Managing Bacterial wilt (*Ralstonia solanacearum*) of Potato (*Solanum tuberosum*) using indigenous biological control

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Introduction

Potato yield in Kenya averages between 6-10 t/ha. These low yields are attributed to among other factors, diseases such as Bacterial wilt (BW). Currently, the disease has spread to all potato growing areas and on-farm management of the seed and soil borne disease has proven futile. It has wide range of host plants with no chemical control. The use of farmer-saved seeds in resource limited, small-land sizes has increased the disease prevalence with yield losses of up to 100%. The devastating lethal effect of the disease with great economic impact requires urgent measures to be taken to curb the disease.

Alternative low-cost and sustainable technical solution to manage the disease is therefore apparent. Integration of biological control agents (BCAs) using beneficial microorganisms in potato farming system is hence a promising approach to sustainable production especially in Kenyan context.

The strategy involves isolation of indigenous Rhizobacteria (BCAs) and screening under invitro and invivo to identify promising isolates and testing in field trials to determine their efficiency and effectiveness. The poster describes method and results of the trials that were done in Kenya.

A B C D

Figure 1. (A) Healthy potato plant (B) healthy tubers (C) BW Infected potato (D) BW infected tubers

Materials and Methods

#300 rhizobacteria were isolated from potato rhizosphere sampled from major potato growing areas in Kenya. They were identified based on their morphological characteristic on specific media and their antagonistic activity tested against BW in vitro by measuring the zones of inhibition as shown in Table 1.The first three isolates in bold were selected for further studies.

Table 1. Promising Rhizobacteria Isolates antagonistic to *Ralstonia solanacearum*

Isolate	Site isolated	Mean diameter (mm)	codes used
P19	**Molo**	**13.00a**	**Pseud**
A104	**Taita**	**10.08b**	**Bac**
B86	**Nyeri**	**10.03bc**	**Azoc**
B36	Molo	10.02c	

Green house trial

The experiment was conducted in a green-house. Bacterial wilt inoculum was prepared and inoculated in solarized soil mixed with coco peat at 1:1 ratio. Well sprouted seed potato of two cultivars; Tigoni (Bw susceptible) and clone 387164.4. (Bw tolerant) were planted 4 plants per experimental unit. Three promising isolates of Rhizobacteria; *Pseudomonas sp, Azoctobacter sp. and Bacillus* sp. in various combinations were evaluated. The BCAs were applied according to the respective treatment except for the control.

This experiment was arranged in randomized complete block design (RCBD) with four replicates. The plants were left for natural disease infestation and rated visually for bacterial wilt severity every week. For the treatment with no disease symptoms latent infection was done using enzyme linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA). The parameters evaluated included disease severity, latent infection and tuber yield. List of treatments and layout arrangements are as shown in table 2 and figure 1 respectively.

Table 2. Biological control treatments

Treatments	codes used
Pseudomonas sp.	Pseud
Bacillus sp.	Bac
Azoctobacter sp.	Azoc
Pseudomonas sp.+ *Bacillus* sp.	Pseud+Bac
Pseudomonas sp.+ *Azoctobacter* sp.	Pseud+Azoc
Bacillus sp. + Azoctobacter sp.	Bac+Azoc
Pseudomonas sp.+*Bacillus* sp.+ *Azoctobacter* sp.	Pseud+Bac + Azoc
Control	control

Figure 2. Arrangement of the treatments in the greenhouse

Field trial

A field study to evaluate these best performing BCAs was conducted for two seasons in 2015. During the first season(March to June), three experiments were established in three locations which were, two highly infected farms with soils containing BW of 10^3 cfu and an artificially inoculated field located at a research quarantine station with different BW inoculum concentration of 10^3, 10^5 and 10^7 cfu. Tolerant (Shangi) and susceptible (Tigoni) potato cultivars were used in this study.

The second season experiment was established during the short rains (October –December) and was carried out only at the quarantine station where BW inoculation (10^6 cfu) was done using only Tigoni cultivar. Parameters evaluated were disease severity, tuber yield and weight.

Green-house results

Area under disease Progress curve (AUDPC)

BCAs reduced expression of bacterial wilt with AUDPC ranging from 0-53 compared with the control with 2051-2900 (table 3). Triplicate mixture of Pseudomonas + Bacillus +Azoctobacter in clone was the only treatment that did not show Bacterial wilting symptoms.

Table 3. AUDPC of the BCA treatments

Treatment	Tigoni	Clone
Pseud	44b	36b
Bac	43b	36b
Azoc	53b	42b
Pseud+Bac	32b	27b
Pseud+Azoc	44b	32b
Bac+Azoc	40b	27b
Pseud+Bac+Azoc	28b	0c
control	2900a	2051a

Latent infection

This was done on the asymptommatic Pseudomonas+Bacillus+Azoctobacter treatment and found to be negative.

Tuber number harvested

Control performed similar to the applied BCAs with the exception of Bacillus+Azoctobacter which had the lowest number of tubers (figure 2)

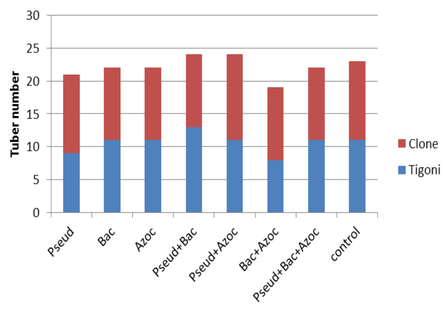
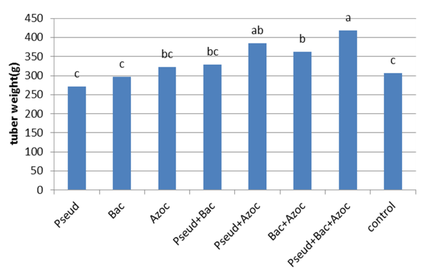


Figure 3. Tuber number as influenced the BCAs

Tuber weight

This was highest in mixed BCAs of Pseudomonas+Bacillus+Azoctobacter, Bacillus+Azoctobacter and in Pseudomonas+Bacillus ranging from 384-418g compared to the control with 307g as shown in figure 3.



Figurė 4. Average tuber weight as influenced by the BCAs

Field trial results

In the first season wilting was minimal ranging from 1-3 wilted plants in all the treatments including the control even in field that was artificially inoculated with bacterial wilt. In the second season the BCAs treatments had 12-21 number of wilted plants compared to the control which had only 8. In both season there was no effect of the BCAs on tuber number and weight when compared with the control.

Discussion and conclusions

In this study use of BCA were effective in reducing bacterial wilt severity under controlled conditions with combined application of Pseudomonas + bacillus +Azoctobacter having no wilt symptoms and tubers had no latent infection.

There was different response of the BCAs in the field trial in both seasons. Bacterial wilt infection was observed even in the promising triple BCAs application of Pseudomonas + Bacillus +Azoctobacter. Low disease incidence on susceptible host plant grown on BW infested soil shows that a field may be infected with the pathogen but have minimal wilting. BCAs may also work contrary to expectation of disease control as was observed in the second season.

In conclusion BCAs under controlled conditions may have promising outcome but in the field due to complex nature of the soil, adverse weather condition and possibility of cross-contamination caused by surface-runoff the result may be varied and inconsistent.

Acknowledgement

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