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SPREAD OF TOMATO AND POTATO WILT EPIDEMIC CAUSED BY *RALSTONIA SOLANACEARUM* IN THE COASTAL AND SOUTHERN HIGHLANDS OF TANZANIA

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ABSTRACT

A survey was carried out to determine tomato and potato farms infected with *Ralstonia solanacearum*, the pathogen responsible for the bacterial wilt disease. During the survey, tomato bacterial wilt disease was found in 21 farms located in Tanga, Morogoro, Pwani and Unguja ukuu (Zanzibar) while 15 potato farms from Njombe, Mbeya rural and Rungwe were also found infected. Tomato farms in Bungu, Tanga had the highest incidence of bacterial wilt (78%). Severe potato wilt was recorded in Lekato B, Rungwe (84%). A total of 41 bacterial isolates were obtained from infected samples. The bacterial DNA was amplified and a 280bp PCR product was viewed in a gel confirming isolates to be of *R. solanacearum*. Pathogenicity test on susceptible hosts was positive. No tomato plants infected with *Ralstonia solanacearum* were found in Mtwara and Lindi.

Keywords: Spread, Distribution, Incidence, Pathogenicity, Detection

1. INTRODUCTION

Ralstonia solanacearum is the causative agent of bacterial wilt in many major crops across the globe. In potatoes the disease commonly known as potato brown rot. The pathogen is a major production constraint with an ability of causing up to 100% yield loss. It can cause wilting in over 300 plant species belonging to over 30 botanical species. The major hosts of *Ralstonia solanacearum* belong to the family Solanaceae (nightshades) which include crops like tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.),

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chilli (*Capsicum spp* L.) and tobacco (*Nicotiana tabbacum*) (Khavkar *et al*., 2011). Other major hosts include banana and groundnut.

The high genetic variation of *Ralstonia solanacearum* led to it being tagged as a species complex (Fegan and Prior 2005). Presence of this pathogen in West Africa was first reported in Cameroon by Mahbou Somo Toukam *et al*., (2005) and in Ghana by Subedi *et al*., (2014) where it was found in tomato, potato and pepper farms. It was earlier spotted in East Africa where reports from Tanzania reflected on the occurrence of the disease on tomatoes and potatoes in Lushoto, Tanga (Black *et al* 1998). However, previous reports of *R. solanacearum* in Tanzania lack in-depth information on location, incidence, and epidemiology.

Plants infected by *Ralstonia solanacearum* appear shrunk with severe leaf epinasty while still freshly green. When stem is vertically split, vascular discoloration can be observed while when laterally sectioned and dipped in water, bacteria can be seen streaming. Infected potato tubers rot and when sliced bacteria can be seen oozing from the vascular bundles.

We conducted a survey to determine areas with tomato and potato farms infected with *Ralstonia solanacearum* along the coast and southern highlands of Tanzania.

This report will help stage measures of interventionsuch as selection of hotspots for trials with resistant materials and pinpointing ineffective measures currently being deployed to control the pathogen.

2.0 MATERIALS AND METHODS

2.1 Determination of disease distribution

The survey was conducted in 8 regions in Tanzania (Table 1). The survey was done in the year 2012 where the Potato fields were surveyed from July to August whereas the Tomato fields was surveyed in October. A total of 21 districts were surveyed. These included three districts from each region along the coast, two districts from Morogoro, Mbeya, Unguja North and a single district in Njombe (Table 1). In each district from regions along the coast, three villages were surveyed and sampling was done at random in three Tomato farms per village. The same village sampling pattern was emulated in Morogoro. In the fields symptoms typical to bacterial wilt in Tomato were observed, plants that showed typical bacterial wilt symptoms a piece of stem (5 cm) was cut from the crown and was immersed in clear water to check for bacterial streaming. The bacteria were allowed to stream in extraction buffer in a mesh bag and an immunostrip test using kits from Agdia was used to confirm the species. Each kit contained 25 immunostrip embedded with monoclonal antibodies specific for *Ralstonia solanacearum*. This Immunostrip test is a rapid means of screening crops and bacterial samples for *Ralstonia solanacearum (*Rs).

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The Rs Immunostrip detects Rs to the species level and cannot differentiate race or biovar. Immunostrip tests require no equipment or expertise to run. Results can be obtained in 30 minutes or less, making Immunostrip perfect for use in the field or greenhouse. The immunostrip Kit components may be stored at room temperature (18 - 30 ℃) or refrigerated (2 - 8 ℃) between uses, and Immunostrip should be tightly sealed in the desiccated container at all times. Before use, allow all kit components to warm to room temperature (18 - 30 ℃). Cold BEB1 extraction buffer will contain a white precipitate or appear cloudy, but will clear upon warming to 18 - 30 ℃

Take a sample from symptomatic leaves, petioles, or stems when possible. When working with stems cut two cross section pieces at the first two internodes from the crown. The stem can be cut into smaller sections for easier grinding. Agdia sample extract bags contain 3 mL of extraction buffer, requiring 0.15 g (approximately 1 inch 2 or the size of the bottom of the Immunostrip container) of tissue for the optimal 1:20 dilution. It is important to note that thick or dense tissues can alter the targeted 1:20 dilution. Samples that test positive due to interaction of the surface antigen of the bacteria with embedded antibodies on the strip show a confirmatory test line and a control line to confirm validity of the test. The tomato plants which tested positive were then placed in a paper bag and labeled appropriately with the date of collection and location. Soil samples and river water samples from such areas were also collected.

Tomato samples collected from coastal Tanzania (soil, plant and water) were taken to the IITA laboratory in Mikocheni Dar es Salaam.

In the Southern Highlands, The Potato fields were surveyed in Njombe, Mbeya Rural and Rungwe districts. The districts are the major potato producing areas in the country. During the survey sampling was done at random in 5 farms per village. Ten (10) villages were surveyed in each district, the distance between one field and another was 3-5 km. Most of the potato fields surveyed were at flowering stage.

In each field 10 rows of about 100 plants were selected randomly, bacterial wilt symptoms were 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.

Bacterial wilt incidence was recorded based on number of plants showing symptoms and were expressed as % of the total number of plants observed.

Disease severity was done by recording on severity score as described by Horita and Tsuchiya (2001) as 1= no symptoms, 2= top young leaves wilted, 3= two leaves wilted, 4= 4 or more leaves wilted and 5= plant died. Two types of samples were collected in each farm, one

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comprised of 25 healthy looking potato tubers and the other of 4 diseased tubers, packed in paper bags and labeled in each farm for further tests. Potato samples from the southern highlands were sent to the Tanzania Official Seed Certification Institute (TOSCI) laboratory located at Sokoine University of Agriculture in Morogoro, During the survey field altitudes and their corresponding geographical position were recorded.

2.2 Isolation of *Ralstonia solanacearum* from diseased tomato plants and soil

Isolation of the bacteria from the plant was carried out according to Kelman (1954). A 5 cm stem piece was cut using a sterile knife and washed with tap water. It was later rinsed with sterile water and sprayed with 70% alcohol in a laminar air flow chamber. After 3 min. the alcohol was rinsed off with sterile water and the stem piece was dried. Upon immersion in sterile water, bacterial streaming from the stem piece was observed. This suspension was streaked on a TZC agar (Casamino acids 1 g, Peptone 10 g, Glucose 2.5 g, Tetrazolium chloride 5 ml, Agar 18 g, distilled water 995 ml) and incubated in 28°C for 48 h. The plates were observed for growth of irregular shaped and mucoid colonies with red/pink centers.

From the soil samples, isolation was done according to Elphinstone (2005). A serial dilution was performed and the suspensions plated on a SMSA (Semi selective South Africa) medium (1% Crystal violet 5 ppm, 1% Polymyxin B Sulphate 100 ppm, 1% Tetrazolium 50 ppm, 1% Bacitracin 25 ppm, 0.1% Penicillin 0.5 ppm, 1% Chloramphenicol 5 ppm). This media included antibiotics in addition to the TZC media to inhibit growth of fungi and unwanted bacteria.

2.3 Isolation of *Ralstonia solanacearum* from diseased potato tubers

Infected potato tubers were thoroughly washed with running tap water then dipped in 70% alcohol for 5 minutes for surface sterilization. The tubers were then dried with tissue paper and cut into half by a sterile knife. The vascular ring removed from the tuber and then placed in test tubes containing 5 ml of sterile water The bacteria was allowed to flow from the vascular bundles for 5 minutes.

One loopful of bacterial suspension was streaked into 2, 3, 5 Triphenyl Tetrazolium Chloride (TZC) agar medium and incubated at 28°C for 48 h. Single colonies of *Ralstonia solanacearum* showing virulent, fluidal, irregular and creamy white with pink centres were picked from the TZC Petri-plates and streaked onto CPG (Casamino acid, Peptone and Glucose) medium. These were incubated for 48 h. Virulent cultures were maintained in distilled water in screw capped tubes at room temperature after 48 h of incubation.

2.4 Serological detection of *Ralstonia solanacearum* in river water

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Detection of *R. solanacearum* was carried out by a Double Antibody Sandwich – Enzyme Linked Immunosorbent Assay (DAS-ELISA). This was performed according to Priou *et al.*, (2010) with few modifications. The protocol, which was meant for detection of *Ralstonia solanacearum* in stems of asymptomatic potato plants was modified to suit detection of the pathogen in river water. River water was collected in a 50 ml screw cap tube. The sample aliquot of 25 ml was mixed with 25 ml of CPG broth (casamino acids 1 g, peptone 10 g, glucose 5 g, distilled water 1000 ml) instead of SMSA broth and incubated for 48 h in 28°C. This would enrich the bacteria to a detectable population density in case of their presence. From this sample, 500 µl was transferred to a microfuge tube containing 50 µl of the general extraction buffer and mixed thoroughly. Using a spectrophotometer at the absorbance of 600 nm the cells were adjusted to a concentration of 10^5cfu/ml.

An aliquot of 100 µl of this sample was added to a microtiter well coated with anti-*Ralstonia solanacearum* antibodies. The plate was incubated for one hour at room temperature in a humid chamber. After incubation the wells were dumped with a quick flipping motion without mixing the contents. The wells were then washed with 1X PBST wash buffer eight times and tapped firmly on a folded paper towel to remove any remaining drops. A hundred microliters of peroxidase enzyme conjugate per well was added and the plate was again incubated for one hour in a humid box. The wells were dumped again after incubation and washed eight times with PBST buffer. The plate was later moved to a dark humid chamber and 100 µl of TMB substrate was added to each well. The plate was incubated for fifteen minutes. Thereafter, the colorless aliquots in the plate were observed for change to the color blue

2.5 Pathogenicity test

Inoculum was prepared according to Pradhang *et al.* (2000) by streaking *Ralstonia solanacearum* on sterile CPG media (casamino acids 0.5 g, peptone 10 g, glucose 5 g, distilled water 1000 ml, bactoagar 18 g) in 90 mm diameter Petri dishes. The petri dishes were incubated for 48 hours at 28°C. *Ralstonia solanacearum* was then suspended in 500 ml of sterile water. The suspension was set to $5x10^8$ c.f.u/mL by measuring the absorbance at 600 nm and setting the optical density to 0.3 O. D using a spectrophotometer. Inoculation was carried out by soil drenching. Pots filled with 80 g of potting mixture with three weeks old seedlings were drenched with 80 ml of *R. solanacearum* inoculum and observed once per week for wilting symptoms.

2.6 Molecular confirmation of *Ralstonia solanacearum*

With the help of a sterile microtip, a small bacterial sample was transferred from CPG agar plates to a 25µl PCR premix containing AU 759/760 species specific primers for *Ralstonia solanacearum*. The concentration of the primers in the master mix was 0.5 µM. Amplification

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was carried out in one cycle of initial denaturation at 94°C for 3 mins, 35 cycles of denaturation at 94°C, annealing at 60°C and elongation at 72°C each for 30 s. This was followed by one cycle of a final elongation step at 72°C for 5 mins. The reaction was held at 4°C. PCR products were run against a 250 bp – 1kb ladder at 110V in a 1.2% agarose gel stained with gel red for 40 mins and later observed under UV light for the expected band size (280bp). The procedures used for molecular characterization used for confirming the presence of *Ralstonia solanacearum* were as follows:

2.6.1 DNA Extraction

Bacterial DNA was extracted as follows; two tubers per sample were washed under running water and air dried for an hour. The skin was removed at the heel end of the tuber with a clean disinfected vegetable knife so that the vascular tissues become visible. A small conical core (3-5 mm diameter) of vascular tissue at the heel end was carefully cut out. A sample of a heel end was crushed with the help of pestle and mortar and collected in a sterile Falcon tube containing 5 ml of double distilled water The content was allowed to stand for 30 min. The supernatant was then centrifuged for 2 min in 1.2 ml microfuge tube. The pellets were resuspended in a 500 µl tube by vortexing. Then 30 µl of Sodium Dodecyl Sulphate (SDS) and 3 µl of 20 mg/ml Proteinase K was added. The mixture was vortexed and incubated at 35°C for one hour. One hundred microlitre (100 µl) of 5M CTAB/NaCl solution was added and the mixture was incubated at 65°C for 10 min. Phenol, chloroform and isoamyl was added in the ratio (25:24:1) and the tubes were centrifuged for 5 min. The supernatants were then transferred to new microfuge tubes and 0.6 volume of Isopropanol was added. Centrifugation was again done for 5 min at 16000 g. The supernatant was then discarded and the DNA pellets were resuspended in 100 µl of 1X T.E. buffer. (Grover *et al*., 2012).

2.6.2 Species specific PCR

Species specific PCR was done by using a Random Amplified Polymorphic DNA (RAPD) Primer set AU 759/760. Master mix (readymade) from Thermo Scientific containing 0.4 Mm of dATP, dCTP, dGTP, dTTP, 4 Mm M_gCl_2 and 5 U/µlTaq Polymerase was used. Primer concentration in the master mix was 0.1 Mm. One µl of 10 ngμl^{-1} DNA The reaction volume was 25 µl.

The PCR conditions were set as one cycle of 94°C for 3 min., 53°C for 1 min. and 72°C for 1.30 min., followed by 30 cycles of 94°C for 15 s, 72°C for 15 s, one cycle of 72°C for 5 min and held at 4°C. Gel electrophoresis was done for 40 minutes in which 1.2% Agarose gel in TAE buffer stained with gel red was prepared and the samples were run against 1 kb DNA ladder for 45 min.

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2.6.3 Phylotype determination

Phylo-typing was determined by Multiplex PCR (pmx PCR). The reaction involved four forward primers, one reverse primer and a species-specific primer. The four forward primers involved were Nmult21:1F, Nmult21:2F, Nmult23: AF, and Nmult22:1nF, one reverse primer used was Nmult 22: RR and species-specific primer pairs AU 759/760. The reaction mixture contained 6 p moles of the forward primers, 12 p moles of the reverse primer, 4 p moles of species-specific primer, 12.5 µl of Thermoscientific Taq Green Mastermix and 1 µl of 20 ngµl¯ DNA templates making a total 25 µl volume of reaction mixture. The PCR conditions were set as one cycle of 96°C for 5 min., 59°C for 30 s and 72°C for 30 s. The final extension was done at 72°C for 10 min and samples were held at 4°C. For 45 minutes Agarose gel electrophoresis was done in which 1% of Agarose gel was prepared in TAE buffer stained with gel red and the samples were run against a 1 kb plus DNA ladder.

3.0 RESULTS AND DISCUSSION

3.1 Determination of the distribution of *Ralstonia solanacearum*

Some tomato farms in Tanga, Morogoro, Pwani and Unguja ukuu were highly infected with *Ralstonia solanacearum.* Potato bacterial wilt disease was found in farms in Mbeya, Iringa and Njombe (Table 1). An exemplary symptomatic wilting tomato plant is shown in Figure 1 and an image of an immunostrip specific to *Ralstonia solanacearum* with positive results as was observed in the fieldis displayed on Figure 2. There were no tomato plants infected with *Ralstonia solanacearum* in farms sampled in Mtwara and Lindi. The highest wilt incidence in tomato was recorded in Bungu, Korogwe, Tanga (78 %) as depicted in Figure 5.

Potato bacterial wilt was sighted in both Njombe, Mbeya rural and Njombe. Disease incidence was highest in Lukata B in Rungwe with an incidence of 84% (Figure 6).

3.2 Isolation of *Ralstonia solanacearum* from diseased plants and soils

After 48 h of incubation in 28°C, growth of irregular shaped mucoid colonies with pink centers was observed on the TZC (Figure 3) and SMSA agar plates. This is characteristic of *Ralstonia solanacearum*. White mucoid colonies grew on CPG media (Figure 4).

3.3 Pathogenicity test

After three weeks, all inoculated potato and tomato seedlings inoculated with *Ralstonia solanacearum* wilted (Figure 5). The isolates proved to be pathogenic to their respective hosts and symptoms were consistent to those seen in the field.

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3.4 Molecular confirmation of *Ralstonia solanacearum*

PCR products of 280bp were observed in the gel (Figure 8). This is the expected base pair sizehence confirming *Ralstonia solanacearum* as the source of wilting in the sites where samples were collected during the survey.

Positive samples of tubers that were infected by Ralstonia solanacearum that were detected by NCM-ELISA were subjected to molecular characterization to species specific PCR to confirm if they were really *Ralstonia solanacearum*.

Out of 15 potato strains tested from each district;7,4 and 3 from Rungwe, Njombe and Mbeya Rural respectively were confirmed to be *Ralstonia solanacearum* under specie specific PCR in which 280bp band was observed, which is the expected universal expected band size for specificity of *Ralstonia solanacearum.*

Phylotype specific multiplex PCR revealed that the potato strains belonged to Phylotype III as 280 and 91 bp amplicon was observed in all the strains when Pmx-PCR products of these strains where subjected to electrophoresis on 1.2% Agarose gel.

The results obtained correspond to those of Fegan and Prior (2005) whom described a phylotypic classification system consisting of four phylotypes, in which Phylotype 1 are those from Asiaand are characterized by production of 280 and 144 bp amplicon, Phylotype II strains are from America and they produce 280 and 372 bp amplicons. Phylotype III are mainly from Africa and nearby islands such as Reunion and Madagascar which produce 280 and 91 bp and Phylotype IV strains which are from Indonesia, Japan and Australia which produce 280 and 213 bp amplicon. Phylotyping helps in the concept of integrated disease management where the use of resistant cultivar is of prime importance. A variety may be resistant to one phylotype but susceptible to another therefore it is very important to know the diversity of local strain of the pathogen (Sagar *et al*., 2014).

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Figure 1: A symptomatic wilted tomato plant which was found in Tanga.

Figure 2: An immunostrip test showing positive for *Ralstonia solanacearum* in the field.

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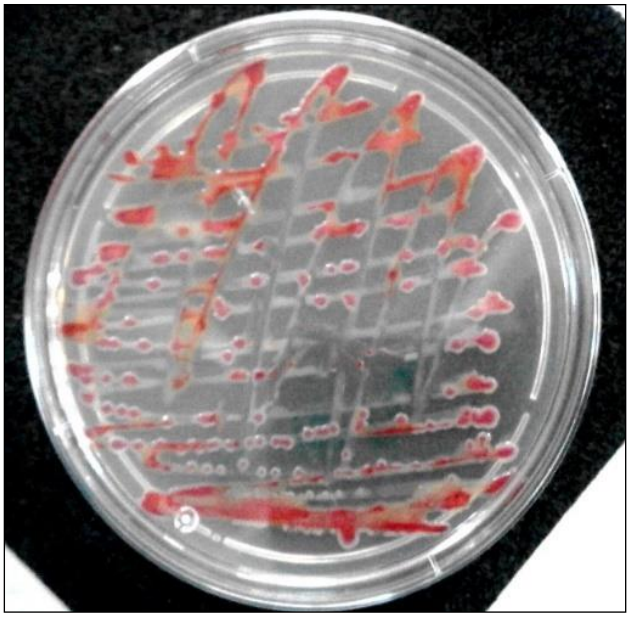

Figure 3: *Ralstonia solanacearum* colonies with pink/red centres on TZC medium

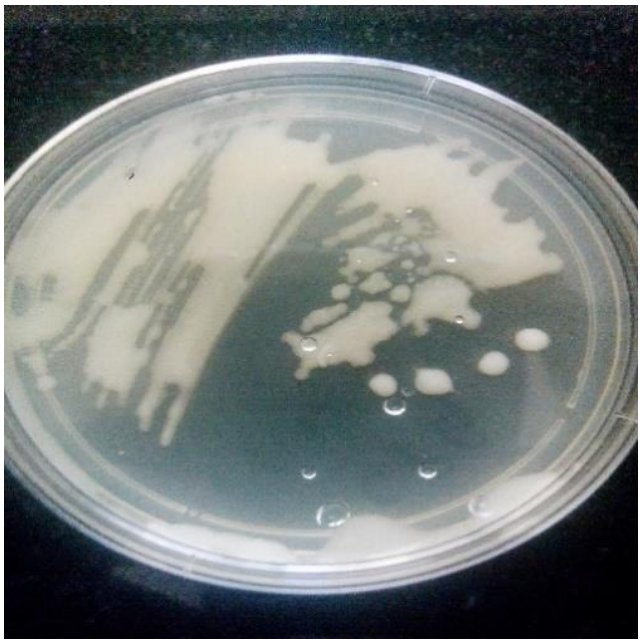

Figure 4: White mucoid *Ralstonia solanacearum* colonies on CPG medium

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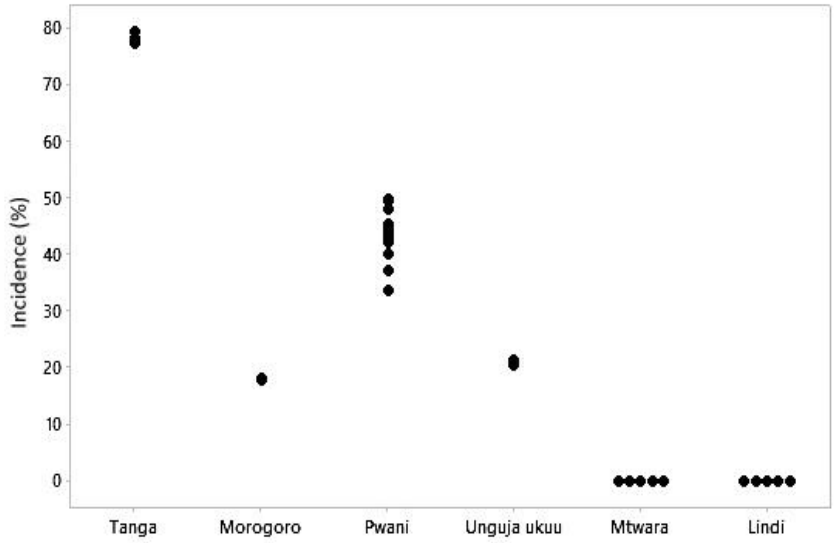

Figure 5: Individual value plots of incidence of tomato bacterial wilt in farms along coastal Tanzania.

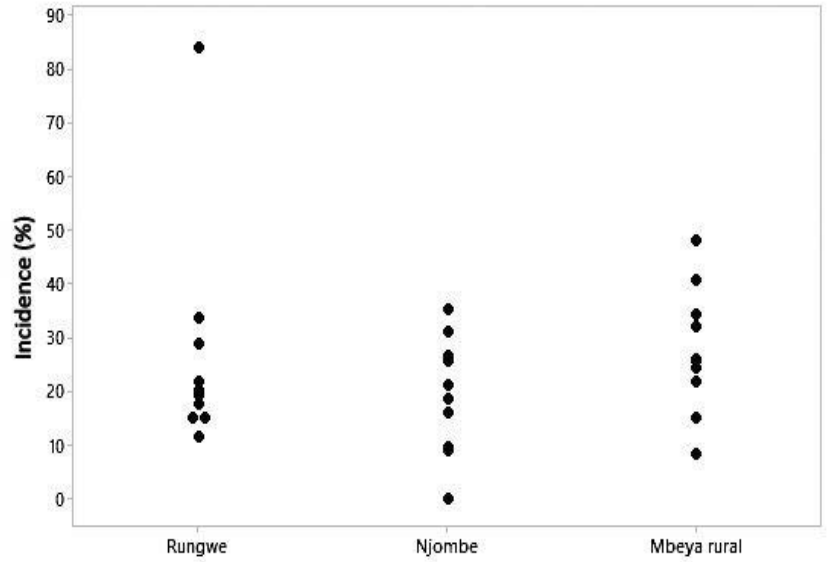

Figure 6: Plots of incidence of potato brown rot in farms located in the southern highlands of Tanzania

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Figure 7: Wilted tomato plant after inoculation with *Ralstonia solanacearum*

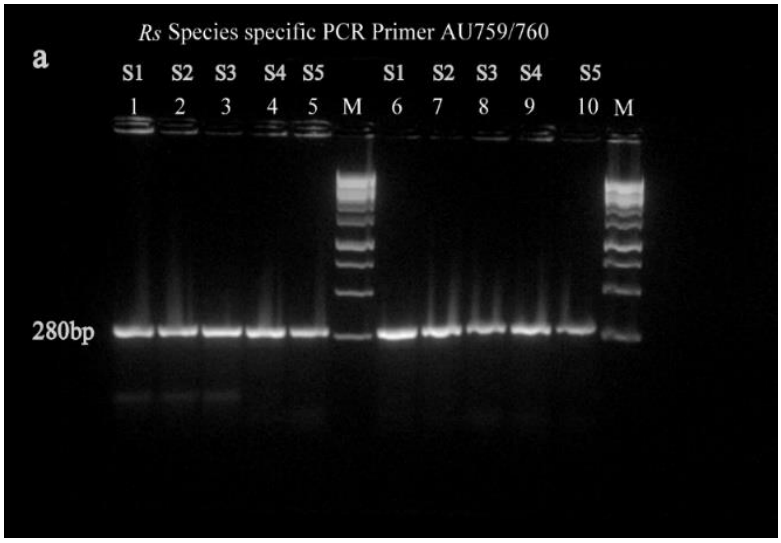

Figure 8: Gel image of AU 759/760 PCR products with 280bp bands as is consistent with *R. solanacearum*

3.5 Serological detection of *Ralstonia solanacearum* in river water

DAS ELISA plates specific for *Ralstonia solanacearum* detected *Ralstonia solanacearum* from samples collected from rivers Rufiji in Pwani and minor rivers in Wami, Kwa bululu and Vuje in

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Tanga. The solution turned from colorless to blue to reveal positive results for the mentioned water samples.

Table 1: *Ralstonia. solanacearum* hotspots

Region	District	Village	Crop
Mbeya	Rungwe	Ndaga	Potato
		Lukata b	Potato
Njombe	Njombe	Welela	Potato
		Mtwango	Potato
		Ulembwe	Potato
		Usalule	Potato
		Magoda	Potato
Mbeya	Mbeya Rural	Itizi	Potato
		Sanje	Potato
		Jojo	Potato
Tanga	Korogwe	Bungu	Tomato
	Handeni	Pogwe	Tomato
		Msaje	Tomato
Pwani	Kibaha	Mpiji	Tomato
	Bagamoyo	Mtoni	Tomato
		Chemichemi	Tomato
	Mkuranga	Kisse	Tomato
		Hoyoyo	Tomato
Unguja	North	Donge	Tomato
Morogoro	Mvomero	Misufini	Tomato

NB: Results are based on disease incidence (≥ 30%)

The establishment of disease is the result of successful interaction between a susceptible host and a virulent pathogen under a conducive environment (Jeger 2009). The main environmental factors affecting soil borne pathogens like *Ralstonia solanacearum* are temperature and soil moisture (Hayward, 1991). Most strains of *Ralstonia solanacearum* are pathogenic at 25-30°C (Ghini *et al*., 2007), a temperature range corresponding to that of Tanga, Morogoro, Zanzibar and Pwani in the month of October (TMA, 2013) when tomato plants begin flowering. Some strains of *R. solanacearum* are known to cause disease at lower temperatures but most strains are

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non-pathogenic below 20°C. Race 3 biovar 2 strains survive in 18°C can infect potatoes in cold regions such as Mbeya and Njombe. This is supported by proteomic studies which showed the effect of temperature on the expression of hrp B and hrp G genes, key determinants of pathogenicity in *Ralstonia solanacearum* (Bocsanczy *et al*., 2014). The failure to isolate the bacteria from soil in Mtwara and Lindi also doesn't conclusively advocate the absence of the pathogen. *Ralstonia solanacearum* enters into a viable but non culturable (VBNC) state when subjected to unfavorable conditions including lower temperatures. The VBNC state was attributed to its ability to survive for a long time and resuscitate in soil (Kong *et al*., 2014).

Bacterial wilt disease of Potato and Tomato is generally favored by temperatures between 25 and 37 degrees Celsius, it usually does not cause problem in areas where mean soil temperature is below 15 degrees Celsius. Under conditions of optimal temperature, infection is favored by wetness of soil. Once infection has occurred, symptoms will often be more severe with hot and dry conditions which hastens wilting. The wilting disease occurs generally in humid conditions with relatively high temperatures.

Smallholder tomato farmers in Bungu, Tanga and Bagamoyo, Pwani have resorted to crop rotation much to their disappointment. The detection of *R. solanacearum* in river water by DAS ELISA means that farmers have been using *R. solanacearum* infected water for irrigation. *Ralstonia solanacearum* can survive for over four years in a nutrient depleted environmental water microcosm and maintain its pathogenic status (Belena *et al*., 2008) which makes one year rotation program ineffective. Sources of irrigation water can harbor plant pathogens. In general, surface water such as rivers, creeks and ponds have a higher likelihood to be contaminated with plant pathogens when compared with water from sealed wells (Salamana, 2014). By choosing a clean source of **water**, the risk of introducing plant pathogens carried by the irrigation water to the production fields is decreased. Water not only plays a role in dispersing waterborne pathogens, but also can provide the moisture that favors the infection and disease development of airborne pathogens. In order for disease to occur, a susceptible host, virulent pathogen and favorable environmental conditions are needed. Irrigation provides moisture and leaf wetness so that if spores of the pathogen have landed on a host plant, an irrigation event allows the infection process to begin. As the disease progresses, overhead irrigation can splash disperse pathogens from infected plants to nearby healthy plants, allowing the epidemic to continue to develop in the field. It is therefore necessary to make sure that quality irrigation water is used in terms of cleanliness and amount used; well water is best in term of cleanness. It is further recommended to apply recommended water according to crop growth stage as pathogen is favored by water, hence more water more disease spreading.

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Most potato farmers in the Southern highlands of Tanzania know the bacterial wilt disease and its symptoms but do not know how to control it and thus do nothing to contain the disease hence the disease spread in the region.

4.0 CONCLUSION

The study showed that the bacterial wilt disease symptoms were observed in both potato and tomato farms all areas of the survey. Due to importance of Potato and Tomato crops to farmers economies in Tanzania; we recommended farmers make use of certified tomato seeds or seedlings together with certified potato seed tubers which are available in the country. Cooling soil temperature by grass mulching can help reduce infection. Weeding is also very important as some weeds such as *Solanum dulcamara*, *Ipomoea* sp. and *Portulaca oleraceae* are good hosts of *R. solanacearum* and remain asymptomatic (Pradhang *et al.,* 2001). They aid in long term survival of the pathogen in the soil. Use of wells as source of irrigation water instead of rivers would significantly reduce infection. The burning of plant debris would deny the bacteria of a source of food as it enters the saprophytic stage of its life cycle. Farmers training on disease control is strongly recommended.

Further studies are needed to develop an epidemiological model of the bacterial wilt disease. Studies on soil chemical composition and how soil amendment can be done to hamper invasion would also be useful. Yadessa *et al*. (2010) proposed amending top soil with coco peat, farmyard manure and compost to control *Ralstonia solanacearum*. Grafting of susceptible varieties onto resistant rootstocks has shown great promise in controlling the pathogen. The rootstocks of *Solanum sisymbrifolium*, *S. integralism* and *S. torvum* proved to be resistant to *Ralstonia solanacearum* in Bangladesh and seedlings successfully grafted onto these rootstocks gave rise to healthy plants (Rashid and Zaman, 2005). Grafting tomato on *Solanum sisymbrifolium* also increases marketable yield even under disease free conditions (Miller *et al.,* 2005).

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