# PREVALENCE AND MANAGEMENT OF TOMATO BACTERIAL WILT USING SELECTED RESISTANT VARIETIES IN MOROGORO REGION, TANZANIA

**AGAPE PALILO**

**A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.**

**2019**

## EXTENDED ABSTRACT

Bacterial wilt disease is a very serious problem to Tomato growers of Morogoro region. The objective of this study was to identify the status and pathogenicity of *Ralstonia solanacearum* causing Tomato bacterial wilt disease in Morogoro region. Most of the farmers 71.7% had a farm of less than 1 ha. Majority of the farmers 32% were growing both hybrid and OPV while the reasons for variety preference differed across the villages. Most of respondents 79% were knowledgeable with the disease and considered uprooting affected plants as the only major control measure on the disease. Most of the farmers 35.5% neither burn nor bury the remains of uprooted infected plants and the remained 32.6% bury while 31.9% burn the crop remains. The results showed the highest disease incidence and severity were 42.32% and 3.1 recorded in Kiberengeni and the lowest were 13.76% and 1.75 Nyandira respectively. Majority of the farmers are using seedbed and furrow irrigation system contrary to seedling trays and drip irrigation system. Assila F1 recorded the lowest disease scores, then Rio safari variety while Cal J recorded the highest. Root drenching+wounding inoculation technique recorded significantly highest disease scores and was fatal compared to the other inoculation techniques. Most of Tomato growing farmers84.8% don't manage the disease properly in Morogoro. Equipping farmers with knowledge of good agronomic practices will enhance proper management techniques of the disease. The use of certified and disease free seeds, resistant or tolerant varieties, good agronomic practices can help in proper management of the disease across Morogoro region. The bacterial inoculum isolated from Morogoro was confirmed as *R. solanacearum* using biochemical tests, Serological test and pathogenicity test. From above such results it was observed that Assila F1 tomato variety was significantly resistant compared to Rio safari and the susceptible Cal J. The use of Asilla

F1, followed by Rio safari variety with proper agronomic methods can help farmers to minimize the disease new infections and ultimately spread.

# DECLARATION

I, Agape Palilo do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

……………………………                                        …………………………

**Agape Palilo**                                        **Date**

**MSc. Crop Science Candidate**

The above declaration is confirmed by

……………………………                                        …………………………

**Dr. Newton L Kilasi**                                        **Date**

**Supervisor**

# COPYRIGHT

## ACKNOWLEDGEMENTS

I would like to thank my family for their unending inspiration and the support they have given me in the pursuit of this study, especially my parents and siblings whose love and guidance are with me in whatever I pursue.

I am greatly indebted to my supervisor Dr. Newton Lwilisyo Kilasi and my academic adviser Prof. R. B Mabagala, who have been supportive and worked actively to provide me with guidance to achieve my academic goals. Thank you and God bless you.

I wish to gratefully acknowledge the professional guidance, follow ups and constructive criticisms of Dr Mpoki Shimwela and Prof. D. P Mamiro during the report writing. Their support enabled me to perform the work properly and to collect large amounts of reliable information and data for my dissertation.

Last but not least, I would also like to thank all my classmates for their support as well as the farmers in Nyandira, Mlali, and Kiberengeni for their hard work. Not forgetting the Agricultural and village officers in Mvomero and Morogoro municipality who worked tirelessly to facilitate this work.

This work would not have been possible without the financial and material support of SANGA-PALILO’s Family.

# DEDICATION

I dedicate this work to all farmers worldwide who are working tirelessly in the field to ensure world's food security; they have the utmost patience in a prudent path of being warriors in the garden rather than the gardeners in a war.

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| | | |
|---|---|---|
| SUA | - | Sokoine University of Agriculture |
| FAO | - | Food and Agriculture Organization |
| FAOSTAT | - | Food and Agriculture Organization statistics |
| TZC | - | Tetrazolium chloride |
| NCM | - | Nitrocellulose membrane |
| ELISA | - | Enzyme Linked Immunosorbent Assay |
| DSU | - | Disease severity units |
| AUDPC | - | Area under disease progress curve |
| EPS | - | Extracellular polysaccharide |
| EG | - | Endoglucanase |
| TOSCI | - | Tanzania official seed certification institute |
| OPV | - | Open pollinated varieties |
| $\beta$ | - | Beta |

# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION

### 1.1 Background

The Bacterial wilt disease of Tomato caused by *Ralstonia solanacearum* (Smith, 1896) is the most destructive disease that threatens Tomato production in Morogoro region (Minja *et al.*, 2011). The bacteria itself is a complex species being divided into races, biovars, genomovars, phylotypes and sequevars (Fegan and Prior, 2005). The disease is spread by floods that transfer the bacteria from infected to uninfected plants or fields causing dispersal and multiplication of inoculum which leads to severe infestation (Loreti *et al.*, 2007; EPPO, 2004).

The bacteria invade a plant through wounds created on the lateral roots (Yao and Allen, 2007; Hardoim *et al.*, 2008, Czajkowski *et al.*, 2010). The root exudates released initiate the bacteria's chemotactic movements towards the injured roots (Wei *et al.*, 2018). This renders injured plant roots to much more infection more than other uninfected ones.

The disease symptoms are often severe and which include wilting and death of whole plant. During hottest part of day, wilting of youngest leaves occurs at the end of branches. Under favorable conditions, quick wilting of whole plant occurs and dehydration of leaves took place but dried leaves remain green. Later on, wilting and yellowing of foliage leads to entire plant death (Wei *et al.*, 2018).

Tomato (*Lycopersicon esculentum* Mill.) is considered as the most widely grown warm season vegetable crop due to its wider adaptability and high yield potential. It is originated from Peru-Ecuador region and firstly was domesticated in Europe. It is primarily a selfpollinated crop belongs to Solanaceae family. It is an annual and short lived herbaceous plant which gives better performance under short day condition (Blanca *et al.*, 2012).

Tomato is grown in a wide variety of climate but it best adapted to warm, dry environment, usually during the hot-wet season yields are low due to poor fruit-setting caused by very high temperatures, as well as many severe disease problems (Ofori, 2015).

Among diseases, Tomato bacterial wilt disease is usually the most damaging. In some areas the yield loss is almost 100% during hot and rainy season and especially in low lying areas (Yuquing *et al.*, 2018; Xue *et al.*, 2009).

The use of host resistance appears to be the best option for controlling the bacterial wilt of Tomato (Nion and Toyota, 2015.). Bacterial wilt of Tomato is difficult to control because the pathogen is soil-borne, has a wide host range, widespread distribution, and vast genetic variability (Timila and Joshi, 2007). The disease has many alternate hosts ranging from weeds to crops families (EPPO, 2004). Often tomato cultivars resistant to Tomato bacterial wilt disease in one location have been susceptible in other locations. The crop has shown different levels of resistance and susceptibility to Tomato bacterial disease (Vanitha *et al.*, 2009; Rivard and Louws, 2008; McAvoy *et al.*, 2012)

So there is a need to screen for resistance the commercially Tomato varieties grown by farmers in Morogoro region.

## 1.2 Justification

The Bacterial wilt disease of tomato is one of the most destructive diseases that threaten eastern tomato growing zone (Minja *et al.*, 2011). The disease is difficult to control because the pathogen is soil-borne with a wide host range, widespread distribution, and vast genetic variability (Timila and Joshi, 2007; Yadeta and Thomma, 2013).

Once introduced in a field *R. solanacearum* is difficult to eradicate but can be suppressed through soil sterilization (fumigation and solarization), crop rotation, flooding and use of soil amendments (Momma, 2008). Crop rotation and flooding are not feasible options due to dwindling arable farm sizes (Tahat and Sijam, 2010; Ishihara et al., 2012). The use of host resistance appears to be the best option for controlling the Tomato bacterial wilt disease (Lin *et al.*, 2008; Loreti *et al.*, 2008).

Tomato is the most important vegetable crop in Tanzania so the disease poses adverse impacts on the livelihood of small holder farmers in the country. There is a constant growing demand of vegetable produce in Tanzania. The average tomato yield under smallholder farming ranges from 2.2 to 16 t/ha which is far below the world average of 36.7 tons/ha (FAOSTAT, 2017). The low tomato yield under small-scale farming system is largely due to diseases such as Fusarium wilt (*Fusarium oxysporum*), Late blight (*Phytophthora infestans*), Early blight (*Alternaria solani*), and bacterial wilt (*Ralstonia solanacearum*) (Msogoya and Mamiro, 2016; Muthoni, and Nyamongo, 2009). To increase productivity, there is a need to identify and recommend stress-tolerant varieties adaptable to the coastal areas especially Morogoro region (Minja *et al.*, 2011). Resistant tomato plant material identified can be established for grafting and future breeding programs. Resistant varieties can be sorted out from the screening process of the prevalent varieties grown by farmers in Morogoro.

## 1.3 Objectives

### 1.3.1 Overall objective

Identification of the status and response of Tomato varieties to Bacterial wilt disease in Morogoro region.

### 1.3.2 Specific objectives

i. To evaluate smallholder farmers' knowledge, perception and cultural practices for controlling Tomato bacterial wilt disease in Morogoro region.
ii. To determine the occurrence and distribution of Tomato bacterial wilt disease of Tomato in Morogoro region
iii. To assess the performance of selected Tomato varieties from Morogoro region for resistance to Bacterial wilt disease.

**References**


Blanca, J., Cañizares, J., Cordero, L., Pascual, L., Diez, M.J. and Nuez, F., 2012. Variation revealed by SNP genotyping and morphology provides insight into the origin of the tomato. PloS one, 7(10), p.e48198.

Czajkowski, R., de Boer, W.J., Velvis, H. and van der Wolf, J.M., 2010. Systemic colonization of potato plants by a soilborne, green fluorescent protein-tagged strain of Dickeya sp. biovar 3. Phytopathology, 100(2), pp.134-142.

EPPO. (2004). Diagnostic protocols for regulated pests; Ralstonia solanacearum.pp. 173-178.

FAOSTAT (2017). FAO Statistic database. http://faostat.fao.org (accessed on 20 June 2018).

Goftishu, M., Seid, A., and Dechassa, N. (2014). Occurrence and Population Dynamics of Tomato Leaf Miner (*Tuta absoluta* (Meyrick), Lepidoptera: Gelechiidae) in Eastern Ethiopia. *East African Journal of Science* 8(1): 59-64

Haile, A., and Safawo, T. (2018). Shelf life and quality of tomato (*Lycopersicon esculentum* Mill.) fruits as affected by different Packaging Materials. *African Journal of Food Science* 12(2): 21-27.

Hardoim, P.R., van Overbeek, L.S. and van Elsas, J.D., 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends in microbiology, 16(10), pp.463-471.

Ishihara, T., Mitsuhara, I. and Nakaho, K. (2012). Transcriptome analysis of Quantitative Resistance-Specific Response upon Ralstonia solanacearum in Tomato. PLoS ONE,7(10): doi:10.1371/journal.pone.0046763.

Lin, C.H., Hsu, S.T., Tzeng, K.C. and Wang, J.F., 2008. Application of a preliminary screen to select locally adapted resistant rootstock and soil amendment for integrated management of tomato bacterial wilt in Taiwan. Plant disease, 92(6), pp.909-916.

Loreti, S., Fiori, M., Simone, D. D., Falchi, G., Gallelli, A., Schiaffino, A., and Ena, S. (2008). Bacterial wilt caused by Ralstonia solanacearum, on tomato in Italy. *Plant pathology* 57(2).

Match Marker Associates (2008). *Fresh fruits and vegetables subsector/value chain analysis in Tanzania; Small & medium Enterprise Competitive Facility*. March 2008:33 pp.

McAvoy, T., Freeman, J.H., Rideout, S.L., Olson, S.M. and Paret, M.L., 2012. Evaluation of grafting using hybrid rootstocks for management of bacterial wilt in field tomato production. HortScience, 47(5), pp.621-625.

Minja, R. R., Ndee, A., Swai, I. S., and Ojiewo, C. O. (2011). Promising improved tomato varieties for eastern Tanzania. *African Journal of Horticultural Science* 4(1); 17- 21.

Momma, N., 2008. Biological soil disinfestation (BSD) of soilborne pathogens and its possible mechanisms. Japan Agricultural Research Quarterly: JARQ, 42(1), pp.7-12.

Msogoya, T.J. and Mamiro, D. P. (2016). Effect of improved tomato cultivars on productivity and profitability in Morogoro region, Tanzania. *Journal of Animal and Plant Sciences* 30(3): 4774-4780.

Muthoni, J. and Nyamongo, D.O., 2009. A review of constraints to ware Irish potatoes production in Kenya. Journal of Horticulture and Forestry, 1(7), pp.98-102.

Nion, Y.A. and Toyota, K.. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes and environments* 30: 1, 1-11, 2015

Ofori, E., 2015. Influence of Grafting On the Growth, Yield, Quality and Shelf Life of Tomatoes (Solanum Lycopersicum L.) Grafted Onto Three Solanum Species (Doctoral dissertation, University of Ghana).

Piras, S., Vittuari, M., Möllers, J., and Herzfeld, T. (2018). Remittance inflow and smallholder farming practices. The case of Moldova. *Land use policy* 70: 654-665.

Rivard, C.L. and Louws, F.J., 2008. Grafting to manage soilborne diseases in heirloom tomato production. HortScience, 43(7), pp.2104-2111.

Schreinemachers, P., Balasubramaniam, S., Boopathi, N. M., Viet Ha, C., Kenyon, L., Praneetvatakul, S., and Srinivasan, R. M. (2015). Farmers' perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. *Crop Protection* 75: 115-123.

Sujarwo, S. (2016). Incorporating Entrepreneurship in a Production Function. *Agricultural Socio-Economics Journal* 16(2): 79-86.

Tahat, M. M. and Sijam, K. (2010). Ralstonia solanacearum: The bacterial wilt causal agent. Asian Journal of Plant Science 9: 385-393.

Timila, R.D. and Joshi, S., 2007. Participatory evaluation of some tomato genotypes for resistance to bacterial Wilt. Nepal Agriculture Research Journal, 8, pp.50-55.

Vanitha, S.C., Niranjana, S.R., Mortensen, C.N. and Umesha, S., 2009. Bacterial wilt of tomato in Karnataka and its management by Pseudomonas fluorescens. Biocontrol, 54(5), pp.685-695.

Wei, Y., Caceres-Moreno, C., Jimenez-Gongora, T., Wang, K., Sang, Y., Lozano-Duran, R. and Macho, A.P., 2018. The Ralstonia solanacearum csp22 peptide, but not flagellin-derived peptides, is perceived by plants from the Solanaceae family. *Plant biotechnology journal* 16(7): 1349-1362.

Xue, Q.Y., Chen, Y., Li, S.M., Chen, L.F., Ding, G.C., Guo, D.W. and Guo, J.H., 2009. Evaluation of the strains of Acinetobacter and Enterobacter as potential biocontrol agents against Ralstonia wilt of tomato. Biological Control, 48(3), pp.252-258.

Yadeta, K. and Thomma, B., 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Frontiers in plant science, 4, p.97.

Yuqing, W., Zhang, Y., Zhipeng, G. and Wencai, Y., 2018. Breeding for resistance to tomato bacterial diseases in China: challenges and prospects. Horticultural Plant Journal, 4(5), pp.193-207.

Smith, E. F. (1896). A bacterial disease of the tomato, eggplant and Irish potato (Bacillus solanacearum nov. sp). U. S. Department of Agriculture Division of Vegetable Physiology and Pathology pp 1854-1927.

# CHAPTER TWO

## 2.0 SMALLHOLDER FARMERS' KNOWLEDGE AND PERCEPTION OF TOMATO BACTERIAL WILT DISEASE MANAGEMENT

### 2.1 Abstract

Smallholder farmers' knowledge and perception on disease management give an insight and technical knowledge a farmer possesses. The objective of this study was to evaluate farmers' knowledge and perception involved in controlling Tomato bacterial wilt disease in selected villages of the Morogoro region. Face-to-face interview using a semi-structured questionnaire was conducted in three villages of Morogoro municipality and Mvomero districts of Morogoro region, from September to December 2018. The distribution of farm sizes was the same across categories of the village ($\chi2 = 124.68$; $P < 0.01$). Majority of the farmers (56%) were growing both hybrid and OPV while the reasons for variety preference differed across the villages. Most of respondents (73.2%) were knowledgeable with the disease and considered uprooting affected plants as the only major control measure on the disease. Most of the farmers (61.6%) were uprooting the infected plants as a means to control the disease. Most of the farmers 35.5% neither burn nor bury the remains of uprooted infected plants and 32.6% were burying the crop remains. The remaining 31.9% are burning the crop residues. Most of Tomato growing farmers don't manage the disease properly in Morogoro. Equipping farmers with knowledge of good agronomic practices will enhance proper management techniques of the Tomato bacterial wilt disease.

**Keywords:** *Ralstonia solanacearum*, Knowledge, Good agronomic practices, Morogoro and Tomato.

## 2.2 Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the most widely cultivated and consumed vegetable crop in the world (Haile and Safawo, 2018). Tomato increased production and productivity within the country is very important for food and nutritional security. In Tanzania, the average Tomato yield under smallholder farming ranges from 2.2 to 16 t/ha while the world average is about 36.7 t/ha (FAOSTAT, 2017). Morogoro region is among the largest producers of Tomato in the country with the area under tomato cultivation of 6519 ha (Match Marker Associates, 2008).

The low tomato yield under small-scale farming is largely due to different stresses such as insect pests, drought, and diseases, Tomato bacterial wilt disease being one of the most common diseases in the Morogoro region. The Tomato bacterial wilt disease (*Ralstonia solanacearum*) can cause yield loss of up to 100% during the hot rainy season (Yuqing *et al.*, 2018; EPPO, 2004;Xue et al., 2009).

Persistence of the disease is attributed to a lack of knowledge on good agronomic practices, disease infection cycles and farmers' perception of synthetic pesticides as the only option to control the disease. Majority of smallholder farmers don't adopt recommended cultural and chemical management practices due to the high cost of implementation or ineffectiveness of the method.

Despite the importance of farmers' knowledge on disease management in crops, there is a scarcity of information on this knowledge in Tanzania. The assessment of farmers' perception and knowledge in the management of the Tomato bacterial wilt disease is essential in designing appropriate control options that meet farmer's needs and demands. Therefore, this study was designed together vital information about farmers' knowledge and perception of Tomato bacterial wilt disease and their preferred Tomato cultivars. So the main objective was to evaluate farmers' knowledge and perception of Tomato bacterial wilt disease management in Morogoro Region, Tanzania.

## 2.3 Material and Methods

### 2.3.1 Description of the study site

The study was conducted in the three villages (Kiberengeni, Mlali and Nyandira) from the three agro-ecological zones of the two districts Viz. Morogoro municipality and Mvomero of Morogoro region. These districts are located at 06°49′20″S, 037°39′55″E and 6°18′0″ S, 37°27′0″ E respectively with an altitude of 400-1500 m above sea level.

### 2.3.2 Sample Selection

Interviewed farmers were selected using a multi-stage random sampling procedure as per Schreinemachers *et al.*, (2015). These districts were selected based on their long history of Tomato production. In Mvomero two villages were purposively selected i.e. Nyandira and Mlali while in Morogoro municipality one village Kiberengeni was selected. The sample size (n = number of farmers to be interviewed) was determined as per Wonnacott and Wonnaccot, (1990) with the following equation.

$$N = \frac{Z^2 P(1-P)}{Q^2} = \frac{1.96^2(1-0.9)}{0.05^2} = 138 \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where, N = required sample size, Z = confidence level at 95% (standard value of 1.96), p = estimated proportion of an attribute (average percent of Tomato farmers in a population of horticultural farmers in the villages), estimated at 90% and the Q = margin of error at 5% (standard value of 0.05). One hundred thirty eight (138) farmers in three villages, forty six farmers were chosen per village.

### 2.3.4 Data collection

Data were collected through face-to-face interviews and filled in a semi-structured questionnaire. The questionnaire based on factors related to farmers' preferences in type of varieties grown, farmer's practices involved in management of the Tomato bacterial wilt disease. The data collected included farmers' socioeconomic profiles (e.g. age, gender, and education), preferred Tomato varieties and knowledge and perception on the Tomato bacterial wilt disease management.

## 2.4 Data Analysis

Quantitative and qualitative data were coded and subjected to statistical analysis using the Statistical Package for Social Sciences software (IBM SPSS Statistics 20). Cross-tabulations tables were constructed and descriptive statistics were calculated to summarize data from the questionnaires. To make statistical inferences, contingency chi-square tests were computed at $P \leq 0.05$ levels of significance to analyze relationships between variables.

## 2.5 Results

### 2.5.1 Description of the demographic characteristics

There was a significant difference ($\chi2 = 1.38$; $P = 0.01$) across surveyed villages on the sex of the farmers (Table 1). Among the farmers interviewed about 72.5% were males and 27.5% were females.

There was a significant difference ($\chi2 = 18.47$; $P = 0.01$) across surveyed villages on age of the farmers (Table 1). The age of the farmers ranged from 19 to 63 years.

There was no significant difference (χ2 = 6.17; P = 0.404) across surveyed villages on the education level of the farmers (Table 1). About 30.4% of the interviewed farmers had primary school education, 32.6.7% had secondary school education, 7.2% had at least a college education and 29.7 had no formal education at all.

There was no significant difference (χ2 = 2.79; P = 0.248) across surveyed villages on the farm sizes of the farmers (Table 1). For most of the farmers (71.7%) The amount of land devoted to Tomato production was less than one hectare.

There was a significant difference (χ2 = 42.89; P < 0.01) across surveyed villages on the Tomato yield per hectare. Nyandira had the highest average yield 7.3 Tons/Ha of the surveyed three villages of the Morogoro region (Figure 1).

**Table 2.1: Demographic characteristics of farmers in the surveyed area (n = 138)**

| Characteristic | Villages | | | | df | χ2 | *P*-value |
|---|---|---|---|---|---|---|---|
| | Kiberengeni (n=46) | Mlali (n=46) | Nyandira (n=46) | Mean (%) | | | |
| *Age (Years)* | | | | | | | |
| 18-35 | 41.3 | 60.9 | 28.3 | 43.5 | | | |
| 36-45 | 28.3 | 37.0 | 45.7 | 37 | 4 | 18.47 | 0.01 |
| >46 | 30.4 | 2.2 | 26.1 | 19.6 | | | |
| *Sex* | | | | | | | |
| Male | 67.4 | 71.7 | 78.3 | 72.5 | 2 | 1.38 | 0.01 |
| Female | 32.6 | 28.3 | 21.7 | 27.5 | | | |
| *Education level* | | | | | | | |
| No formal education | 23.9 | 34.8 | 30.4 | 29.7 | | | |
| Primary | 37 | 19.6 | 34.8 | 30.4 | 4 | 6.17 | 0.404 |
| Secondary | 30.4 | 41.3 | 26.1 | 32.6 | | | |
| College | 8.7 | 4.3 | 78.7 | 7.2 | | | |
| *Farm size (Ha)* | | | | | | | |
| 0-1 | 69.6 | 65.2 | 80.4 | 71.7 | 2 | 2.79 | 0.248 |
| >1.1 | 30.4 | 34.8 | 19.6 | 28.3 | | | |

Note. df = degree of freedom, χ2 = Chi-Square test, P ≤ 0.05 shows there was a significant difference.

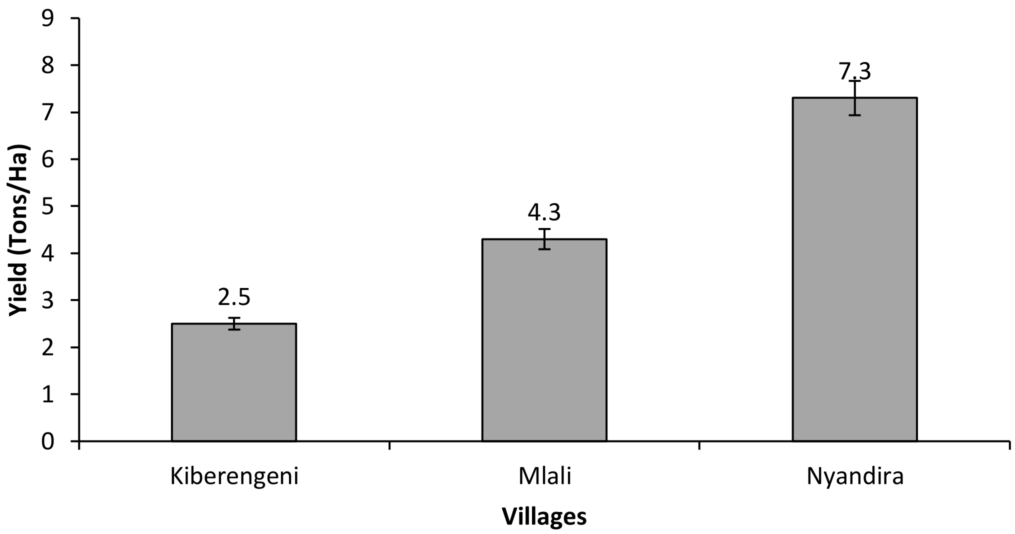



**Figure 2.1: Mean Tomato yield across villages**

### 2.5.2 Tomato varieties preferred by farmers

There was a significant difference ($\chi2 = 56.9$; $P < 0.01$) of preferred varieties across the surveyed villages. Farmers interviewed were growing Tomato varieties as follows; in kiberengeni Rio safari, Rio Grande and Kipato F1. In Mlali: Rio safari, Asila F1 and Imara F1. In Nyandira: Anna F1, Assila F1, and Eden F1. Nyandira had the highest percentage of farmers (63%) growing the hybrid Tomato seeds (Figure 2.2).

The significant difference on the reasons for variety preference was detected ($\chi2 = 128.97$; $P < 0.01$) across categories of the surveyed villages. The variety preference was influenced by different reasons such as market, disease resistance, price affordability etc.

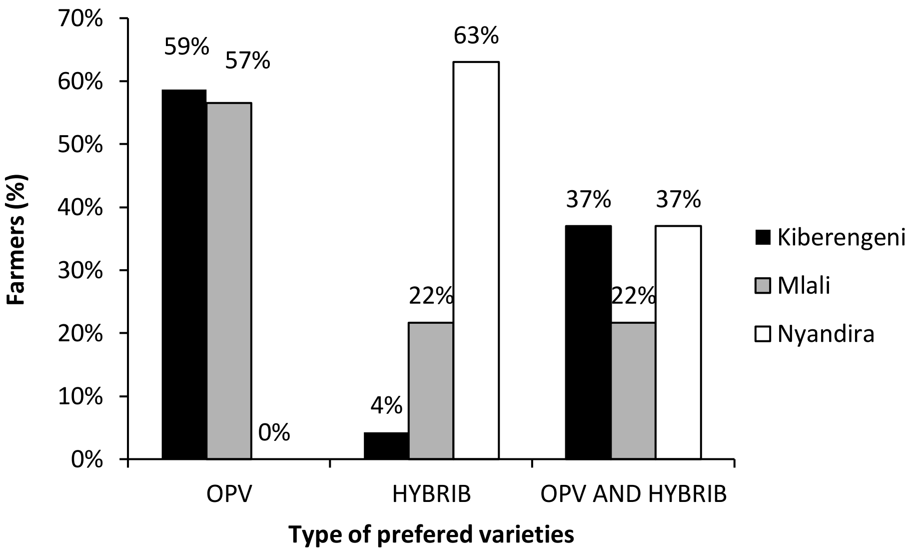



**Figure 2.2**: **The preference of Tomato varieties across villages.**

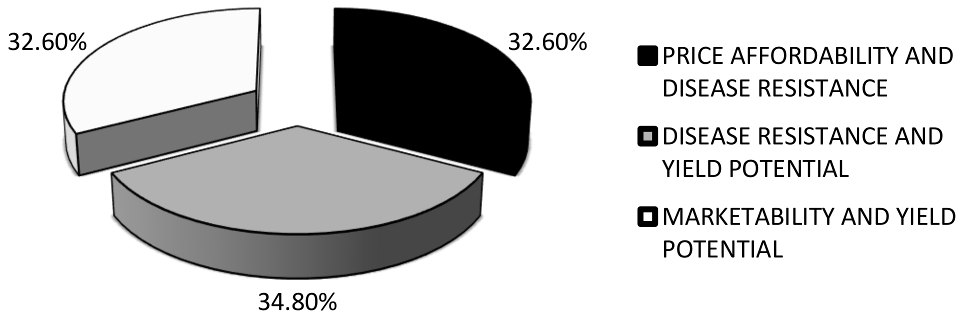



**Figure 2 3**: **The reasons for variety preference in the study area**

### 2.5.3 Farmers' knowledge and perception

There was a significant difference ($\chi 2 = 1.14$; $P = 0.01$) observed among farmers on the knowledge of disease across surveyed villages. Most of the farmers 79% of them were well knowledgeable with the disease and the remaining 21% were unknowledgeable about the disease (Table 2.2).

There was no significant difference ($\chi 2 = 0.33$; $P = 0.778$) across the surveyed villages on the control measure used. About 61.6% of interviewed tomato farmers were uprooting the infected plants and 15.2% were liming and 23.2% were not controlling at all (Table 2.2). There was a significant difference ($\chi2$ = 18.69; P = 0.01) observed among farmers on disposal method of infected crop residues across surveyed villages. Most of the farmers 35.5% neither burn nor bury the remains of uprooted infected plants and 32.6% were burying the crop remains. The remaining 31.9% are burning the crop residues (Table 2.2).

**Table 2.2: Knowledge and perception on management of Tomato bacterial wilt disease**

| **Knowledge and perception** | **Villages** | | | **Mean (%)** | **df** | **$\chi2$** | ***P*-value** |
|---|---|---|---|---|---|---|---|
| | **Kiberengeni (n=46)** | **Mlali (n=46)** | **Nyandira (n=46)** | | | | |
| Knowledge on disease | | | | | | | |
| *Yes* | 80.4 | 82.6 | 73.9 | 79 | 2 | 1.14 | 0.01 |
| *No* | 19.6 | 17.4 | 26.1 | 21 | | | |
| Control measure | | | | | | | |
| *Uprooting* | 58.7 | 58.7 | 67.4 | 61.6 | | | |
| *No control* | 26.1 | 21.7 | 21.7 | 23.2 | 4 | 1.77 | 0.778 |
| *Liming* | 15.2 | 19.6 | 10.9 | 15.2 | | | |
| Disposal method | | | | | | | |
| *Burning* | 19.6 | 34.8 | 41.3 | 31.9 | | | |
| *Burying* | 47.8 | 13 | 37 | 32.6 | 4 | 18.69 | 0.01 |
| *Don't burn or bury* | 32.6 | 52.2 | 21.7 | 35.5 | | | |

Note. df = degree of freedom, $\chi2$ = Chi-Square test, $P \leq 0.05$ shows there was a significant difference.

## 2.6 DISCUSSION

Most of the farmers were males and this implies they are the dominant primary owners of land in the farming community across the region. This has been contributed by stereotypic perception built in the society where by men are believed to be primary owners of resources as supported by Doss *et al.,* (2013) that men are more likely five times to own a land than women.

The highest percentage of farmers occurs within the age range 18-35; this shows that in surveyed villages the youths are participating actively in agriculture activities especially in the horticulture industry. The situation may have been caused by intensification of production in horticulture industry. This correlates with the studies from Nyalulu, (2015) which shows that most of the youths participate in horticulture crop production.

The highest percentage of farmers still had primary school education and some no formal education at all. Most of the farmers have low level education which may technically impair the good agricultural practice literacy. This correlates with the report from Piras *et al.*, (2018) which indicates that majority of farmers engaged in semi-subsistence farming have relatively low levels of education and many have not completed even a primary school education.

Nyandira has the highest yield per hectare because most of the farmers are using hybrid seeds which will, in turn, leads to high production and productivity. This can be supported by the study from Sujarwo, (2016) which shows that improved vegetable seeds lead to increased production and productivity.

The reasons for variety preference were distributed according to the interests by the farmers as dictated by locality. The highest percentage of price affordability and disease resistance especially in Kiberengeni, Marketability and yield potential was recorded in Nyandira. The farms in Nyandira are located in the mountainous area leading to the farming plots being extra small, so due to this the only option to generate profit is to increase yield per unit area. This can be supported by the studies from Norton, (2016) which states that farmers in marginalized land tend to increase the productivity of their farms.

Most of the farmers are uprooting the infected plants only as a measure to control the disease but the dead plants are not properly disposed, Instead they are just thrown in the field. This renders the region to disease spread and much more infections as the pathogen itself is both soil and seed borne. The lack of knowledge of proper disposal of infected plants leads to an increase in disease spread. This can be supported with the study from Neindow *et al*., (2018) which shows that farmers who lack in technical knowledge have no capacity in controlling disease infections in the field.

Although most of the farmers are knowledgeable with the disease but its only symptoms, so there is still much more need for technical knowledge. This also contributes to the disease spread in the Morogoro region. This can be supported with the study from Neindow *et' al*, (2018) which shows that farmers can minimize disease infections in the field if they are well knowledgeable and follow GAP-good agronomic practices.

Generally, the amount of knowledge, technical know-how and perception of the farmer has an impact on the preferred varieties grown and productivity of Tomato in the study area. There are some farming practice used by the farmers in the region which tends to impair control and management of the disease in the region. So to minimize the disease spread in the region farmers need to be more agronomy literate and follow Tomato Good agronomic practices (GAPs). The IPM strategy should also be followed to manage the disease appropriately.

**References**

Doss, C., Kovarik, C., Peterman, A., Quisumbing, A., and van den Bold, M. (2015). Gender inequalities in ownership and control of land in Africa: myth and reality. *Agricultural Economics* 46(3): 403-434.

FAOSTAT (2017). FAO Statistic database. http://faostat.fao.org (accessed on 20 June 2018).

Haile, A., and Safawo, T. (2018). Shelf life and quality of tomato (*Lycopersicon esculentum* Mill.) fruits as affected by different Packaging Materials. *African Journal of Food Science* 12(2): 21-27.

Match Marker Associates (2008). *Fresh fruits and vegetables subsector/value chain analysis in Tanzania; Small & medium Enterprise Competitive Facility*. March 2008:33 pp.

Neindow, M., Sowely, E. N. K., and Abubakari, A. H. (2018). Farmers' knowledge and perceptions of leaf spot disease of groundnut and its management in the Northern Region of Ghana. *Journal of Agricultural Biotechnology and Sustainable Development* 10(9): 170 - 177.

Norton, R. D. (2016). The Competitiveness of Tropical Agriculture: A Guide to Competitive Potential with Case Studies. Academic Press.

Nyalulu Gulamiwa, G. (2015). Youth participation in holticulture and Poverty reduction in rural areas: a case study Of horticultural production in Mvomero District, Tanzania (Doctoral dissertation, Mzumbe University).

Piras, S., Vittuari, M., Möllers, J., and Herzfeld, T. (2018). Remittance inflow and smallholder farming practices. The case of Moldova. *Land use policy* 70: 654-665.

Schreinemachers, P., Balasubramaniam, S., Boopathi, N. M., Viet Ha, C., Kenyon, L., Praneetvatakul, S., and Srinivasan, R. M. (2015). Farmers' perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. *Crop Protection* 75: 115-123.

Sujarwo, S (2016). Incorporating Entrepreneurship in a Production Function. *Agricultural Socio-Economics Journal* 16(2): 79-86.

Wonnacott, T. H., and Wonnacott, R. J. (1990). Introductory statistics (Vol. 5). New York: Wiley.

Yuqing, W. A. N. G., Zhang, Y., Zhipeng, G. A. O., and Wencai, Y. A. N. G. (2018). Breeding for Resistance to Tomato Bacterial Diseases in China: Challenges and Prospects. *Horticultural Plant Journal* 4(5): 193-207.

# CHAPTER THREE

## 3.0 THE OCCURRENCE AND DISTRIBUTION OF TOMATO BACTERIAL WILT DISEASE

### 3.1 Abstract

A field survey was carried out in two Tomato growing districts of the Morogoro region in Tanzania to determine incidence, severity and symptom parameters of Tomato bacterial wilt caused by *Ralstonia solanacearum.* A total of 138 field grown Tomato crop were surveyed. Three villages from each agro ecological zone were purposefully selected. The plants were sampled by using a standard quadrat (1 x 1)$m^2$ and bacterial wilt symptoms identified. Wilting incidence was recorded based on number of plants showing symptoms and expressed as percentage of the total number of plants observed. Disease severity was recorded as a fraction of the amount of diseased plant tissue and then rated on a scale of 1 to 5. Although the disease was observed to occur in all three surveyed villages, there was a significance difference on disease incidence ($p<0.01$) and severity ($p<0.01$) across surveyed villages of the study area. The results showed the highest disease incidence and severity were 42.32% and 3.1 recorded in Kiberengeni and the lowest were 13.76% and 1.75 Nyandira respectively. There was a significance difference on type of irrigation system ($p<0.01$) and type of nursery ($p<0.01$) used by the farmers across surveyed villages. Majority of the farmers are using seedbed and furrow irrigation system contrary to seedling trays and drip irrigation system. The study has found out that the disease is present in all three agro ecological zones of the study area.

**Keywords:** *Ralstonia solanacearum***,** Agro ecological zones, Good agronomic practices, Disease scores, Morogoro.

## 3.2 Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important edible and nutritious vegetable crop around the world which belongs to the solanaceae family. In Tanzania tomato is grown on 39251 hectares with the production of 565441 tonnes, the average yield under smallholder farming ranges from 2.2 to 16 t/ha which is far below the world average yield of 36.7 t/ha (FAOSTAT, 2017). The crop is prone to a number of bacterial diseases, among which bacterial wilt caused by *Ralstonia solanacearum* (Smith), is one of the most economic important diseases (EPPO, 2004). The pathogen is a major constraint to production of tomato in the wet tropics and subtropics region of the world. The bacterium can spread through transportation of infested seeds, farm implements, irrigation (drainage) water and infected soils to mention a few (Tahat and Sijam, 2010; Ishihara et al., 2012).

The disease control is difficult because it survives in water and in residues of infected plants in the soil and has a wide host range, wide geographical distribution, and vast genetic variability of the pathogen (Wei *et al.*, 2018). Due to this the pathogen has been considered as an important quarantine organism in many countries (Kaaya *et al*., 2003, EPPO 2004).

The pathogen enters plant roots through natural openings or wounds created by pests such as nematodes, colonizes the intercellular space of the root cortex and vascular parenchyma, and eventually enters the xylem vessel and spreads into the stem and leaves (Yuliar *et al.*, 2015, Wei *et al.*, 2018). The bacterial wilt symptoms in tomato are characterized by initial wilting of upper leaves and within a few days followed by complete wilting of the plants. The vascular tissues of the infected stem have brown discoloration and, if the stem is cut crosswise, white or yellowish bacterial ooze may be visible (Wei et al., 2018).

Once introduced in the field, *R. solanacearum* is difficult to control, but various measures have been suggested to manage the disease. These include certified disease free seeds, use of resistant cultivars, physical methods i.e solarization and hot water treatment, crop rotation, biological control, and use of soil amendments (Yuliar *et al.,* 2015). Often tomato cultivars resistant to disease at one location have been susceptible at other locations (Loreti *et al.,* 2008, Yuliar *et al*., 2015).

Abiotic factors being favourable weather condition for the tomato bacterial wilt disease will tend to fever spread and affect the occurrence and distribution across the regions. Hot and wet conditions, and low land areas tend to be affected intensely with the tomato bacterial wilt disease (Loreti *et al.,* 2008). However, the disease occurrence and distribution is affected by pathovar, hosts and specific environmental conditions. Although *R. solanacearum* has been one of the major diseases of tomato growing area of Morogoro (Msogoya and Mamiro, 2016), But no information has been published in the occurrence and distribution of *R. solanacearum* in major tomato growing areas of Morogoro. So the study was carried to assess the disease occurrence and distribution across the three agro ecological zones of Morogoro region.

## 3.3 Material and Methods

### 3.3.1 Description of the Study Site

The field survey was conducted in the three villages of the three agro-ecological zones of Morogoro region from September 2018 to January 2019. The three villages were selected from Morogoro municipality and Mvomero districts. These districts are located at 06°49′20″S, 037°39′55″E and 6°18′0″ S, 37°27′0″ E respectively with an altitude of 400-1500 m above sea level. Farms surveyed at Kiberengeni village are at an average altitude of 491 m a s l, Mlali village are at an average altitude of 570 m a s l and Nyandira village are at an average altitude of 1632 m a s l. The areas selected are major Tomato producing areas in the region.

### 3.3.2 Scoring Tomato bacterial wilt disease incidence and severity in the surveyed farms

A transect walk was made across 46 farmers' fields per village in three villages Viz. Kiberengeni, Mlali and Nyandira to assess the incidence and severity of bacterial wilt disease of Tomato. A total of 138 fields grown Tomato crop were surveyed. Destructive sampling was applied for observation of typical Tomato bacterial wilt disease symptoms. Plants assessed for disease severity and incidence were sampled by using a standard quadrat (1 x 1)$m^2$. Severity of wilting symptoms of individual plants were being rated on a scale of 1 to 5, where: 1 = no visible symptoms; 2 = one to less than half of the foliage wilting; 3 = about half of the foliage wilting; 4 = nearly all of the foliage wilting; 5 = the whole plant wilting and dead as per (Horita and Tsuchiya, 2001). The scores were an average in each quadrat, an average in the individual field and ultimately an average in each selected area.

$$\text{Disease severity} = \frac{\sum \text{severity scores in a quadrat}}{Number\ of\ plants\ sampled} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(2)}$$

Disease incidence was calculated as the ratio number of diseased plants to a total number of plants sampled multiplied by 100. The incidence of bacterial wilt disease in each area was calculated as an average of the scores of the selected farms.

$$\text{Disease incidence (\%)} = \frac{Number\ of\ plants\ infected}{Total\ number\ of\ plants\ sampled} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(3)}$$

### 3.3.3 Farm characteristics survey

Data were collected through face-to-face interviews and filled in a checklist. The checklist based on factors related to farmers' agronomic practices and farm technologies involved in a production. The data collected were type of irrigation system, type of nursery used, days to total plant death, type of disease signs or symptoms and crop growth stage of symptoms occurrence.

### 3.4 Data Analysis

Quantitative and qualitative data including disease scores (incidence and severity) were coded and subjected to statistical analysis using the Statistical Package for Social Sciences software (IBM SPSS Statistics 21). Cross-tabulations tables were constructed and descriptive statistics were calculated to summarize data from the checklist. To make statistical inferences, contingency chi-square tests were computed at $P \leq 0.05$ levels of significance to analyze relationships between variables. This allowed empirical analyses and description of the association between the collected parameters across the surveyed villages of the three agro ecological zones of Morogoro region.

### 3.5 Results

#### 3.5.1 Disease severity scores across the region

There was a significance difference ($\chi2 = 75.47$; $P < 0.01$) across surveyed villages on disease severity. The distribution of disease severity was different across the villages of the three agro ecological zones. The lowest average disease severity was 1.75 recorded in Nyandira and the highest average disease severity was 3.1 recorded in Kiberengeni (Table 3.1).

#### 3.5.2 Disease incidence scores across the region

There was a significance difference ($\chi2 = 78.36$; $P < 0.01$) across surveyed villages on disease incidence. The distribution of disease incidence was different across the surveyed villages of the three agro ecological zones. The lowest average disease incidence was 13.76% recorded in Nyandira at the highest altitude of the three villages and the highest average disease severity was 42.32% recorded in Kiberengeni at the lowest altitude of the three villages (Table 3.1).

### 3.5.3 Type of disease symptom and sign

There was a significance difference ($\chi 2 = 135.8$; $P < 0.01$) across surveyed villages on type of disease symptom and sign occurred. The distribution of the type of disease symptoms and signs was different across the surveyed villages of the three agro ecological zones. Disease symptoms and signs varied according to the areas with different altitudes from the sea level. The highest percentage of white milky slime flow was 34.8% recorded in kiberengeni, Sterm rotting was 48.2% recorded in Mlali, and yellowing of older leaves was 22.5% in Nyandira (Table 3.1).

### 3.5.4 The crop growth stage of symptom occurrence

The type of crop growth stage of symptom occurrence varied significantly ($\chi 2 = 135.8$; $P < 0.01$) across villages. The distribution of crop growth stage of symptom occurrence was different across the surveyed villages of the three agro ecological zones. The symptom occurrence at vegetative stage was 82.1% in kiberengeni, Flowering stage was 78.6% at Mlali and Fruiting stage was 82.1% at Nyandira (Table 3.1).

### 3.5.5 The number of days to total plant death

The number of days to total plant death varied significantly ($\chi 2 = 53.8$; $P < 0.01$) across villages. The distribution of days to total plant death was different across the surveyed villages of the three agro ecological zones. The average number of days to total plant death was 4 in Kiberengeni which was at the lowest altitude, 11 days in Mlali which was at the mid-latitude and 20 in Nyandira which was at the highest altitude of the three surveyed areas (Table 3.1).

**Table 3.1: Tomato bacterial wilt symptom distribution across the villages (n = 138)**

| | Villages | | | | | | |
|---|---|---|---|---|---|---|---|
| **Symptom and sign parameter** | **Kiberengeni** | **Mlali** | **Nyandira** | **Mean (%)** | **df** | **χ2** | ***P*-value** |
| *Disease severity* | *n = 46* | *n = 46* | *n = 46* | | | | |
| 0-2 | 10.9 | 23.9 | 50 | 28.3 | | | |
| 2.1-3 | 15.2 | 63 | 50 | 42.8 | 4 | 75.4 | <0.01 |
| 3.1-5 | 73.9 | 13 | 0 | 29 | | | |
| *Disease incidence* | | | | | | | |
| 0-20 | 8.7 | 8.7 | 39.1 | 18.8 | | | |
| 21-40 | 13 | 10.9 | 45.7 | 23.2 | | | |
| 41-60 | 13 | 52.2 | 15.2 | 26.8 | 8 | 78.3 | <0.01 |
| 61-80 | 50 | 21.7 | 0 | 23.9 | | | |
| 81-100 | 15.2 | 6.5 | 0 | 7.2 | | | |
| *Type of symptom* | | | | | | | |
| White-milky slime flow | 80.4 | 13 | 75.0 | 34.1 | | | |
| Sterm rotting | 19.6 | 87 | 17.9 | 43.5 | 4 | 135.8 | <0.01 |
| Yellowing of older leaves | 8.7 | 23.9 | 67.4 | 22.5 | | | |
| *Growth stage infected* | | | | | | | |
| Vegetative | 76.1 | 10.9 | 0 | 29 | | | |
| Flowering | 15.2 | 84.8 | 23.9 | 41.3 | 4 | 135.8 | <0.01 |
| Fruiting | 8.7 | 4.3 | 76.1 | 29.7 | | | |
| *Days to plant death* | | | | | | | |
| <7 | 67.4 | 32.6 | 4.3 | 34.2 | | | |
| 8-14 | 17.4 | 56.5 | 43.5 | 39.1 | 4 | 53.8 | <0.01 |
| >15 | 15.2 | 10.9 | 52.2 | 26.1 | | | |

Note. df = degree of freedom, χ2 = Chi-Square test, P ≤ 0.05 shows there was a significant difference.

### 3.5.6 The disease severity in different irrigation systems

There was no significance (χ2 = 5.76; P = 0.056) relationship of disease severity scores across the type of irrigation system used by the farmers. The distribution of disease severity scores on the type of irrigation system was the same across the surveyed villages of the three agro ecological zones (Table3.2).

### 3.5.7 The disease incidence in different irrigation systems

The disease incidence scores varied significantly (χ2 = 11.38; P = 0.023) across the type of irrigation system. The distribution of disease incidence scores on irrigation system was different across the surveyed villages of the three agro ecological zones. Among the respondents interviewed about 9.3% of farmers using furrow irrigation system had a disease incidence ranging from 81 to 100 while for drip system were 0% (Table 3.2).

**Table 3.2: The disease scores in different types of irrigation system**

| Disease scores | Percentage of respondents | | df | χ2 | *P*-value |
|---|---|---|---|---|---|
| | Drip | Furrow | | | |
| *Disease incidence* | | | | | |
| 0-20 | 35.5 | 14 | | | |
| 21-40 | 12.9 | 26.2 | | | |
| 41-60 | 32.3 | 25.2 | 3 | 11.38 | 0.023 |
| 61-80 | 19.4 | 25.2 | | | |
| 81-100 | 0 | 9.3 | | | |
| *Disease severity* | | | | | |
| 0-2 | 29 | 28 | | | |
| 2.1-3 | 58.1 | 38.3 | 2 | 5.76 | 0.056 |
| 3.1-5 | 12.9 | 33.6 | | | |

Note. df = degree of freedom, χ2 = Chi-Square test, $P \leq 0.05$ shows there was a significant difference.

### 3.5.8 The disease severity in different type of nurseries

The disease severity scores didn't vary significantly ($\chi2 = 2.43$; $P = 0.296$) across the type of nursery used by the farmer. The distribution of disease severity scores on the type of irrigation system was the same across the surveyed villages of the three agro ecological zones (Table 3.3).

### 3.5.9 The disease incidence in different type of nurseries

The disease incidence scores didn't vary significantly ($\chi2 = 4.82$; $P = 0.305$) across the type of nursery used by the farmer. The distribution of disease incidence scores on the type of nursery was the same across the surveyed villages of the three agro ecological zones (Table 3.3).

**Table 3.3: The distribution of disease scores across the type of nursery**

| Disease scores | Percentage of respondents | | df | χ2 | *P*-value |
|---|---|---|---|---|---|
| | Trays | Seedbed | | | |
| *Disease incidence* | | | | | |
| 0-20 | 30.4 | 16.5 | | | |
| 21-40 | 26.1 | 22.6 | 4 | 4.82 | 0.305 |
| 41-60 | 26.1 | 27 | | | |
| 61-80 | 8.7 | 27 | | | |
| 81-100 | 8.7 | 7 | | | |
| *Disease severity* | | | | | |
| 0-2 | 39.1 | 26.1 | | | |
| 2.1-3 | 43.5 | 42.6 | 2 | 2.43 | 0.296 |
| 3.1-5 | 17.4 | 31.3 | | | |

Note. df = degree of freedom, χ2 = Chi-Square test, P ≤ 0.05 shows there was a significant difference.

**Table 3.4: The average disease scores in the study areas**

| Villages | Disease scores | |
|---|---|---|
| | Disease incidence (%) | Disease severity |
| *Nyandira* | | |
| Mlali | | |
| Kiberengeni | | |

## 3.6 Discussion

According to the disease scores from the survey the disease is much more dominant at Kiberengeni compared to other surveyed villages. The disease incidence and severity is much more distributed and occurring in areas of the low altitudes across the three agro ecological zones. This is because most of the pathogen races causing the disease become more virulent in areas of high temperature and low altitude. This correlates with the study from (Loreti *et al.,* 2008) which justifies that the disease becomes more distributed in areas of low altitudes due to increase in virulence of the pathogen. The villages of low altitude are also affected by floods during rainy days that transfer bacteria from infected upper areas to low lying fields of Kiberengeni. The condition leads to stagnant water and consequently causing deposition of disease inoculum from high altitude infected areas into the farms across the villages.

According to the scores from the survey the disease is less dominant at Nyandira compared to other surveyed villages. The disease incidence and severity is less occurring and distributed in areas of high altitude across the agro ecological zones of Morogoro. This is because the pathogen *Ralstonia solanacearum* become less virulent areas with high altitude and cool temperatures. This correlates with the study from (EPPO, 2004) which justifies that the disease becomes less occurring and distributed in areas of high altitude and cool temperatures.

The type of symptoms differed greatly across the surveyed villages of the three agro ecological zones. White milky slime flow mostly occurs in low altitudes, Sterm rotting mostly occurs in mid altitudes and yellowing of older leaves mostly occurs in high altitudes. This is caused by the difference in virulence of the pathogen due to variation in temperature and altitude from the sea level. This is supported by the study from (EPPO, 2004) which shows that the virulence of the pathogen varies according to the temperature and mean altitude from the sea level.

Most of the farmers grow their seedlings by using a seedbed nursery contrary to nursery trays. Smallholder farmers across Morogoro region are characterized by low levels of technology; this contributes to the spread of Tomato bacterial wilt disease infections in the farms across the region. This has been supported with the study from (Durner *et al.*, 2002) which justifies that the soil-borne disease infections becomes more dominant to farmers with poor agriculture practices.

The crop growth stage at which the plant started to display infections was very early (at vegetative) in kiberengeni, intermediate (at flowering) in Mlali and very late (at fruiting) in Nyandira. This is caused by the difference in virulence of the pathogen according to atmospheric characteristics of the different agro ecological zones. This is supported by the study from (EPPO, 2004) which shows that the virulence of the pathogen varies according to the temperature and mean altitude from the sea level.

The number of days to total plant death was very few in kiberengeni, followed by Mlali and somehow many in Nyandira. This is caused by the difference in virulence of the pathogen according to atmospheric characteristics of the different agro ecological zones. This is supported by the study from (EPPO, 2004) which shows that the virulence of the pathogen varies according to the temperature and mean altitude from the sea level.

Most of the farmers are using furrow irrigation system contrary to a drip irrigation system. This has been attributed by the fact that smallholder farmers across Morogoro region are characterized by low levels of technology. This has contributed to an increase in disease spread across the region. The claims can be supported with the study from (Onduso, 2014) which suggests that the soil-borne disease infections are much more dominant in areas where the furrow irrigation system is practiced contrary to drip irrigation system.

It was generally observed that farmers using drip had a little disease incidence compared to farmers using furrow irrigation system. This is because in drip irrigation system farmers have a little possibility of causing root injuries since fertigation is employed contrary to furrow irrigation system. The claims can be supported with the study from (Onduso, 2014) which shows that the soil-borne disease infections are less distributed in areas where the drip irrigation system is practiced contrary to furrow irrigation system.

The study has found out that the disease is present in all three agro ecological zones of the Tomato growing area of Morogoro region. The results show that locational attributes like atmospheric difference (Humidity and temperature) between locations have significant influence on the disease occurrence and distribution in Morogoro region. The disease scores differed significantly across the surveyed villages with the highest scores recorded in Kiberengeni at 491 m a s l followed by Mlali at 570 m a s l and lastly Nyandira at 1632 m a s l.

**References**

Durner, E. F., Poling, E. B., and Maas, J. L. (2002). Recent advances in strawberry plug transplant technology. *Horticulture Technology* 12(4): 545-550.

EPPO, (2004). Ralstonia solanacearum. European and Mediterranean. Plant Protection Organization Bulletin 34: 173-178

FAOSTAT (2017). FAO Statistic database. http://faostat.fao.org (accessed on 20 June 2018).

Goftishu, M., Seid, A., and Dechassa, N. (2014). Occurrence and Population Dynamics of Tomato Leaf Miner (*Tuta absoluta* (Meyrick), Lepidoptera: Gelechiidae) in Eastern Ethiopia. *East African Journal of Science* 8(1): 59-64

Hanson, P. M., Licardo, O., Wang, J. F., and Chen, J. T. (1998). Diallel analysis of bacterial wilt resistance in tomato derived from different sources. *Plant disease 82*(1): 74-78.

Horita, M and Tsuchiya, K. (2001). Genetic diversity of Japanese strains of *Ralstonia solanacearum*. *Phytopathology* 91: 399-407.

Kaaya, N. K., Mortensen, C. N., Massomo, S. M. S., & Mabagala, R. B. (2003). A guide on seed-borne bacterial diseases of tomato in Tanzania: Technical Bulletin.

Loreti, S., Fiori, M., Simone, D. D., Falchi, G., Gallelli, A., Schiaffino, A., and Ena, S. (2008). Bacterial wilt caused by Ralstonia solanacearum, on tomato in Italy. *Plant pathology* 57(2).

Msogoya, T.J. and Mamiro, D., 2016. Effect of improved tomato cultivars on productivity and profitability in Morogoro region, Tanzania. *Journal of Animal and Plant Sciences* 30(3): 4774-4780.

Nion, Y.A. and Toyota, K., 2015. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes and environments* 30: 1, 1-11, 2015

Onduso, J. N. (2014). Management of bacterial wilt of tomato by use of resistant rootstock (Doctoral dissertation, Thesis. University of Nairobi, Nairobi, Kenya).

Wei, Y., Caceres-Moreno, C., Jimenez-Gongora, T., Wang, K., Sang, Y., Lozano-Duran, R. and Macho, A.P., 2018. The Ralstonia solanacearum csp22 peptide, but not flagellin-derived peptides, is perceived by plants from the Solanaceae family. *Plant biotechnology journal* 16(7): pp.1349-1362.

# CHAPTER FOUR

## 4.0 THE RESPONSE OF SELECTED TOMATO VARIETIES FROM MOROGORO REGION TO BACTERIAL WILT DISEASE

### 4.1 Abstract

Bacterial wilt disease is a very serious problem to Tomato growers of Morogoro region. The objective of this study was to assess the ability of selected Tomato varieties from Morogoro region to resist infection by Bacterial wilt disease causing pathogen. Three Tomato varieties Viz; Rio safari, Assila F1, Cal J were evaluated for their performance against *Ralstonia solanacearum* with three Inoculation techniques Viz Root drenching, Root drenching+wounding and No inoculation. The experiment was laid out in a 3x 3 Factorial experiment in RCBD with four replications. Assila F1 recorded the lowest disease scores, followed by Rio safari variety while Cal J recorded the highest disease scores of all. Root drenching+wounding inoculation technique recorded significantly highest disease scores and was fatal compared to the other inoculation techniques i.e. Root drenching, No inoculation. The highest average area under disease progress curve was 100 Disease severity units (DSU) observed from Cal J variety, followed by 65 DSU from Rio safari and the lowest was 60 DSU recorded from Asilla F1 variety. The bacterial inoculum isolated from Morogoro was confirmed as *R. solanacearum* using biochemical tests, Serological test and pathogenicity test. From above such results it was observed that Assila F1 tomato variety was significantly resistant compared to Rio safari and the susceptible Cal J. The use of Asilla F1, followed by Rio safari variety with proper agronomic methods can help farmers to minimize the disease new infections and ultimately spread.

**Keywords:** Root injury, Performance, Tomato varieties, Resistance, *Ralstonia solanacearum*, Morogoro.

## 4.2 Introduction

Tomato bacterial wilt disease caused by bacterium *Ralstonia solanacearum* is a devastating disease that is quickly spreading and causing reduction in yields and income to farmers of Morogoro region. The disease occurs in almost all climatic regions of the world but usually severe damages are observed in wet tropical regions (Loreti *et al.,* 2008). It has been observed that the yield loss is more than 60% and sometimes 100% during hot-rainfall seasons at Kiberengeni in Morogoro urban district.

The pathogen is both soil-borne and seed-borne although the later has been reported to display minimal loses. The pathogen is mainly disseminated through soil and enters roots through wounds or natural openings mainly through lateral roots. It multiplies after infection and moves up through the vascular system, and finally blocks water transport in the xylem vessels causing plant wilting.

Water movement in the plant is impaired as a result of bacterial infection build up in the xylem vessels. Water moves in a continuum as explained by cohesion-adhesion hypothesis. As a result of bacterial infection in water transportation system there will be formation of tyloses which helps also to prevent pathogen movement up the xylem vessel (Agrios, 2005).

The bacteria itself tends to produce polysaccharides complex materials which aids its movement up the xylem vessel. These entire polysaccharide complex produced by both the pathogen and plant itself will tend to hinder water movement and consequently wilting (Wei *et al.,* 2018).

It has been shown from the infected stems that, the major factor which cause wilting symptoms is the high-molecular-mass acidic extracellular polysaccharide (EPS I), but the β-1,4-endoglucanase (EG) contributes to virulence of the pathogen (Wei *et al.*, 2018). The control methods such as soil amendment, crop rotation, biological control, and field sanitation are often not very effective compared to host resistance. The stability of Bacterial wilt resistance in Tomato is highly affected by pathogen density, pathogen strains, temperature, soil moisture, and presence of root injuries especially on the lateral roots of the plant (Loreti *et al.*, 2008).

Farmers during their farm operations such as fertilizer application and weeding tend to cause root injury on Tomato plants during. Such injury can aid easy penetration of the bacterial into plant systems through the injuries especially lateral roots.

Other than chemical fumigants, there is no commercial pesticide available for control of the disease. The method is unfeasible due to environmental concerns and health of the consumers; this leaves the host resistance as the sole technique to control the pathogen. Thus, this study was carried out to evaluate the performance of Tomato varieties under inoculation with the bacterial wilt disease causing pathogen from the selected varieties grown by the farmers of Morogoro region.

## 4.3 Material and Methods

### 4.3.1 Description of the study site

The Study was conducted at the Tanzania Official Seed Certification Institute (TOSCI) pathology laboratories and Crop Science Department screen houses located at Sokoine University of Agriculture (SUA) compound. The area is located at latitude of 06`50`S, longitude of 37`39`E and altitude of 526 m. above sea level.

### 4.3.2 Seed sowing and seedling growth

Seeds of selected tomato varieties Viz Asilla F1, Rio safari, and control Cal J were sown in the plastic trays. The pit moth was used as a growing media. Recommended agronomical practices were followed to ensure good and healthy seedling stand.

### 4.3.3 Tomato seedling transplanting

The seedlings were transplanted 30 days after sowing in the pots in a screenhouse in a 3x3 Factorial experiment laid out in Randomised complete block design with four replications. Sterilized soil was used for the experiments. Fertilizers were applied as per the recommendation of tomato fertilizer program of 115Nkg/ha, 100kg$P_2O_5$/ha and 60Kg $K_2O$/ha in Morogoro. Irrigation followed on regular interval to raise a good crop stand.

### 4.3.4 Isolation and culturing *Ralstonia solanacearum* inoculum

The infected Tomato plant tissues were collected from farmers fields at Kiberengeni village in Morogoro urban District. The samples were brought to the TOSCI pathology laboratory then washed by tape running water to remove debris. After that, each sample was cut to expose its vascular tissues and then left to release bacterial ooze into the testtube for 5 to 10 minutes. The bacterial suspension was streaked onto a semi-selective medium tetrazolium chloride (TZC) agar which was prepared as per (Kelman, 1954). The agar plates were incubated at room temperature 29.3°C for 48 hours in an inverted position to prevent water condensation. Separately growing colonies were picked and sub-cultured onto fresh media to obtain pure cultures. *Ralstonia solanacearum* appeared as mucoid, Pink-whitish colonies after 48 hours as per Mortensen (1997).

### 4.3.5 Biochemical tests of the pathogen

#### 4.3.5.1 Gram staining

A loop full of bacterium was spread on a glass side and fixed by heating on a very slow flame. Aqueous crystal violet solution of 0.5 % was then spread over the smear for 30 seconds, and then washed with running tape water for 60 seconds. Then Iodine (95%) was flooded for a minute followed by rinsing with tap water. The slides were decolorized with 95% ethanol until colorless runoff. The slides were then counter stained with safranin for 10 seconds and washed with water. The slides were dried under the laminar flow cabinet and placed under the light microscohspe at 10X, 40X and 100X for observation (Schaad, 1980).

#### 4.3.5.2 Potassium hydroxide test

Bacterial suspension was picked from petri-plates by wire loop and placed on glass side containing a drop of 3% KOH solution, stirred for 10 seconds and observed for the formation of slime threads as described by Suslow *et al.* (1982).

#### 4.3.5.3 Catalase oxidase test

A loop full of bacterial culture obtained from young agar cultures of 18-24 h were mixed with a 3% hydrogen peroxide ($H_2O_2$) on a glass slide to observe production of gas bubbles with a naked eye and under a dissecting magnification of 25X as per (Schaad, 1980).

#### 4.3.5.4 Kovacs oxidase test

Oxidase reagent (1% tetra-methyl-p-phenyl diamine dihydrochloride) solution of 100ml was prepared and kept in rubber stoppered dark bottle. A drop of the oxidase reagent was added onto a piece of filter paper placed within a glass Petri dish. Small quantity of inoculum was rubbed on the filter paper containing oxidase reagent solution. Bacteria were then observed for the development of purple color in 10-60 seconds. Kovacs oxidase test was performed as per (Mortensen, 1997).

#### 4.3.5.5 Serological test

A sample of the bacterial inoculum was tested using procedures outlined in CIP, NCM-ELISA (Enzyme Linked Immunosorbent Assay on Nitrocellulose Membrane) protocol as per (Priou, 2001).

#### 4.3.5.6 Hypersenstive test

The Tobbaco plant was used in Hypersenstive test as outlined by (Mortensen, 1997) in Seed bacteriology laboratory guide. An aqueous suspension of bacterial at a concetration of 3.0 x $10^8$ cfu/ml was injected into the mesophyll of the leaf blade through a 0.4mm diameter needle. Then also a sterile distilled water was injected into a separate area of the leaf as a control.

### 4.3.6 Innoculation of Ralstonia solanacearum on Tomato varieties

The Bacteria (*R.solanacearum*) suspension was prepared at a concentration 1.0 x$10^8$ cfu/ ml then seedlings with four to five fully expanded true leaves (four weeks old) were inoculated by using three techniques of inoculation R. solanacearum suspension, viz., i) Root dip with wounding ii) Root dip without wounding iii) No inoculation. Inoculated seedlings were maintained in screen house at a temperature range of 25-30°C for disease parameters assesment.

#### 4.3.6.1 Root drenching with wounding technique

In this technique, 5.0ml of bacterial suspension was inoculated to each of the seedlings by drenching the soil around the root zone with the help of micro pipette. Before inoculation, the roots were slightly severed by inserting a sharp knife 1.0cm away from the stem. Root severing was done to ensure bacterial penetration through roots.

#### 4.3.6.2 Root drenching without wounding technique

In this technique, 5.0ml of bacterial suspension was inoculated to each of the seedlings by drenching the soil around the root zone with the help of micro pipette. No Root severing was done in this technique.

### 4.3.7 Assessment of bacterial wilt disease incidence and severity on tomato varieties

Evaluation of disease incidence and severity was done at 0, 5, 10, 20, and 30 days after inoculation. Severity was based on wilting symptoms of individual inoculated plants and was rated using a scale of 1 to 5, where: 1 = no visible symptoms; 2 = one to less than half of the foliage wilting; 3 = about half of he foliage wilting; 4 = nearly all of the foliage wilting; 5 = the whole plant wilting and dead based on (Horita and Tsuchiya, 2001). Disease incidence was scored as the ratio number of diseased plants to total number of plants sampled multiplied by 100.

### 4.3.8 Assessment of the level of resistance of Tomato varieties.

The disease progress curves for each Tomato variety were obtained where by disease severity was plotted against time in days. The area under disease progree curve AUDPC was calculated as per Simko and Piepho, (2012) by the formula as follows;

$$\text{AUDPC}=\sum_{i=1}^{n}\left[\frac{(D_i+D_{i+1})}{2}\right][t_{i+1}-t_i] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Where by AUDPC= Area under disease progress curve, D= disease score, t=time

#### 4.3.9 Data analysis

The experiment was Randomized Complete Block Design (RCBD) laid out in 3x3 Factorial with four replications. Data on disease incidence, disease severity, incubation period, days to total plant death, and survival period were subjected to two-ways analysis of variance (2 WAY ANOVA) using GENSTAT statistical software. Treatment mean separation was conducted using Duncan MCT at $P < 5$ %.

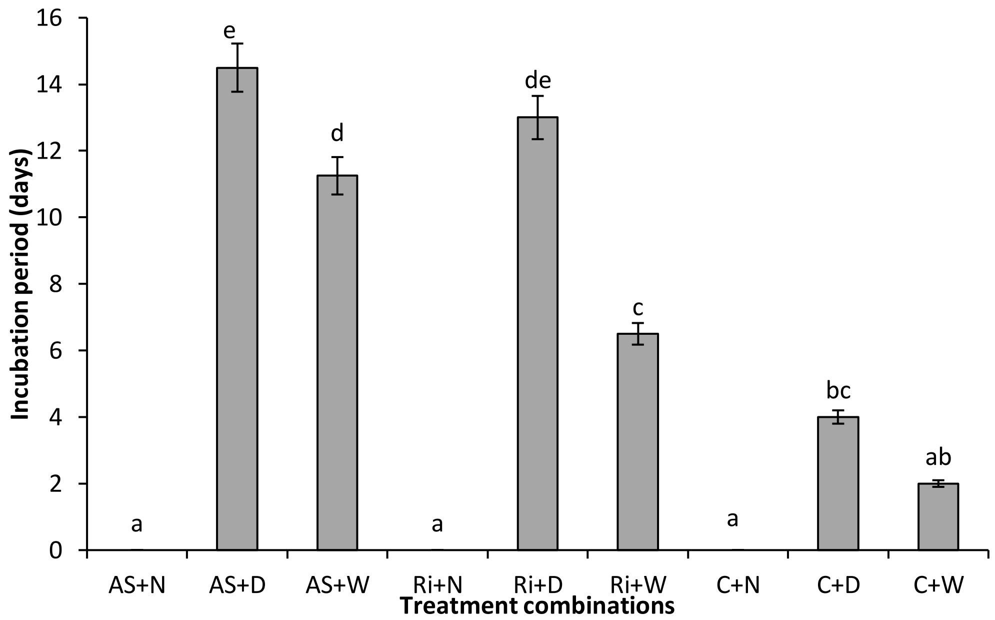
## 4.4 RESULTS

### 4.4.1 Incubation period

There was a significant effect ($F_{3,24}$=22.44, P<0.01) of incubation period among selected Tomato varieties. All varieties showed the wilting symptom from 2 to 20 days after inoculation (Table 1). The fastest incubation period was observed on Cal J with 1 day after inoculation. Meanwhile the longest incubation period was observed on Asilla F1 with 20 days after inoculation. All treatments showed wilt symptom started from the upper leaves and later spread to lower leaves. Finally, the plant losses turgor and died completely (Table 6).

There was also a significant effect ($F_{3,24}$=55.79, P<0.01) of incubation period across inoculation techniques. The lowest incubation period was 1 day observed from Drenching and wounding inoculation technique while the highest was 18 days from root drenching inoculation technique (Table 7).

Significant effects ($F_{3,24}$=6.5, P<0.01) of the combination between inoculation technique and variety was detected among incubation period. The longest incubation period was 14.5 days recorded on treatment combination between Assila F1 variety and root drenching while the shortest was 2 days recorded between Cal J variety and root drenching with wounding.



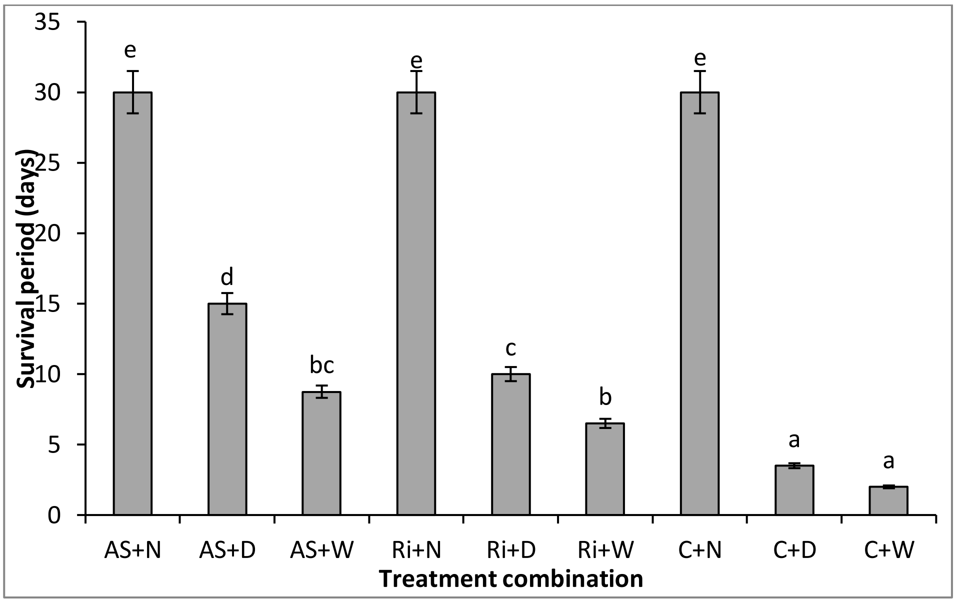

AS=Asilla F1, Ri=Rio safari, C=Cal J, N=No inoculation, D=Root drenching, W=Root drenching with wound.

**Figure 4.1: The mean incubation period at each treatment combination (Bars that do not share a letter represent significantly different means by Duncan's MCT)**

### 4.4.2 Survival period

There was a significant effect ($F_{3,24}$=44.7, P<0.01) of survival period observed among Tomato varieties. The highest survival period was 20 days observed from Asilla F1 variety and the lowest was 2 days recorded from Cal J (Table 6).

There was also a significant effect ($F_{3,24}$=811.89, P<0.01) of survival period observed across inoculation techniques. The lowest survival period was 1 day observed from Drenching and wounding inoculation technique while the highest was 30 days recorded from no inoculation then 14 days from root drenching inoculation technique (Table 7). Significant effects ($F_{3,24}$=13.43, P<0.01) of the combination between inoculation techniques and varieties was detected among survival period. The longest survival period was 30 days recorded on treatment combination between all tomato varieties with no inoculation then 15 days recorded on Assila F1 variety and root drenching while the shortest was 2 days recorded between Cal J variety and root drenching with wounding.




AS=Asilla F1, Ri=Rio safari, C=Cal J, N=No inoculation, D=Root drenching, W=Root drenching with wound.

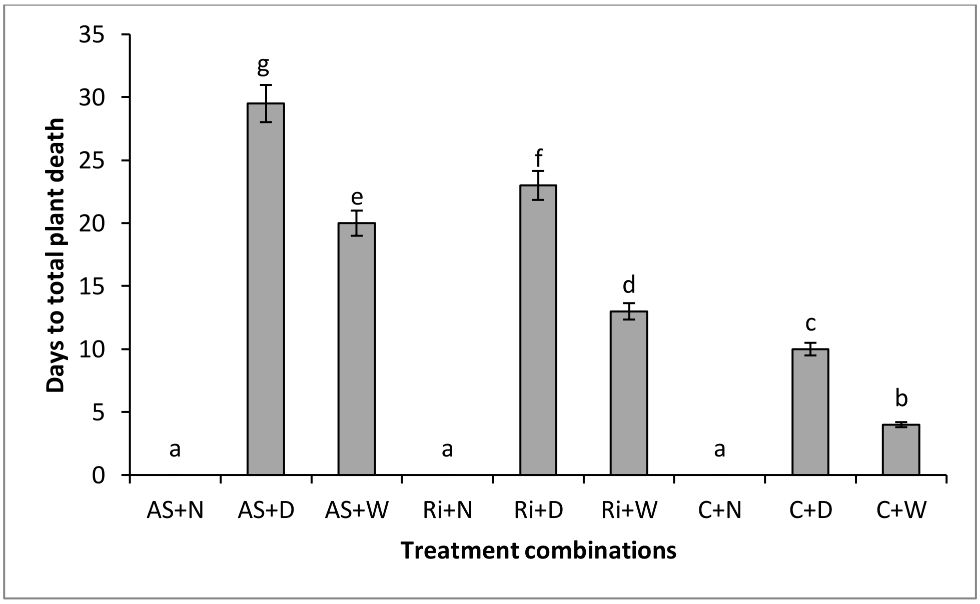
**Figure 4.2: The mean survival period at each treatment combination (Bars that do not share a letter represent significantly different means by Duncan's MCT).**

### 4.4.3 Days to total plant death

There was a significant effect ($F_{3,24}$=75.16, P<0.01) of days to total plant death observed among Tomato varieties. Depending on their resistance varieties wilted and completely died at different days although all of them wilted and completely died 30 days after inoculation. The highest number of days to total plant death was 30 observed from Asilla F1 variety and the lowest was 2 days recorded from Cal J (Table 6).

There was a significant effect ($F_{3,24}$=185.59, P<0.01) of days to total plant death observed among inoculation techniques. The highest number of days to total plant death was 30 observed from root drenching together with no inoculation and the lowest was 2 days recorded from drenching and wounding (Table 7).

Significant effects ($F_{3,24}$=20.78, P<0.01) of treatment combination between inoculation techniques and varieties was detected among number of days to total plant death. The highest number of days to total plant death was 30 recorded on treatment combination between all varieties and no inoculation followed by 29.5 days recorded on Assila F1 variety and root drenching while the shortest was 4 days recorded between Cal J variety and root drenching with wounding.




AS=Asilla F1, Ri=Rio safari, C=Cal J, N=No inoculation, D=Root drenching, W=Root drenching with wound.

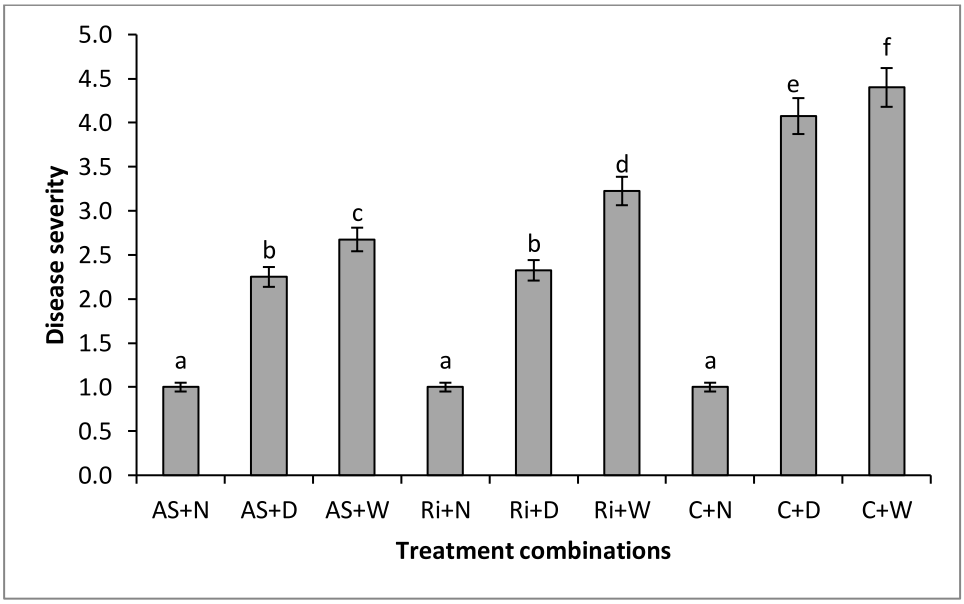
**Figure 4.3: The mean days to total plant death at each treatment combination (Bars that do not share a letter represent significantly different means by Duncan's MCT)**

### 4.4.4 Disease severity

There was a significant effect ($F_{3,24}$=243.37, P<0.01) of disease severity among selected Tomato varieties. All varieties showed the wilted tissues although at different levels of disease severities depending on their resistance. The highest average disease severity was 4.4 observed from Cal J variety and the lowest was 2.2 recorded from Asilla F1 (Table 6).

There was a significant effect ($F_{3,24}$=993.15, P<0.01) of disease severity observed among inoculation techniques. The lowest disease severity was 1 observed from no inoculation then 2.9 observed from root drenching and the highest was 3.4 days recorded from drenching and wounding (Table 7).

Significant effects ($F_{3,24}$=65.64, P<0.01) of treatment combination between inoculation techniques and varieties was detected on disease severity. The highest disease severity was 4.4 recorded on Cal J variety and root drenching with wounding while the lowest was 1 recorded on all tomato varieties with no inoculation then 2.3 recorded on Assila F1 and Root drenching together with Rio safari and root drenching.




AS=Asilla F1, Ri=Rio safari, C=Cal J, N=No inoculation, D=Root drenching, W=Root drenching with wound.

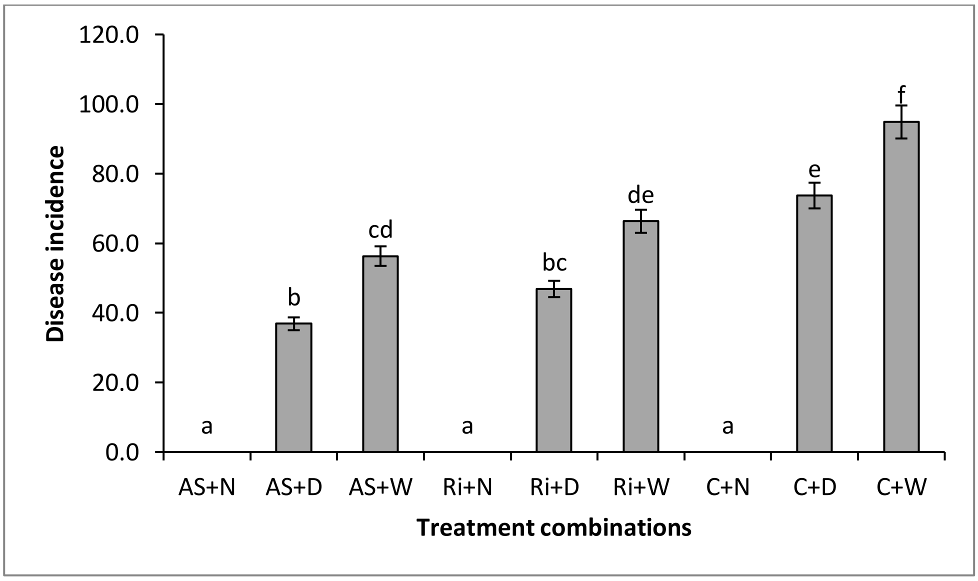
**Figure 4.4: The mean disease severity at each treatment combination (Bars that do not share a letter represent significantly different means by Duncan's MCT)**

### 5.4.5 Disease incidence

There was a significant effect ($F_{3,24}$=28.3, P<0.01) of disease incidence observed on Tomato varieties. All varieties showed the wilted tissues although at different levels of disease severities depending on their resistance. The highest average disease incidence was 53.1% observed from Cal J variety and the lowest was 30.9% recorded from Asilla F1 (Table 6).

There was a significant effect ($F_{3,24}$=285.53, P<0.01) of disease incidence observed among inoculation techniques. The lowest disease incidence was 0% observed from no inoculation then 52.4% observed from root drenching and the highest was 69.3% recorded from drenching and wounding (Table 7).

Significant effects ($F_{3,24}$=7.4, P<0.01) of treatment combination between inoculation techniques and varieties was also detected among disease incidence. The highest disease incidence was 94.9% recorded on Cal J variety and root drenching with wounding while the lowest was 0% recorded on all varieties with no inoculation then 36.9% recorded on Assila F1 and Root drenching.




AS=Asilla F1, Ri=Rio safari, C=Cal J, N=No inoculation, D=Root drenching, W=Root drenching with wound.

**Figure 4.5: The mean disease incidence at each treatment combination (Bars that do not share a letter represent significantly different means by Duncan's MCT)**

**Table: 4.1: The relationship between disease parameter and Tomato varieties**

| Parameter | Tomato variety | | | F-value | *P*-value |
|---|---|---|---|---|---|
| | ***Assila F1*** | ***Rio safari*** | ***Cal J*** | | |
| Disease incidence (%) | 30.9a | 37.7b | 53.1c | 28.3 | <.001 |
| Disease severity | 1.975a | 2.183b | 3.158c | 243.37 | <.001 |
| Survival period (days) | 17.92c | 15.50b | 11.83a | 44.7 | <.001 |
| Incubation period (days) | 8.25c | 6.50b | 2.00a | 31.93 | <.001 |
| Days to total plant death | 16.17c | 12.00b | 3.83a | 112.11 | <.001 |

Note. P ≤ 0.05 shows there was a significant difference.

**Table 4.2: The relationship between disease parameter and inoculations techniques**

| Parameter | Inoculation techniques | | | F-value | *P*-value |
|---|---|---|---|---|---|
| | ***No inoculation*** | ***Root drenching*** | ***Root drenching and wounding*** | | |
| Disease incidence (%) | 0.00a | 52.47b | 69.25c | 285.53 | <.001 |
| Disease severity | 1.000a | 2.883b | 3.433c | 993.15 | <.001 |
| Survival period (days) | 30.00c | 9.50b | 5.75a | 811.89 | <.001 |
| Incubation period (days) | 0.00a | 10.50c | 6.25b | 85.67 | <.001 |
| Days to total plant death | 0.00a | 20.00c | 12.00b | 288.63 | <.001 |

Note. P ≤ 0.05 shows there was a significant difference.

### 4.4.5 Area under disease progress curve (AUDPC)

There was a significant effect ($F_{3,24}$=321.1, P<0.01) of area under disease progress curve observed on Tomato varieties. The varieties displayed different area under disease progress curve depending on their resistance. The highest average area under disease progress curve was 100 Disease severity units (DSU) observed from Cal J variety and the lowest was 60 DSU recorded from Asilla F1 variety.

There was a significant effect ($F_{3,24}$=972.6, P<0.01) of area under disease progress curve observed among inoculation techniques. The lowest area under disease progress curve was 30 DSU observed from no inoculation, followed by 85.88 DSU observed from sole root drenching and the highest was 105.8 DSU recorded from drenching and wounding. Significant effects ($F_{3,24}$=86.8, P<0.01) of interaction between inoculation techniques and varieties was detected on area under disease progress curve. The highest area under disease progress curve was 140 DSU recorded on Cal J variety and root drenching with wounding while the lowest was 30 DSU recorded on all the varieties with no inoculation.

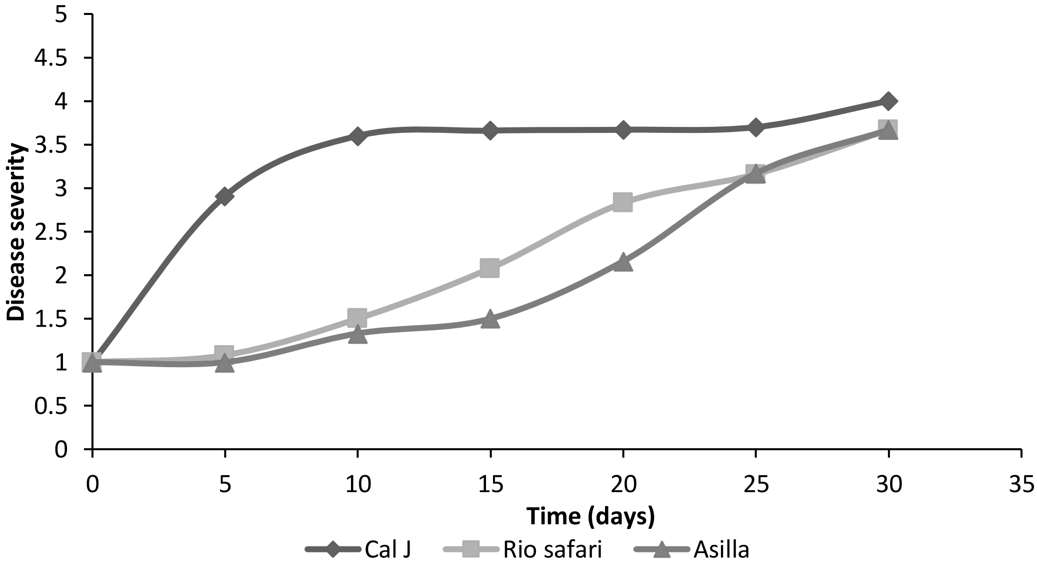



**Figure 4.6: Disease progress curve used to estimate the resistance Tomato varieties to *Ralstonia solanacearum*, Asilla: AUDPC=60DSU Rio safari: AUDPC=65DSU and Cal J: AUDPC=100DSU**

### 4.4.6 Morphological characteristics of the pathogen

#### 4.4.6.1 Bacterial colony color and shape

When bacterial suspensions that had vivid symptoms of the disease were streaked to TZC medium large and elevated fluidal, colonies which were creamy with pinkish center were observed indicating that they were virulent cultures. The shape of bacteria colonies was fluidal and irregular.

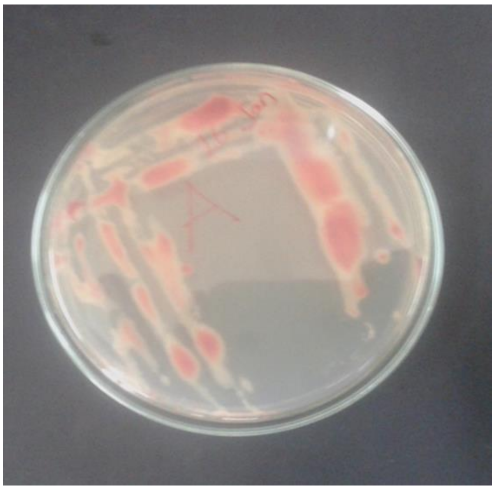


**Figure 4.7: Virulent colony of *R. solanacearum* on TZC medium**

#### 4.4.6.2 Biochemical tests of the pathogen

##### 4.4.6.2.1 Gram staining

The isolate gave negative response when tested for Gram staining. Bacteria retained reddish pink colony color when counter stained with safranin. This showed that the bacteria were Gram negative.

##### 4.4.6.2.2 KOH test

The inoculum tested negative on KOH loop test as they formed slime threads when the bacterial cultures (48 h) were mixed with 3 % KOH solution. The test tends to characterize the flaccidity of the cell of the bacteria cell.

##### 4.4.6.2.3 Catalase oxidase test

The bacterial isolates tested produced gas bubbles when mixed with a drop of $H_2O_2$ on a glass slide, indicating that they might be R. solanacearum. This is common for all gram negative bacteria including R. solanacearum.

#### 4.4.6.2.4 Kovacs Oxidase Test

The bacterial inoculum tested positive on the development of purple colour after being rubbed with Kovacs oxidase reagent. The inoculum produced the purple colour within 10 seconds.

#### 4.4.6.2.5 Serological Detection of the pathogen

The inoculum tested serologically by NCM-ELISA was found to be Ralstonia solanacearum. The bacterial concentration was observed in the positive samples ranged from $10^7$to $10^8$ cfu/ ml. This concentration was based on color intensity of the positive and negative control strips of the NCM-ELISA kit.

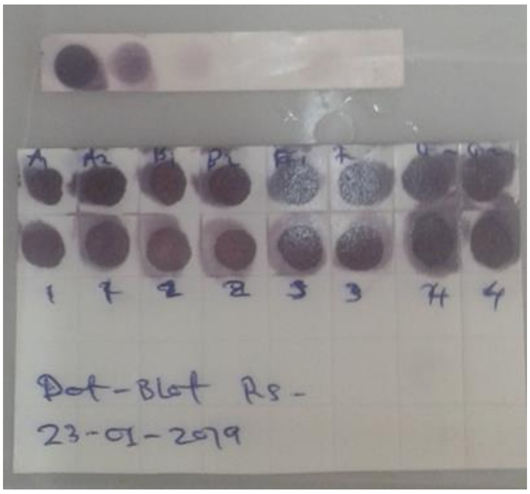



**Figure 4.8: Below, Dot blotting of *R.solanacearum* on NCM ELISA membrane. Above, control strip**

#### 4.4.6.2.6 Hypersensitive test

For the area of the lamina injected with Bacterial inoculum, there was a rapid collapse and water soaking of inoculated tissue within 36 hours followed by dry, light-brown localized necrosis within three days. This indicates the positive hypersensitive test. For the area of the lamina injected with sterile distilled water as a control, there was a no visible reaction. This indicates that the negative hypersensitive test.

## 4.5 DISCUSSION

Bacterial strains were fluidal and irregular with pinkish or light red centre on TZC Media. The similarity in shapes of the bacterial strain is similar to the shapes described in the study from Dhital *et al.*, (2000) which describes *R.solanacearum* with the same color and shape.

The bacterium was gram negative which is similar to protocol described by (Schaad, 1980). The protocol described a gram reaction where the bacteria retaining reddish pink colony colour are gram negative (G-ve) while stain the blue violet colour gram positive (G+ve).

Gram negative bacteria have relatively fragile cell walls which are bounded by an outer membrane. The outer membrane is disrupted by exposing it to 3 % KOH solution which results in releasing slime threads which is actually the viscous DNA. Where by, the Gram positive bacteria possess thick and more rigid cell wall which resists the disruptive effect of KOH. The KOH technique is easier and faster to distinguish gram negative and positive bacteria than the traditional gram stain in which dyes are employed (Suslow *et al.*, 1982).

Production of gas bubbles is a characteristic importance of all Gram negative bacteria. The character is an indication for presence of aerobic and facultative anaerobic bacteria (Schaad, 1980).

In Kovac's oxidase test bacterial inoculum which give purple color when mass of bacterial growth was rubbed on filter paper impregnated with oxidase reagent were categorized as positive whereas negative ones do not produce purple color. This test is useful for differentiating aerobic and anaerobic bacteria and is particularly important for differentiating gram negative bacteria. *R. solanacearum* gives a positive reaction (Kovacs, 1956).

The area of the lamina injected with Bacterial inoculum indicated the positive hypersensitive test. Positive hypersensitive test confirms the presence of *Solanacearum* in the inoculum. The results are the same as in hypersensitive test as outlined by (Mortensen, 1997) in Seed bacteriology laboratory guide.

The longest incubation period on Tomato varieties was observed on Assila F1 while the shortest was observed on Cal J. The longest incubation period on inoculation techniques was recorded on root drenching while the shortest was recorded on root drenching and wounding. The longest incubation period on treatment combination between Tomato variety and inoculation technique was observed on all Tomato varieties and no inoculation then on Assila F1 variety and root drenching while the shortest was recorded between Cal J variety and root drenching with wounding. This is because improved varieties and especially hybrids tend to have some tolerance on the disease compared to OPV varieties. This can be supported with a study from (Onduso, 2014) in which an improved variety showed Tolerance to Bacterial wilt disease compared to Open pollinated varieties.

The longest survival period on Tomato varieties was observed on Assila F1 while the shortest was observed on Cal J. The longest survival period on inoculation technique was recorded on no inoculation followed by root drenching while the shortest was recorded on root drenching and wounding. The longest survival period on treatment combination between Tomato variety and inoculation technique was recorded on all Tomato varieties and no inoculation then on Assila F1 and root drenching while the shortest was recorded between Cal J and root drenching with wounding. This is because open pollinated varieties tend to have less tolerance on the biotic stresses compared to hybrids varieties. This can be supported with a study from (Timila and Joshi., 2007) in which a hybrid variety showed survived to Bacterial wilt disease compared to open pollinated varieties.

The longest days to total plant death on Tomato varieties was observed on Assila F1 while the shortest was observed on Cal J. The longest days to total plant death on inoculation technique was recorded on no inoculation followed by root drenching while the shortest was recorded on root drenching and wounding. The longest days to total plant death on treatment combination between Tomato variety and inoculation technique was recorded on all tomato varieties and no inoculation then Assila F1 and root drenching while the shortest was recorded between Cal J and root drenching with wounding. This is because improved varieties and especially hybrids tend to have long life when subjected to the inoculum of wilting diseases compared to open pollinated varieties. This can be supported with a study from (Techawongstien *et al.,* 2007) in which the hybrid varieties lived longer compared to other Open pollinated varieties.

The lowest disease severity on Tomato varieties was observed on Assila F1 while the highest was observed on Cal J. The lowest disease severity on inoculation technique was recorded on no inoculation followed by root drenching while the highest was recorded on root drenching and wounding. The lowest disease severity on treatment combination was observed on all tomato varieties and no inoculation then on Assila F1 and root drenching while the highest was recorded between Cal J and root drenching with wounding. This is because improved varieties and especially hybrids tend to have low tissue destruction on the diseases compared to open pollinated varieties (OPV) which showed very high plant tissue destruction. This can be supported with a study from (Baitan, 2017) in which Cal J variety showed very high tissue destruction to Bacterial wilt disease compared to other improved bred lines.

The highest disease incidence on Tomato varieties was observed on Cal J while the lowest was observed on Assila F1. The lowest disease incidence on inoculation technique was recorded on no inoculation then on root drenching while the highest was recorded on root drenching and wounding. The lowest disease incidence on treatment combination between Tomato variety and inoculation technique was recorded on all Tomato varieties and no inoculation then Assila F1 and root drenching while the highest was recorded between Cal J and root drenching with wounding. This is because improved crop varieties and especially hybrids tend to have low disease occurrence compared to open pollinated varieties which showed very high disease occurrence. This can be supported with a study from (Baitan, 2017) in which Cal J variety displayed very high Bacterial wilt disease occurrence compared to other improved bred lines.

The largest area under disease progress curve (AUDPC) on Tomato varieties was observed on Cal J then Rio safari and the lowest was recorded on Assila F1. This is because improved crop varieties and especially hybrids tend to be less susceptible to the diseases compared to open pollinated varieties. This can be supported with a study from (Baitan, 2017) in which Cal J variety was observed to have a very small AUDPC compared to other improved bred lines.

The study has found out that the isolated inoculum was confirmed to be *Ralstonia solanacearum* through different biochemical, hypersensitive and serological tests. The variation of disease scores between Tomato varieties and inoculation techniques was significant. This is due to the differences in resistance levels of the varieties and fatality level of inoculation techniques.

The resistance level of the Tomato varieties differed in degrees starting with Assila F1 the highest, followed by Rio safari moderate and the lowest was Cal J. The fatality level of the inoculation techniques differed in degrees starting with Root drenching and wounding the highest, followed by root drenching without wounding and the lowest was no inoculation. The treatment combinations had varying responses to Ralstonia solanacearum Assila F1 and Root drenching performed the best while Cal J and Root drenching and wounding performed the worst. Farmers are encouraged to use seedlings trays, drip irrigation system so as to minimise lateral root injuries and consequently minimising disease occurrence in their fields. Farmers are also advised to building drainage canals especially during rainy seasons so as to prevent water stagnancy.

**References**

Agrios, E. N. (2005). Plant pathology (5th ed.). Miami: Elsevier Academic Press. 252pp.

EPPO, (2004). *Ralstonia solanacearum*. European and Mediterranean. Plant Protection Organization Bulletin 34: 173-178

Horita, M and Tsuchiya, K. (2001). Genetic diversity of Japanese strains of *Ralstonia solanacearum*. Phytopathology 91: 399-407.

Kelman, A. (1954). The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on tetrazolium medium. Phytopathology 64: 693-695.

Kovacs, N. (1956). Identification of Pseudomonas pyocyanea by the Oxidase reaction. Nature 178: 703.

Pradhanang, P. M., Elphinstone, J.G and Fox, R.T. V. (2000). Sensitive detection of Ralstonia solanacearum in soil: a comparison of different detection techniques. *Plant Pathololology* 49: 414–422

Priou, S., Gutarra, L., Aley, F. P., Mendiburu, D. and Llique, R. (2010). Detection of *Ralstonia solanacearum* (biovar 2A) in stems of symptomless plants before harvest of the potato crop using post-enrichment DAS-ELISA. Plant Pathology 59: 59-67.

Saile, E., McGarvey, J. A., Schell, M. A., & Denny, T. P. (1997). Role of extracellular polysaccharide and endoglucanase in root invasion and colonization of tomato plants by Ralstonia solanacearum. Phytopathology, 87(12), 1264-1271.

Schaad, N. W. (1980). Laboratory guide for the identification of plant pathogenic bacteria. St. Paul, Minnesota: American Phytopathological Society.

Suslow, T. V., Schroth, M. N. and Isaka, M. (1982). Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. Physiopathology 72: 917-918.

Priou, S (2001). CIP NCM-ELISA kit for the detection of Ralstonia solanacearum in potato. Instruction for use. International Potato Centre (CIP).

Mortensen C.N. 1997. Seed Bacteriology Laboratory Guide. Danish Government Institute of Seed Pathology for Developing Countries (DGISP), 102 pp.

Dhital S. P., Thaveechai, N and Shrestha, S. K. (2000). Characteristics of Ralstonia Solanacearum Strains of Potato Wilt Disease from Nepal and Thailand1. Nepal Agric. Res. J., Vol. 4.

Techawongstien, S., Thummabenjapone, P. and Bolkan, H., (2007). Screening tomato varieties for their bacterial wilt resistance over seasons in Northeast Thailand. In II International Symposium on Tomato Diseases 808 (pp. 263-268).

Timila, R.D. and Joshi, S., 2007. Participatory evaluation of some tomato genotypes for resistance to bacterial Wilt. Nepal Agriculture Research Journal, 8, pp.50-55.

# CHAPTER FIVE

## 5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Conclusions

i. The amount of knowledge, technical know-how and perception of the farmer has an impact on the spread, distribution and occurrence of the Tomato bacterial wilt disease in Morogoro region.

ii. There are some farming practice done by farmers such as burying without burning of crop residuals, in the region which tends to impair control and management of the disease.

iii. The study has found out that the disease is present in all three villages visited in the current study. This implies that the disease is of economic importance and needs control options as pointed out in this study.

iv. The variation of disease scores between sites was significant. This is due to the differences in epidemiological factors and farmers practice. However, the scores of the disease were the highest in Kiberengeni village and the lowest in Nyandira.

v. The study has found out that the isolated inoculum was confirmed to be Ralstonia solanacearum through different biochemical, hypersensitive and serological tests.

vi. The variation of disease occurence between Tomato varieties and inoculation techniques was significant. This is due to the differences in resistance levels of the varieties and fatality level of inoculation techniques.

vii. The resistance level of the Tomato varieties differed in degrees starting with Assila F1 the highest, followed by Rio safari moderate and the lowest was Cal J.

viii. The fatality level of the inoculation techniques differed in degrees starting with Root drenching and wounding the highest, followed by root drenching without wounding and the lowest was no inoculation.

ix. The treatment combinations had varying responses to *Ralstonia solanacearum*. Assila F1 and Root drenching performed the best while Cal J and Root drenching and wounding performed the worst.

## 5.2 Recommendations

i. Farmers are encouraged to use screened varieties as found from the current study which showed resistance against the disease.

ii. The current study used determinate and semi-determinate varieties to determine resistance against Tomato Bacterial wilt disease, there is a need to for further studies on indeterminate varieties as the sources of variation.

iii. The current study was conducted under controlled environment (Screen house) and the results found were based on such environment. There is a need to conduct more research under field conditions for better recommendations of the varieties to the Farmers.

iv. The current study screened only three varieties against the disease. Further studies are encouraged for more varieties available in the market so that farmers could have a wide range of choice.

v. There is a need for more studies on the bio control of indigenous species of *Pseudomonas fluorescens* which has been used to control *Ralstonia solanacearum* the pathogen of Tomato bacterial wilt disease in other countries.