouse by selected soil amendments

ABSTRACT

Aims: The aim of this study was to establish the effect of selected soil amendments on ~~the incidences of~~ *Ralstonia solanacearum* isolates in greenhouse on selected solaneceous crops.

Study design: The study was laid out as randomized complete block design (RCBD) in split pot arrangement for two seasons in the greenhouse.

Place and Duration of Study: The experiment was carried out in Kenyattta University situated in Kiambu County about 20 km from Nairobi city along Nairobi-Thika road between July, 2017- September, 2017 and between November, 2017- January, 2018.

Methodology: The three host crops of interest (potatoes, tomatoes and capsicum) were inoculated with prepared pure bacterial isolates; 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land), 83 (2T-Kirinyaga-Highland) and MX (18/71/67/83). The experiment had a total of 450 pots having a total area of 6.93 M^2. The treatments were Chalim^{TM}, Super-hydro-grow polymer + Metham sodium, Metham sodium, Metham sodium & Orange peel, Super-hydro-grow polymer, Brassica tissues, Chalim^{TM} + Super-hydro-grow polymer, Brassica tissue + Orange peel, Metham sodium + Super-hydro-grow polymer and Control (no amendments).

Comment [Anon2]: ????

Results: There ~~was~~ were significant differences ($P \leq 0.05$) in the bacterial wilt incidences in selected solaneceous crops between control and all the soil amendments used in season 1 and 2. Brassica tissue + Super-hydro-grow polymer was superior in reducing bacterial wilt incidences in selected solaneceous crops in all the *R. solanacearum* isolates from Kenyan highlands and lowlands both in season 1 and 2.

Conclusion: Organic and inorganic soil amendments could serve as a viable control of bacterial wilt in solaneceous crops caused by *R. solanacearum* in *the* greenhouse.

Key: Bacterial wilt, incidences, Ralstonia solanacearum, solaneceous crops

1. INTRODUCTION

The increasing global population coupled with the challenges of environmental degradation and an increasingly variable climate have created a world-wide need for improved food security [1, 2]. Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the most important constraints in production of vegetables in the tropical and sub-tropical regions [3]. The pathogen *R. solanacearum* is widespread in tropical, sub-tropical and warm temperate regions and infects more than 450 plant species in 54 families [4, 5]. *R. solanacearum*, the causal agent of bacterial wilt disease, is considered one of the most destructive bacterial pathogens due to its lethality, unusually wide host range, persistence and broad geographical distribution ~~Wei *et al.*~~ [6]. *R. solanacearum* is a diverse species that differs in host range, geographical distribution, pathogenicity and biochemical and physiological properties [7]. This pathogenic species has been divided into five races based on host range [8] and six biovars based on metabolic profiles, related to the ability to metabolize three sugar alcohols and three disaccharides. These globally dispersed and heterogeneous strains cause loss of productivity of many crops, which have major socio-economic impacts [9]

Tim *et al.* [10] and Joshi *et al.* [11] reported that bacterial wilt disease is affected by environmental conditions like soil temperature, soil moisture, and soil type (which influences soil moisture and microbial populations).The bacterium enters host plant roots from the soil and colonizes the xylem vessels in the vascular system [12]. Infected plants suffer yellowing, stunting and wilting, and often die rapidly [13]. Symptoms of *R. solanacearum* on tomato include wilting and necrosis as well as vascular browning [14]. Typically, stem and tuber cross-sections ooze whitish bacterial exudates [15]. Ramesh *et al.* [16] further reported that *R. solanacearum* infection could spread through contaminated water and weeds in the Solanaceae family. *Ralstonia solanacearum* can be disseminated by farm implements, pollinator insects in banana, irrigation water, infested soil, plant debris, latently infected vegetative propagation materials (such as *Pelargonium* cuttings, potato tubers and banana corms) and through roots damaged by nematodes [17, 18]. Latent infection is widespread and has been identified in several asymptomatic host plants including tomato, geranium, squash, and potato [19, 20]. An incidence of 55 % and 25 % has been recorded on chili and potato crops respectively from the major chili and potato producing regions of Ethiopia [21]. Assefa *et al.* [22] (2015) reported that~~,~~ ~~in Ethiopia~~ the percentage wilt incidence of bacterial wilt in Ethiopia was as high as 63 % on potato, 55 % on tomato and 100 % on pepper. Singh *et al.* [23] observed in a study in India that crop losses of up to 90% ~~was~~ were reported in the greenhouse compared to losses of 25-60 % reported for open field tomato.

Bacterial wilt control in various pathosystems has been possible through use of a combination of diverse methods such as~~,~~ host resistance, biofumigation, fertiliser application, soil solarisation, biological control, chemical control, ~~other~~and other cultural practices and integrated disease management schemes [24,25]. However, traditional control measures are not always effective in the control of bacterial wilt, such as the application of bactericides, disease-resistant cultivars and crop rotation [26], thus making it compelling to find a potential soil-borne disease control method to reduce economic loss. Synthetic chemicals have been used for many years to control agricultural biological agents, however, considerable problems have arisen from the continued application of these chemicals, including development of resistance by the pathogen, high cost, residual effect on soil, pollution of the environment and hazard from handling toxic compounds [27]. The use of soil amendments (SAs) is a widespread means to control soil-borne disease. It has been reported that an SA, composed of urea and calcium oxide (CaO), is effective to control the bacterial wilt of tomato by affecting the pH and nitrite accumulation in the field [28, 29]. Calcium carbonate ($CaCO_3$) could not only serve as a soil amendment to change soil pH but also increase soil Ca^{2+} content. Polymers are widely used for many applications in agriculture: to combat viruses and other crop pathogens, and functionalized polymers are employed to increase the efficiency of pesticides and herbicides, allowing the application of lower doses and thus indirectly protecting the environment [30]. Amendments provide energy and nutrients to soil, drastically changing the environment for the growth and survival of crops and microorganisms [31]. In the current study, the aim of this study was to establish the effect of selected soil amendments on ~~the incidences of~~ *R. solanacearum* isolates in greenhouse on selected solaneceous crops. This current study will help to reduce bacteria wilt in very important selected solaneceous crops by using organic and inorganic amendments.

2. MATERIAL AND METHODS

2.1 Study Area

The experiment was carried out in Kenyattta University situated in Kiambu County about 20 km from Nairobi city along Nairobi-Thika road. The county enjoys a warm climate with temperatures ranging between 12°C and 18.7°C. The rainfall aggregate for the county is 1000 mm each year. Its geographical coordinates are 1° 10' 0" South, 36° 50' 0" East. Low fertility soils are mainly found in the middle zone and the eastern part of the county which form part of the semi-arid areas. The soils in the midland zone are dissected and are easily eroded. The soils are sandy or clay and can support drought resistant crops such as soya beans and sunflower as well as ranching. The elevation of the main campus is 1720 meters above sea level (ASL) [32].

2.2 Eperimental Design and Treatments

The experiment was carried out between July, 2017- September, 2017 and between November, 2017- January, 2018 and was replicated three times for the two seasons. The experiment was laid out in randomized complete block design (RCBD) in split plot arrangement in the greenhouse. Potatoes, tomatoes and capsicum were planted in pots each with a radius of 0.07 M (area 0.015 M^2). The experiment had a total of 450 pots having a total area of 6.93 M^2. The treatments were Chalim^{TM}, Super-hydro-grow polymer and Metham sodium, Metham sodium +Orange peel, Super-hydro-grow polymer, Control, Brassica tissue, Chalim^{TM} + Super-hydro-grow polymer, Brassica tissue + Orange peel and Metham sodium + Super-hydro-grow polymer. The three host crops of interest (potatoes, tomatoes and capsicum) were inoculated with prepared pure bacterial isolates; 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land), 83 (2T-Kirinyaga-Highland) and MX (18/71/67/83). All agronomic practices including, watering, fertilization, weeds, pests and disease control were well managed.

Comment [Anon3]: What is "M"? do you mean metre (m) ? -> change for the entire manuscript

2.3 Preparation of Pot Soil Amendments

Fresh leaves of cabbage plant residues were finely chopped and incorporated into the soil at the rate of 0.01 kg per pot of an area of 0.015 M^2. The inoculated soil was thoroughly mixed with the finely chopped cabbage plant residue, ensuring that all the residues were well incorporated in the soil pot. Metham sodium, a chemical fumigant was applied in pot of an area of 0.015 M^2 at the rate of 3.08 ml per pot. This was the positive control. Chalim™ effect was assessed in the inoculated pots after application at the rate of 0.3 g per pot of an area of 0.015 M^2. Super-hydro-grow polymer was applied in plot of 0.015 M^{2}at the rate of 0.0003 ml per pot using knap-sack sprayer. Combination of Chalim^{TM} + Super-hydro-grow polymer was applied at the rate of 0.3 g per pot and 0.0003 ml per the same pot of an area of 0.015 M^2 respectively. Metham sodium + Super-hydro-grow polymer was applied in a pot of an area of 0.015 M^2 at the rate of 3.08 ml per pot and 0.0003 ml per the same pot respectively.

Metham sodium +Orange peel treatment was applied in a pot of an area of 0.015 M^2 at the rate of 3.08 ml per pot and Orange peel rate of 0.01 kg per the same pot respectively. Brassica tissue + Orange peel treatment were applied at a rate 0.01 kg per pot of an area of 0.015 M^2 and Orange peel at a rate of 0.01 kg per the same pot respectively.

2.4 Greenhouse inoculation

The positively identified potato tubers and stem tubers of capsicum and tomato were used to isolate *R. solanacearum*. The five pure bacterial isolates were 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land), 83 (2T-Kirinyaga-Highland) and MX (2T-L/2A-L/2A-H/2T-H(18/71/67/83). The bacteria was purified, multiplied and then harvested to make a composite bacterial inoculum. Pure bacterial was harvested (30 plates per plant sample) into a 10 L of sterile distilled water to make composite bacterial inoculate to be sprayed in 450 pots. The experimental plant pots was performed in glasshouse where the 450 pots were inoculated each with 10 mL of 3.05 x 10^9 cfu/mL of *R. solanacearum* isolates 18, 71, 67, 83 and MX. Metham sodium, a known fumigant was used as a positive control. Randomized complete split plot design was used in the pot layout.

Comment [Anon4]: how were the isolated multiplied?

2.5 Data Collection and Analysis

Three sample crops (tomato, capsicum and potato) were used. The plants were rated weekly, each Wednesday for bacterial wilt disease incidence from the 18th day after planting where wilted plants were uprooted upon total foliage wilt and recorded though only the incidence at 4th, 7th and 10th weeks after planting (WAP) was considered for evaluation. For proper key diagnostic identification of *R. solanacearum* in the field and to distinguish bacterial wilt from vascular wilts caused by fungal pathogens, bacterial wilt symptoms was identified by visual observation of typical bacterial wilt disease symptoms such as wilting, vascular discoloration, bacterial streaming in glass of water and browning of the vascular bundles of the tuber. Milky white strands containing bacteria and extracellular polysaccharide was oozed out from the cut ends of the xylem. The diseased samples were brought to the laboratory and subjected aseptically for detection and confirmation of *Ralstonia solanacearum*. The assessment of the incidences of *R. solanacearum was* estimated using the formulas as adapted from Mwaniki *et al.,* (2016).

Comment [Anon5]: Not in ref list

$$Disease\ incidence = \frac{\text{Total asymptomatic Plants}}{\text{Total Plants per area}} \times 100\%$$

2.8 Data analysis

Data that was obtained from soil amendments effect of incidences of *R. solanacearum* on selected solaneceous plants was statistically analyzed by statistical package for social sciences (SPSS) software for Windows, ver. 23 (SPSS, IBM, USA). Chi-square was done to measure the strength of associations between variables. A p-value of <0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Influence of Organic and Inorganic Soil Amendments on Disease Incidence on Potatoes

The results of incidences of bacterial wilt on potatoes grown under greenhouse for season 1 and 2 are shown by figure 1 and 2 respectively.

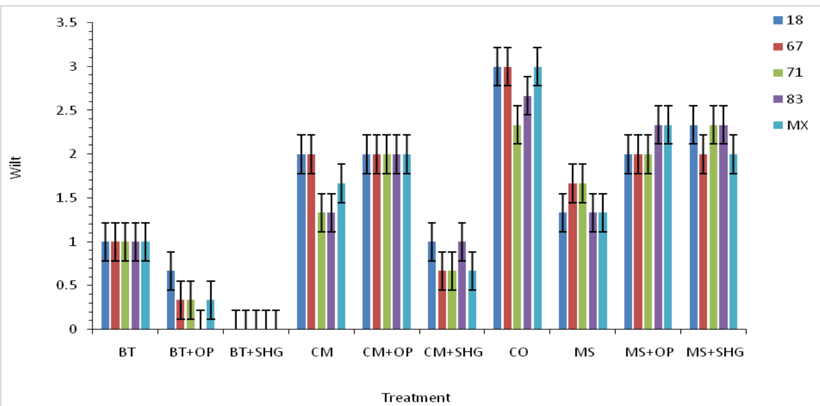
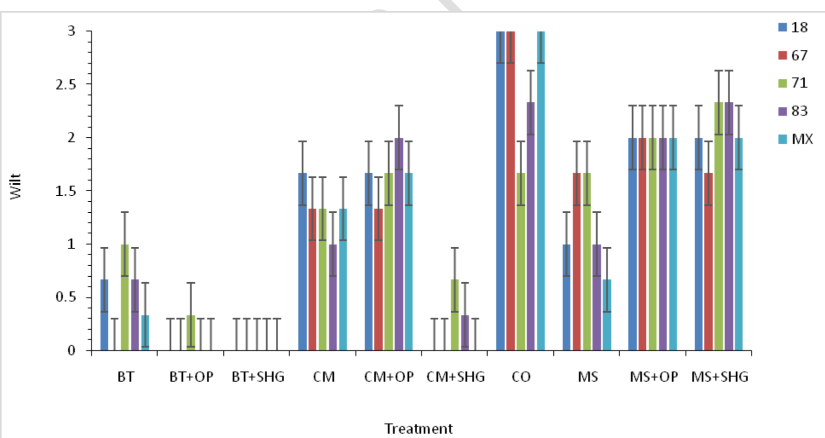


Figure 1. Greenhouse Bacterial wilt incidence in potatoes season 1

[1]



[1] *BT-Brassicae Tissue, BT+OP -Brassica tissue+Orange peel, BT+SHG- Brassicae Tissue+ Super-hydro-grow polymer, CM- Chalim^{TM}, CM+OP- Chalim^{TM}+ Orange peel, CM+SHG- Chalim^{TM}+ Super-hydro-grow polymer,MS- Metham sodium, Ms+OP- Metham sodium+ Orange peel, MS+SHG- Metham sodium+ Super-hydro-grow polymer : 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land),83 (2T-Kirinyaga-Highland) and MX (2T-L/2A-L/2A-H/2T-H(18/71/67/83).*

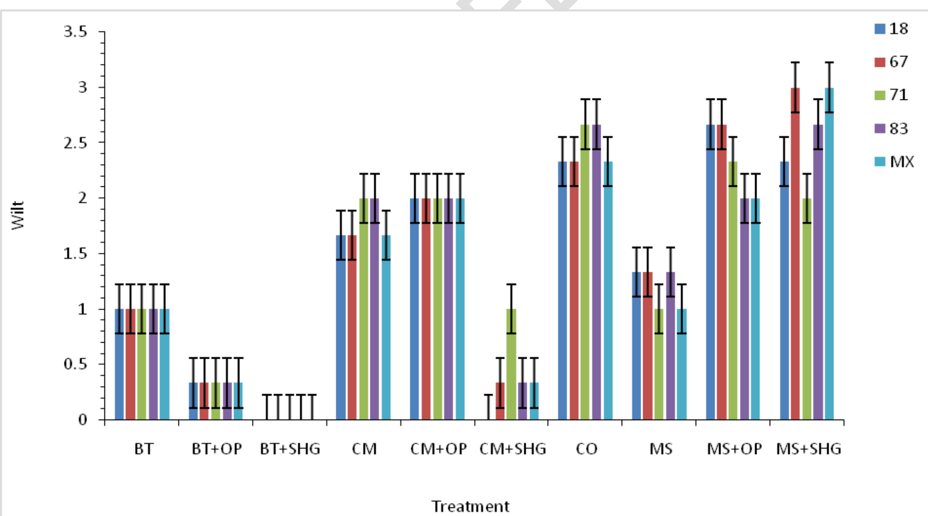
Figure 2. Greenhouse Bacterial wilt incidence in potatoes season 2

Significant differences (*P*≤0.05) were revealed in the bacterial wilt incidences in potatoes between control and all the soil amendments used in season 1 and 2 in the five *R. solanacearum* isolate from Kenyan highlands and lowlands. These results indicate the suppressive effect of organic and inorganic treatments used in this study. The Brassica tissue + Super-hydro-grow polymer was superior in reducing bacterial wilt incidences in potatoes in all the *R. solanacearum* isolates from Kenyan highlands and lowlands. Brassica species produce glucosinolates which are nematocidal and biocidal. The biocidal action of isothiocyanates produced by Brassica tissue and their potential to manage and suppress phytopathogens has been reported by Brown & Morra [33] and Matthiessen & Kirkegaard [34]. Significant differences (*P*≤0.05) were found in the bacterial wilt incidences in potatoes between Brassica tissue alone and Brassica tissue + Orange peel and Brassica tissue + Super-hydro-grow polymer soil amendments used in both season 1 and 2. Brassica tissue + orange peels, Brassica tissue + Super-hydro-grow polymer and Chalim[TM] + Super-hydro-grow polymer *had* a synergetic effect in eliminating *R. solanacearum* in potatoes as opposed to Brassica tissue and Chalim[TM] used individually as seen in Figure 1 and 2. Chalim ($CaCO_3$) in soil reduced bacterial wilt incidence, which was accord with the results of Heyman *et al.* [35]. The population of *R. solanacearum* was reduced significantly in the soil with $CaCO_3$ treatment, which suggested that the effect of $CaCO_3$ on *R. solanacearum* was mainly related to the role of Ca^{2+} in the greenhouse experiment which increased activity of peroxidase (POD) and polyphenoloxidase (PPO) thus reducing incidences of *R. solanacearum*. Isolate 71 was resistant to Brassica tissue + Orange peel soil amendment causing bacterial wilt while the other isolates were susceptible hence no incidence of bacterial wilt in potatoes to the same treatment in season 2. Variations in the incidence of bacterial wilt are attributable to the diversity of *R. solanacearum* strains, variations in soil types in different agro ecological zones. There ~~was~~ were no significant differences (*P*≤0.05) revealed in the bacterial wilt incidences among the isolates in the greenhouse control experiment in potatoes. There was decline in the incidences of bacterial wilt in the second season with subsquent treatment with the same soil ammendement except for greenhouse control experi~~e~~ment.

[2]

3.2 Influence of Organic and Inorganic Soil Amendments on Disease Incidence on Capsicum

The results of incidences of bacterial wilt on capsicum grown ~~under~~ in the green-house for season 1 and 2 are shown by figure 3 and 4 respectively.



[2] *BT-Brassicae Tissue, BT+OP -Brassica tissue+Orange peel, BT+SHG- Brassicae Tissue+ Super-hydro-grow polymer, CM- Chalim[TM], CM+OP- Chalim[TM]+ Orange peel, CM+SHG- Chalim[TM]+ Super-hydro-grow polymer,MS- Metham sodium, Ms+OP- Metham sodium+ Orange peel, MS+SHG- Metham sodium+ Super-hydro-grow polymer: 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land),83 (2T-Kirinyaga-Highland) and MX (2T-L/2A-L/2A-H/2T-H(18/71/67/83).*

Figure 3. Greenhouse Bacterial wilt incidence in Capsicum season 1

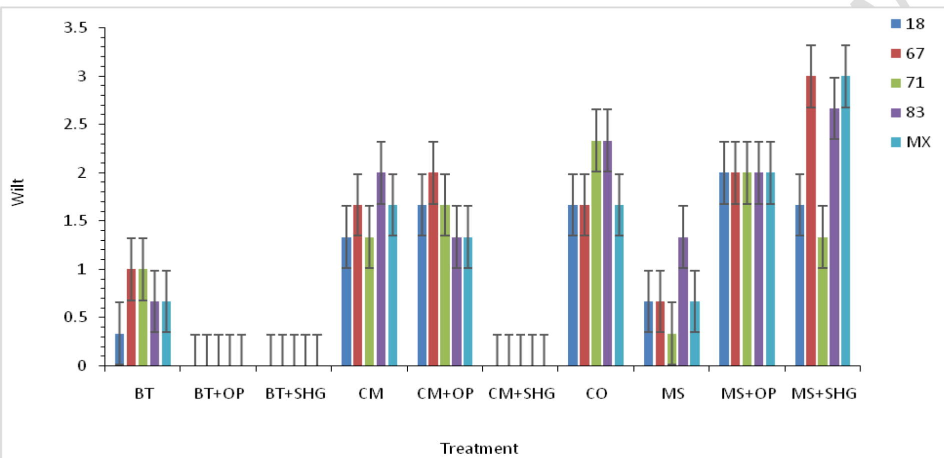


Figure 3. Greenhouse Bacterial wilt incidence in Capsicum season 2

There was no significant differences (*P*≤0.05) revealed in the bacterial wilt incidences between control and Metham sodium + Super-hydro-grow polymer and Metham sodium + Orange peel in the greenhouse control experiment in capsicum in both season 1 and 2. The Brassica tissue + Super-hydro-grow polymer was superior in reducing bacterial wilt incidences in capsicum in all the *R. solanacearum* isolates from Kenyan highlands and lowlands both in season 1 and 2. Soil amendment in season 2 with Brassica tissue + Orange peel, Brassica tissue + Super-hydro-grow polymer and Chalim™ + Super-hydro-grow polymer recorded zero incidence of bacterial wilt in capsicum in all the isolates. The use of Brassica tissue plant residue in the control of bacterial wilt has been conducted and reported to be effective in a study by [36]. The use of plant tissue in controlling of bacterial wilt has been reported to be eco-friendly relative to the use of inorganic chemicals as well as being readily available to the resource-poor farmers [37]. Combination of Chalim™ + Super-hydro-grow polymer significantly (*P*≤0.05) reduced bacterial wilt incidences in capsicum as opposed to use of Chalim™ alone in the two seasons. Metham sodium soil amendment significantly (*P*≤0.05) reduced bacterial incidences as opposed to use a combination of Metham sodium + Super-hydro-grow polymer and Metham sodium + Orange peel in the two seasons.

3.3 Influence of Organic and Inorganic Soil Amendments on Disease Incidence on Tomatoes

The results of incidences of bacterial wilt on tomatoes grown under greenhouse for season 1 and 2 are shown by figure 5 and 6 respectively.
[3]

[3] *BT-Brassicae Tissue, BT+OP -Brassica tissue+Orange peel, BT+SHG- Brassicae Tissue+ Super-hydro-grow polymer, CM- Chalim™, CM+OP- Chalim™+ Orange peel, CM+SHG- Chalim™+ Super-hydro-grow polymer,MS- Metham sodium, Ms+OP- Metham sodium+*

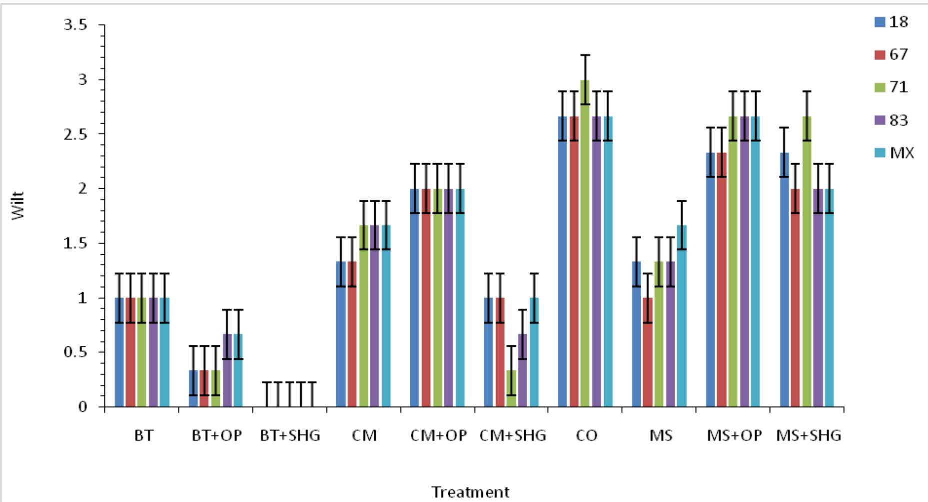


Figure 5. Greenhouse Bacterial wilt incidence in Tomatoes season 1

[4]

Orange peel, MS+SHG- Metham sodium+ Super-hydro-grow polymer: 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land),83 (2T-Kirinyaga-Highland) and MX (2T-L/2A-L/2A-H/2T-H(18/71/67/83).

[4] *BT-Brassicae Tissue, BT+OP -Brassica tissue+Orange peel, BT+SHG- Brassicae Tissue+ Super-hydro-grow polymer, CM- Chalim^{TM}, CM+OP- Chalim^{TM}+ Orange peel, CM+SHG- Chalim^{TM}+ Super-hydro-grow polymer,MS- Metham sodium, Ms+OP- Metham sodium+ Orange peel, MS+SHG- Metham sodium+ Super-hydro-grow polymer : 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land),83 (2T-Kirinyaga-Highland) and MX (2T-L/2A-L/2A-H/2T-H(18/71/67/83).*

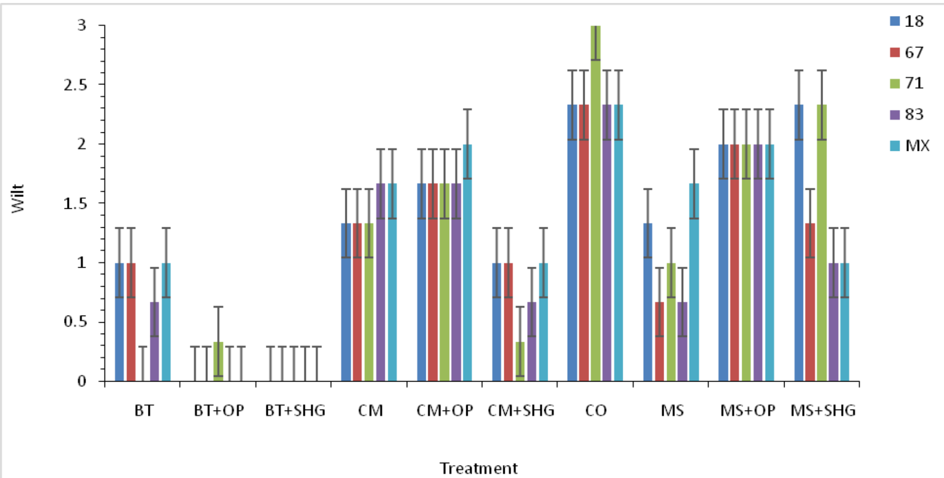


Figure 6. Greenhouse Bacterial wilt incidence in Tomatoes season 2

Significant differences ($P \leq 0.05$) were revealed in the bacterial wilt incidences in Tomatoes between control and all the soil amendments used in season 1 and 2 except in season 1 where Metham sodium + Orange peel and control did not have significant ($P \leq 0.05$) difference as shown on figure 5 and 6. The Brassica tissue + Super-hydro-grow polymer was superior

in reducing bacterial wilt incidences in tomatoes in all the *R. solanacearum* isolates from Kenyan highlands and lowlands both in season 1 and 2. Brassicaceous materials have been reported to have allellopathic effects as well as biofumigation effects to soil biota that includes plant parasitic nematodes [36, 37, 38, 39]. The production of biofumigation products including isothiocyanates (ITCs) that has an active ingredient related to that of Metham sodium and dazomet have been reported to be highly toxic to pests and pathogens. Kim *et al.* [40] reported that, Bacterial wilt of tomatoes caused by *R. solanacearum* is a devastating disease that limits the production of tomato in Korea. Tomato plants are grown worldwide in the field or greenhouse [41]. Isolate 71 was resistant to Brassica tissue + Orange peel soil amendment causing bacterial wilt while the other isolates were susceptible hence no incidence of bacterial wilt in tomatoes to the same treatment in season 2**.** There was significant difference ($P \leq 0.05$) in bacterial wilt incidence in tomatoes for soil amendment between Brassica tissue soil amendment alone compared to a combination of Brassica tissue + Orange peel and Brassica tissue + Super-hydro-grow polymer in both season 1 and 2. Previous studies have shown that essential oils in citrus, protopine and corydaline alkaloids, lactons, polyacetylene, acyclic sesquiterpenes, hypericin and pseudohypericin compounds are effective toward various bacteria including *R. solanacearum* [42] (Maruti *et al.,* 2011).

[5]

In this study, all representative isolates from Kenyan highland and lowland used were pathogenic to tomato seedlings in the greenhouse to varying degrees. Singh *et al.* [23] showed that the micro-climate inside the greenhouse (Temperatures of 28^{0}C-30^{0}C, 80%-90% RH and wet soil) favors rapid pathogen multiplication and disease. Various findings have reported Ralstonia strains as differing in their virulence [43, 44]. Morais *et al.* [45] reported that information on the pathogenicity and molecular variability of Ralstonia strains will improve our knowledge on the epidemiology and ecology of these pathogens. This is particularly true with respect to latency, survival and aggressiveness of each strain. Bacterial wilt caused by *R. solanacearum* is one of the major diseases of tomato and the disease causes concern for tomato production because it can drastically reduce tomato up to 90% [46, 47].

[5] *BT-Brassicae Tissue, BT+OP -Brassica tissue+Orange peel, BT+SHG- Brassicae Tissue+ Super-hydro-grow polymer, CM- Chalim™, CM+OP- Chalim™+ Orange peel, CM+SHG- Chalim™+ Super-hydro-grow polymer,MS- Metham sodium, Ms+OP- Metham sodium+ Orange peel, MS+SHG- Metham sodium+ Super-hydro-grow polymer: 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land),83 (2T-Kirinyaga-Highland) and MX (2T-L/2A-L/2A-H/2T-H(18/71/67/83).*

Chalim soil amendment in tomatoes minimally reduced bacterial wilt between the two seasons. Meanwhile, higher Ca^{2+} content in tobacco was associated with less disease, which was agreement with the results of Sugimoto *et al.* [48]; the mechanism may be related to the increased activity of peroxidase (POD) and polyphenoloxidase (PPO) as reported by Jiang *et al.* [49] that the severity of tomato wilt can be reduced by increasing activity of POD and PPO in tomato with the increased calcium concentration in tomato tissues which concurs with our current findings. Mondal *et al.* [50] also found that Disease incidence in tomato crop was higher compared to other solanaceous crops like brinjal, chilli, capsicum and potato.

4. CONCLUSION

In conclusion, our findings showed that organic and inorganic soil amendments could serve as a viable control of bacterial wilt in solaneceous crops caused by *R. solanacearum in the* greenhouse. Brassica tissue + Super-hydro-grow polymer was superior in reducing bacterial wilt incidences in selected solaneceous crops in all the *R. solanacearum* isolates from Kenyan highlands and lowlands both in season 1 and 2. The data presented in this study substantiate the findings that, various *R. solanacearum* isolates from both the Kenyan highland and lowland are causing bacteria wilt disease in various important solanaceous crops grown in the country.

COMPETING INTERESTS

The authors have no competing interests to declare

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