Original Research Article

Prevalence of Bacterial Wilt DiseaseCaused by*Ralstoniasolanacearum*in TomatoeswithinMeru South Sub-County

ABSTRACT

Tomato (*Solanumlycopersicon*) is among the most consumed vegetables across the globe. In most parts of Kenya, tomato production is characterized by low quality and huge losses due to pests and diseases. The most prevelance tomato diseases are caused by bacteria and fungi, most common being bacterial wilt. This study determined the prevalence of *Ralstoniasolanacearum* in tomato farms in Meru South sub-county. The Prevalence of bacterial wilt was carried out by conducting a survey across agroecological zones in MeruSouth. Study farms were randomly selected. Data on the frequency of occurrence, severity, and impacts of the disease were collected by observation and administration of questionnaires to the farmers. The study broadens the understanding of *R. solancaerum*prevalence in Meru South sub-county**.**

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

I. INTRODUCTION

Tomato (*Solanumlycopersicon*) belongs to *Solanaceae* family and is among the most consumed vegetables across the globe. The crop (tomato) is grown in over 5 million hectares globally with production of up to 170 million tonnes per annum with an average of 34 tonnes per hectare (Samberg *et al*., 2016; FAOSTAT 2021). In Africa, Egypt leads in tomato production followed by Nigeria (FAOSTAT, 2021). In Kenya, tomato production accounts for 14% of the total vegetable produce and about 7% of the total horticultural crops (Wanjohi*et al*., 2018). Despite tomato being a major vegetable, its production in Kenya (average of 20.4 tonnes/ha) has remained below the global average (59.4 tonnes/ha) and the production by other African countries like South Africa (67.1 tonnes/ha) (FAOSTAT, 2021).Tomato farming is a popular economic activity among farmers in Kenya. The crop performs best in medium to lower zones in major tomato growing areas such as Mwea in Kirinyaga County, Ngurumani in Kajiado County and parts of Rift Valley and western regions (Nguetti*et*

al., 2018). Between 2012 and 2014, the total national production was 400,204 MT valued at Kenya shillings 11.8 billion (Neema & Fredrick, 2019). According to Kinuthia (2019), the leading counties in tomato production in Kenya in 2021 were Bungoma (50,399 MT), Kirinyaga (48,560) and Kajiado (47368 MT) in 2014. TharakaNithi produces approximately (8,184 MT) according to TharakaNithi crop projection while Meru south sub-county production is approximately 1,650 MT, which is quite low comparedto other counties. TharakaNithi County is ranked position 29 of 47 counties with a total area of 120 hectares (Njonjo *et al*., 2019). Low production is contributed by abiotic factors, (high temperatures, erratic rainfall and poor soils) as well as biotic factors which include, arthropod pests, fungal, bacterial and viral diseases (Dube & Maphosa, 2020).

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

Bacteria wilt of tomato is mainly attributed to*Ralstoniasolanacearum*, a serious soil-borne pathogen (Wu, 2013). Studies have shown that the disease causes 70-80% yield loss when grown in greenhouse and field environments (Huang *et al*., 2013). *Ralstoniasolanacearum*is a sophisticatedphytopathogen with a number of hosts, widely distributed, contagious, epidemiological associations and physiological effects (Siri*et al*., 2011). Bacterial wilts limit the production of tomatoes in warmer areas, sub-tropics and tropics temperatures in the world (Rowles*et al*., 2020). The bacteria can persist in soil medium and plant tissues for several years, thus hard to eradicate. Due to its wide distribution it's expected to have different strains adapted to different

ecological zones. Hence, it is important to frequently characterise this pathogen in different agro- ecological Zones.

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

The findings of Bawa, (2016) reported that various cultural methods involved in controlling and management of *Ralstoniasolanacearum*include crop protection, use of resistant cultivars. However, the report differed fromthe findings of Mamphogoro*et al*., (2020) whopurported that shifting cultivation is unfeasible since the time involved in preparing other seedbed is a lot and therefore, lead to a waste of time in moving from one field to the next.This study determined the prevalence of *Ralstoniasolanacearum* in tomato farms in Meru South sub-county.

II. METHODOLOGY

The study was conducted in January-March 2022 using descriptive survey and experimental designs. The survey was descriptive in nature to assess the prevalence of *R. solanaceaarum*on tomato farms in the study area. Disease prevalence in this study was the number of cases of *Ralstoniasolanacearum* in MeruSouth during this study season.

Tomato farms were selected using purposive sampling based on tomato production status whereby the size of land under tomato production in each farm was ¼ ha and above to ensure adequate mapping to constitute the target population. The target population was distributed within LM1, UMI, UM2, UM3, and LM3 agro ecological zones in Meru South Sub-county. There were 250 estimated tomato farms that

satisfied the conditions of at least ¼ ha farm under tomato production within these agro ecological zones (Tharaka-Nithi County; Table 1). The sample size was calculated using the formula documented by (Israel, 2009) as shown;

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

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

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

Therefore, n = 154

Sampling Procedure

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

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

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

Data Collection

The sampled tomato farms were surveyed for bacterial wilt disease caused by *Ralstoniasolanacearum*. Tomato farms in the study area weresurveyed for bacterial wilt disease. Questionnaires wereadministered to the sampled farmers and additional information was collected on the disease's impacts on tomato production. Data on disease prevalence was obtained by determining the number of infected plants and disease prevalence data recorded. The study was done in rows of tomato from rows 3, 5, 7, 9, and 11 whereby at least 10 rows of tomatoes were sampled for every selected farm and assessed for bacterial wilt disease infections. One hundred and fifty-four

tomato farms in Meru south sub-county were surveyed for the prevalence of bacterial wilt by counting ten plants in each sampled farm along the selected rows and the prevalence of bacterial wilt in every plant assessed. The results were recorded based on the presence or absence of disease symptoms. Infected plant stems were collected for bacterial isolation in the laboratory.

Disease prevalence in this study was the number of cases of bacterial wilt by *Ralstoniasolanacearum* in MeruSouth during this study season. To determine the disease prevalence, thirty tomato plants wererandomly selected from each established sampling farm.

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

Isolation of Bacterial Pathogen from Infected Plants

The bacterial wilt causative agent (*R. solanacearum*) was isolated from the collected samples according to Oljira and Berta, (2020). The collection of samples was done from the surveyed farms in Meru south –sub county.Small tomato plant stem portions were cut into bits, their surfaces were cleaned with a 70% alcohol solution, and they were then put in a petri dish with moist conditions to encourage the growth of bacteria oozing. Surface streaking of the bacteria oozing that was visible on the stem was done on Kelman`s medium containing 0.01% of TZC (Triphenyltetrazolium chloride). A fluidal colony with a pink patch or a white colony on the plate was chosen as a probable candidate after 48 hours of incubation at 28^{0} C.

Culturing of Bacterial Pathogen

Pathogen was isolated from collected samples on Kelman'smedia in the laboratory according to Sharma & Singh, (2019).Kelman's media was prepared by mixing various components where 1 gram of Casamino acid or (meat extract), 10 grams of

Peptone water, 5 ml Glycerol, 20 grams of Agar, 10 grams of Dextrose. The dissolved media components were then autoclaved at 121ºC for 15 minutes at a pressure of 15 psi in an autoclave model (Model X280A). The media was allowed to cool to 50 ºC in a water bath. 5 ml of 1% stock solution of TTC was added then filter sterilized. Thereafter, 5mg of Crystal violet, was dispensed in Petri dishes and allowed to solidify. Upon isolation, morphological characteristics and biochemical tests were performed identify the pathogen.

Data Analysis

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

III.RESULTS AND DISCUSSION

Prevalence of *Ralstoniasolanacearum* in Tomato Farms

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

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

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

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

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

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

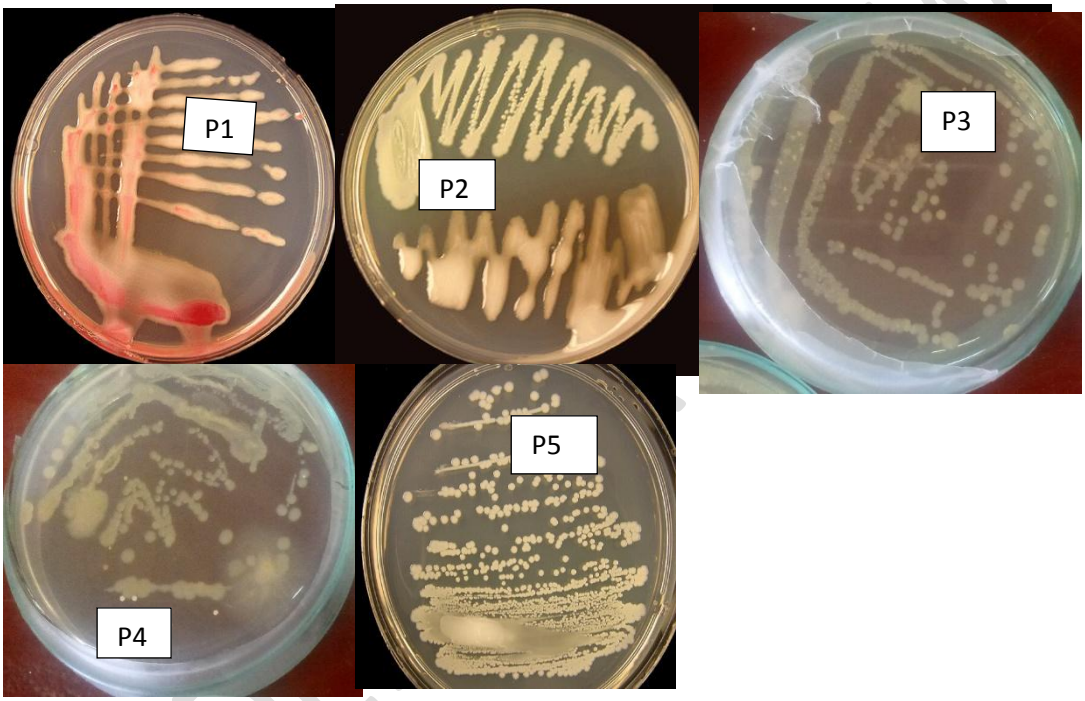
Table 3:Average groupings for means of isolates from the agro-ecological zones of the study area

AEZ	Isolate ID	Mean of Colony Count
LM3	Plate 1	69.056[a]
UM3	Plate 2	57.520[b]
UM1	Plate 3	55.588[b]
UM2	Plate 4	45.456[c]
LM1	Plate 5	20.662[d]
	LSD	4.3103
	Mean	49.65640
	CV (%)	6.579512

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

There was a difference in colony morphology traits (Plate 1). The virulent isolates had pinkish coloured colonies with characteristic red centre and whitish margin, non-fluidal and dry-texture colonies on TZC (P1) whereas the isolates obtained on nutrient agar were characterized by white-cream and fluidal wet texture after 24 h of incubation (P3). Isolate P 1 of zone LM3 had pinkish colonies which were fluidal in nature. Isolate P2 from zone UM3 displayed white, small colonies and wet colonies. Isolate P3 from zone UM1 was creamy with dry texture and small round colonies. On the other hand, there was notable similarity of isolate P4 and P3 which showed almost the same characteristics. Isolate P4 was obtained from zone UM2. However, isolate P5 obtained from zone LM1 was whitish in colour with widely dispersed colonies.

The variation of cultures isolated from different agro ecological zones was linked to different climatic conditions observed in the zones. Temperature fluctuations could have influenced mutations of *R.solanacearum* pathogen to change to different strains. The result was similar to the findings of Caruso *et al*. (2005) who recordedfound surface water samples infected with *R. solanacearum* ranged in temperature from 9 to 20°C, differing significantly across all sites examined. Temperature variations determines biochemical and metabolic processes of microbes. Higher temperature has been found to be beneficial to bacterial mutations and evolutionary processes.



Plates P1-P5: Morphological characteristics of 24 hour old cultures of *Rsolanacearum* pathogen isolates of TZC agar and Nutrient agar.p1-plate 1 bacteria isolates of LM3,P2-plate 2 bacteria isolates of UM3, p3-plate 3 bacteria isolates of UM1,p4 -plate 4 bacteria isolates of UM2,p5-plate 5 bacteria isolates of LM1.

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

AEZ	Isolates ID	Mean Count(24ours)	Mean Count(48 hours)	Mean Count(72 hours)
LM3	Plate 1	61.270[a]	69.920[a]	75.982[a]

UM3	Plate 2	47.002[c]	59.198[b]	66.312[b]
UM1	Plate 3	39.718[d]	46.730[c]	49.930[d]
UM2	Plate 4	51.322[b]	54.954[b]	60.488[c]
LM1	Plate 5	15.258[e]	20.956[d]	25.774[e]
	LSD	2.9881	5.2293	5.6132
	Mean	42.926	50.35160	55.69720
	CV(%)	5.276415	7.872106	7.639133

After 48 hours of incubation at 28°C, all visible colonies were counted using colony counter and there were more than 200 colonies per plate. Additionally, it was observed that plates from samples were generally dominated by small pinkish colonies in addition to white colonies. The results of this study revealed that the average colony count recorded ranged from plate 5 (LM1) with 20.956 to 69.920 of plate 1[(LM3) Table 4] This was followed by plate 2 (UM3) with a value count of 59.198, plate 4 (UM2) with colony count of 54.954 and plate 3 (UM1) with a colony count of 46.730. The variation of the number of colonies counted within 48 hours could have been because of difference of strains of *R.solanacearum* isolated from various agro ecological zones. Also noted, was the increase of the number of colony count with increase to incubation period.

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

Conclusion

It can be concluded that the prevalence of the bacterial wilt diseases of tomato differed in different AEZ of Meru South Sub-county thathighest rate of infection was recorded in zone LM3 with mean infection value of 62.7% followed UM3 51.33 %, followed by UM1 43.33% then UM2 41.33% while lowest rate of infection was recorded in zone LM1 with a mean infection rate of 32.0%. This was attributed to the variation of cultures isolated from different agro ecological zones which was linked to

different climatic conditions observed in the zones.Temperature fluctuations could have influenced mutations of *R.solanacearum* pathogen to change to different strains difference of strains hence difference in theirvirulence.

References.

Akbar, A., Hussain, S., Ullah, K., Fahim, M., & Ali, G. (2018). Detection, virulence and genetic diversity of Fusarium species infecting tomato in Northern Pakistan. *PloS one,, 13(9)*, 203-613.

Arogundade, O., Balogun, S., & Fawole, B. (2007). INCIDENCE AND SEVERITY OF COMMON VIRAL AND FUNGAL DISEASES OF DRY SEASON TOMATO CROP IN A SOUTHERN GUINEA SAVANNAH AGROECOLOGY. *Agrosearch, 9*, 53 - 60.

Bamazi, B., Banito, A., Ayisah, K., Sikirou, R., & Paret, L. (2022). Distribution and Incidence of Tomato Bacterial Wilt Caused by Ralstonia solanacearum in the Central Region of Togo. *APA Publications*, 1-4.

Bawa, I. (2016). MANAGEMENT STRATEGIES OF FUSARIUM WILT DISEASE OF TOMATO INCITED BY Fusarium oxysporum f. sp. lycopersici (Sacc.). *International Journal of Advanced Academic Research*, 2488-9849.

Caruso, P., Palomo, J., Bertolini, E., Álvarez, B., López, M., & Biosca, G. (2005). Seasonal Variation of Ralstonia solanacearum Biovar 2 Populations in a Spanish River: Recovery of Stressed Cells at Low Temperatures. *Appl Environ Microbiol. Jan, 71(1)*, 140–148.

Dube, B., & Maphosa, V. (2020). Tomato breeding in sub-Saharan Africa-Challenges andopportunities during the COVID-19. International Journal of Higher Education,. *International Journal of Higher Education,, 9(5)*, 84-93.

Huang, X., Zeng, Z., & Zhang, H. (2013). Metal dichalcogenide nanosheets: preparation, properties and applications. . *Chemical Society Reviews, 42(5),*, 1934-1946.

John, V., Maurya, A., Murmu, R., & Pan, H. (2020). WILT DISEASE OF TOMATO (LYCOPERSICON ESCULENTUM L.) AND IT'S MANAGEMENT. *110ISBN : 978-81-923535-7-9Three Major dimensions of life : Environment, Agriculture and Health*, 1-10.

Kinuthia, C. (2019). *Determinants of pesticide use and uptake of alternative pest control methods among small scale tomato farmers in Nakuru County, Kenya (No. 634-2020-156).*

Koki, T. (2004). Effect of Moisture Conditions and PreIncubation at Low Temperature on Bacterial Wilt of Tomato Caused by Ralstonia solanacearum. *Microbes and Environments, 19(3)*, 244-247.

Mamphogoro, T., Babalola, O., & Aiyegoro, A. (2020). Sustainable management strategies for bacterial wilt of sweet peppers (Capsicum annuum) and other Solanaceous crops. *Journal of Applied Microbiology, 129(3)*, 496-508.

Mengesha, W., Powell, S., Evans, K., & Barry, K. (2017). Diverse microbial communities in non-aerated compost teas suppress bacterial wilt. *World Journal of Microbiology and Biotechnology,, 33(3),*, 49-67.

Neema, D., & Fredrick, O. (2019). Incidence and Severity of Solanum lycopersicum Bacterial leaf spot Caused by Xanthomonas species in Farms in Wanguru, Mwea,Kirinyaga County, Kenya.

Nguetti, J., Imungi, J., Okoth, M., Wang'ombe, J., Mbacham, W., & Mitema, S. (2018). Assessment of the knowledge and use of pesticides by the tomato farmers in Mwea Region, Kenya. *African Journal of Agricultural Research, 13(8),* , 379-388.

Nion, Y., & Toyota, K. (2015). *Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes and environmen.*

Njonjo, M., Muthomi, J., & Mwang'ombe, A. (2019). Production Practices, Postharvest Handling, and Quality of Cowpea Seed Used by Farmers in Makueni and Taita Taveta Counties in Kenya. *International Journal of Agronomy*, 23-43.

Oljira, T., & Berta, S. (2020). Isolation and Characterization of Wilt-Causing Pathogens of Local Growing Pepper (Capsicum annuum L.) in Gurage Zone, Ethiopia. . *International Journal of Agronomy*, 1-2.

Onduso, J., Muthomi, w., & Mutitu, E. (2018). Management of bacterial wilt of tomato using resistant rootstocks. *RUFORUM Working Document Series*, 807-810.

Otieno, M. (2019). Reproducing human and cross-species drug toxicities using a Liver-Chip. . *Science translational medicine,, 11(517)*, 1-10.

Rowles, J., Smith, J., Applegate, C., Miller, R., Wallig, M., Kaur, A., & Erdman, J. (2020). Dietary Tomato or Lycopene Do Not Reduce Castration-Resistant Prostate Cancer Progression in a Murine Model. *The Journal of Nutrition.*, 36-88.

Samberg, H., Gerber, J., Ramankutty, N., Herrero, M., & West, P. (2016). Subnational distribution of average farm size and smallholder contributions to global food production. *Environsmental Research Letters.*, 1-44.

Sarfo, F. (2018). Tele-rehabilitation after stroke: an updated systematic review of the literature. *Journal of Stroke and Cerebrovascular Diseases, 27(9)* , 2306-2318.

Sharma, D., & Singh, Y. (2019). Characterization of Ralstonia solanacearum isolates using biochemical, cultural, molecular methods and pathogenicity tests. *Journal of Pharmacognosy and Phytochemistry, 8(4)*, 2884-2889.

Silva, Y., Ferreira, T., Jiao, & Brooks, M. (2019). Sustainable approach for lycopene extraction from tomato processing by-product using hydrophobic. *eutectic solvents. Journal of food science and technology,, 56(3)*, 1649-1654.

Singh, V., Kishore, A., & Ajay. (2017). Disease management of tomato through PGPB. *Current trends and future perspective, 7(4)*, 1-11.

Siri, C., Santangelo, G., Cilia, R., Poletti, M., Canesi, M., & Barone, P. (2011). Impulsivity and compulsivity in drug-naïve patients with Parkinson's disease. *Movement Disorders, 26(3)*, 464-468.

Waghunde, R., Shelake, R., & Sabalpara, A. (2016). Trichoderma: A significant fungus for agriculture and environment. *African journal of agricultural research, 11(22)*, 1952-1965.

Wanjohi, J., Wafula, O., & Macharia, C. (2018). Integrated management of Fusarium wilt-root knot nematode complex on tomato in central highlands of Kenya. *Kenya. Sustainable Agriculture Research, 7(526-2020-469)*, 8-18.

Wu, D. J. (2013). Advances on the Molecular Mechanism of the Interaction between Ralstonia solanacearum and Hosts. *Agricultural Science and Technology,* , 1698-1701.