Original Research Article

Prevalence of Bacterial Wilt DiseaseCaused by*Ralstonia solanacearum* on Tomatoesin Meru South Sub-County.

ABSTRACT

Tomato (*Solanumlycopersicon*) is among the most consumed vegetables across the globe. In most parts of Kenya, tomato production is characterized by low quality and huge losses due to pest and diseases. The most prevelance tomato diseases are caused by bacteria, most common being bacterial wilt. This study determined the prevalence of *Ralstoniasolanacearum* in tomato farms in Meru South sub-county. The Prevalence of bacterial wilt was carried out by conducting a survey across agroecological zones in Meru south. Study farms were randomly selected. Data on the frequency of occurrence, severity, and impacts of the disease were collected by observation and administration of questionnaires to the farmers. The disease symptoms was scored using a scale of 0 – 5. The prevalence of bacterial wilt was higher in the study area. However, there was a variation in the level of infection of the pathogen within the surveyed farms in the agro-ecological zones. The disease prevalence ranged from a mean of 15.53% to 62.66%. All groups of isolates of *R. solanacearum* were gram-negative. *Trichoderma* had a significant ($p < 0.05$) effect on the growth of bacteria. There was a strong activity of controlling fungi depending on the concentration. Study broadens the understanding of *R. solancaerum*prevalence in Meru South sub-county'

Keywords: Prevalence; Bacterial wilt; agro-ecological zones.

I. INTRODUCTION.

Tomato (*Solanumlycopersicon*) belong to *Solanaceae* family and is among the most consumed vegetables across the globe. The crop (tomato) is grown in over 5 million hectares globally with production of up to 170 million tonnes per annum with an average of 34 tonnes per hectare (Samberg *et al*., 2016; FAOSTAT 2021). In Africa, Egypt leads in tomato production followed by Nigeria (FAOSTAT, 2021). In Kenya, tomato production accounts for 14% of the total vegetable produce and about 7% of the total horticultural crops (Wanjohi *et al*., 2018). Despite tomato being a major

vegetable, its production in Kenya (average of 20.4 tonnes/ha) has remained below the global average (59.4 tonnes/ha) and the production by other African countries like South Africa (67.1 tonnes/ha) (FAOSTAT, 2021).

Tomato farming is a popular economic activity among farmers in Kenya. The crop performs best in medium to lower zones in major tomato growing areas such as Mwea in Kirinyaga County, Ngurumani in Kajiado County and parts of Rift Valley and western regions (Nguetti*et al*., 2018). In between 2012 and 2014, the total national production was 400,204 MT valued at Kenya shillings 11.8 billion (Neema & Fredrick, 2019). According to Kinuthia (2019), the leading counties in tomato production in Kenya in 2021 were Bungoma (50,399 MT), Kirinyaga (48,560) and Kajiado (47368 MT) in 2014. Tharaka Nithi produces approximately (8,184 MT) according to Tharaka Nithi crop projection while Meru south subcounty production is approximately 1,650 MT, which is quite low comparing to other counties. Tharaka Nithi County is ranked position 29 of 47 counties with a total area of 120 hectares (Njonjo *et al*., 2019). Low production is contributed by abiotic factors, (high temperatures, erratic rainfall and poor soils) as well as biotic factors which include, arthropod pests, fungal, bacterial and viral diseases (Dube & Maphosa, 2020).

Tomato have several health benefits, which include essential amino acids, sugars, carotene that have anti-cancer and antioxidants properties, dietary fibre, vitamins such as A, B, C, mineral ions like iron, phosphorous and lycopene (Silva *et al*., 2019). Demand for tomato continues to grow due to rapidly increasing population (Akbar *et al*., 2018). However, in Kenya tomato demand outweighs its production (John *et al*., 2020). This has been attributed to attacks by insect pests and phytopathogens, such as, viruses, fungi and bacteria (Mengesha *et al*., 2017). The main bacterial diseases of tomatoes include bacterial leaf spots, tomato wilts and cankers. Bacterial disease causes 70 - 80% yield loss when grown in greenhouse and field environments (Singh *et al*., 2017).

Bacteria wilt of tomato is mainly attributed by *Ralstonia solanacearum*, a serious soil borne pathogen (Wu, 2013). Studies have shown that the disease causes 70-80% yield loss when grown in greenhouse and field environment (Huang *et al*., 2013). *Ralstonia*

*solanacearum*is a sophsticated phytopathogen with a number of host, widely distributed, contagious, epidemiological association and physiological effects (Siri *et al*., 2011). Bacterial wilts limit production of tomatoes in warmer areas, sub-tropics and tropics temperatures in the world (Rowles *et al*., 2020). The bacteria can persist in soil medium and plant tissues for several years, thus hard to eradicate. Due to its wide distribution it's expected to have different strains adapted to different ecological zones. Hence, it is important to frequently characterise this pathogen in different agro-ecological Zones.

According to Sarfo, (2018) chemical control using pesticides (for example, fumigants, dichloropropene, and chloropicrin) and plant activators produce a physiological resistance on the tomato have been utilized to minimise bacterial wilt. However, overreliance on pesticides or misuse of chemicals leads to accumulation of pesticide residues in soils and plant tissues (Otieno, 2019). This results in environmental contamination and reduced quality of the produce, which negatively affects their market. Hence, chemical control method may not be the best alternative to control bacteria wilt in tomatoes (Ondus*oet al*, 2018). Therefore, there is need to use a more effective and sustainable bacteria wilt management options such as use bio agents.

The findings of Bawa, (2016) reported that various cultural methods involved in controlling and management of *Ralstonia solanacearum* include crop protection, use of resistant cultivars. However, the report differed with the findings of Mamphogoro *et al*., (2020) that purported that shifting cultivation is unfeasible since the time involved in preparing other seedbed is a lot and therefore, lead to wastage of time in moving from one field to the next.

II. METHODOLOGY.

The study was conducted using descriptive survey and experimental designs. The survey was descriptive in nature to assess the prevalence of *R. solanaceaarum*on tomato farms in the study area. Disease prevalence in this study was the number of cases of *Ralstonia solanacearum* in Meru south during this study season.

Tomato farms were selected using purposive sampling based on tomato production status whereby the size of land under tomato production in each farm was ¼ ha and

above to ensure adequate mapping to constitute the target population. The target population was distributed within LM1, UMI, UM2, UM3, and LM3 agro ecological zones in Meru South Sub-county. There were 250 estimated tomato farms that satisfied the conditions of at least ¼ ha farm under tomato production within these agro ecological zones (Tharaka-Nithi County; Table 1). The sample size was calculated using the formula documented by (Israel, 2009) as shown;

$$n = \frac{N}{1 + N(e)^2}$$

Where, n is the sample size, N is the estimated population size, and e is the level of precision. Given, N = 250 and *e* = 0.05.

$$n = \frac{250}{1 + 250(0.05)^2} = 153.846$$

Therefore, n = 154

Sampling Procedure

The study area was divided into five agro ecological zones, LM1, UMI, UM2, and UM3 and, LM3.Then a clustered random sampling method was used to select farms growing tomatoes (Table 1).

Table 1: Sample Size Distribution among the five Agro ecological Zones

Agro ecological Zones	Farms	Sample size
LM1	45	30
UM1	30	30
UM2	70	34
UM3	35	30
LM3	70	30
Total	250	154

Data Collection

The sampled tomato farms were surveyed for bacterial wilt disease caused by *Ralstoniasolanacearum*. Tomato farms in the study area was surveyed for bacterial wilt disease. Questionnaires was administered to the sampled farmers and additional information collected on the disease impacts on tomato production. Data on disease prevalence was obtained by determining the number of infected plants and disease

prevalence data recorded. The study was done in rows of tomato from row 3, 5, 7, 9, 11 whereby at least 10 rows of tomatoes were sampled for every selected farms and assessed for bacterial wilt disease infections. One hundred and fifty-four tomato farms in Meru south sub -county were surveyed for the prevalence of bacterial wilt by counting ten plants in each sampled farm along the selected rows and the prevalence of bacterial wilt in every plant assessed. The results were recorded based on the presence or absence of disease symptoms. Infected plant stems were collected for bacterial isolation in the laboratory.

Disease prevalence in this study was the number of cases of bacterial wilt by *Ralstonia solanacearum* in Meru south during this study season. To determine the disease prevalence, thirty tomato plants was randomly selected from each established sampling farm. From each of the ten tomatoes selected from every farm, five stems were randomly picked from each selected row and used for disease severity for bacterial wilt. Scale of 0 – 5 was used to score disease severity where 0 = no wilting symptom, 1 = less than 10% wilt, 2 = 11-25%, 3 = 26 - 50%, 4 = 51-75% plants wilted, 5 = 75% plants wilted (Aslam *et al*., 2017). Disease score was converted into percentages as shown in the formula below.

$$\text{Disease prevalence (\%)} = \frac{\text{Sum of number of individual rating}}{\text{Number of stems examined x maximum disease grade}}\ x\ 100$$

Tomato plants with typical bacterial infection symptoms were collected from the farms during the survey. Infected samples were cut using sterile scalpel and placed in a sterile zip lock bags. The sample in the zip lock labelled with field accession numbers starting with the abbreviation for different agro ecological zones and sample number. The samples were carried to Chuka university botany laboratory and kept at 4^{o}c prior to pathogen isolation.

Isolation of Bacterial Pathogen from Infected Plants

The bacterial wilt causative agent (*R. solanacearum*) was isolated from the collected samples according to Oljira and Berta, (2020). The collection of samples was done

from the surveyed farms in Meru south –sub county.Small tomato plant stem portions were cut into bits, their surfaces were cleaned with a 70% alcohol solution, and they were then put in a petri dish with moist conditions to encourage the growth of bacteria oozing. Surface streaking of the bacteria oozing that was visible on the stem was done on Kelman`s medium containing 0.01% of TZC (Triphenyl tetrazolium chloride). A fluidal colony with a pink patch or a white colony on the plate was chosen as a probable candidate after 48 hours of incubation at 280 C.

Culturing of Bacterial Pathogen

Pathogen was isolated from collected samples on Kelman's agar in the laboratory according to Sharma & Singh, (2019). Kelman's media was prepared by mixing various components where 1 gram of Casamino acid or (meat extract), 10 grams of Peptone water, 5 ml Glycerol, 20 grams of Agar Agar, 10 grams of Dextrose. The dissolved media components were then autoclaved at 121ºC for 15 minutes at a pressure of 15 psi in an autoclave model (Model X280A). The media was allowed to cool to 50 ºC in a water bath. 5 ml of 1% stock solution of TTC was added then filter sterilized. Thereafter, 5mg of Crystal violet, 100 gm of Polymoxin B Sulphate, 25 mg Bacitracin, 5mg Chloromycetin, 0.5 mg Penicillin was added. Culture media 15 ml was dispensed in Petri dishes and allowed to solidify. Upon isolation, morphological characteristics and biochemical tests were performed identify the pathogen.

Data Analysis

To determine the prevalence of various diseases, descriptive statistics were used to summarize the data into means, percentages, and graphs.Analysis of variance determined if there is difference between isolates of bacteria obtained from different agro ecological zones.Analysis of variance were further used to test the prevalence of bacterial wilt disease and the significant effect of *T. harzianum*on *R. solanacearum* in vitro.Using Least Significance Difference (LSD) at = 0.05, significant means were separated. Data obtained was subjected to ANOVA and analysed using SAS version 9.4 and SPSS version 21 for survey data. The data was presented using frequencies, distribution tables, graphs and percentages.

III.RESULTS AND DISCUSSION

Prevalence of *Ralstonia solanacearum* in Tomato Farms

Five major agro-ecological zones within the tomato production areas viz. LM3, UM3, UM1, UM2, and LM1 were surveyed to assess the prevalence and severity levels of bacterial wilt in Meru South Sub-county. The analysis of variance indicated that the model fitted was adequate [($p < 0.0001$). The study revealed that there was a significance variation ($p < 0.0001$) in the bacterial wilt prevalence among the various farms in different agro-ecological zones within the study area. The highest rate of infection was recorded in zone LM3 with mean infection value of 62.7% while lowest rate of infection was recorded in zone LM1 with a mean infection rate of 32.0%, respectively (Table 2). UM3 recorded a mean infection of 51.330 whereas UM1 and UM2 recorded a mean infection of 43.33 and 41.33 each respectively. It can be concluded that the prevalence of the bacterial wilt of diseases of tomato differed in different AEZ of Meru South Sub-county.

Table 2: The mean percentage prevalence of *R. solanacearum* in various AEZ in Meru South Sub-county

Agro-ecological zones(AEZ)	Mean infection
LM3	62.664[a]
UM3	51.330[c]
UM1	43.330[bc]
UM2	41.330[bd]
LM1	31.996[d]
LSD	9.4515
Mean	46.1300
CV(%)	15.53039
R-squared	0.721196

[a]Means followed by the same letters are not significantly different at 5% probability level.

The prevalence of bacterial wilt by the test pathogen was found to be higher in the farms where tomatoes were grown in the study area. However, there was notable

variation of the level of infection of the pathogen within the surveyed farms in the agro-ecological zones. The disease prevalence ranged from a mean of 15.5% to 62.7%. This was also reported by Bamazi*et al*. (2022) who found that bacterial wilt prevalence is high in tomato farming zones. This is because infected plants would never recover and farmers do not use the diseased plants for any reason, the mean percentage of infected plants was equal to the standard loss.Bamazi*et al*. (2022) reported that continuous use of synthetic chemicals for a long period of time has led to the development of resistance and alters the biological properties of the soil and pH of the soil that harbours bacteria. Some cultural farming practices favours the inoculum of the disease in plant debris hence growth and survival. Lack of hygienic disposal of tomato plants wastes, mono-cropping, poor irrigation methods were reported to increase bacterial inoculum hence high wilting prevalence and severity. Mamphogoro *et al.,* (2020) showed that as a latent infection, *R.solanecearum* was able to survive longer in the soil and on vegetation.

The bacterial wilt illness in tomato crops was said to have developed, spread, and been distributed primarily due to differences in environmental conditions. Weather conditions such as rainfall and temperature variation have a substantial effect on disease development and have influenced bacterial infection hence outbreaks. Koki (2004) reported that wilt prevalence was lower under warm soil conditions unlike water-holding wet soils. Bamazi*et al*., (2022) also reported a high prevalence of bacterial wilt caused by *R. solanacearum* in tomato farms were contributed to heavy rains in the study area during the survey period and the condition favoured the pathogen sporulation.

Table 3:Average groupings for means of isolates from the agro-ecological zones of the study area

AEZ	Isolate ID	Mean of Colony Count
LM3	Plate 1	69.056[a]
UM3	Plate 2	57.520[b]
UM1	Plate 3	55.588[b]
UM2	Plate 4	45.456[c]
LM1	Plate 5	20.662[d]

	LSD	4.3103
	Mean	49.65640
	CV (%)	6.579512

[a]Means followed by the same letter are not significantly different at 5% probability level.

There was a difference in colony morphology traits (Plate 1). The virulent isolates had pinkish coloured colonies with characteristic red centre and whitish margin, non-fluidal and dry-texture colonies on TZC (P1) whereas the isolates obtained on nutrient agar were characterized by white-cream and fluidal wet texture after 24 h of incubation (P3). Isolate P 1 of zone LM3 had pinkish colonies which were fluidal in nature. Isolate P2 from zone UM3 displayed white, small colonies and wet colonies. Isolate P3 from zone UM1 was creamy with dry texture and small round colonies. On the other hand, there was notable similarity of isolate P4 and P3 which showed almost the same characteristics. Isolate P4 was obtained from zone UM2. However, isolate P5 obtained from zone LM1 was whitish in colour with widely dispersed colonies. The variation of cultures isolated from different agro ecological zones was linked to different climatic conditions observed in the zones. Temperature fluctuations could have influenced mutations of *R.solanacearum* pathogen to change to different strains. The result was similar to the findings of Caruso *et al*. (2005) who recordedfound surface water samples infected with *R. solanacearum* ranged in temperature from 9 to 20°C, differing significantly across all sites examined. Temperature variations determines biochemical and metabolic processes of microbes. Higher temperature has been found to be beneficial to bacterial mutations and evolutionary processes.

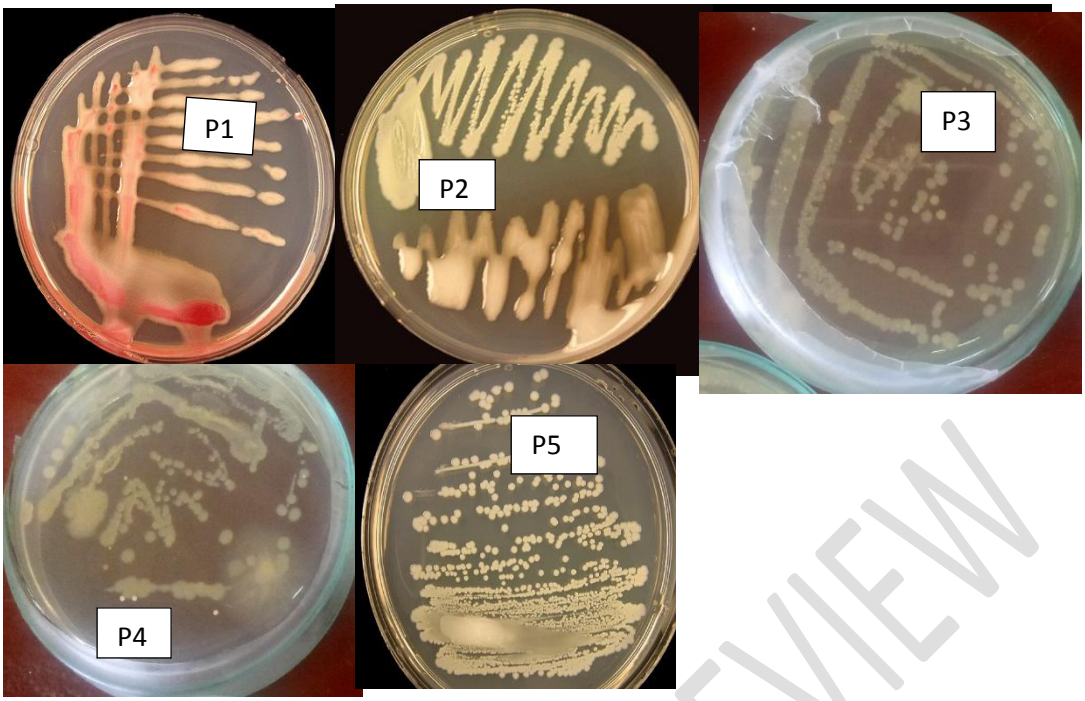

Plate 1: Morphological characteristics of 24 hour old cultures of *R solanacearum* pathogen isolates of TZC agar and Nutrient agar.

Table 4:Mean grouping of colony count isolates after an interval time of 24hours, 48 hours and 72 hours

AEZ	Isolates ID	Mean Count(24ours)	Mean Count(48 hours)	Mean Count(72 hours)
LM3	Plate 1	61.270[a]	69.920[a]	75.982[a]
UM3	Plate 2	47.002[c]	59.198[b]	66.312[b]
UM1	Plate 3	39.718[d]	46.730[c]	49.930[d]
UM2	Plate 4	51.322[b]	54.954[b]	60.488[c]
LM1	Plate 5	15.258[e]	20.956[d]	25.774[e]
	LSD	2.9881	5.2293	5.6132
	Mean	42.926	50.35160	55.69720
	CV(%)	5.276415	7.872106	7.639133

After 48 hours of incubation at 37 °C, all visible colonies were counted using colony counter and there were more than 200 colonies per plate. Additionally, it was observed that plates from samples were generally dominated by small pinkish

colonies in addition to white colonies. The results of this study revealed that the average colony count recorded ranged from plate 5 (LM1) with 20.956 to 69.920 of plate 1[(LM3) Table 4] This was followed by plate 2 (UM3) with a value count of 59.198, plate 4 (UM2) with colony count of 54.954 and plate 3 (UM1) with a colony count of 46.730. The variation of the number of colonies counted within 48 hours could have been because of difference of strains of *R. solanacearum* isolated from various agro ecological zones. Also noted, was the increase of the number of colony count with increase to incubation period.

The results indicated that the average colony count recorded ranged from plate 5 (LM1) with 25.774 to 75.982 of plate 1 [(LM1) Table 4]. This was followed by plate 2 (UM3) with a value count of 66.312, plate 4 (UM2) with colony count of 60.488 and plate 3 (UM1) with a colony count of 49.930. The variation of the number of colonies counted within 24hours could have been as a result of difference of strains of *R. solanacearum* isolated from various agro ecological zones. Also noted, was the increase of the number of colony count with increase to incubation period.

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