Identification and Molecular Characterization of Fungi Associated with Rotting *Solanum lycopersicum* Fruits Obtained from Certain Markets in Oyo and Osun State, Nigeria

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Dedication

This research work is dedicated to God Almighty.

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Abstract

Solanum lycopersicum is the second most important crop after potato across the globe. This important crop is however highly perishable and subjected to rot due to the effect of microorganism. Fungi has been described as the most destructive phytopathogens of *Solanum lycopersicum* which can appear symptomless on the crop and they have been attributed to 41% loss in Nigeria. The molecular characterization of fungi associated with *Solanum lycopersicum* fruits rot was studied in certain markets of Oyo and Osun state using two varieties namely; Royal and Cherry (Cerasiforme)and the objective of this study was to detect and identify molecular characterization of fungi responsible for *Solanum lycopersicum* fruits rot. Thirty-two rotting *Solanum lycopersicum* fruits were assigned to four groups with 8 samples in each group. Direct culture plate method was used to isolate fungi and the gDNA was analyzed using PCR, Sanger sequencing method and result was obtained using Blast on NCBI data base. The result from the Blast identified 6 different fungi from both varieties and the four locations. *Geotrichum candidum* and *Rhizopus delemar* were isolated from Eleekara market of Oyo state from both varieties with frequency of 4 out the 4 samples and a percentage of 18.2%. *Aspergillus flavus* and *Pichia kudriavzevii* were identified from Sasa market of Oyo state with the frequency of 4 out 4 samples and a percentage of 18.2%. *Aspergillus niger* and *Penicillium citrinum* were identified from Oluwo market of Osun state with frequency of 3 out of 4 samples and percentage of 13.6% for both. Special preventive methods like resistant cultivar, basic sanitary rules, sun drying and organic preservative to minimize *Solanum lycopersicum* fruits rots caused by fungal organism is very paramount. The storage of tomato should be done at a temperature and relative humidity that does not favour the growth of fungi.

Keywords: *Solanum lycopersicum*, Blast, gDNA, PCR, Fungi

Word Count: 297

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List of Acronyms

Abbreviation	Meaning
PCR	Polymerase Chain Reaction
gDNA	Genomic Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
BLAST	Basic Local Alignment Search Tools
NCBI	National Centre for Biotechnology Information
Grp	Group
PDA	Potato Dextrose Agar
NASBA	Nucleic Acid Sequence Based Amplification
LAMP	Loop-Mediated Isothermal Amplification
RAPD	Random Amplified Polymorphic DNA
ELISA	Enzyme Linked Immunosorbent Assay
rRNA	ribosomal Ribonucleic Acid

Chapter One

Introduction

1.1 Background to the Study

Phytopathogenic fungi species has been attributed to enormous loss of *Solanum lycopersicum* yields and this has been a major economic issue globally[1]. *Solanum lycopersicum* (Tomato) is popularly referred to as tomato and is considered the second most important crop after potato[1]. A whole lot of fungi like *Alternaria alternata, Colletotrichum truncatum, Phytophthora infestans, Geotrichum* species and *Fusarium* species had been detected in *Solanum lycopersicum* fruits using direct plate methods or conventional method[1]. They are capable of causing diseases with different signs and symptoms[1.] These fungi reduce *Solanum lycopersicum* produce and quality leading to the severe economic loss and they can lead to health problem in human if infected fruits are consumed because some of the fungi can produce mycotoxin as reported in some studies[1]. *Alternaria solani, Rhizopus stolonifer* and *Aspergillus niger* have been tagged as the most common pathogenic fungi across the globe and they have been traced to causing about loss of 52.7%, 35.9% and 25% respectively in *Solanum lycopersicum* fruits rots in Egypt being the number one largest producer of *Solanum lycopersicum* in Africa[1.]

Fungi have the history of poor growth using direct plate method or conventional method coupled with identification challenge of different species and ones with closely related characteristics which requires expertise[1]. Correct, accurate, timely, precise and rapid detection and identification of fungi infecting *Solanum lycopersicum* fruits is highly essential to prevent health problems that may result from effect of mycotoxin[2]. Also, to facilitate effective management from tomato fruits rots diseases in order to improve quality and quantity of the tomato produce and as well boosting the economy[2]. Molecular

techniques have become popular methods for correct and accurate plant disease diagnosis which has since inception reduce identification challenge which require expertise of fungi from old conventional method[2]. Recently, developments in standard and variants of polymerase chain reaction (PCR) assays including nested, multiplex, quantitative, bio and magnetic-capture hybridization PCR techniques, post and isothermal amplification methods, DNA and RNA based probe development, and next-generation sequencing has provided novel tools in molecular diagnosis of fungal detection and differentiation fields[2].

Solanum lycopersicum history has its origins traced back to early Aztecs around 700 AD as it is believed that it is native to Americas[2]. It was introduced to Europeans around 16th century when early explorers set to sail in order to discover new lands throughout Southern Europe[2]. This plant was quickly accepted into the kitchen as it moved to Northern Europe[2]. The British admired it for its beauty but they believed it was poisonous because it looks similar to poisonous wolf peach[2]. *Solanum lycopersicum* is popularly referred to as Tomato and it is an herbaceous annual plant belonging to the family *Solanaceae* grown for its edible fruits[2]. The plant can be erect with short stems or vine-like with long spreading stems[2]. Most of the time it has its stems covered in coarse hairs where the leaves are arranged spirallys[2]. It is called Tomatl in Nahuti language, Tomate in Spanish language and it is known as Tomato in English language although originated from Greek[3]. Tomato plant is classified as one of the members of *Solanaceae* family which comprises most utilized and important plants like potato, all peppers, ground cherries and eggplant[3]. They are often referred to as nightshade family as they also comprise some deadly toxic plants represented by belladonna, mandrake, Jimson weed, henbane and tobacco[3].

Solanum lycopersicum was first referred to as fruit because people at that time were only eating it raw and there was a belief that only fruits can be eaten raw[4]. But later called vegetable when they began adding it to dishes in cooked form[4]. *Solanum lycopersicum* has

over 3000 varieties with about seven most popular and important species used in different dishes and delicacies like puree, salad and many more all over the world[4]. The *Solanaceae* family members have potent psychoactive Alkaloids as they are plant with veritable chemical soup of desirable and toxic compound known as tropane alkaloids[4]. These chemicals include scopolamine, nicotine, solanine, capsaicin, atropine and hyoscyamine which have been used as healing drugs in small doses or as an addictive drug when the dosage is abused and has been used also as pesticides including warfare agents (e.g. sarin)[5]. *Solanum lycopersicum* has been described as a vegetable that has major important antioxidant called lycopene which gives the vegetable its redness common colour and protection of the skin against ultraviolet ray of sun[5]. It plays a substantial role in human health against some health issues like risk of heart diseases, healthy blood pressure, reduce blood glucose level in people with diabetics, eye sight against light-induced damage and certain cancer[5].

This antioxidant serves as good and abundant source of vitamins (C, B, E), potassium, folate, simple calories, water, protein and fibre in appropriate values per serving[6]. Despite the aforementioned functions, benefits and importance of lycopene in the tomato there is still major challenge of the whole plant diseases and fruits rot mostly caused by certain pathogenic agents such as fungi[6]. These fungi is capable of causing rot as it attacks this important vegetable[7]. It poses a very big threat against all the health benefits of the vegetable to human and reduction in tomato quality and quantity[7]. .*Alternaria alternata, Aspergillus niger, Aspergillus flavus, Geotrichum candidum, Alternaria solani* and *Fusarium oxysporum* are fungi that have been most of the time isolated from *Solanum lycopersicum* fruits rots and attributed to most of rots diseases[8]. The most favourable temperature for fungi attack is between 27- 31^{0}C but fungi disease severity and prevalence are at the peak when tomato has its fruits[8].

The other signs and symptoms of fungi infection on *Solanum lycopersicum* plant from the root to the apex of the plant include -; lesions, dark spot, leaf darkening, foliage turning yellow, a gray to white moldy growth, fruits turn greasy, olivaceous-brown decay and powdery mildews[8].Pathogenic fungi cause damage and rots in most edible plants of importance and health benefit of which *Solanum lycopersicum* is one[8]. Although other microorganisms have their contribution but this study will concentrate on the tomato fruits rot caused by fungi, its identification and the molecular analysis to each rot that resulted from the fungi infections[9]. Fungi are kingdom of usually multicellular and sometimes unicellular eukaryotic organism that are heterotrophs[9].They have symbiotic association too with plant and bacteria and can also function in nutrients cycling in an ecosystem[9]. Fungi are among the dominant causative agents of plants diseases found in *Solanum lycopersicum*[9]. Phytopathogenic fungi use diverse strategies to either kill the host and feed on them or colonize the living plant for successful invasion till plants manifest infections and died off[9]. The rot of this plant can be described as a broad term used to describe the visible effects that certain pathogenic microorganism or non-biotic factors have on the plants and its fruits[10]. The effect those rot-causing diseases have on the plant and its fruits is the sickly-look, the decay and colour depicting appearance[10].

Tomato rot typically makes the plant and fruit get progressively more decayed with dark spots and patches, weakened and shriveled plant parts and that of the fruits are further evidence[10]. The diseases of tomato caused by fungi are immense and a major threat to survival of the plant including the health benefits derived from the plant by human[11]. The rots diseases caused by Fungi in *Solanum lycopersicum* are anthracnose fruits rot, early blight, Septoria leaf spot, late blight, buckeye rot, Fusarium wilt and rot, Verticillium wilt and soil rot of fruit and many other ones[11].Fungi infections is most common and most destructive pathogen in *Solanum lycopersicum* as the infection occurs rapidly when the

weather condition is warm and wet[12]. *Alternaria alternata*, *Aspergillus niger, Aspergillus flavus, Geotrichum candidum, Alternaria solani and Fusarium oxysporum* are fungi that have been most of the time isolated from *Solanum lycopersicum* fruits rots and attributed to most of rots diseases[12]. The major postharvest tomato fruits rot caused by fungi are Sour rot, Rhizopus rot, Buckeye rot and Black mold rot. The most favourable temperature for fungi attack is between 27- 31^{0}C but fungi disease severity and prevalence are at the peak when tomato has its fruits[12].

The other signs and symptoms of fungi infection on *Solanum lycopersicum* plant from the root to the apex of the plant include -; lesions, dark spot, leaf darkening, foliage turning yellow, a gray to white moldy growth, fruits turn greasy, olivaceous-brown decay and powdery mildews[12]. Farming of *Solanum lycopersicum* is practice throughout Nigeria[12]. Kano, Borno, Benue, Sokoto, Katsina, Bauchi and Kwara are major producer states of the Northern region of the variety known as royal while Oyo and Osun of Southwest region are the major states producer of Yoruba local variety[12]. Most of these producing states lie between latitudes 8.3°N and 14° with temperature range of 21 – 33°C which favour the cultivation of tomato[12]. The cultivation temperature of tomato in the South-South region is not really favourable due to heavy downpour and too much rain or water is not good for tomato leading to low production of tomato from the region[12].

1.2 Statement of the Problem

Solanum lycopersicum has been described as second most important crop after potato across the globe[12]. It is an important income generating crop and also contain abundant antioxidant that is very beneficial to human health against some diseases[12]. As important as this crop is, it is highly subjected to rot due to the effect of microorganism mostly and Fungi has been described as the most destructive phytopathogens of *Solanum lycopersicum*

which can appear symptomless on the crop at initial stage and eventually cause irreversible damage of the entire plant over time[12]. There have been incidence of 40-45% loss with severity index of 2.0-2.5 of fungi effect on *Solanum lycopersicum* fruits in Nigeria[12]. This havoc reduces the yield quantity, quality, nutritional values and the economic growth. There are have been reports on causative organisms responsible for *Solanum lycopersicum* fruits rot across different countries and some states in Nigeria but there is dearth of information on fungi responsible for *solanum lycopersicum* fruits rot in Oyo and Osun, Nigeria[12]. This called for the need to isolate, identify and characterize fungi associated with *Solanum lycopersicum* fruits rot in certain markets of Oyo and Osun state. Correct, accurate, timely detection and identification of fungi associated with this rot using molecular method will play important role about prevention and management measures to the menace.

1.3 Aim and Specific Objectives of the Study

The aim of this study is to identify fungi associated with *Solanum lycopersicum* fruits rot using molecular tools in major vegetable markets of Oyo and Osun state.

The specific objectives are as follows:-

i. To isolate the fungi associated with *Solanum lycopersicum* fruits rots in Osun and Oyo State.
ii. To identify and characterize the isolates using molecular method.
iii. To identify the fungi to species level by using Sanger sequencing and BLAST search (NCBI) and
iv. To establish the peculiarities of fungi isolated to their location.

1.4 Research Questions

1. Are fungi associated with the Tomato Rot?

2. Are fungi isolated really responsible for the rot observed on the samples?

3. Are fungi isolated from rotting tomato samples variety based?

4. Are the fungi isolated from both tomato varieties location dependant?

1.5 Justification of the Study

Solanum lycopersicum fruits rot caused by fungi is a big threat to second most important crop causing huge economy loss as revealed by Geodesign optimization problem (GOP) in 2016 and 2018. The increasing importance and health benefit of *Solanum lycopersicum* actually call for combat on organisms responsible for its rot which brings about greater loss during cultivation and storage[13].

Due to challenges of fungi identification which require sound knowledge and expertise in fungal plant pathology and taxonomy when conventional method is used makes molecular technique an alternative better option[13]. Molecular method of detection, identification and characterization of fungi has been of great help since inception for growers, crop agronomists and plant pathologist to detect fungi even when symptomless because their attack on plants manifest very late when little or no remedy can be done[13]. Molecular techniques have potential to permit early detection of phytopathogenic fungi, prompt prevention, control and management measures which may in return improve quality and quantity as availability is a major components of food security because a nation with secured food is a nation half –way to healthy living[13].

1.6 Significance of the Study

Fungi attack on *Solanum lycopersicum* has been attributed to the largest percentage of tomato fruits rot all over the world and detecting fungi using conventional method only has been very challenging due to expertise required[13]. The need to characterize fungi using

molecular techniques cannot be over-emphasized because it identifies organism to their very basic fundamental molecular unit as no two organisms share the same order of nucleotides arrangement, each organism with its own unique genetic expression[13]. There have been reports on organisms responsible for *Solanum lycopersicum* fruits rot across different countries and some states in Nigeria but there is dearth of information on fungi responsible for *Solanum lycopersicum* fruits rot in Oyo and Osun, Nigeria. This called for the need to isolate, identify and characterize fungi associated with *Solanum lycopersicum* fruits rot in certain markets of Oyo and Osun state.

1.7 Scope of the Study

This study was carried out on fungi isolated from *Solanum lycopersicum* fruits rot from certain vegetable markets of Oyo and Osun state between February 2022 and March, 2022.

1.8 Limitation of the Study

This study was carried out on only two varieties of *Solanum lycopersicum* fruits from certain major vegetable markets of Oyo and Osun state. Polymerase chain reaction, Sanger sequencing method and Blast were the only molecular tools used.

1.9 Operational Definition of Terms

PCR - Polymerase Chain reaction

gDNA- Genomic deoxyribonucleic acid

DNA- Deoxyribonucleic acid

BLAST- Basic Local Alignment Search Tool

NCBI- National Centre for Biotechnology Information.

Endnotes

1. A. Casadevall. *Fungal Diseases in the 21st Century: The Near and Far Horizons*. **Pathogen. Immunology**. 3, 2018. 183–196.

2. N.N. Zakawa, K.F. Channya, B. Magga, & T.M. Akesa, Antifungal *Effect of Neem (Azadirachta Indica) Leaf Extracts on Mango Fruit Post-Harvest Rot Agents in Yola, Adamawa State.* **Journal of Pharmacognosy and Phytochemistry 7**, 2018. 23-26.

3. K. Stein, D. Coulibaly, K. Stenchly, D. Goetze, S. Porembski, & A. Lindner, *Bee Pollination Increases Yield Quantity and Quality of Cash Crops in Burkina Faso,* **West Africa Science Report**. 7, 2017. 17691.

4. P. Abrahamian, J.M Klein, J. B Jones, G.E Vallad & R.A Melanson. *First Report of Bacterial Spot of Tomato Caused by Xanthomonas Perforans in Mississippi. **Plant Disease** 103(1), 2019. 147.*

5. E.J. Andersen, S. Ali, E. Byamukama, Y. Yen & M.P. Nepal, *Disease Resistance Mechanisms in Plants. Genes. (Switzerland).* 9(7), 2018. 339.

6. M.K. Ávila & H.M. Romero, *Plant Responses to Pathogen Attack: Molecular Basis of Qualitative Resistance.* **Rev. Facultad Nacional De Agronomía**. (Colombia). 70(2), 2017. 8225-8235.

7. V.C. Biju, L. Fokkens, P.M. Houterman, M. Rep & B.J.C.Cornelissen. *Multiple Evolutionary Trajectories Have Led to the Emergence Of Races in Fusarium Oxysporum F. Sp. Lycopersici.* **Applied and Environmental Microbiology.** (United States). 83(4), 2017. 02548-16.

8. V. Botero, L. Hoyos-Carvajal & J. Marín. *Detection of Asymptomatic Plants of Solanum lycopersicum L. Infected with Fusarium Oxysporum Using VIS Reflectance Spectroscopy.* **Ciencias Hortícolas**. (Colombia). 12(2), 2018. 436-446.

9. M. Camagna & D. Takemoto, *Hypersensitive Response in Plants.* Els. John Wiley and Sons, Ltd *(*Chichester, UK). 2018.1-7.

10. S.L.Carmona, D. Burbano-David, M. Gómez, W. López, N. Ceballos, J. Castaño-Zapata, J. Simbaqueba & M. Soto-Suárez, .*Characterization Of Pathogenic and Nonpathogenic Fusarium Oxysporum Isolates Associated with Commercial Tomato Crops in the Andean Region of Colombia. Pathogen*s. (Switzerland). 9(70), 2020. 1-23.

11. N. Ceballos-Aguirre, W. López, M. Orozcocárdenas, Y. Morillo & F. Vallejo-Cabrera*, Use Of Microsatellites for Evaluation of Genetic Diversity in Cherry Tomato. Bragantia. (Brazil).* 76(2), 2017. 220-228

12. · N. Khan, M. Maymon & A.M. Hirsch, Combating *Fusarium* Infection Using Bacillus- Based Antimicrobials. Microorganisms. (Switzerland). 5(4), 2017. 75.

13. S. Gautam, A.Acedo, P .S Chreinemachers & Bhishma P. Subedi, *Volume and Value of Postharvest Losses: the Case of Tomato in Nepal*, **British Food Journal,** 119(2), 2017, 2547-2558.

14. Abhay K. P, Abhishek Kumar, K.Dinesh, Richa Varshney & Pranab Dutta: *The Hunt for Beneficial Fungi for Tomato Crop Improvement – Advantages and Perspectives.* **Plant –Stress** 6.2022.

15. Christian J S, Casper Van Den A, Isabel Ortega- Salazar, Victor P, Jaclyn A A, Duoduo W, Clare L. C, Graham B .S, & Barbara B.U, *Host Susceptibility Factors Render Ripe Tomato Fruit Vulnerable to Fungal Disease Despite. Active Immune Responses.* **Journal of Experimental Botany,** 72(7), 2021, 2696-2709.

16. Evelyn E &Villanueva G. *An Overview of Recent Studies of Tomato (Solanum Lycopersicum Spp) from a Social, Biochemical and Genetic Perspective on Quality Parameters*, Alnarp-**Sweden: Sveriges, Lantbruksuniversitet.** 3, 2018.

17. Elham A. K, Sajeewa S.N. Maharachchikumbura, Velazhahan R, Hamed Al-M & Abdullah M.A.S. *Fungi Diversity In Tomato Rhizosphere Soil Under Conventional and Desert Farming Systems*. **Frontires in Microbiology**, 2017.01462.

18. Leander D. M, Agyemang D, Samuel K. Offei, K.O, Eric Danquah & Micheal O*, Review on Tomato (Solanum Lycopersicum,L) Improvement Programmes in Ghana,* 2019. Doi: 10.5772/Intechopen

19. Martina S, Marta R, Laura G, Giada D' E & Sheridan L.W. *Endophytic Fungi of Tomato and Potential Application for Crop Improvement,* **Agriculture,** 10(12), 2020, 587.

20. Mehrunisa S, Samina M, Muhammad B.S, *Prevalence of Fungi in Fresh Tomatoes and their Control by Chitosan and Sweet Orange (Citrus Sinensis) Peel Essential Oil Coating.* **Journal of The Science Of Food And Agriculture,** 101(15), 2021, 6248-6257.

21. Muhammad S.M, Shabeer H, Abdul Rehman, Shafiq U.R, Muhammad J & Muhammad A, *Prevalence of Fungi in Fresh Tomatoes and their Control by Chitosan and Sweet Orange (Citrus Sinensis) Peel Essential Oil Coating,* **Journal of Basic Microbiology**, 62, 2022: 48-62.

22. Rabab S & Lorenzo B, *Fungal Diseases on Tomato Plant Under Greenhouse Condition,* **European Journal Of Biological Research Review**, 2017, 2449-8955, Article ISSN.

23. Tinde Van A, Rutger A.V, Ewout M & Anastasia S, *Sixteenth-Century Tomatoes in Europe: Who Saw them, What they Looked Like, and Where this hey Came From.* **Journal For Life And Environment,10,** 2017, E12790

Chapter Two

Literature Review

2.1 Origin and History of *Solanum lycopersicum*

Solanum Lycopersicum has been around for centuries as the first wild species originated in the Andes of Mountains of Western south and Central America[1]. Its name was derived from Nahuatl (Aztec) word tomati which literally translate to "a plump thing with navel" but it was introduced to Europe by the Spanish in the early 16th century[1]. Spanish and Italians seem to have been the first Europeans to adopt it as food which now become a vegetable of the globe[1]. It was considered deadly poisonous of which people were not getting closer to let alone consumption because it resembles poisonous wolf peach[1]. Folklore had it that if tomato is eaten, its poison would turn human blood into acid and instead the colonists grew tomatoes purely for decoration[1]. In France and Northern Europe, the tomato was initially grown as ornamental plant and was regarded with suspicion as a food because botanists recognized a relative of the poisonous belladonna and deadly nightshade[2].

Although, the root and leaves of tomato plant are said to be poisonous because it contains neurotoxin solanine[2]. The Italians called tomato *pomodoro* (golden Apple) which has given rise to speculation that the first known tomatoes to Europeans were yellow and it has been said that in French language, it is called *pomme d'amour* (love apple) because it was thought to have aphrodisiacal properties[2]. A French Botanist, Joseph Pitton de Tournefort gave the Latin botanical name l*ycopersicum esculentum* to tomato which portray it to be exactly like wolf peach[2]. Modern age of commercially grown tomato started with the effort of an American botanist cum scientist who dedicated much of his life upgrading tomato with selective breeding into the most commonly known forms of today[2]. *Solanum lycopersicum* was botanically classified as fruit while United State of America tariff law of

1887 classified it as vegetable on the basis that it's often served with dinner and not a dessert[3]. There are more than 7000 varieties of tomatoes and they all represent only one species of tomato that is cultivated as *Solanum lycopersicum*[3].

2.2 Taxonomical Classification of *Solanum lycopersicum*[4]

Tomato is classified as follows-;

Kingdom	***Plantae***
Subkingdom	*Viridiplantae*
Infrakingdom	S*treptophyta*
Superdivision	*Embryophyta*
Division	*Tracheophyta*
Subdivision	Spermatophytina
Class	*Magnoliopsida*
Super order	*Asteranae*
Order	*Solanales*
Family	*Solanaceae*
Genus	*Solanum*
Species	*lycopersicum*

2.3 Description of *Solanum lycopersicum*

Tomato plants are generally much branched spreading 60-180 cm (24-72inches) and somewhat trailing when fruiting but a few forms are compact and upright[4]. The tomato

leaves are more or less hairy, strongly odorous and pinnately compound up to 45cm long[4]. It has five petals yellow flowers, fruits are berries that vary in diameter from 1.5 to 7.5cm or more and fruits are usually red, scarlet, or yellow[4]. Though green and purple varieties do exist and they vary in shape from almost spherical to oval, elongate to pear-shaped, each fruits contains at least two cells of seeds surrounded by jelly pulp[4].*Solanum lycopersicum* requires relatively warm weather and much sunlight but grown chiefly in hot house when climates is cooler and usually staked, tied or caged to keep the stems and fruits off the ground[5]. Consistent watering is necessary to avoid blossom – end rot and cracking of the fruits[5]. The plant is susceptible to a number of pests and diseases though many of these problems can be solved with crop rotation, fungicides, pesticides usage and planting genetically engineered resistant varieties[5].

Cultivating tomato plant yield best at optimum temperature of 21–24 degree celsius and it requires medium rainfall, warm and cool climate with minimum light intensity which affect pigmentation, fruits colour and fruits set[5]. Although different climatic condition is require at different stages of seed germination, seedling growth, flower, fruits set and fruits quality[5]. The *Solanum lycopersicum* produces yellow flowers which can develop into a cyme of 3-12 usually a round fruit (berry) which is fleshy, smooth skinned and can grow up to 0.7-2m in height as an annual plant which can be harvested after only one growing season[5].

2.4 Tomato Varieties

There are thousands varieties of tomatoes all over the world that can be a wide range of colours from pink to purple, yellow to white, and sometimes even dark as black but most common colour is red[9]. Determinate (bush) tomato are varieties of tomato that have been purposefully bred to grow vertically and remain relatively compact as it stops growing once fruits begins developing at the terminal shoot[9]. All the fruits of the determinate varieties ripen at around the same time[9]. In contrast, indeterminate (vining) tomato varieties spread

laterally and will continue to grow and produce fruits until end of season begin[9]. Indeterminate can produce fruits all season and fruits will develop and ripe at the same time[9]. Heirloom tomatoes are generally open-pollinated varieties which have been conserved over many generation due to certain desirable characteristics such as flavor[10]. They are hybrid tomatoes, the product of cross-pollination between two parents with desirable characteristics such as high yield, early maturation, improved flavor or resistance to diseases[10]. There are different varieties of tomatoes namely-; Beefsteak, Roma, Salad, Chery and Grape[10].

2.5 Descriptions and Appearances of Different Varieties of Tomatoes and their Sub-varieties.

A. Beefsteak Tomatoes

Beefsteak Tomatoes are known for their size, 6 inches in diameter, weighing about 1-3pounds, hence, they are very large [10]. It requires long growing season and it is thick and meaty making them perfect slicing tomatoes[10].

Fig 2.1: Beefsteak Tomato Fruits[10]

B. Cherry and Grape Tomatoes

This type of tomato is easy to grow and it has small size with 1 inch diameter[11]. It is very resistance to diseases and do well even in the cases of drought or otherwise poor soil [11]. This variety do well in containers which is one the reasons it is recommended for first time farmer[11].

Fig 2.2: Cherry Tomato[11]

C. **Roma Tomatoes**

They are also called plump tomatoes, larger than Cherry and Grape tomato but still not large enough for convenient slicing[12]. One Roma tomato contains 11 grams of Calories with 1 gram of fibre and they are naturally sweet and juicy though very little which enables them for long time storage[12].

Fig 2. 3: Roma Tomatoes Fruits[12]

D. **Salad Tomatoes**

Salad tomato normally get up to 3 inches in diameter, a little bit tart and juicier than cherry or beefsteak, easy to slice and good for sauce, especially when cooked down to a good consistency[12]. In fact, they are the best combination of tartness, juiciness and acidity which balances one another out to create a perfect tomato taste as this variety is best eaten raw[12]**.**

Fig 2.4: Salad Tomato[12]

2.6 Nutritional Benefits of *Solanum lycopersicum*

Solanum lycopersicum is highly nutritious, its water content is about 95% while the other 5% contain mainly carbohydrate and fibre[19]. 100g of raw tomato contain 3.89g of

carbohydrate, 0.9g protein, sugars (glucose and fructose) 2.6g, fibre 1.2g, fat 0.2g and a good source of vitamins and minerals (C, K, B, potassium)[19]. There is also presence of some compounds like Lycopene, Beta carotene, Naringenin and Chlorogenic acid[19].

2.7 Lycopene

Lycopene is a non-provitamin A and a type of organic pigment called carotenoid related to beta-carotene which gives some vegetable and fruits red colour[19]. It is a natural powerful antioxidant that might help protect red plant cells from damage and it's found in tomato, watermelon, red orange, pink grapefruits, apricot, rose hip and guava[19]. Antioxidants are molecules that fight free radicals in the system of organisms which build up naturally as cell ages and it can increases on the influence of environmental and behavioral factors like pollution and smoking[20]. High level of free radicals in the human system can lead to serious health conditions like cancer, diabetes, cardiovascular problem and antioxidant is believed to be playing stabilizing role in the aforementioned health issues[20]. Although, researches are still on going to establish the major role of lycopene in addressing health issues[20].

2.8 Naringenin

Naringenin is a flavonoid belonging to flavanones subclass and is widely distributed in several citrus fruits like bergamot but very abundant in tomato[21]. It is believed to poses antiviral, anti-inflammatory, antiadipogenic and also has cardio protective effect[21].

2.9 Health Benefits of *Solanum lycopersicum*

Consumption of tomatoes and its product has been linked to some health benefits like skin health by reducing sunburn, lower risk of cardiovascular disease and cancer prevention or risk reducer[22].

2.10 Heart Benefit

American journal of clinical nutrition has reported that antioxidant in tomato play major role in reducing LDL and increase HDL which in turn able to lower risk of heart attack, clogged arteries, reduce blood pressure and other cardiovascular diseases[22].

2.11 Skin Health

There have been claims in some quarters that raw tomato direct application on skin prevents aging, skin cancer and sunburn, this was established and reported in a journal named "scientific reports nature search" of year 2017[23]. Some hairless and immunocompetent mice were fed with tomato powder for 35 weeks and also exposed to Ultraviolet light to reveal the effectiveness of lycopene against cancer, sunburn and aging, the result established that dietary lycopene mitigate against the ultraviolet effect and the mice skin appeared unaffected[23].

2.12 Cancer Prevention

It was reported in an American cancer society journal of year 2015 that consumption of tomatoes and tomato products has been found to be associated with a reduced incidence of a number of different types of cancer notably prostate, lung and stomach[23]. Another study also observed that high concentration of carotenoids found in tomatoes may protect against breast cancer and skin cancer too if incorporated in daily skin care routine[23].

2.13 Diseases

Diseases has been described as disorder or a particular abnormal condition that negatively affects the structure or function of all or certain part(s) of an organism or plants especially with manifestation of specific signs and symptoms[24].

2.14 Diseases of Tomato Plant

Solanum lycopersicum disease can be described as any problem that leads to a reduction in yield, well-being, appearance and deviation from normal functioning of physiological processes due to the effect of the pathogens that can be virus, bacteria, fungi, nematodes and some other physiological and environmental conditions[24].

2.15 Pathogens

They are described as microbes or microorganism that can cause harm, disorder or damages in the susceptible host like animate and inanimate[25]. The damaging effect comes to manifestation once there is favorable environmental condition[25].

The *Solanum lycopersicum* fruits rots associated with the fungi are as listed below[26]; -

i. Alternaria Stem Canker

This rot is caused by *Alternaria alternata and* it can affect stem, leaves, and fruits[27]. The symptoms appear on tomato plants as dark to brown cankers with concentric zonation on stems near the soil line or a bit above ground[28]. The cankers can become enlarge and then girdle the stem before the harvest which mostly kill the plant although, the vascular tissue above and below the cankers exhibit brown streaks[29]. There is presence of dark brown to black areas of dead tissue between leaf veins which is caused by toxin produced by the fungus[29]. Also, there may be dark brown sunken lesion with characteristic concentric rings developed on green fruits either on plant or during postharvest of ripening fruit[29].

Fig 2.5: Alternaria Stem Canker of Tomato [29]

ii. Anthracnose

This is a serious rot of overripe tomatoes caused by several species of fungus *Colletotrichum (C. dermatium, C. gloeosporioides, C. coccodes and Glomerella cingulata) and* it can affect green fruits too but symptom may not really show until fruits begin to ripe[30]. The symptom usually start as small spot, circular and depressed which may enlarge over time to concentric rings and the Centre become very dark[30]. Tomato is referred to as a cull if it has more than two Anthracnos*e* lesions aggregating more than a circle as the causal organism produces spore- containing structures known as microsclerotia and acervuli[30]. These spores are dispersed mostly by wind to other plant on the field when the weather is wet or humid as the other fruits nearest to ground are most affected and sometimes root are not spared[30].

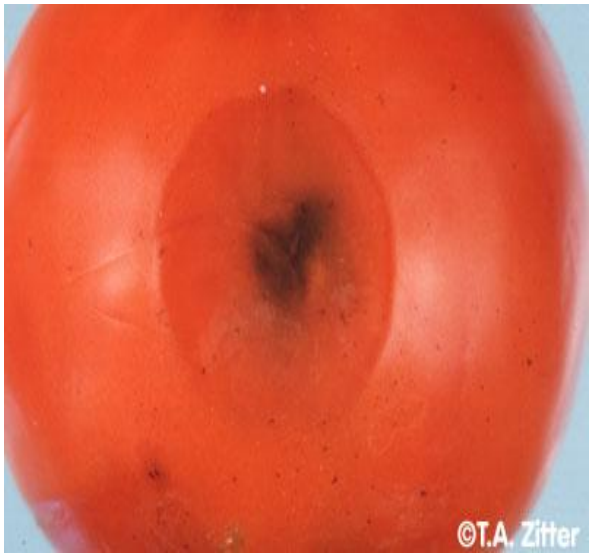

Fig 2.6: Anthracnose on Tomato Fruit[30]

iii. Black Mold Rot

Different fungi are responsible for this disease but the most common is *Alternaria alternata* and others like *Stemphylium botryosum, Stemphylium herbarum, Pleospora herbarum, Ulociadium consortiale*[31]. This rot is characterized by obvious lesion that appear on the surface of a ripe tomato fruit as the lesion grows from light to dark brown[32]. The lesion varies from small flecks affecting only epidermal tissue to large sunken lesions with decay extending into the carpel wall and as well as seed locule[33]. The causal organism may sporulate to form a black, velvet like layer on the surface of the sunken lesion during warm or humid weather and this disease has higher incidence with late season rain because it only takes 3-5 hours of wetness for the causal organism to begin actions[34]

Fig 2.7: Black Mold Rot on Tomato Fruit[34]

vi. Buckeye Rot

This rot is caused by *Phytophthora species (P. capsici, P. drechsleri, P. nicotianae var. parasitica)* and the species attack on tomato plant varies based on the region[38]. This disease is most common in the Southeast and Central of United States and the major symptoms of the infection start from the seed or transplant as it begins to show on the fruits near the soil[39]. Buckeye rot disease is favoured by prolonged warm wet weather and non – staked tomato plant are most affected[40]. The spot that resulted from this infection begins as small brown on the fruits which grow to large, round, oblong lesion with alternating concentric ring of light to dark brown coloration as the lesions are firm with smooth margin but later become soft and rot away[41].

Fig 2.8: Buckeye Rot on a Tomato Fruit[41].

viii. Charcoal Rot

This rot is also known as dry weather wilt, it is a soil borne disease of tomato caused by fungus *Macrophomina phaseolina*[44]. The plant exhibit wilting symptoms, drying and death of older leaves while the younger ones appears unaffected at start but later collapse eventually[45]. When crowns are cut opened, they show dark brown necrotic areas in the internal cortex and vascular tissue because it is a disease that attacks roots, stem and fruits[46]. The stem develops basal cankers that girdle the stem resulting in yellowing of foliage, wilting and death of the whole tomato plant[47].

Fig: 2.9: Internal Crown and Vascular Charcoal Rot of Tomato[43]

x. Early Blight of Tomato

Alternaria species (A. solani, linariae) are the main fungi of tomato early blight disease *and* it can affect leaves, stem and fruits as the symptoms can occur at any stage of development[48]. The lesion first develops at lower leaves as small, brownish-black spots which can expand in diameter with characteristic concentric rings in the darkened area[49]. The area surrounding the lesion may become yellow as the disease progresses and it may turn the entire leaf to colour yellow[50]. Lesion may appear at upper leave and defoliation may occur in lower part of the plant leaving the fruits susceptible to sunscald[51]. The infected plant may not die but become weaken leading to abnormal set of tomato fruit[52]. Generally, early blight disease affects older tomato but at times can still affect seedlings and stressed plant[53].

Fig 2.10: Early blight with Initial Infection on a Tomato Leaf [54]

Fig 2.10.1: Early blight with Concentric Rings on a Tomato Leaf [54]**.**

Fig 2.10.2: Early blight on tomato stem[54]

xi. Fusarium Wilt, Crown and Root Rot of Tomato

This disease is the most damaging soil-borne infection of tomato caused by *Fusarium oxysporum and* it was first discovered in Florida in the year 1974[55]. The disease is a soil – borne which makes his way through the plant root which may resulted in clogging and blocking of the xylem preventing movement of water and other important nutrient to the stem[56]. Branches and leaves of the tomato plant will become weakened and less productive[57].The causative agent of Fusarium wilt may remain in the soil for years which makes it a tough disease of tomato and it can also infect the resistant variety[58].

The symptoms of the tomatoes infected by Fusarium wilt often start with single leaf near the top of the plant showing yellow coloration as well as lower leaves and mostly at only one side[59]. The wilted leaves tend to dry and eventually fall off but, if the stem is cut opened, dark brown streak discoloration will be observed which is the typical characteristics of Fusarium infected tomato plant[60].The disease can spread from root – to - root contact, air spread, the surface of equipments and from workers touch[61].

Fig 2.11: Fusarium Wilt on Tomato plant[61] **Fig 2.11.1 Fusarium Crown and Root Rot**[61]

xii. Gray Leaf Spot

Stemphylium species (S. lycopersici, S. botryosum, S. solani) is the causative organism of tomato gray leaf spot leaving the tomato leaves with lesions that become glazed in the Center and crack[62]. This in turn produces shot holes and as the disease progresses, the lesion can grow up to 0.31cm across the affected leaves[62]. Stems may also develop spots and primarily, young stems and petioles are infected as the organism can hide under the debris or other nightshade plant to infect tomato[63]. Like most of tomato diseases, moist, warm condition favors the organism to attack while consistent dropped leaves expose the fruits to sunscald thereby affecting the quality and quantity of tomato fruits yield[63].

Fig: 2.12: Gray leaf Spot of Tomato[63]

xiii. Late Blight

It is a potentially devastating disease of tomato which can infect the leaves, stems, fruits and spreads quickly in the field resulting in total crop failure if prompt action is not taken[64]. This disease is caused by *Phytophthora infestans,* a fungus –like organism also called water mold but not true fungi[64]. Its symptoms on tomato leaves manifest appearance of large dark brown blotches with a green gray edge which not is confined by major leaf veins but as the disease progresses, large section of dry brown foliage results through leaflets and petioles[65]. Stem infections are firm, dark brown and a rounded edge with circular spots growing to cover large parts of the fruits while the spot may become mushy as secondary bacteria invade[66].

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Fig 2.13: Late blight Tomato on leaves [66] **Fig 2.13.1: Late blight Tomato fruit**[66.]

xv. Powdery Mildew

It is a disease of tomato plant worldwide, mostly occur when the weather is warm as the disease is caused by *Leveillula taurica* which brings about dryness and brittle[69]. The symptom first appear on tomato leaves as pale yellow spot which is later covered with spots as if it is has been dusted with white flour and it progresses till the leaves turn brown and shrivel[70]. It is a disease of late season, although, stressed tomato plant are the most susceptible[70].The damaging effect of powdery mildew is that the fungus clog up the leaf pores which blocks light and affect photosynthesis process[70]. The tomato plants become weaken as a result of inability to use light for energy and in turn stops growth[70]. It causes

the fall off of the old leaves as the plants struggle to live and the fruits produced by infected plants of this disease most of the time lack flavour[71].

Fig: 2.14: Powdery Mildew on Tomato leaf[71]

xvi. **Damping – off and Fruits Rot**

This disease is caused *Pythium species (P .aphanidermatum, P. arrhenomanes, Debaryanum, P. myriotylum, P. ultimum)* and some other fungi[72]. Damping off of tomato occurs in two stages, the pre-emergence and the post-emergence phase[72]. In the pre-emergence phase, the seedlings are killed just before they reach the soil surface with the young radical and the plumule being killed and there is complete rotting of the seedlings[73].The post-emergence phase is characterized by the infection of the young, juvenile tissues of the collar at the ground level where the infected tissues become soft and water soaked as the seedlings toppled over or collapsed[73].Seedlings affected by damping-off fail to emerge or fall over and die soon after emergence while stems usually have a dark, shriveled portion at the soil line[74]. Damping-off is generally limited to areas where drainage is poor or where soil is compacted but whole fields can be affected especially if early

plantings are exposed to rain[75]. Although, other organisms like Phytophthora and Rhizoctonia can also infect tomato seedlings in warmer soils[76]. Once tomato seedlings reach the leaf stage, they are no longer susceptible to infection by Pythium or Rhizoctonia, however, Phytophthora can infect tomato plants at any stage[77].

Fig: 2.15: Damping –off and fruits Rot on Tomato seedling[77]

xviii. Southern Blight

Tomato plants with Southern blight have lesions on the stem at or near the soil line and these lesions develop rapidly girdling the stem resulting in a sudden and permanent wilting of tomato plant[87]. Southern blight, also known as Southern wilt or Southern stem rot, is a serious and frequent disease of tomato which was first reported in North Carolina on tomato plant among nightshades[87]. It was later reported on hundreds of other economically important crops including pepper, bean, cantaloupe, carrot, potato, sweet potato, watermelon, cotton, peanut, tobacco, and soybean[88]. High temperatures of 77 to 95°F, aerobic and moist conditions favor the growth of the causative organism known as *Athelia rolfsii*[88]. The most common symptom of Southern blight occurs as brown to black lesion usually develops on the stem near the soil line which completely girdle the stem and cause a sudden and permanent wilt of every part of tomato plants[89].

Fig 2.16: Southern blight[89].

xix. White Mold -

This disease is caused by *Sclerotinia sclerotiorum and S. minor* which generally appears on tomato plants at flowering stage[100]. The symptoms include water-soaked areas on flowers and at stem joints where senescent flower petals have fallen as the infection can quickly kill stem resulting in dried and bleached appearance[100]. Water-soaked stem lesions may also appear at the soil line if senescent plant debris is present around the plant showing white infected fruits turn gray and rot[101]. The pathogen is monocyclic and thus does not spread from an infection site during a season and it can survive for years in soil[101].

Fig 2.17: White Mold on Stem[101]

Fig 2.17.1: White Mold on Tomato Fruits[101]

xx. Sour Rot

Sour rot of tomato is caused by *Geotrichum candidum* and a *Galactomyces geotrichum,* they are common soil borne fungi that cause the disease not only in tomatoes, but also in citrus fruits and some other vegetables[102]. The lesion from this disease may be initially watery but later become coated with fungal growth[103]. This growth resembles a thick gelatinous mass similar in appearance to cottage cheese and remains relatively firm unless a secondary infection by a soft rot bacterium occurs[103].The odor of the lesion is distinctive and is similar to that produced by lactic acid bacteria, hence the disease name, sour rot[104]. Survival and Spread of *G. candidum* and other species of its kind are opportunistic pathogen that can live on plant debris in the soil[104]. Saprophyte and ubiquitous in the natural environment are found in almost all soil and tomatoes are most resistant when they are relatively dry and firm but mature green tomatoes are more resistant to this disease than ripe fruits[105].

Fig: 2.18: Sour Rot of Tomato Fruit[105]

xxi. Target Spot-

Corynespora cassiicola is the causative organism of Target spot of tomato disease which often cause necrotic lesions[106]. It appears in a concentric pattern similar to early blight and is favored by temperatures of 68- 82°F[107]. The fungus can survive in host residue for a long period of time and it attacks older leaves which spread upward and its first sign is irregular-shaped spots less than 1mm with a yellow margin[107]. Some of the spots enlarges up to 10 mm and show characteristics rings, hence the name of 'target spot'[108]. It spreads to all leaves causing the leaves to turn yellow, collapse and die while spots can also occur on the fruits, stems as long, thin, small light brown with dark margins[108].

Fig: 2.19: Target Spot on Tomato Leaf[108]

2.15 Management of *Solanum lycopersicum* Diseases Caused by Fungi

Solanum lycopersicum, like any other plants can be infected with diseases, this is a major limiting factor for tomato production in the world considering the economic importance and health benefits of it[109]. Tomato may be produced in large scale for commercial purpose or small scale for individual use[109]. The abnormal condition of a tomato is majorly in two phases, the emergence phase where the leaf is covered with irregular spot and the mature phase where the stem or fruit is withered[109]. The infectious microorganism is contagious and can spread from plant to plant in a field[109]. This most times spread very rapidly when environmental conditions are favorable[109].

The non-infectious diseases of *Solanum lycopersicum* such as adverse environmental factors, nutritional or physiological disorders are not contagious like the ones caused by pathogen[109].

In the effective disease management of *Solanum lycopersicum,* it is very important to first identify the disease and its symptoms[110]. Then, begin prevention at early phase to avoid diseases or delay its occurrence at the emerging phase than controlling the disease at maturity stage[110]. When symptoms of diseases are confusing, the sample of the infected plant can be taken for laboratory diagnosis for accurate and proper identification of causative agent[110]. Some of the tomato diseases and mode of management include: -

2.16 Early Blight

This disease is caused by *Alternaria linariae* (formally known as *A. solani*) and is first observed on the plants as small brown lesions mostly on the older foliage with spots becoming enlarge and concentric rings in a bull's-eye pattern in the center of the infected area[111].

Prevention, Treatment and Management: Resistant or tolerant tomato cultivars, pathogen-free seeds, crop rotation, weeds eradication and volunteer tomato plants should be prioritize[111]. Spacing, mulch plants and fertilizer of proper chemical combination should be used[111.] Tomato foliage wetting should be avoided as well as trimming off and disposing infected lower branches and leaves to reduce disease severity should be regularly done[111]. The garden soil must be tested annually in which a sufficient level of potassium must be maintained, liming of the soil according to soil test results and side dressing of tomato plants monthly with calcium nitrate for adequate growth[111]. But if it is too severe to the level of using chemical control, one of the following fungicides can be selected; mancozeb, chlorothalonil or copper fungicides[112].

2.17 Late Blight

Late blight is a potentially serious disease of potato and tomato and is caused by the water mold pathogen known as *Phytophthora infestans*[113]. Late blight is especially damaging during cool, wet weather and this pathogen can affect all parts of tomato plants[113].

Prevention, Treatment and Management: The following guidelines should be followed to minimize late blight problems:

a. Tomato foliage must be kept dry by locating the garden where it will receive morning sun[113].
b. Allowing extra room between the plants and avoidance of overhead watering, especially late in the day[113.]
c. Certified disease-free seeds and plants will reduce the incident of the disease to the minimum if planted[113].
d. Destroy volunteer tomato and potato plants as well as nightshade family weeds such as Carolina horsenettle or black nightshade which may harbor the fungus[113].
e. Plant resistant cultivars[113].

2.18 Septoria Leaf Spot

This destructive disease of tomato foliage, petioles, and stems (fruit is not infected) is caused by *Septoria lycopersici* and infection usually occurs on the lower leaves near the ground after plants begin to set fruit[114]. Numerous small, circular spots with dark borders surrounding a beige-colored center appear on the older leaves[114].

Prevention, Treatment and Management: Most currently grown tomato cultivars are susceptible to Septoria leaf spot but crop rotation of 3 years and sanitation (removal of crop

debris) will reduce the amount of inoculum[114]. But avoidance of overhead irrigation and repeated fungicide applications can keep the disease in check[114].

2.19 Leaf Mold

Passalora fulva causes leaf mold and it is first observed on older leaves of tomatoes near the soil where air movement is poor and humidity is high[115]. The initial symptoms are pale green or yellowish spots on the upper leaf surface which enlarge and turn a distinctive yellow[115].

Prevention and Treatment of Leaf Mold: Infected crop residue should be removed from the field while Staking and pruning should be done to increase air circulation[115]. In order to control the disease, spacing tomato plants further apart for better air circulation between plants should be observed[115].

2.20 Anthracnose

Anthracnose on tomatoes is caused by a group of fungi within the genus *Colletotrichum* and these species are primarily pathogens of the tomato fruits[116]. As the fruit are ripening the symptoms first become noticeable as small, circular indented areas which later develop darkened centers[116]. The diseased spots continue to grow larger with time as each infection site also spreads deeper into the fruits[116]. With warm, moist, and humid weather the fungus produces salmon-colored spores that are exuded from the black fungal material in the center of the spots and these spores are spread by splashing water[116].

Prevention, Treatment and Management of Anthracnose

Purchasing of disease-free seed as the fungus that causes anthracnose of tomato may be within the seed[117]. Tomato seed may be treated by soaking them in hot water (122 ºF) for 25 minutes to destroy the fungus but some varieties of tomatoes have resistance to anthracnose[117]. Some of the resistance cultivar is chef's Choice Orange Hybrid which

dwell best in a sunny site[117]. Staking or caging tomato plants to provide better air movement should be put in place[117]. Fungal spores can remain in the soil to infect plants the following year and so, proper clearing, mulching, help create a barrier between the soil surface and the fruit to reduce infections[118].

2.21 Fusarium Wilt

This is a warm-weather disease caused by the *Fusarium oxysporum* and the first indication of disease in small plants is a drooping and wilting of lower leaves with a loss of green color[119]. This is then followed by wilting and death of the plant while long-distance spread is through seed and transplants[119].

Prevention, Treatment and Management of Fusarium wilt: Pathogen-free soil, disease-free transplants and growing only cultivars with at least resistance to races 1 and 2 of Fusarium wilt is the first step to preventing the attack of the disease on tomato plant[119]. Then, raising the soil pH to 6.5 – 7.0 and the use of nitrate nitrogen such as in calcium nitrate rather than ammoniacal nitrogen will retard disease development because no chemical control is available[119].

2.22 Damping-off (Seedling Disease)

The fungi *Pythium* and *Rhizoctonia* cause damping-off where seedlings fail to emerge from the soil in the greenhouse or small seedlings wilt and die soon after emergence or transplanting [119]. There is appearance of water-soaked areas on the stem close to the soil line of any surviving tomato plant[119].

Prevention, Treatment and Management of Damping- off: This disease is often a problem in tomato that are planted too early in the spring as the fungi are more active in cool, wet, rich soil [119]. To prevent damping-off, these precautions are necessary[120]: -

i. Seeds should be started indoors in a sterilized mix pot or new and clean containers.

ii. Avoidance of planting seeds in soil that has a high nitrogen level but nitrogen fertilizer can be added after the seedlings have produced their first true leaves.

iii. The surface of the soil should be allowed to dry between watering.

2.23 Summary of General Management and Control of Fungal Associated with Tomato Rot

Solanum lycopersicum is susceptible to some diseases caused by pathogenic fungi being the second most cultivated vegetable, it is exposed to these pathogens during cultivation or post-harvest storage[121]. So, it is a necessity to manage and control its diseases to avoid its benefit and importance being defeated because tomato rots is one of the major factors limiting or influencing the production of tomatoes and tomato products[121].

Soil Testing- Soil testing should be the first management and control of tomato disease before planting because the soil is the habitat which will aid the cultivation of tomato and anything fall short of the best soil quality will jeopardize whatever planted[121]. The soil should be tested for pH, soil drainage, soil minerals and nutrients adequacy should be ascertained[110]. Making sure the soil is in good condition will set everything in motion for planting tomato seed or transplanting[121].

Prompt Time Planting – Planting of *Solanum lycopersicum* should be timely which will prevent it from certain harsh weather conditions like high temperature and too much moisture aid the pathogen to thrive well on the plant[121].

Resistant Cultivars – Molecular Biologist have done some great research analysis to improve tomato varieties quality for it to be able to resist pathogen in order to increase the yield seasonally[122]. Planting improved cultivar is another way of controlling and managing tomato rot[122].

Weed Clearing-Clearing of weeds from the tomato farm goes a long way of preventing the plant from attacks of pathogen because the weeds harbors some of these pathogens that in turn affects tomato[123]**.**

Chemical Control- Fungicide management should be used and control of fungi infected tomato that appears spreading fast should be permanently removed[123].

Space Planting- Spacing out one tomato plant a certain centimeter from another is a way of curbing, controlling and managing the spread within the farm space[124].

Certified Seed – some seeds are treated and certified resistance to pathogen attacks using such seed will minimize or eradicate some forms of pathogen[124].

Crop Rotation- Rotating crop on a piece of land where tomato has been previously planted helps in controlling and managing fungi attacks, some fungi can survive in the soil for 3 years after tomato has been harvested, planting tomato back immediately to same piece of land may be disastrous[125].

Proper Irrigation- Irrigating tomato farm land from overhead can contribute to its pathogenic attacks since they dwell beautifully well in moisture environment[126]. Wetting or irrigation of tomato plant should be at the base in order not to create enabling environment for fungi infection[125]**.**

Daily Harvest- Harvesting on daily basis when tomato fruits has matured or ripe will manage and control the action of pathogens [126]. Leaving or neglecting the mature and ripe fruits of tomato will expose them to pathogen because some fungi only attacks overripe and mature tomato fruits[126].

Hand Picking- Picking of infected plant is the only solution to some fungi infection due to the fact that they cannot be controlled with the aforementioned action but to only hand-picked before other plants become infected[127].

2.24 Molecular Tools for Detecting Fungi Associated with *Solanum lycopersicum* Rots

Some fungi cannot be taxonomically detected and identified from phenotypic characteristics[127.] Recently, detecting and identifying fungi associated with *Solanum lycopersicum* diseases is based on the application of genotypic techniques using highly specific probes and nucleic acid amplification which is far different from conventional ways of detection[128]. Precise and rapid detection and identification of phytopathogens infecting plant are essential to facilitate effective management of fungi diseases[128]. But recent developments in standard and variant polymerase chain reaction (PCR) assays which include nested, multiplex, quantitative, bio and magnetic-capture hybridization PCR techniques, post and isothermal amplification methods, DNA and RNA based probe development and next-generation sequencing has provided novel tools in molecular diagnosis against phytopathogen particularly fungi being the most destructive pathogen of *Solanum lycopersicum*[128].

These molecular based detection techniques are effective in detecting symptomatic and asymptomatic diseases of both culturable and unculturable fungi in sole and co-infections[129]. Although, the molecular diagnostic approaches have expanded substantially in the recent past but there is still a long way to go in the development and application of molecular diagnosis in plant diseases[129]. Molecular techniques or tools used in Phytofungal diseases diagnosis are said to be more reliable, faster and easier than conventional methods[129]. Recent advancement in the improvement and application of molecular methods for diagnosing the widespread and emerging plant fungi infection in *Solanum lycopersicum* diseases cannot be overemphazised[129].

Basic conventional methods used to detect pathogenic organism mostly rely on microscopy, culture, morphological and biochemical identification approaches that require extensive time, labour and classical taxonomical knowledge [130]. This approach though being the cornerstone of microbial diagnosis is cumbersome and at the same time can lead to unreliable results due to the problems of identification[130]. Then, experts and specialist with a practice in microbial identification are required for its effectiveness[130]. Then, due to the conventional methods limitations, molecular techniques came into existence for the investigation, identification and classification of microorganism characteristics to species level[130].

High varieties of molecular methods are increasingly becoming valuable tools in all aspects of Phytopathology diagnosis and these techniques include immunological methods, nucleic acid-based probe technology and polymerase chain reaction technology[130]. The former method rely upon phenotypic characters while the latter is based on genotypic characters which give fast, highly specific, effective and potentially more accurate results in contrast to the basic methods of organisms isolation[131]. But molecular techniques also require bioinformatics databases known as GenBank like National Centre for Biotechnology Information (NCBI), Nucleotide Sequence Database Collaboration at the European Bioinformatics Institute (EBI) and MycoBank as they are platforms for referencing, documenting microorganism nomenclatural novelties, storing and retrieving facilities of nucleotide sequences[131].

The enormous effect of fungi infecting tomato plants accelerate the use of molecular tools to potentially diagnose and perform species delimitation among existing and evolving different species of microorganism[131]. Rapidly emerging and novel fungi threaten the global economy hence rapid and accurate detection and identification of fungi in tomato plant and its fruits is crucial being most consumed and popular plant after potato[132]. The molecular

markers are no longer looked upon as simple DNA fingerprinting markers in variability studies or as mere forensic tools but they are constantly being modified to enhance their utility and to bring about automation in the process of genomic analysis[132].

This is in order to offer a number of evolutionary new insights into the detection of phytopathogens and also to provide information on identifying unknown or novel species from their DNA or RNA sequences[133.] Detection and identification of diseases in tomato fruits can be realized through both direct and indirect methods as discussed earlier[133]. Direct detection of diseases includes conventional and molecular methods that involve high-throughput analysis when large numbers of samples are needed to be analysed[133]. In these direct methods, the disease-causing pathogens such as bacteria, fungi, insects and viruses are directly detected by providing accurate identification of the pathogens responsible for the disease[133].

But on the other hand, indirect methods of identifying tomato plant diseases are through various parameters such as morphological changes, temperature changes, transpirational rate changes and volatile organic compounds released by infected tomato plants[134]. The molecular diagnostic tools have been designed to detect diseases and identify organisms genetically in plants such as tomatoes which is needed to monitor the occurrence and development of pathogens[134]. Also, to come up with proper and judicious ways in establishing management strategies aimed at combating and limiting the occurrence of tomatoes diseases[134].

2.25 The Polymerase Chain Reaction (PCR)

In the years of 1984 and 1993, Nobel prices were won by some researchers for the development of monoclonal antibodies and amplification of nucleic acid sequences respectively using the technology of polymerase chain reaction (PCR)[135]. Based on the fidelity of DNA hybridization and replication, PCR was initially used for highly specific

detection of clinical diseases caused by bacteria and viruses[135]. Now, it has been widely used for the detection of other plants pathogens as well[135]. In addition to the basic PCR technology, advanced PCR methods such as reverse-transcription PCR (RT-PCR) has also been used for plant pathogen identification due to its high sensitivity[135]. Multiplex PCR was proposed to enable simultaneous detection of different DNA or RNA by running a single reaction[135]. Real-time PCR platforms have also been used for on-site, rapid diagnosis of plant diseases based on the bacterial, fungal and viral nucleic acids [135].

Pcr technique can provide high sensitivity and specificity due to the fidelity of DNA amplification but it is also limited by lack of operational robustness because it depends on the efficacy of DNA extraction[135]. The performance is affected by inhibitors present in the sample assay, polymerase activity, PCR buffer and concentration of deoxynucleoside triphosphate [136]. In addition, application of PCR for pathogen detection requires designing a primer to initiate DNA replication which could limit the practical applicability of this technique for field sampling of diseases[136]. The tools used in DNA diagnosis are well appreciated among the techniques because it is an extremely sensitive Invitro method and capable of amplifying trace amounts of microorganism nucleic acid in samples to a detectable levels[136].

2.26 Nested PCR

Sensitivity and specificity problems associated with conventional PCR and RT-PCR can be reduced by using nested PCR-based methods[137]. It is based on two consecutive rounds of amplification and usually the products of the first amplification are transferred to another tube before the nested PCR is carried out [137]. It uses one or two internal primers as heminested or nested amplification respectively[137]. The potential of nested-PCR in plant pathology has been reported and there are many published examples of its application to

fungi detection in plants [137]. It uses small volume of reagent which could increase susceptibility to inhibitors and requiring a previous RNA extraction to reach a good sensitivity in detection[137]. The nested PCR was performed to diagnose the *Pilidiella granati* infection in the pomegranate samples that were collected from the different areas of Anhui Province, China to validate the protocol, artificially infected pomegranate fruits were also used[137]. The genomic DNA were isolated from naturally infected, artificially infected and healthy control fruits and subjected to the nested PCR assay. Both the naturally infected and artificially infected samples were found to be positive for *P. granati* as a 450-bp PCR product was obtained on the agarose gel. Whereas, no PCR products were obtained with DNA from the control samples[137].

2.27 Multiplex or Duplex PCR

The simultaneous detection of two or more DNA or RNA targets can be afforded by duplex or multiplex PCR in a single reaction with several specific primers included[138]. Multiplex PCR is very useful in plant pathology because different fungi, bacteria or viruses frequently infect a single crop or host can be detected and identified[138]. This methodology has demonstrated to be a valuable tool for detection and identification purposes and there are several examples of simultaneous detection of viruses, bacteria and fungi at the same time[138].

The design of a multiplex RT-PCR is based on the use of compatible primers specific to different targets, which must be evaluated theoretically *in silico* and empirically tested *in vitro* [138]. It is worth noting that the use of general and common primers to amplify different targets such as those based on 16SrRNA gene sequence is not appropriate because the targets are competing and the reaction will be displaced to the most abundant target thereby making detection of the less abundant ones more difficult[138].

2.28 Multiplex Nested PCR

This technology was first used by Chamberlain in the year 1998 for the diagnosis of Duchenne muscular dystrophy[139]. A Multiplex PCR System has been used for the Specific detection of Cylindrocarpon liriodendri, C. macrodidymum, and C. pauciseptatum from Grapevine at Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, and Spain[139]. Multiplex nested PCR method in a single tube combines the advantages of the multiplex PCR with the sensitivity and reliability of the nested PCR[139]. It saves time and reagent costs because two reactions are sequentially performed using a single reaction cocktail and it enables simultaneous detection of RNA and DNA targets[139]. The accurate design of compatible primers is necessary to avoid hairpins and primer-dimer formation[139]. Although there are some examples in which multiplex nested PCR has been used for detection of Phytoplasma, fungi and viruses in plant[139].

2.29 Real-time PCR

Conventional PCR has demonstrated its sensitivity and specificity under optimized and controlled conditions[140]. However, it does not provide information about the amount of the pathogen in the sample and users must employ agarose gel electrophoresis, hybridisation or colorimetric detection as the endpoint analysis[140]. On the contrary, real-time PCR allows the monitoring of the reaction while it is in course, thus avoiding the need to manipulate amplicons that implies high risk of contamination[140]. At the same time the method requires fewer reagents and less time and also allows additional studies to be performed during detection i.e quantification of original target population and detection of several variants of a pathogen or point mutations in a gene[141]. Among the different variants of PCR, real-time PCR represents a quantum leap and is a tool that has proven indispensable in a wide range of molecular biology protocols[141]. In the detection field this high throughput technique has improved the systems by achieving very accurate speed, specificity and reliability[141].

2.30 BIO-PCR

BIO-PCR assay is a modification of end-point PCR technique which involves a pre-assay incubation step in a diseased sample to increase the biomass of the causal agent [142]. This technique is mainly used to concentrate target pathogens by growing the target pathogen in a growing media that prevent the growth of non-target microorganisms to improve detection and has been effectively used to detect seed-borne fungal pathogen[142]. A seed and airborne lupin anthracnose disease caused by *Colletotrichum lupini* was diagnosed using the BIO-PCR method[142]. Incubation of the seeds with amended Yeast Malt Broth was done to enrich *C. lupine* biomass and a species-specific primer pair was developed based on rDNA IGS sequence[143]. The seed-borne fungal pathogens *Alternaria alternata, A. radicina, and A. dauci* were detected using specific primers of Internals Transcribed Spacer in rDNA with the help of a deep-freeze blotter method during the BIO-PCR[143]. High sensitivity, elimination of PCR inhibitors and detection of living cells to avoid false positives are the advantages over endpoint PCR techniques[143]. Limitations of this technique is that it is time consuming and costs incurred when selective media is used for the assay[143].

2.31 Quantitative PCR

Quantitative PCR (qPCR), permits detection and quantification of particular DNA or RNA sequences of phytopathogenic fungi in a PCR reaction mixture in real-time[144]. The relative number of copies of target DNA and RNA sequences can be estimated by projecting a cycle threshold value of the fungal samples using sequence-specific primers[144]. Fluorescent dyes such as SYBR Green I, Eva Green, Molecular Beacons or sequence-specific fluorescence-labeled reporter probes such as TaqMan are used to monitor the reaction during amplification steps[144]. The basic principle is that the fluorescent signal is proportional to the amount of amplicons produced in each cycle and can be generated by an intercalating dye or from the breakdown of a dye-labeled reporter probe during amplification[145]. An

emerging fungal pathogen *Ramularia collo-cygni* shows typical symptoms of small, brown spots on leaves, sheaths, and awns which made it difficult to accurately diagnose this disease using conventional techniques[146].

qPCR test was developed and submitted as the first report on molecular detection of *R. collo-cygni* in barley seed .and an aggressive and emerging British Verticillium longisporum that was diagnosed with a qPCR approach[146]. Also, qPCR was able to distinguish and quantify *Diaporthe helianthi and D. gulyae,* the fungal pathogens of Phomopsis stem canker in sunflower[147]. This technique is fast and very sensitive and can provide reliable information on pathogen load and high throughput quantification of target DNA in biological areas[147] However, qPCR needs a specialized instrument and cost of the instrument and probe can be very high[147.]

2.32 Magnetic-Capture Hybridization PCR

Magnetic-capture hybridization PCR (MCH-PCR) uses DNA isolation with a purification phase that contains hybridization with single stranded DNA (ssDNA) probe on magnetic beads followed by the PCR amplification of target DNA sequences[148].This PCR assay was chiefly established to deal with PCR inhibitors in plant extracts during DNA isolation steps[148]. The magnetic beads used are coated with a biotiny lated oligonucleotide that is specific to a DNA region of the pathogen of interest[148]. The hybridization of double stranded DNA (dsDNA) and magnetic beads allows for separation of the complex from inhibitors[148]. Designing of a capture probe used in MCH-PCR involves selection of oligonucleotide probe sequence from highly conserved regions of pathogens and the selected sequence can be examined in *silico f*or specificity using BLAST[149]. The 5' end of the probe is biotin-labeled to allow attachment with streptavidin-coated magnetic beads which would decrease the total detection time, increase PCR sensitivity and remove most of the inhibitors of the amplification reaction and excess of non- target DNA[149].

2.33 Post Amplification Techniques

DNA Microarray

Development of a DNA Microarray-Based Assay has been used for the detection of Sugar Beet Root Rot Pathogens[140]. A DNA microarray (DNA chip, gene chip, or biochip) is an assemblage of microscopic DNA spots attached to a solid surface usually glass in defined positions[150]. The microscopic spots consist of thousands of specific DNA probes that are used to hybridize a cDNA (target) sample[150]. Hybridized probecDNA systems can be detected and quantified using fluorophores, silver, or chemiluminescence-labeled targets to define the comparative abundance of transcripts in the sample[150]. Advancement of DNA microarray technology has led to high throughput and multiple detection of various phytopathogens including viruses, viroid, bacteria, and fungi[151].

A novel microarray assay (ArrayTube) using marker genes ITS, TEF-1a and 16SrDNA with well performing probes allowed the detection of various pathogens[151]. DNA microarrays can be produced batch wise on standard microscope slides in a rapid, easy, consistent, and cost-efficient way[151]. A major drawback of microarray is that it can only detect sequences that the array is designed to identify[151]. Microarray technique is used for the simultaneous detection of multiple pathogens infecting plants in a single reaction[151]. This method uses pathogen-specific oligos immobilized on a membrane or glass slide as probe[151]. The total RNA isolated from infected plant is converted into cDNA and amplified through PCR using pathogen-specific primers and labelled using suitable molecules for detection[151].

2.34 DNA or RNA Probe Based Assays

In Situ Hybridization

In situ hybridization (ISH) technique functions to detect mRNAs present in the fixed sample. Designing of an antisense ssRNA probe aimed to bind target mRNA (sequence of interest) is the main step of this assay[152]. However, synthetic oligonucleotide probes and cDNA probes can also be used typically because probes are labeled with the radioactive isotopes 35S, 125I, and 32P as they are very sensitive and easily quantified for detection[152]. Non-isotopic probes can use biotin, digoxigenin, tyramide, alkaline phosphatase, or bromodeoxyuridine for probe labeling as Photography, autoradiography with X-ray film, liquid emulsion, and microscopic techniques can be used in signal detection[152]. The rust fungi in their respective paraffin fixed plant tissues using the ISH technique has been detected and identified[152]. Main limitations of ISH are cost and hazardous nature of radioactive probes with the difficulty in identification when the target has low concentrations of DNA and RNA[152].

2.35 Next-Generation Sequencing

Next-generation sequencing (NGS) or high throughput sequencing (HTS) is a new approach for diagnostics[152]. The development of NGS technologies has fueled innovative ways for detection and Identification of phytopathogens[152]. Isolation and fragmentation of DNA, library preparation, massive parallel sequencing, bioinformatics analysis, and variant/mutation annotation and interpretation are the major steps involved in DNA based NGS[152]. Massively parallel signature sequencing, pyrosequencing, polony sequencing and

sequencing by oligonucleotide ligation detection (SOLID) are some commonly available advanced sequencing methods in HTS, RNA-Sequencing (RNA-Seq) offers advanced coverage and greater resolution of the dynamic nature of the transcriptome[152]. Illumina HiSeq platform is the most universally functional NGS platform for RNA-Seq and has established the standard for NGS[152]. The platform more recently released a desktop sequencer, MiSeq and RNA-Seq based NGS that can be used in the rapid identification of phytopathogens inducing novel diseases[152]. A whole-genome sequencing approach using Illumina MiSeq was established to detect the Sarcococca blight-causing novel fungal pathogen, Calonectria pseudonaviculata in ornamental plants[152]. Comparative genomics using RNA-Seq of close relative species of P. humuli identified seven specific regions in P. cubensis that allowed for the development of diagnostic markers and was also used on Monilinia fructicola, a brown rot disease caused by fungal pathogen[152]. The sequenced reference genomes can be used to study the genome biology and evolution with other species and the arrival of a novel pathogen is an instance where the target cannot be well-defined[158]. Since NGS involves no prior knowledge of pathogen sequences, the whole genome of the causal organism may be sequenced without using specific primer pairs and PCR amplification[152]. The major limitation in NGS is the time consumption incurred during assembly and analysis of large amounts of sequence data[153]. Next generation applications are often restricted by low RNA yield or integrity, RNA stability, and impurities with DNA, salts, or chemicals[153]. Bioinformatics are necessary for NGS analysis, even if the data can be easily and quickly obtained, knowledge about bioinformatics analyses are mandatory to avoid any misinterpretation[153].

2.36 Nucleic Acid Sequence Based Amplification (NASBA)

NASBA is an isothermal amplification method that can be used to detect RNA targets and the reaction requires the use of three enzymes[153]. The enzymes are AMV-RT for reverse

transcription and to obtain double stranded cDNA, RNase H to hydrolyze the RNA fragment of the hybrid molecule, DNA RNA and T7 RNA polymerase to produce a large amount of anti-sense, single strand RNA transcripts corresponding to the original RNA target[153]. It can be achieved by using two specific primers, one of them including at 5' end the T7 promoter, NTPs and also dNTPs but the entire NASBA process is performed at 41ºC for 60 min[153]. The detection of NASBA products can be assessed by chemi-luminescent or colorimetric detection using an internal specific probe digoxigenin labelled or in a real-time assay using molecular beacons (Amplidet RNA)[153]. NASBA-beacon assay offers the advantages that no contaminating DNA is amplified and it is performed at 41ºC without the need of a thermal-cycler[153]. This reaction affords high levels of sensitivity and superior in some cases to real-time PCR[153].

2.37 Fluorescence In-situ Hybridization

Another type of molecular detection technique is fluorescence *in-situ* hybridization (FISH), which is applied for bacterial detection in combination with microscopy and hybridization of DNA probes and target gene from plant samples[153]. Due to the presence of pathogen-specific ribosomal RNA (rRNA) sequences in plants, recognizing this specific information by FISH can help detect the pathogen responsible for infections in plants[153]. In addition to bacterial pathogens, FISH could also be used to detect fungi and viruses and other Endosymbiotic bacteria that can infect plant because its high affinity and specificity of DNA probes provide high single-cell sensitivity in FISH as the probe will bind to each of the ribosomes in the sample[153].

However, the practical limit of detection lies in the range of around 10^3 CFU/mL and in addition to the detection of culturable microorganisms that cause plant diseases, FISH could also be used to detect yet-to-be cultured (unculturable) organisms in order to investigate

complex microbial communities[164]. Some pitfalls compromise the technique's potency for plant disease detection like false positive results with auto fluorescence materials is a common problem that often lowers its specificity [165]. Accuracy and reliability of FISH is highly dependent on the specificity of the nucleotide probes, insufficient penetration and photo bleaching[153].

2.38 Enzyme Linked Immunosorbent Assay (ELISA)

The enzyme-linked Immunosorbent assay (ELISA) was first introduced in plant virology by Voller, Clarks and Adams as another method for identification of diseases based on antibodies and colour change in the assay[154]. In this method, the target epitopes (antigens) from the viruses, bacteria and fungi are made to specifically bind with antibodies conjugated to an enzyme and the detection can be visualized based on colour changes resulting from the interaction between the substrate and the immobilized enzyme[154]. The performance of ELISA can be improved greatly with the application of specific monoclonal and recombinant antibodies which are commercially available as the specific monoclonal antibodies have been used in ELISA to achieve lower limits of detection in the order of 10^5–10^6 CFU/mL for plant disease detection[154]. Tissue print-ELISA and lateral flow devices that enable detection have been fabricated for on-site detection, however, the sensitivity for bacteria is relatively low (10^5–10^6 CFU/mL) but ELISA technique detects tomato mosaic virus perfectly[154].

2.39 Loop-Mediated Isothermal Amplification (LAMP)

It is a powerful molecular diagnostic tool and innovative gene amplification technique that amplifies nucleic acid at a very rapid pace, maintaining high sensitivity, specificity and efficiency[155]. An attack by disease causing organisms generates a complex immune response in a plant and this may bring about the production of disease-specific proteins

involved in plant defence for detection[155]. Crop damage can be minimized if plant diseases are diagnosed with right tools designed to detect plant diseases early either by identifying the presence of the pathogen in the plant or the molecules (proteins) produced by either the pathogen or the plant during an infection as a response in order to protect the plant[155].

2.40 Random Amplified Polymorphic DNA (RAPD)

RAPD is based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence[156]. These primers detect polymorphisms in the absence of specific nucleotide sequence information and the polymorphisms function as genetic markers which can be used to construct genetic maps[156]. Since most of the RAPD markers are dominant, it is not possible to distinguish whether the amplified DNA segment is heterozygous (two different copies) or homozygous (two identical copies) at a particular locus[156]. In rare cases, co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus may be detected[156]. The basic technique of RAPD involves extraction of highly pure DNA, addition of single arbitrary primer, polymerase chain reaction (PCR), separation of fragments by gel electrophoresis, visualization of RAPD-PCR fragments after ethidium bromide staining under UV light and determination of fragment size comparing with known molecular marker with the help of gel analysis software[156]. It is important to note that RAPD technique requires maintaining strictly consistent reaction conditions in order to achieve reproducible profiles[156]. In practice, band profiles can be difficult to reproduce between (and even within) laboratories, if personnel, equipment or conditions are changed[156]. Despite these limitations, the enormous attraction of this technique is that there is no requirement for DNA probes or sequence information for primer design as the

procedure involves no blotting or hybridizing steps[156]. The technique is quick, simple and efficient and requires only the purchase of a thermocycling machine and agarose gel apparatus with relevant chemicals which are available as commercial kits (e.g., Ready-To-Go RAPD analysis beads; GE Healthcare, Buckinghamshire, UK)[156]. Another advantage is that it requires only small amounts of DNA (10–100 ng per reaction) [156].

Endnotes

1. P. Concetta, L. Margherita , R. De Prisco,, E. Coppola,, E. Grilli, C. Russo & M. Isidori, *Tomato Plants (Solanum Lycopersicum L.) Grown In Experimental Contaminated Soil: Bioconcentration of Potentially Toxic Elements and Free Radical Scavenging Evaluation*. 13 (10), 2020, 1371.

2. R. Bulgari, G. Franzoni & A. Ferranti, *Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions.* **Agronomy.** 306(9) 2019: 1–3.

3. A. Przybylska- Balcerek., J. Frankowski & K.Stuper- Szablewska. *The Influence of Weather Conditions on Bioactive Compound Content in Sorghum Grain.* **European. Food Research. Technology**. 246, 2020: 13–22.

4. C. Piscitelli , M. Lavorgna , M. Isidori , C. Russo, R. De Prisco & G.R. Abbamondi , *Antioxidant And Antiproliferative Activities Of Different Cultivars of Tomatoes (Lycopersicon Esculentum) On Tumoral Cell Lines.* **Journal of Advance Biology**. 2(10) 2017:2061–2072.

5. F. Rutigliano, R. Marzaioli, S. De Crescenzo & M. Trifuoggi, *Human Health Risk from Consumption of Two Common Crops Grown In Polluted Soils*. **Science Total Environment** 691 2019; 195–204.

6. A. Zwolak, M. Sarzyńska, E. Szpyrka & K. Stawarczyk, Sources *Of Soil Pollution By Heavy Metals And Their Accumulation In Vegetables: A Review.* **Water Air Soil Pollution**,; 230 2019:1–9

7. M. Lavorgna , E. Orlo., R. Nugnes , C. Piscitelli , C. Russo C. & Isidori M. *Capsaicin In Hot Chili Peppers: In Vitro Evaluation of Its Antiradical, Antiproliferative and Apoptotic Activities*. **Plant Foods and Human Nutrition**. 74(2) 2019: 164–170.

8. D.P. Godinho., H.C. Serrano, A.B. Da Silva, C. Branquinho & S. Magalhaes. *Effect of Cadmium Accumulation on the Performance of Plants and Of Herbivores that Cope Differently with Organic Defenses.* **Frontier Plant Science.** 9, 2018; 1–12.

9. G. Rao, S. Huang, U. Ashraf, Z. Mo, M. Duan & S. Pan, *Ultrasonic Seed Treatment Improved Cadmium (Cd) Tolerance in* Brassica Napus L. **Ecotoxicology Environmental Safe.** 185, 2019:1–6.

10. U. Ashraf, X. Tang, *Yield and Quality Responses, Plant Metabolism and Metal Distribution Pattern in Aromatic Rice under Lead (Pb) Toxicity*. **Chemosphere**, 176: 141–155. 2017

11. J. Trebolazabala, M. Maguregui, H. Morillas, Z. García-Fernandez, A.De Diego, J.M. Madariaga, *Uptake Of Metals By Tomato Plants (*Solanum Lycopersicum*) And Distribution Inside The Plant: Field Experiments In Biscay (Basque Country)*. **Journal of Food Composition Analysis**. 59, 161–169, 2017.

12. B. Magaji, F. Fai, F.F. Yirankiyuki & S.Y. Simon. *Accumulation of Heavy Metals By Vegetables Grown In Kembu Farms, Gombe, Nigeria.* **Irjpac.** 21(5) 2020: 1–5.

13. S. Raptis, D.Gasparatos, M. Economou-Eliopoulos, & A. Petridis, *Chromium Uptake by Lettuce as Affected by the Application of Organic Matter And Cr(Vi)-Irrigation Water: Implications to the Land Use and Water Management*. **Chemosphere.** 2018, 597–606.

14. H.A. Hashem, A.I. Shouman & R.A. Hassanein, *Physico–Biochemical Properties of Tomato (Solanum Lycopersicum) Grown In Heavy–Metal Contaminated Soil*. **Acta Agr**. 68(4), 2017, 334–341.

15. D. Kisa, *Expressions Of Glutathione-Related Genes And Activities of Their Corresponding Enzymes In Leaves of Tomato Exposed to Heavy Metal. Russ*. **Journal of Plant Physiology**. 64(6), 2017, 876–882.

16. H. Sbartai , I. Sbartai , M.R. Djebar & H. Berrebbah, *Phytoremediation of Contaminated Soils by Heavy Metals—"Case Tomato".* **Ishs Acta Hortic.** 2017; 1159: 95–100.

17. K. Elisabeth , S. Roberto , C.Gabriele & S. Wilfried , *Effect Of Tomato Variety, Cultivation, Climate And Processing on Sola L 4, An Allergen from Solanum Lycopersicum* 14 (10), 2018, 1371.

18. R. Massantini , E. Radicetti , M.T. Frangipane & E. Campiglia, *Quality of Tomato (Solanum Lycopersicum L.) Changes under Different Cover Crops, Soil Tillage and Nitrogen Fertilization Management.* **Agriculture.** 11(2) 2021:106.

19. E. Hermann, V. Lecomte & C. Current Status of Fusarium Oxysporum Formed Species and Races. **Phytopathology.** 109(4) 2019:512-530.

20. Common Diseases of Tomatoes/ Mississippi State University Extension; Https://Www. Plantvillage.Psu.Edu>Info.

21. M. Abadi."A Tomato Is Actually A Fruit — *But It's A Vegetable At the Same Time". Business* Insider. Retrieved 21, 2019.

22. D. A. Franco, J.F Arango, A. Hurtado-Salazar & N. Ceballos-Aguirre, *Development, Production, and Quality of "Chonto" Type Tomato Grafted on Cherry Tomato Introductions*. 65(2) 2018:150-157.

23. E.L. García-Enciso, A. Benavides-Mendoza, M.L. Flores-López, A. Robledo-Olivo, A. Juárez-Maldonado, & S. González-Morales. *A Molecular Vision of the Interaction of Tomato Plants and Fusarium Oxysporum Lycopersici*. 2017.

24. T. Gordon, *Fusarium Oxysporum and the Fusarium Wilt Syndrome. Annual Review* **Phytopathology**. 2017, 55:23-39.

25. Vegetable: Tomato, Pith Necrosis / Centre for Agriculture, Food, and Environment at Umass Amherst. Https//Ag.Umass.Edu>Facts-Sheets.

26. Collar Rot And Alternaria Stem Canker of Tomato/ Nc State Extension Publications, Https://Www.Contents.Ces.Nscu.Edu>Collar Rot and Alternaria Canker

27. How To Manage Tomato Anthracnose Symptoms- Gardening Know How, Https:Www.Gardening Know How Come.

28. . Li, J. Fokkens, L. Conneely, L.J. & Rep, M, *Partial Pathogenicity Chromosomes in Fusarium Oxysporum are Sufficient to Cause Disease and Can be Horizontally Transferred.* **Environmental Microbiology**. 22(12) 2020: 4985-500

29. . Li, J. Gao, M. Gabriel, D.W. Liang & W. L. Song, *Secretome-Wide Analysis Of Lysine Acetylation In Fusarium Oxysporum F. Sp. Lycopersici Provides Novel Insights Into Infection-Related Proteins.* **Frontiers in Microbiology** 2020b, 11:559440.

30. P. Murillo-Gómez, R. Hoyos, & P. *Chavarriaga, Organogenesis In-Vitro Using Three Tissue Types Of Tree Tomato* **Agronomía Colombiana.** 35(1) 2017: 5-11.

31. L. Murugan, N. Krishnan, V. Venkataravanappa, S. Saha, A.K. Mishra, B.K. Sharma, & A.B Rai, *Molecular Characterization and Race Identification of Fusarium Oxysporum F. Sp. Lycopersici Infecting Tomato In India. Biotechnology*. 10 (11) 2020.

32. S.N. Rampersad, *Pathogenomics and Management of Fusarium Diseases in Plants.* **Pathogens.** (340) 2020. 9:21.

33. Black Dot Root Rot- Seldom Seen Tomato Disease Found In High Tunnel/ University of Maryland Extension. Https://Www.Extension.Umd.Edu>Resources.

34. W, M. Rodríguez-Ortega, V. Martínez, M. Nieves, I. Simón, V. Lidón, J.C Fernández-Zapata, N.J.J Martínez & Z.J. F Cámara- Garciasánchez, *Agricultural and Physiological Responses of Tomato Plants Grown In Different Soilless Culture*

Systems With Saline Water Under Greenhouse Conditions. **Scientific Reports** 9(6733) 2019: 1-13.

35. D. Segorbe, A. Di Pietro, E. Pérez-Nadales & D. Turrà, *Three Fusarium Oxysporum Mitogenactivated Protein Kinases (Mapks) have Distinct and Complementary Roles In Stress Adaptation and Crosskingdom Pathogenicity.* **Molecular Plant Pathology**. 18(7) 2017: 912-924.

36. Buckeye Fruit Rot On Tomato /Vegetable Pathology-Long Island Horticultural Research And Extension Centre. Https://Www.Blogs.Cornell.Edu>Tomato.

37. Cercospora Leaf Mold- *Plant Pathology/Purdue.* University Vegetable Crops Hotline. Https://Www.Vegcropshotline.Org.

38. V.K Singh, H.B. Singh & R.S. Upadhyay, *Role of Fusaric Acid in the Development of 'Fusarium Wilt'symptoms in Tomato: Physiological, Biochemical and Proteomic Perspectives.* **Plant Physiology and Biochemistry**. 2017, 118:320-332.

39. .C. Srinivas, D. Nirmala, K. Narasimha Murthy, C.D. Mohan, T.R. Lakshmeesha, B.Singh, N.K. Kalagatur, S.R. Niranjana, A. Hashem, A.A. Alqarawi, B. Tabassum, E.F Abd_Allah, S. Chandra Nayaka,& R.K Srivastava*, .Fusarium Oxysporum F. Sp. Lycopersici Causal Agent of Vascular Wilt Disease of Tomato: Biology to Diversity– A Review.* **Saudi Journal of Biological Sciences**. 26(7) 2019:1315-1324.

40. L.V.Trong, L.Q. Tuong, B.B.Thinh, N.T Khoi, & V.T Trong, *Physiological And Biochemical Changesin Tomato Fruit (Solanum Lycopersicum L.) During Growth Ripening Cultivated In Vietnam.* **Bioscience Research.** 16(2) 2019:1736-1744.

41. H.C. Van Der Does, M.E. Constantin, P.M. Houterman, F.L.Takken, B.J Cornelissen, M.A Haring, H.A Van Der Burg, & M. Rep, *Fusarium Oxysporum Colonizes the Stem of Resistant Tomato Plants, the Extent Varying With The R-Gene Present.* **European Journal of Plant Pathology**. 2019. 154:55-65.

42. B.Wang, H. Yu, Y. Jia, Q. Dong, C. Steinberg, C. Alabouvette, V. Edel-Hermann, C. Kistler, K.Ye, L.J Ma & L. Guo, *Chromosome-Scale Genome Assembly of Fusarium Oxysporum Strain Fo47, A Fungal Endophyte and Biocontrol Agent.* **Molecular Plant Microbe Interaction**. 33(9) 2020:1108-1111.

43. G. Michelle & O. Angela, *Fruits and Vegetable Farming; University of Minnesota Extension* 2018.

44. S. Hyder, R. Gondal, S.T Ahmed, A. Sahi & A. Hannan. *First Report of Charcoal Rot in Tomato Caused by Macrophomina Phaseolina from Pakistan*. 2018.

45. A.L Testen, A. Chala, F. Azerefegne & S.A. Miller. *First Report of Corky Root Rot of Tomato Caused by Pathogen Pyrenochaeta Lycopersici in Africa* 2019.

46. F. Baysal- Gurel*, Pseudocercospora Fuligena (Black Leaf Mold). Invasive Species of Compendium, Wallingford,* 2020.

47. A. Lookabaugh, B. Thomas, B. Shew, S.C Butler, & Louws F.J ;, *First Report Of Black Leaf Mold of Tomato Caused By Pseudocercospora Fuligena In North Carolina,* **American Phytophathological Society**, 2017, 10.1094.

48. G. Kaashima, *Tomato Rot Diseases: Causes, Types, Treatment And Prevention*, (15)18 2021:31.

49. H. .Aksoy, Y. Kaya , M. Ozturk , Z. Secgin , H. Onder & A. Okumus*, Pseudomonas Putida Induced Response In Phenolic Profile of Tomato Seedlings (Solanum Lycopersicum L.) Infected by Clavibacter Michiganensis Subsp. Michiganensis*. **Biological Control**. 2017, 105:6–12.

50. K. Alexandria, *Wisconsin Horticulture Division of Extension, Madison Plant Pathology*, 2017, Xht1250.

51. A.L.Testen, A. Chala, F. Azerefegne & S.A Miller*, First Report of Corky Root Rot of Tomato Caused by Pathogen Pyrenochaeta Lycopersici In Africa.* 2019.

52. Z. Ning, G. Yang, Y. Pan, X.Yang, L. Chen, & C. Zhao. "*A Review of Advanced Technologies and Development for Hyperspectral-Based Plant Disease Detection in the Past Three Decades*." **Remote Sensing** 12, 19 (2020): 3188.

53. J.E. Holland, A.E Bennett, A.C Newton, P.J White, B.M Mckenzie, T.S George, R.J Pakeman, J.S Bailey, D.A Fornara, & R.C Hayes. "*Liming Impacts on Soils, Crops and Biodiversity in the United Kingdom:* A Review." **Science of the Total Environment**. 610, 2018, 316-332.

54. H. Maryam, D. Tabet, M. Sandroni, C. Benavent-Celma, J. Seematti, C. B. Andersen, & L. J Grenville-Briggs. "*The Hunt for Sustainable Biocontrol of Oomycete Plant Pathogens,* a *Case Study of Phytophthora Infestans*. **Fungal Biology Reviews**, 2021.

55. K. Bhimanagoud, R. Mahmood, S.N Nagesha, M.S Nagaraja, D.G Prashant, O.Z Kerima, A. Karosiya & M. Chavan. "Field Application of Bacillus Subtilis Isolates for Controlling Late Blight Disease of Potato Caused By Phytophthora Infestans." **Biocatalysis And Agricultural Biotechnology** (22), 2019, 101366

56. S Pavan, A Srinivasulu & K. R Babu. "Symptomology of Major Fungal Diseases on Tomato and Its Management." **Journal of Pharmacognosy and Phytochemistry** 7(6) 2018, 1817-1821.

57. P. Rautela, S. Gupta, C.S .Azad & R. P. Singh. "Diseases of Tomato Crops and Their Management." In Diseases Of Fruits And Vegetable Crops, Apple Academic Press, 181-209: 2020.

58. C. S. Azad, P. Rautela, S. Gupta & R.P Singh, "Major Diseases of Chili and Their Management." In Diseases Of Fruits And Vegetable Crops, Apple Academic Press, 353-377: 2020.

59. .L. D. Ray. D. Ray, Langham, & A. Kimberly "*Fungi, Oomycetes, Bacteria, and Viruses Associated With Sesame (Sesamum Indicum L.*). Cochran December 2021." (2021).

60. Q. Kang, Q. Liu, Y. Huang, Y. Xia & S. Zhang. *"Management Of Bacterial Spot of Tomato Caused By Copper-Resistant Xanthomonas Perforans Using A Small Molecule Compound Carvacrol." Crop Protection* 132, (2020): 105114.

61. .B. Eduardo. "Development of Tomato (S. Lycopersicum) Lines with Resistance to Xanthomonas Spp. and Use of Genetic Resources to Characterize Infection and Diversity in Pathogen Populations." The Ohio State University, 2020.

62. B. Rishi. "Exploring the Drivers of Xanthomonas Population Dynamics on Tomato and Pepper." (2019).

63. S.K. Gupta and M. Gupta. "*Diseases of Vegetables under Protected Cultivation Conditions." Plant Disease Research*. 33, No. 1 (2018): 1-14.

64. Y. Wang, Y.X. Zhang, G. Zhipeng and Y. Wencai. "*Breeding for Resistance to Tomato Bacterial Diseases in China: Challenges and Prospects*." Horticultural Plant Journal 4(5) 2018, 193-207.

65. M. Sandra, Z.Gonzaga, G. Rodgers, A.Goldwater, L.Borines, R. Gerona, M. Neil Seriño, M. Labonite, N. Gonzaga & Ms V. Justo. *"Project Integrated Crop Management (Icm) to Enhance Vegetable Profitability and Food Security In the Southern Philippines and Australia." 2019.*

66. P. Leonel, K. Bahcevandziev & N. H Joshi. *Seaweeds as Plant Fertilizer, Agricultural Biostimulants and Animal Fodde*r: Crc Press, 2019.

67. V. Devappa, T.C Archith, A. Bhattacharyya, B.N. Chakraborty, R.N. Pandey, & D. Singh, "*Wilt Diseases of Ornamental Crops and their Management." Wilt Diseases of Crops; Dubey, Science, Edition:* 2021, 141-164.

68. M. Sabrine, H. Jabnoun-Khiareddine, B. Nasraoui & M. Daami-Remadi. "*Biocontrol Of Pythium Damping-Off on Pepper (Capsicum Annuum) With Selected Fungal and Rhizobacterial Agents."* **International Journal of Phytopathology** 9(1) .2020, 29-42.

69. B. Ozgur, T. A. Turini, M. Lestrange, S. Stoddard, G. Miyao, B. J. Aegerter, L.F. Chen, N. Mcroberts, D. E. Ullman, & R. L. Gilbertson. "*Development of An Ipm Strategy for Thrips and Tomato Spotted wilt Virus in Processing Tomatoes in the Central Valley of California."* **Pathogens**. 9(8). (2020), 636.

70. .N. Claudia, M. Noorlander & M. A. Hubbell. "*Tomato Spotted Wilt Virus of Tomato and Pepper." (2019).*

71. .S.R. Stuart, G. Yulin , K. William Dj, M. S. Hoddle, K. A. Leiss & J. E. Funderburk, "*Invasion Biology, Ecology, and Management of Western Flower Thrip*s." **Annual Review of Entomology** 65, 2020 17-37.

72. D. Bhabesh & A. Babu. *"Chapter-3 Thrips (Scirtothrips Dorsalis, Hood): Vectors of Tospoviruses in Agricultural Crops*." **Essentials of Science,** 35.2020.

73. A.O. Ogunsiji, T. O Ibrahim & F.A Odusanya. *"Management Strategies of Forest Plant Diseases: A Review."* **International Journal of Plant & Soil Science**, 2020, 87-95.

74. H. Djangsou, E.Francia, D. Ronga & B. Matteo, *"Blossom End-Rot In Tomato (Solanum Lycopersicum L.): A Multi-Disciplinary Overview Of Inducing Factors and Control Strategies*." **Scientia Horticulturae**, 2019, 49-58.

75. R.F. Nicholas & E. J. Mitcham. *"Validation and Demonstration of A Pericarp Disc System for Studying Blossom-End Rot Of Tomatoes*." **Plant Methods.** 17(1), 2021 1-10.

76. .H.E.Wenshu, C. Baysal, M. L. Gómez, X. Huang, D. Alvarez, C. Zhu, V.Armario-Najera, A.B. Perera, P.C. Bennaser & A. Saba-Mayoral. "*Contributions Of The International Plant Science Community to the Fight Against Infectious Diseases In Humans—Part 2: Affordable Drugs In Edible Plants For Endemic And Re-Emerging Diseases*." **Plant Biotechnology Journal**. 19(10), 2021, 1921-1936.

77. .Martini, Marta, D. Delić, L. Liefting, & H. Montano. *"Phytoplasmas Infecting Vegetable, Pulse and Oil Crops." In Phytoplasmas: Plant Pathogenic Bacteria-1*, **Springer,** 2018, 31-65.

78. .Satta, Eleonora, S. Paltrinieri, & A. Bertaccini, *"Phytoplasma Transmission by Seed." In Phytoplasmas: Plant Pathogenic Bacteria*-Ii**, Springer**. 2019, 131-147.

79. S. Shenghui, R. Zhou, Lyahao , Bo Liu, P. Guoxiang , Q. Liu, Q. Xiong, X.Wang, X.Xia, & J. Tu. *"Bacterium, Fungus, and Virus Microorganisms for Energy Storage and Conversion."* **Small Methods**. 3 (12), 2019. 1900596.

80. M. Dipanwita. *"Application of Microbes in Synthesis of Electrode Materials for Supercapacitors." In Application of Microbes In Environmental and Microbial Biotechnology,* 39-92: **Springer,** 2022.

81. C. Shaoqing, P. Ling, H.Zhu, & H. M. Keener. *"Plant Pest Detection Using an Artificial Nose System: A Review."* **Sensors** 18(2) 2018, 378.

82. .O. Kenneth, I. Ekong, I. Okon Markson & K. Enwere. *"Fingerprint Biometric System Hygiene and the Risk of Covid-19 Transmission."* **Jmir Biomedical Engineering** 5, (1) 2020, 19623.

83. Khakimov, A, I Salakhutdinov, A Omolikov & S Utaganov. *"Traditional and Current-Prospective Methods of Agricultural Plant Diseases Detection: A Review." In Iop Conference Series*: **Earth and Environmental Science**, 2022. 951, 012002.

84. .Luchi, Nicola, Renaud Ioos & Alberto Santini. "Fast and Reliable Molecular Methods to Detect Fungal Pathogens in Woody Plants." **Applied Microbiology and Biotechnology,** 104 (6) (2020): 2453-2468.

85. Kumar, Harsh. "*Biotechnology: Discoveries and Their Applications In Societal Welfare." Biotechnology Business-Concept to Delivery*. **Springer**, 2020, 3-44.

86. G. Riveron, T.Javier & G.Aquino-Jarquin. *"Crispr/Cas13-Based Approaches for Ultrasensitive and Specific Detection of Micrornas*." **Cells.** 10 (7) 2021), 1655.

87. R. Domenico, D.L.Daniele, A. Panattoni, C.Salemi, G. Cappellini, L. Bartolini & G. Parrella, "*Rapid and Sensitive Detection of Tomato Brown Rugose Fruit Virus in Tomato and Pepper Seeds by Reverse Transcription Loop-Mediated Isothermal Amplification Assays (Real Time and Visual) and Comparison with Rt-Pcr End-Point and Rt-Qpcr Methods."* **Frontiers in Microbiology**. 12, 2021.

88. D.Zaizai, C.Tang, Z.Zhang, W. Zhou, R. Zhao, L. Wang, X. Jiachao, W. Yayun , W. Jiang & X. Zhang. *"Simultaneous Detection of Exosomal Membrane Protein and Rna by Highly Sensitive Aptamer Assisted Multiplex–Pcr*." **Acs Applied Biology Materials**. 3(5) 2019, 2560-2567.

89. P. Rajesh, E. Ostermann & W.Qingshan, *"Advances In Point-Of-Care Nucleic Acid Extraction Technologies for Rapid Diagnosis of Human and Plant Diseases***." Biosensors and Bioelectronics** 169, 2020, 112592.

90. N. Trieu, V. C. Aaydha, S. Z. Andreasen, M. Golabi, Q. T. Linh, D. D. Bang & A.Wolff. *"Point-Of-Care Devices for Pathogen Detections: The Three Most Important Factors to Realize Towards Commercialization."* **Trac Trends in Analytical Chemistry**, 2020.

91. P. Stefano, S. Matić, A.Tiberini, A. G.Caruso, P. Bella, L.Torta, R. Stassi, & S. Davino. *"Loop Mediated Isothermal Amplification: Principles and Applications in Plant Virology*." **Plants**. 9(4), 2020, 461.

92. K.Jong-Tar, L.L Chang, C.Y. Yen, T.H. Tsai, Y.C. Chang, Y.T. Huang, & Y.C. Chung. *"Development of Fluorescence in Situ Hybridization as a Rapid, Accurate Method for Detecting Coliforms in Water Samples."* **Biosensors**. 11(1) 2021.

93. R.A.Kannan, R, A Solaimalai, M Jayakumar, & U Surendran. "*Advance Molecular Tools to Detect Plant Pathogens." In Biopesticides***, Elsevier**, 2022. 401-416.

94. G. Yuan, Y. Zhou, & R.Chandrawati. "Metal and Metal Oxide Nanoparticles to Enhance the Performance of Enzyme-Linked Immunosorbent Assay (Elisa)." **Acs Applied Nano Materials**. 3(1), 2019, 1-21.

95. P.Riikka, R. Barderas, E. Benito-Peña, & M. C Moreno-Bondi. *"Recombinant Antibodies and their Use for Food Immunoanalysis."* **Analytical and Bioanalytical Chemistry**, 2021, 1-25.

96. P. Concetta , L. Margherita , R. De Prisco,, E. Coppola,, E. Grilli, C. Russo & M. Isidori, *Tomato Plants (Solanum Lycopersicum L.) Grown in Experimental Contaminated Soil: Bioconcentration Of Potentially Toxic Elements And Free Radical Scavenging Evaluation*. 13 (10), 2020, 1371.

97. R. Bulgari, G. Franzoni & A. Ferranti, *Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions.* **Agronomy.** 306(9) 2019: 1–3.

98. A. Przybylska- Balcerek., J. Frankowski & K.Stuper- Szablewska. *The Influence of Weather Conditions on Bioactive Compound Content in Sorghum Grain.* **European. Food Research. Technology**. 2020; 246: 13–22.

99. C. Piscitelli , M. Lavorgna , M. Isidori , C. Russo, R. De Prisco & G.R. Abbamondi , *Antioxidant and Antiproliferative Activities of Different Cultivars of Tomatoes (Lycopersicon Esculentum) on Tumoral Cell Lines.* **Journal of Advance Biology**. 2(10), 2017:2061–2072.

.

100. F. Rutigliano, R. Marzaioli, S. De Crescenzo & M. Trifuoggi, *Human Health Risk from Consumption of Two Common Crops Grown In Polluted Soils*. **Science Total Environment** 2019; 691: 195–204.

101. A. Zwolak , M. Sarzyńska , E. Szpyrka & K. Stawarczyk, *Sources Of Soil Pollution by Heavy Metals and their Accumulation In Vegetables: A Review.* **Water Air Soil Pollution**, 2019; 230:1

102. M. Lavorgna , E. Orlo., R. Nugnes , C. Piscitelli , C. Russo C.& Isidori M. *Capsaicin in Hot Chili Peppers: In Vitro Evaluation of Its Antiradical, Antiproliferative and Apoptotic Activities*. **Plant Foods and Human Nutrition**. 74(2) 2019: 164–170.

103. D.P. Godinho., H.C. Serrano, A.B. Da Silva & C. Branquinho, S. Magalhaes. *Effect of Cadmium Accumulation on the Performance of Plants and of Herbivores that Cope Differently with Organic Defenses.* **Frontier Plant Science.** 2018; 9: 1–12.

104. G. Rao, S. Huang, U. Ashraf, Z. Mo, M. Duan & S. Pan, *Ultrasonic Seed Treatment Improved Cadmium (Cd) Tolerance in Brassica Napus L.* **Ecotoxicology Environmental Safe.** 2019; 185:1–6.

105. U. Ashraf & X. Tang, *Yield and Quality Responses, Plant Metabolism and Metal Distribution Pattern in Aromatic Rice under Lead (Pb) Toxicity*. **Chemosphere.** 2017, 176: 141–155.

106. J. Trebolazabala, M. Maguregui, H. Morillas, Z. García-Fernandez, A.De Diego & J.M. Madariaga, *Uptake of Metals by Tomato Plants (Solanum Lycopersicum) and Distribution Inside the Plant: Field Experiments In Biscay (Basque Country).* **Journal of Food Composition Analysis**. 59, 2017, 161–169.

107. B. Magaji, F. Fai, F.F. Yirankiyuki & S.Y. Simon. *Accumulation of Heavy Metals by Vegetables Grown in Kembu Farms, Gombe, Nigeria.* **Irjpac.**21(5) 2020: 1–5.

108. S. Raptis, D.Gasparatos, M. Economou-Eliopoulos & A. Petridis, Chromium *Uptake by Lettuce as Affected by the Application of Organic Matter and Cr(Vi)-Irrigation Water: Implications to the Land Use and Water Management*. **Chemosphere.** 210, 2018, 597–606.

109. H.A. Hashem, A.I. Shouman & R.A. Hassanein, *Physico–Biochemical Properties of Tomato (Solanum Lycopersicum) Grown In Heavy–Metal Contaminated Soil*. **Acta Agr**. 68(4), 2017, 334–341.

110. D. Kisa, *Expressions Of Glutathione-Related Genes And Activities of their Corresponding Enzymes In Leaves of Tomato Exposed to Heavy Metal. Russ*. **Journal of Plant Physiology**. 64(6), 2017, 876–882.

111. K. Elisabeth , S. Roberto , C.Gabriele & S. Wilfried , *Effect of Tomato Variety, Cultivation, Climate And Processing on Sola L 4, An Allergen from Solanum Lycopersicum* 14 (10), 2018, 1371.

112. R. Massantini , E. Radicetti , M.T. Frangipane & E. Campiglia, *Quality of Tomato (Solanum Lycopersicum L.) Changes under Different Cover Crops, Soil Tillage and Nitrogen Fertilization Management.* ***Agriculture.*** 11(2) 2021:106.

113. E. Hermann & V. Lecomte, C. *Current Status of Fusarium Oxysporum Formed Species and Races.* **Phytopathology**. 109(4) 2019:512-530.

114. M. Abadi."A Tomato Is Actually A Fruit — But It's A Vegetable At the Same Time". Business **Insider**. Retrieved 21, 2019.

115. D. A. Franco, J.F Arango, A. Hurtado-Salazar & N. Ceballos-Aguirre, *Development, Production, and Quality of "Chonto" Type Tomato Grafted on Cherry Tomato Introductions.* 65(2) 2018:150-157.

116. E.L. García-Enciso, A. Benavides-Mendoza, M.L. Flores-López, A. Robledo-Olivo, A. Juárez-Maldonado & S. González-Morales. *A Molecular Vision of the Interaction of Tomato Plants and Fusarium Oxysporum Lycopersici*. 2017.

117. L. Kator, A.C Iheanacho, K.P Aloho: *Isolation, Identification and Pathogenicity of Fungal Organisms Causing Postharvest Spoilage of Tomato Fruits During Storage* ***Annual Research & Review In Biology*****,** 26(6), 2018, 1-7

118. A. Sagar, *Potato Dextrose Agar, Principle, Uses, Composition, Procedure and Colony Characteristics*.2019.

119. S. Aslam, T. Aisha, M. F. Aslam, W.A. Muhammad , A. A. Shedayi, & S. Sadia, *Recent Advances in Molecular Techniques for the Identification Of Phytopathogenic Fungi – A Mini Review,* **Journal of Plant Interactions**, 12(1). 2017,493-504.

120. S.B.Marthur, & O. Kongsdal, "*Common Laboratory Seed Health Testing Methods for Detecting Fungi, Danish Government Institute of Seed and Pathology for Developing Countries, Copenhagen,* 2003.

121. G. Hariharan, K. Prasannath, *Recent Advances in Molecular Diagnostics of Fungal Plant Pathogens: A Mini Review*.2021.

122. B. Aslam, M. Basit, A. M. Nisar, & M. Khursid, *Proteomics: Technology and their Applications*.2017.

123. Nizamani, S, A.A. Khaskheli, A.M. Jiskani, S.A. Khaskheli, A.J. Khaskheli, G.B. Poussio, H. Jamro, M.I. & Khaskheli: *Isolation and Identification of the Fungi Causing Tomato Fruit Rot Disease in the Vicinity of Tandojam, Sindh*: **Agricultural Science Digest**. 41: 2021, 186-190.Doi: 10.18805/Ag.D-269

124. Hussain A, S. Wali Khan, S. Qasim, F.Faiz & A. Ali, *Geostatistical Analysis of Tomato Fruits Rot and Diversity of Associated Fungal Species*, **Journal Of Animal And Plant Sciences**. 31(4), 2020.

125. Ávila M.K. & Romero H.M., *Plant Responses to Pathogen Attack: Molecular Basis of Qualitative Resistance*. **Review Facultad Nacional De Agronomía**. 70(2) 2017, 8225-8235

126. Franco D. A., Arango J.F, Hurtado-Salazar A & Ceballos-Aguirre N., *Development, Production, and Quality of "Chonto" Type Tomato Grafted on Cherry Tomato Introductions.* 2018, 65(2):150-157.

127. . S Pavan Kumar S, A Srinivasulu & K Raja Babu, *Symptomology of Major Fungal Diseases on Tomato and Its Management*: **Journal of Pharmacognosy and Phytochemistry**; 7(6): 2018, 1817-1821.

128. Holland, Je, Ae Bennett, Ac Newton, Pj White, Bm Mckenzie, Ts George, Rj Pakeman, Js Bailey, Da Fornara & Rc Hayes. "*Liming Impacts on Soils, Crops and Biodiversity in the Uk: A Review."* **Science of The Total Environment**. 610, (2018): 316-332.

129. Christian J S, Casper Van Den A, Isabel Ortega- Salazar, Victor P, Jaclyn A A, Duoduo W, Clare L. C, Graham B .S, & Barbara B.U, *Host Susceptibility Factors Render Ripe Tomato Fruit Vulnerable to Fungal Disease Despite. Active Immune Responses.* **Journal of Experimental Botany,** 72(7), 2021, 2696-2709

130. Evelyn E &Villanueva G. *An Overview of Recent Studies of Tomato (Solanum Lycopersicum Spp) from a Social, Biochemical and Genetic Perspective on Quality Parameters*, Alnarp-**Sweden: Sveriges, Lantbruksuniversitet.** 3, 2018.

131. Elham A. K, Sajeewa S.N. Maharachchikumbura, Velazhahan R, Hamed Al-M & Abdullah M.A.S. *Fungi Diversity in Tomato Rhizosphere Soil Under Conventional and Desert Farming Systems*. **Frontires In Microbiology**, 2017.01462.

132. Tarsicio M., Gabriel A.F, & Jorge G.D, *,Origin And Evolution of Tomato Production Lycopersicon Esculentum In México,* Ciencia Rural,47(3),2017

133. Tinde Van A, Rutger A.V, Ewout M & Anastasia S, *Sixteenth-Century Tomatoes In Europe: Who Saw them, What they Looked Like and Where they Came from.* **Journal for Life and Environment,10,** 2017, E12790

134. Britannica, *The Editors of Encyclopaedia. "Tomato". Encyclopedia Britannica*, 6 Sep. 2022, Https://Www.Britannica.Com/Plant/Tomato.

135. A. Sagar, *Potato Dextrose Agar, Principle, Uses, Composition, Procedure and Colony Characteristics*.2019.

136. Kator L, A.C Iheanacho, K.P Aloho: *Isolation, Identification and Pathogenicity of Fungal Organisms Causing Postharvest Spoilage of Tomato Fruits During Storage* **Annual Research & Review In Biology,** 26(6), 2018, 1-7.

137. Fawole M.O & Oso B.A. Laboratory Manual of Microbiology. Ibadan: Spectrum Books Limited.1998, 26-31.

138. Jaykaran C & Tamoghna B, *How to Calculate Sample Size for Different Study Design in Medical Research,* **Indian Journal of Psychiatric Society South Zonal** Branch: 35(2):2013, 121-126.

139. Leander D. M, Agyemang D, Samuel K. Offei, K.O, Eric Danquah & Micheal O, *Review on Tomato (Solanum Lycopersicum,L) Improvement Programmes In Ghana,* 2019. Doi: 10.5772/Intechopen

140. Martina S, Marta R, Laura G, Giada D' E & Sheridan L.W. *Endophytic Fungi of Tomato and Potential Application for Crop Improvement,* **Agriculture,** 10(12), 2020, 587.

141. Muhammad S.M, Shabeer H, Abdul Rehman, Shafiq U.R, Muhammad J & Muhammad A, *Prevalence of Fungi In Fresh Tomatoes and their Control by Chitosan and Sweet Orange (Citrus Sinensis) Peel Essential Oil Coating,* **Journal of Basic Microbiology**, 62, 2022: 48-62.

142. Rabab S & Lorenzo B, *Fungal Diseases on Tomato Plant Under Greenhouse Condition,* **European Journal of Biological Research Review**, 2017, 2449-8955, Article Issn.

143. Nizamani, S, A.A. Khaskheli, A.M. Jiskani, S.A. Khaskheli, A.J. Khaskheli, G.B. Poussio, H. Jamro, M.I. & Khaskheli: *Isolation and Identification of the Fungi Causing Tomato Fruit Rot Disease In the Vicinity of Tandojam, Sindh*: **Agricultural Science Digest**. 41: 2021, 186-190.Doi: 10.18805/Ag.D-269

144. Hussain A, S. Wali Khan, S. Qasim, F.Faiz & A. Ali, *Geostatistical Analysis of Tomato Fruits Rot and Diversity of Associated Fungal Species*, **Journal Of Animal and Plant Sciences**. 31(4), 2020.

145. Bernal & Eduardo. "*Development Of Tomato (S. Lycopersicum) Lines With Resistance To Xanthomonas Spp. And Use Of Genetic Resources To Characterize Infection And Diversity In Pathogen Populations*." **The Ohio State University**, 2020.

146. Bhimanagoud K., Mahmood R., Nagesha S.N, Nagaraja M.S, Prashant D.G, Kerima O.Z, Karosiya A. & Chavan M.. *"Field Application of Bacillus Subtilis Isolates for Controlling Late Blight Disease of Potato Caused by Phytophthora Infestans."* Biocatalysis and **Agricultural Biotechnology** (22), 2019, 101366

147. Bisegna O, C. P., Swami N.S & Caselli F.. *"Single-Cell Microfluidic Impedance Cytometry: from Raw Signals to Cell Phenotypes Using Data Analytics*." **Lab On Achip** . 21(1), 2021, 22-54.

148. Botero V., Hoyos-Carvajal L. & Marín J., *Detection Of Asymptomatic Plants Of Solanum Lycopersicum L. Infected with Fusarium Oxysporum Using Vis Reflectance Spectroscopy.* **Ciencias Hortícolas** 12(2), 2018, 436-446.

149. Brahimi, M., Arsenovic, M., Laraba, S., Sladojevic, S., Boukhalfa & K., Moussaoui, A "Deep Learning for Plant Diseases: Detection and Saliency Map Visualisation," In *Human and Machine Learning.* Eds. Zhou, J., Chen, F. (Cham, Switzerland: **Springer International Publishing),** 2018, 93–117.

150. Carmona S.L., D. Burbano-David, Gómez M., López W., Ceballos N., Castaño-Zapata J., Simbaqueba J. & Soto-Suárez M., *Characterization of Pathogenic and Non-Pathogenic Fusarium Oxysporum Isolates Associated with Commercial Tomato Crops in the Andean Region of Colombia.* **Pathogens**. 9(70), 2020. 1-23.

151. Raptis S., Gasparatos D. Economou-Eliopoulos, M. & Petridis A., *Chromium Uptake by Lettuce as Affected by the Application of Organic Matter and Cr(Vi)-Irrigation Water: Implications to the Land Use and Water Management*. **Chemosphere**. 210, 2018, 597–606.

152. Ray L. D.. Ray D., Langham & Kimberly A. *"Fungi, Oomycetes, Bacteria, and Viruses Associated with Sesame (Sesamum Indicum L.).* **Cochran** ,2021.

153. Redmon, J & Farhadi, A. Yolo9000: Better, Faster, Stronger. *Ieee Conference on Computer Vision and Pattern Recognition.* 2017, 6517–6525.

154. Reitz, Nicholas F & Elizabeth J Mitcham. "*Validation and Demonstration of A Pericarp Disc System for Studying Blossom-End Rot of Tomatoes*." **Plant Methods**. 17(1), 2021, 1-10.

155. Reitz, Stuart R, Yulin Gao, William Dj Kirk, Mark S Hoddle, Kirsten A Leiss & Joe E Funderburk. *"Invasion Biology, Ecology, and Management of Western Flower Thrips."* **Annual Review Of Entomology**. 65, 2020: 17-37.

156. Ren S., He K., Girshick R., & Sun J., "*Faster R-Cnn: Towards Real-Time Object Detection with Region Proposal Networks,"Ieee Transactions on Pattern Analysis and Machine Intelligence,* 39(4), 2017, 1137–1149.

157. Riikka P., Barderas R., Benito-Peña E. & Moreno-Bondi M. C. "*Recombinant Antibodies and their use for Food Immunoanalysis*." **Analytical And Bioanalytical Chemistry**, 2021, 1-25.

158. Rishi B.. "*Exploring The Drivers of Xanthomonas Population Dynamics on Tomato and Pepper*." (2019).

159. Riveron G., Javier T. & Aquino-Jarquin G.. "*Crispr/Cas13-Based Approaches for Ultrasensitive .and Specific Detection Of Micrornas.*"**Cells**. 10(7) 2021, 1655.

160. Rodríguez-Ortega W.M., Martínez V., Nieves M., Simón I., Lidón V., Fernández-Zapata J.C., Martínez N.J.J.,Cámara-Z J. & Garciasánchez F., *Agricultural and Physiological Responses of Tomato Plants Grown in Different Soil Less Culture Systems with Saline Water Under Greenhouse Conditions.* **Scientific Reports.** 9(6733): 2019, 1-13.

161. Sandra M., Gonzaga Z., Rodgers G., Goldwater A., Borines L., Gerona R., Neil Seriño M., Labonite M., Gonzaga N., & Ms V. Justo. "*Project Integrated Crop Management (Icm) to Enhance Vegetable Profitability and Food Security in the Southern Philippines and Australia*." 2019.

Chapter Three

Methodology

3.1 Sample Collection

The samples were bought and gathered from four different markets of Oyo and Osun states with two markets selected from each state. The four markets are Eleekara and Sasa from Oyo state with Oluwoo and Obada markets from Osun state.

1. Eleekara market, Oyo town (Oyo state) denoted as Market A (A1 and A2 for Cherry and Royal varieties respectively).

2. Sasa market, Ibadan (Oyo state) denoted as Market B (B1 and B2 for Cherry and Royal varieties respectively)

3. Obada market, Ikire (Osun state) denoted as Market C (C1 and C2 for Cherry and Royal varieties respectively)

4. Oluwoo market, Iwo (Osun state) denoted as Market D (D1 and D2 for Cherry and Royal varieties respectively)

3.2. Sample Size Calculation

Sample Size (N) = $\frac{t^2 \times p(1-p)}{m^2}$

N = required sample size,

t = represents confidence level at 95% (standard value of 1.96).

P = represents 2.7% average incidence of fungi tomato rot in previous studies in Nigeria

m = 5 % (standard value of 0.05) margin of error [2]

$$N = \frac{(1.96)^2 \times 0.027(1 - 0.027)}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.027(0.973)}{0.0025}$$

Total = 40.36 Approximately 40.

3.3 Sampling Method Analysis

A total number of 32 *Solanum lycopersicum* fruits samples of two different varieties (Cherry and Royal Varieties) with obvious sign and symptom of rot from different sellers of the four vegetable markets and 8 apparent clean, fresh fruits of *Solanum lycopersicum* fruits samples were used as test and control samples respectively[1]. The samples were cultured using direct plate culture method and the isolated fungi undergone deoxyribonucleic acid (DNA) extraction for further molecular analysis technique to unfold their molecular characterization[1.] The isolated fungi genomic DNA (gDNA) were identified to the species level using Polymerase chain reaction with the appropriate primer designed in forward and reverse forms, Sanger sequencing and Basic Local Alignment Search Tool (BLAST) was used on National Centre for Biotechnology Information (NCBI) data base [2].

Table 3.1 Experimental Design

Markets	Cherry variety	Royal Variety	Control
Market A	(A_1- A_4) four samples	A_2 ($A2_1$-$A2_4$) four samples	two samples
Market B	(B_1-B_4) four samples	B_2 ($B2_1$-$B2_4$) four samples	two samples
Market C	(C_1-C_4) four samples	C_2 ($C2_1$-$C2_4$) four samples	two samples
Market D	(D_1-D_4) four samples	D_2 ($D2_1$-$D2_4$) four samples	two samples
Total = 40	16 samples	16 samples	8 samples

3.4 Study Site

The direct culture method of this study was carried out at the department of microbiology laboratory, Lead City University, Ibadan, Oyo state and the Molecular Analysis of grown organisms was carried out at Inqaba Biotec, Africa Genomics Company, Opposite International Institute of Tropical Agriculture (IITA), Moniya, Ibadan, Oyo State, Nigeria..

3.5 Sterilization of Samples, Glass wares and other Materials

Solanum lycopersicum fruit samples with sign and symptom of rot were sterilized with 3 % sodium hypochlorite and 70 % ethanol, followed by repeated washings with sterile distilled water. Scalpels and inoculating needles used to cut the rotting parts of tomato fruits were sterilized by dipping them into 70% ethanol and passing them over a spirit lamp flame until red hot before using it to culture. The glass wares were soaked in soapy water, washed, Rinsed under running tap water and sterile distilled water. They were drained and dried in an oven at 65^0C before and after use.

3.6 Preparation of Potato Dextrose Agar Media

Thirty – eight point nine grams of Potato dextrose agar was dissolve in 1litre of distilled water according to manufacturer's manual. But the right amount of the agar was measured and dissolve in distilled water according to the volume needed to go round for the experiment. The constituted mixture was heated till boiling in a water bath at 100^0C to have homogenized mixture. Then, the dissolved media mixture was Autoclaved at 121^0C and 15psi for 15minutes. The media mixture was allowed to cool down and streptomycin (10ml of constituted streptomycin to 1000ml of Agar) was added to inhibit the growth of bacteria since Phytopathogenic fungi from tomato fruits rot are the organism of interest. Then, the media was poured into Petri-dishes and was allowed to set or solidified at room temperature before sample were inoculated.

3.7 Isolation of Fungi Pathogen from *Solanum lycopersicum* Fruits Samples

Small sizes of *Solanum lycopersicum* samples with rot signs and symptoms segments were cut with sterilized forceps or cutting tools from the edges in order to isolate the causative fungal organism and inoculated into the already solidified culture media. The sample's media was tapped and covered with foil paper to avoid contamination[3]. They were kept at 28^0C for 7days at Department of Microbiology Laboratory, Lead City University, Ibadan.

3.8 Sub- culture of Isolate to Produce Pure Culture

Isolates from first growth was sub -cultured onto new Potato dextrose agar plates and each culture was inoculated onto potato dextrose broth (PDB) McCartney bottles and incubated for 10 days at 28^oC. The new pure isolates were taken for molecular identification and characterization[3].

3.9 Pathogenicity Test for the Fungi Isolated

Six apparently clean, fresh healthy tomato fruits were properly washed under running tap water to removes dirt and debris, disinfected with 70% alcohol and then rinsed repeatedly with distilled water[3]. Then already Sterilized 6 hole borers were used to create about 2mm

diameter holes in each of the 6 apparently cleaned tomato fruits with the removed part kept intact in order to use them to cover all the holes created after organism inoculation[3]. Each of the 6 already identified fungi were inoculated into the 6 fruits and the earlier removed parts were used to cover the inoculum sites[3]. The surrounding of the holes was sealed with petroleum jelly to prevent environmental contamination[3]. Each of these 6 tomato fruits were placed in a sterile nylon sample bags and incubated for 5days at 28^0C and they were observed for rot[3]. All the 6 samples inoculated with the already identified fungi developed rot signs like original tomato fruits used in this study[3]. These rotting samples were re-inoculated into potato dextrose agar media and the growth observed were compared with the original growth[3]. The growth observed confirmed the pathogenicity of the fungi identified from the original experiment and not contamination from environment.

3.10 Genomic Fungal Extraction Protocol

(Microcentrifuge, Vortex, Cell Disrupter/Pulverizer) Genomic DNA was extracted from pure culture using Quick-DNA™ Fungal/Bacteria Miniprep kit (Zymo Research, Catalogue No.D6005) extraction kit [6]. Then, the following steps were taken

1. 50 – 100 mg fungal cells from pure culture was suspended in up to 200 µl of water or isotonic buffer (e.g., PBS) to a ZR BashingBead™ lysis tube (0.1 mm & 0.5 mm) and 750 µl BashingBead™ Buffer was added to the tube to homogenize the mixture.

2. A bead beater was fitted to a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes and the mixture was centrifuged in a microcentrifuge at 10,000x *g* for 1 minute.

3. The supernatant was transfer to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuge at 8,000 x *g* for 1 minute.

4. 1,200 µl of genomic Lysis Buffer was added to the filtrate in the Collection tube.

5. 800 µl of the mixture from Step 4 was transfer to a Zymo-Spin™ IICR Column 3 in a Collection tube and centrifuge at 10,000 x *g* for 1 minute.

6. The flow through was discarded from the Collection tube and Step 5 was repeated.

7. Another 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IICR Column in a new Collection tube and centrifuge at 10,000 x *g* for 1 minute.

8. Then 500 µl g-DNA wash Buffer was added to the Zymo-Spin™ IICR Column and centrifuge at 10,000 x *g* for 1 minute.

9. The Zymo-Spin™ IICR Column was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl (35 µl minimum) DNA Elution Buffer was added directly to the column matrix, then, it was Centrifuged at 10,000 x *g* for 30 seconds to elute the DNA. Ultra-pure DNA from this extraction was used for Polymerase Chain Reaction Analysis.

3.11 Polymerase Chain Reaction Analysis

Polymerase chain reaction was carried out on pure gDNA from extraction process by following these steps-;

1. **Denaturation:** after the DNA extraction, the genomic DNA was heated to 94° C in order to break the bonds that hold DNA strands together in a helix structure because the process normally act on the two strands when not bonded together. The polymerase chain reaction (Pcr) tube contained the gDNA template, polymerase enzymes, primers, Pcr buffers and they are together referred to as Pcr Mixture.
2. **Annealing:** The mixture was allowed to cool down to 50^0C in order to allows the primers (ITS-1 Ribosomal RNA sequence TCCGTAGGTGAACCTGCGG and Primer ITS-4 Ribosomal RNA sequence TCCTCCGCTTATTGATATGC in

both forward and reverse reaction) to bind or anneal to their complementary sequence in the template DNA.

3. **Extension:** The reaction was heated up to 72° C which is the optimal temperature for DNA polymerase enzymes to add more nucleotides and elongate the template nucleotide to produce maximum samples.

3.12 Gel Electrophoresis of Amplicons

The PCR products or amplicons was run on a agarose gel to ascertain the target segment were actually amplified following this protocol-; The agarose gel was prepared from Agarose powder and casted using comb to create sample wells[4]. The amplicons mixture was applied to agarose gel sample wells alongside ladder to detect the presence of target size DNA fragments. The gel was allowed to run for about 1hour and the movement is usually based on the size of each DNA fragments[4].

3.13 DNA fragments Purification from Agarose Gel

The DNA fragment from Agarose gel was extracted with the Zymoclean™ Gel DNA recovery kit as follows-;

1. The Exo/SAP master mix was prepare and added to a 0.6ml micro-centrifuge tube:
   a. Exonuclease I (Catalogue No. NEB M0293L) 20 U/ul 50 µl
   b. Shrimp Alkaline Phosphatase (Catalogue No. NEB M0371) 1 U/ul 200 µl
2. It was followed by the Preparation of the following reaction mixture:

Amplified PCR Product	10 µl
ExoSAP Mix (step 1)	2.5 µl

3. The whole mixture was Mix well and incubated at **37°C** for **15 min**
4. The reaction was heated for 15minutes and stopped at **80°C**.

3.14 Sequencing Protocol

DNA fragments recovered from agarose gel was sequenced using the Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions. The labelled products was cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053): The cleaned products was injected on the Applied Biosystems ABI 3500XL Genetic Analyser with a 50cm array, using POP7: The purified fragments was analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction of every sample. DNAstar was used to analyze the ab1 files generated by the ABI 3500XL Genetic Analyzer and result was obtained by BLAST (Basic Local Alignment Search Tool) on NCBI (National center for biotechnology information). Sequence chromatogram analysis was performed using Finch TV analysis software.

3.15 Statistical Analysis

Data obtained were statistically analyzed using statistical package for social sciences (SPSS) software version 24.0. Descriptive analysis was used to present prevalence and frequencies of outcomes.

Endnote

1. L. Kator, A.C Iheanacho, K.P Aloho: *Isolation, Identification and Pathogenicity of Fungal Organisms Causing Postharvest Spoilage of Tomato Fruits during Storage* ***Annual Research & Review in Biology*****,** 26(6), 2018, 1-7.

2. A. Sagar, *Potato Dextrose Agar, Principle, Uses, Composition, Procedure and colony Characteristics*.2019.

3. S. Aslam, T. Aisha, M. F. Aslam, W.A. Muhammad , A. A. Shedayi, & S. Sadia, *Recent Advances in molecular techniques for the Identification of Phytopathogenic Fungi – a mini review,* **Journal of Plant Interactions**, 12(1). 2017,493-504.

4. S.B.Marthur, & O. Kongsdal, "*Common Laboratory Seed Health Testing Methods For Detecting Fungi, Danish Government Institute of seed and Pathology for Developing Countries, Copenhagen,* 2003.

5. G. Hariharan, K. Prasannath, *Recent Advances in Molecular Diagnostics of Fungal Plant Pathogens: A mini review*.2021.

6. B. Aslam, M. Basit, A. M. Nisar, & M. Khursid, *Proteomics: Technology and their Applications*.2017.

Chapter Four

Results and Discussion of Findings

4.0 Results

4.1 The Result of the Isolated Fungi

The tomato samples cultured on pototo dextrose agar produce growth that were identified using molecular method as represented in the table 4.1 below. They were identified based on the tomato fruits' variety, the location where they were bought and frequency of the growth. *Geotrichum candidum* was isolated from Royal varitety bought from Eleekara market and *Rhizopus delemar* was isolated, identified and speciate from Cherry variety from Eleekara as well. With frequecy of 4 out of the samples a percentages of 18.2% for organisms. *Aspergillus flavus* was isolated, identified and speciate from Royal variety from Sasa and Pichia kudriavzevii was isolated and identified from Cherry from Sasa with

frequency of 4 out of the 4 samples cultured and percentage of 18.2% for both organisms. *Aspergillus niger* and *Penicillum citrinum* were both isolated and identified from Royal and Cherry respectively with frequncies of 3 out 4 samples cultured and percentage of 13.6% for both organisms from both varieties. No organism was isolated from samples cultured from Obada market in both varieties.

Table 4.1: Shows the Fungi Species Isolated from Tomato fruits Samples from Royal and Cherry Varieties and their Locations

Names of fungi isolated and Identified	Tomato variety	Market Location	Frequency	Percentage %
Geotrichum candidum	Royal	Eleekara (oyo state) A_1	4	18.2 %
Rhizopus delemar	Cherry	Eleekara (Oyo state) A_2	4	18.2%
Aspergillus flavus	Royal	Sasa (Oyo state) B_1	4	18.2%
Pichia kudriavzevii	Cherry	Sasa (Oyo State) B_2	4	18.2%
Aspergillus niger	Royal	Oluwo (Osun state) D_1	3	13.6%

Penicillum citrinum	Cherry	Oluwo (Osun state) D_2	3	13.6%
No isolate	Both varieties	Obada (Osun state) C_1 & C_2	0	0.0 %
Total	-	-	22	100%

(Source: Laboratory result, 2022)

The results from Eight fruits of *solanum lycopersicum* with signs and symptoms of infection asigned to group A with four fruits from Cherry and Royal varieties each with one controls from each varieties were read as indicated below. From all the eight fruits of both varieties, only two pure isolate were recorded one from each variety with frequency of 4 out 4 samples respectively as represented by Table 4.2. The results from conventional culture and sub -culture were as shown in Fig 4.1 and Fig 4.2 respectively. Result from sanger sequencing and Genbank identifiction were represented below with Plate 1, plate 1.1 plate 1.2, Table 4.3 and Table 4.4 respectivey. The two pure isolates were identified as *Geotrichum candidum* for that of Royal variety and that of Cherry as *Rhizopus delemar.*

Table below represent growth observed on direct culture plate method from cherry and royal varieties of the samples gotten from Eleekara market denoted as group A. From the 4 samples of each varieties, growth were observed from all the 4 samples out of the 4 samples cultured.

Table 4.2 Growth Distribution of Pure Isolates from the Cherry and Royal Varieties Samples from Market A, Royal Variety A1(1) - A1(4) and A2, Cherry Variety (A2(1) – A2(4).

A_1	G	NG	A1C	A2	G	NG	A_2C
1 4	+	-	-	1^4	+	_	_
1	+			1	+		
2	+	_		2	+	_	
3	+	_		3	+	_	
4	+	_		4	+	_	

(Source: Laboratory result, 2022)

Key note

A1 - Royal variety , A2- Cherry variety

A1C- Control for Cherry variety, A2C- Control for Royal variety

1_4- number of Royal and Cherry variety growth recorded

NG - No growth

G- Growth

The figure 4.1 and figure 4.2 below represent direct culture method of tomato samples from group A as freshly cultured on potato dextrose agar.

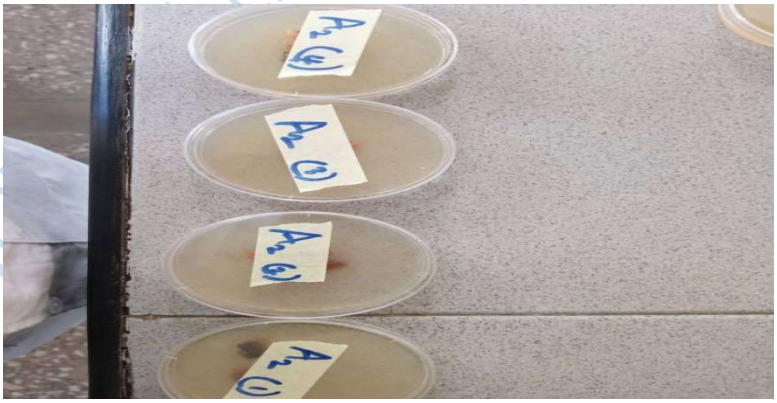
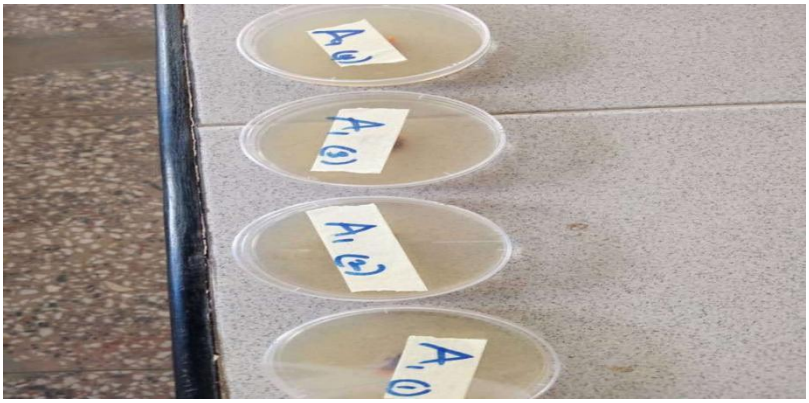
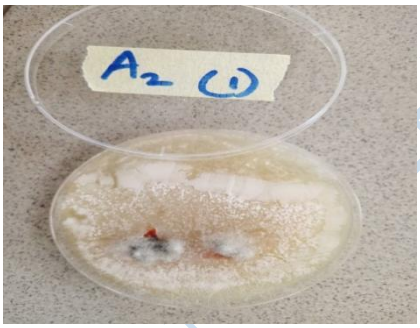


Fig 4.1 Freshly Cultured Royal Variety of *Solanum lycopesricum* on PDA for Grp A2



4.2 Freshly Cultured Cherry Variety of *Solanum lycopesricum* on PDA for Grp A1

The figure 4.3 below represents direct culture method of tomato samples from group A_2 and growth obtained at first culture.



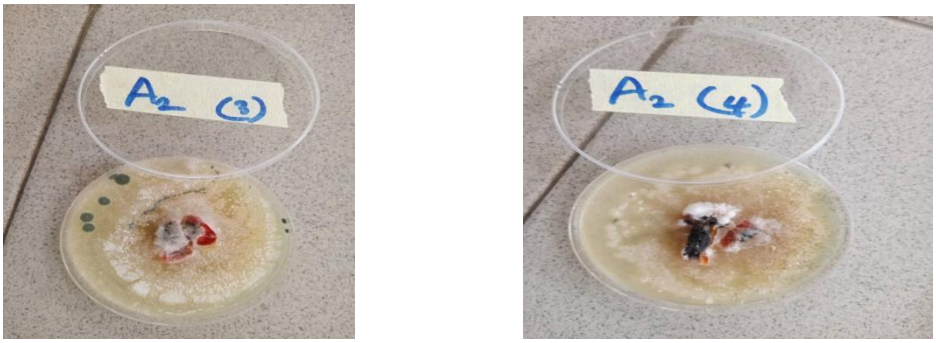


Fig 4.3 Growth Observed after Few Days of First Culture of Grp A_1 and A_2

(Source: Laboratory result, 2022)

The Figure below represents pure growth obtained from tomato samples from group A after sub-culture to obtain pure isolates

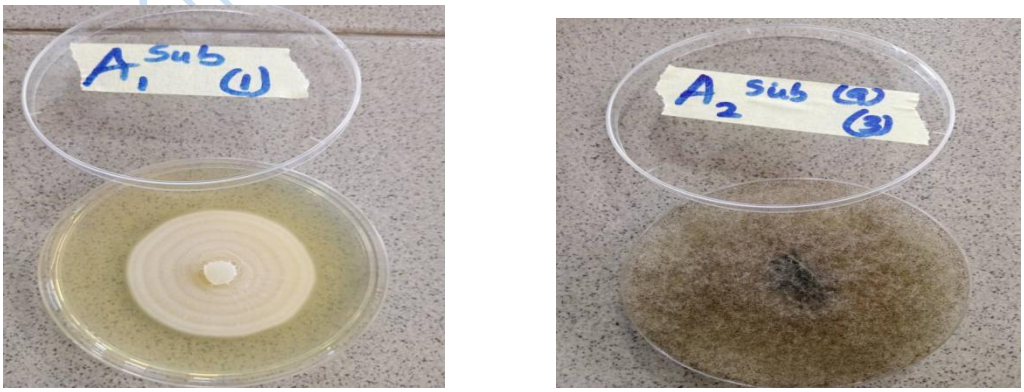


Fig 4.4 Growth Observed after Few Days of Sub- culture of Grp A1 and A2.

(Source: Laboratory result, 2022).

The Plates below Represent the Order of Nucleotides for Each Organism Isolated at the End of Sanger Sequencing Technique.

CCTTKGATYCTGGAGGTTGATAGTGTTGTTTTTCAAACGAATTTGATTCG

AAATTTTAGAAAAGCAATGCAATTCCAAGAGAGAAACAACGCTCAAACAA

GTATACTTTGGGGGATACCCCAAAGTGCAATGTGCGTTCAAAAACTGATG

ATTCACTTCTGCAATTCACAAGAAATATCGCGTTTCGCTGCGTTCTTCAT

CGATACGAGAACCAAGAGATCCATTGTTAAAAGTTTTRATTATTTTTGTT

TTRATTGWWTGATTATGGTTKGYTGKGTAAWTTYCMCAAWTATTATCAAT

TCTWAWTGATCCTTCCSMRGGTTCMCCTACGGAA

Plate 1: Sanger Sequence Result of Speciation for Organism Isolated from Grp A1(1)

(Source: Laboratory Result, 2022)

CAAWWTTTGKGGAATTWYCMCAGCAAACAATAATCATACAATCAAAACAA

AAATAATCAAAACTTTTCCAAGGGATCTCTGGGTTCTCGAATCGATGAAA

AACGCAGSGAAACGCATTATTTCTTGGAATTTGARGAAGKGAATCATCAG

TTTTTGAACGCCCATGGCACTTTGGGGWATCCCCCAAGGWATACTKGTTK

GAGSGTKGTTCCTCTCTTGRAATKGCATTGTTTTTCTAAATTTTCRAATC

AAATTCKTTKGAAAAMCAACACTATTCAACCTCAAATCARGWAGGATTAC

CCSCTGAACTTAASCATATCAWTAACSGRAGAAA

Plate 1.1 : Sanger Sequence Result of Speciation of Fungi Isolated from Grp A1(2)

(Source: Laboratory result, 2022)

TTTWAAGGSSGSCTTACCTCTTAGGGTTTCCTCTGGGGTAAGTGATTGCT

TCTACACTGTGAAAATTTGGCTGAGAGACTCAGACTGGTCATGGGTAGAC

CTATCTGGGGTTTGATCGATGCCACTCCTGGTTTCAGGAGCACCCTTCAT

AATAAACCTAGAAATTCAGTATTATAAAGTTTAATAAAAAACAACTTTTA

ACAATGGATCTCTTGGTTCTCGCATCGATGAAGAACGTAGCAAAGTGCGA

TAACTAGTGTGAATTGCATATTCAGTGAATCATCGAGTCTTTGAACGCAG

CTTGCACTCTATGGTTTTTCTATAGAGTACGCCTGCTTCAGTATCATCAC

AAACCCACACATAACATTTGTTTATGTGGTAATGGGTCGCATCGCTGTTT

TATTACAGTGAGCACCTAAAATGTGTGTGATTTTCTGTCTGGCTTGCTAG

GCAGGAATATTACGCTGGTCTCAGGATCTTTTTCTTTGGTTCGCCCAGGA

AGTAAAGTACAAGAGTATAATCCAGCAACTTTCAAACTATGATCTGAAGT

CAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

Plate 1.2 : Sanger Sequence Result of Isolated Fungi from Grp A2(1)

(Source: Laboratory result, 2022)

Table below represent the prediction of likely organism from genbank when blasted.The predicted organism showed a percentage of 85.36% similarity with information of Geotrichum candidtum present on genbank data base with reference number OM3970721 .

Table 4.3 Fungi Present in Sample from Market A (Royal Variety)

Name of the Sample	A1
Percentage ID	85.36%
Predicted Organism	*Geotrichum candidum*

Genbank Accession	OM397072.1

(Source: Laboratory result, 2022)

Table below represent the prediction of likely organism from genbank when blasted.The predicted organism showed a percentage of 100% similarity with information of *Rhizopus delemar* present on genbank data base with reference number LC514331.

Table 4.4 Fungi Present in Sample from Market A (Cherry variety)

Name of the Sample	A2
Percentage ID	100%
Predicted Organism	*Rhizopus delemar*

Genbank Accession	LC514331.1

(Source: Laboratory result, 2022)

The results from another Eight samples of *solanum lycopersicum* with signs and symptoms of infection asigned to group B with four fruits from Cherry and Royal varieties each with one controls from each varieties were read as indicated in Table 4.3 below. From all the eight samples of both varieties, only two pure isolate were recorded one from each variety with frequency of 4 and 4 samples respectively. The results from two consecutive conventional culture and sub -culture were as shown in Fig 4.5 Fig 4.6 and fig 4.7 respectively. Result from sanger sequencing and Genbank identifiction were represented below with Plate 2, plate 2.1, plate 2.2, Table 4.5 and Table 4.6 respectivey. The two pure isolates were identified as *Aspergillus flavus* for that of Royal variety and *Pichia kudriavzevii* for that of Cherry variety.

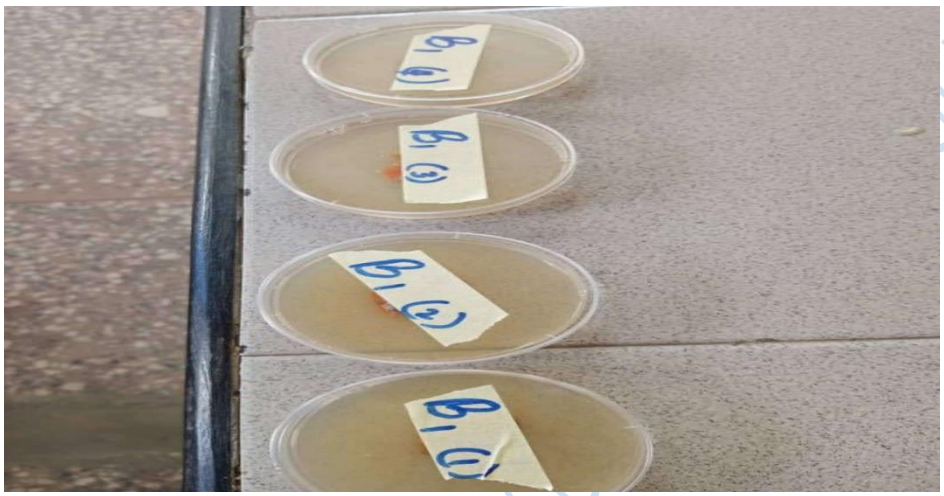


Fig 4.5 Freshly Cultured Royal Variety of *Solanum lycopesricum* on PDA for grp B1

4.6 Freshly Cultured Cherry Variety of *Solanum lycopesricum* on PDA for Grp B2

The Figures 4.7, 4.8 , 4.9 and 4.10 below represent the growth observed from first direct plate culture method to sub –culture and pure isolate of tomato samples from group B.

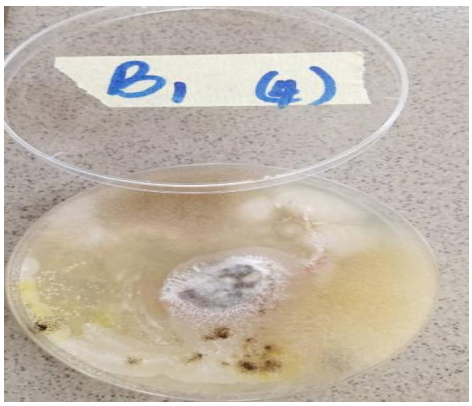
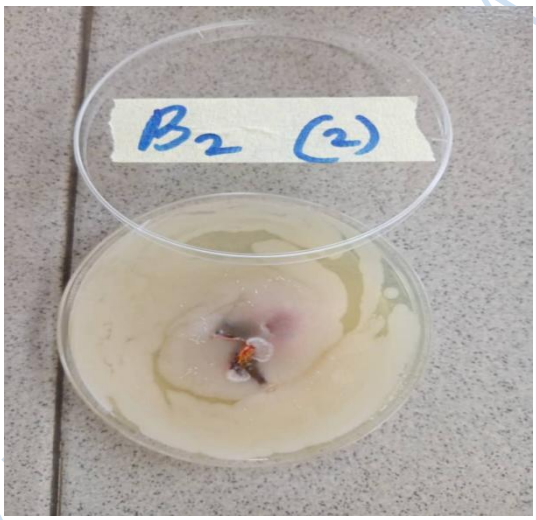


Fig 4.7 Growth Observed after Few Days of Sub- culture of Grp B1 and B2

(Source: Laboratory result, 2022)



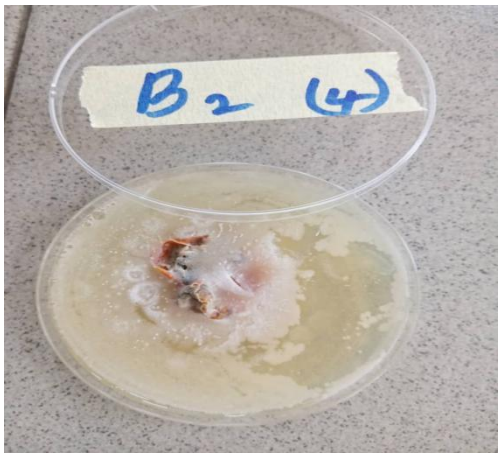
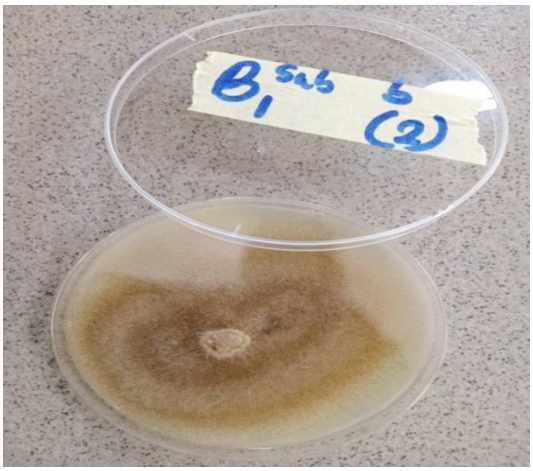
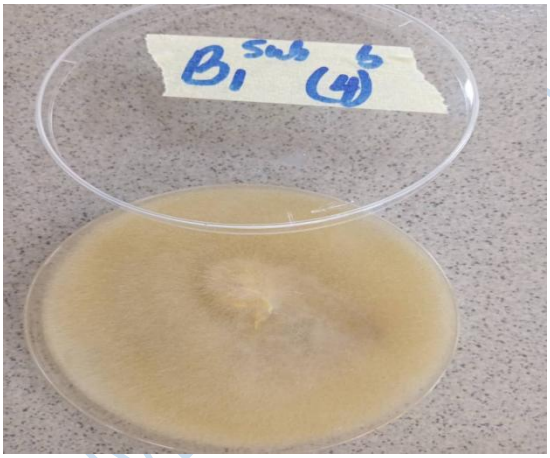


Fig: 4.8 Growth Observed after Few Days of Sub- culture of Grp B1 and B2.

(Source: Laboratory result, 2022)





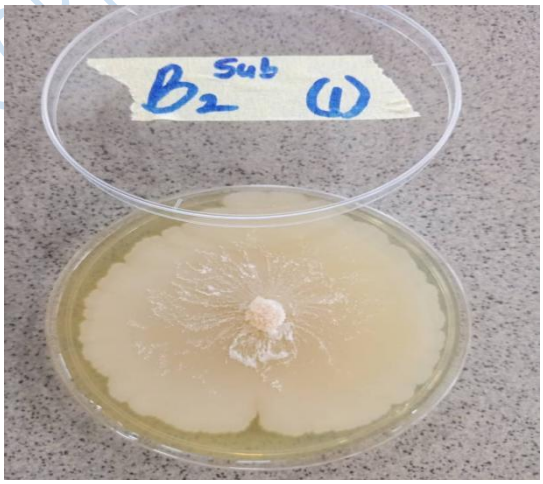


Fig: 4.9 Growth Observed after Few Days of Sub- culture of Grp B1 and B2.

(Source: Laboratory result, 2022)

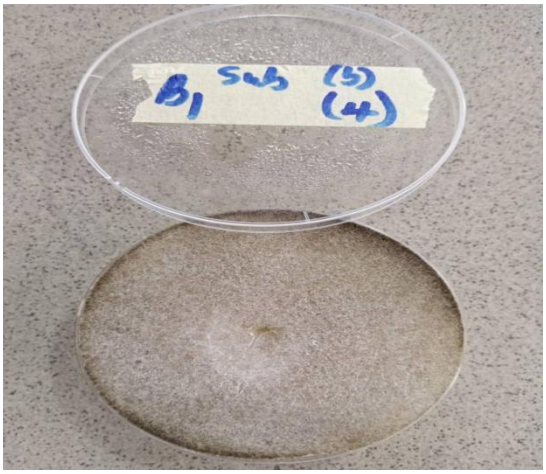


Fig : 4.10 Growth Observed after Few Days of Sub- culture of Grp B1 and B2.

(Source: Laboratory result, 2022)

Table below represent growth observed on direct culture plate method from cherry and royal varieties of the samples gotten from Sasa market denoted as group B. From the 4 samples of each varieties, growth were observed from all the 4 samples out of the 4 samples cultured.

Table 4.5 Growth Distribution of Pure Colonies from the Cherry and Royal Tomato Varieties of Market B1 Royal ($B_1 – B_4$) and B2, Cherry(B_1-B_4)

B_1	G	NG	B_1C	B_2	G	NG	B_2C
1_4	+	-	-	1_4	+	-	-
2	+	_		2	+	_	
3	+	_		3	+	_	
4	+	_		4	+	_	

(Source: Laboratory result, 2022)

Key note

B1 - Royal variety

B2- Cherry variety

B1C- Control for Cherry variety

B2C- Control for Royal variety

14- number of Royal and Cherry variety growth recorded

NG - No growth , G- Growth

The Plates (Plates; 2, 2.1 & 2.2, below Represent the Order of Nucleotides of each Organism Isolated at the End of Sanger Sequencing Techniques for Group B

TCCKGAAYCCGAGGTCAACCTGGAAAAAGATTGATTTGCGTTCGGCAAGC

GCCGGCCGGGCCTWCAGAGCGGGTGACAAAGCCCCATACGCTCGAGGATC

GGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCGGAGAGGGGA

CGACGACCCAACACACAAGCCGTGCTTGATGGGCAGCAATGACGCTCGGA

CAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTC

GATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCG

TTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTG

ATTGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGT

GTCTCCGGCGGGCGCGGGCCCGGGGCTGAGAGCCCCCGGCGGCCATGAAT

GGCGGGCCCGCCGAAGCAACTAAGGTACAGTAAACACGGGTGGGAGGTTG

GGCTCGCTAGGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCACCT

ACGGAA

Plate 2 : Sanger Sequence Result of Speciation for Organism Isolated from Grp B1(1)

(Source: Laboratory result, 2022)

TCWTTWGWMTACACTGCGTGAGCGGACGAAAACAACAACACCTAAAATGT

GGAATATAGCATATAGTCGACAAGAGAAATCTACGAAAAACMAACMAAAC

TTTCAACMACGGATCTCTTGGKTCTCSCATCSATGAARARCGCAGCGAAA

TGSGATACCTARTGKGAATTGCAGCCATCGTGAATCATCSAGTTCTTGAA

CGCACMTTGSGCCCCTCSGSATTCCGGGGGGSATGSCTGKTTGARCGKCG

TTTCCATCTTGCGCGTGCGCAGAGTTGGGGGAGCGGAGCGGACSACSTGK

AAAGAGCGKCGGARCTGCGACTCSCCTGAAAGGGARCGAAGCTGGCCSAR

CGAACTARACTTTTTTTCMGGGACSCTTGGCGGCCSARARCGAGTGKTGC

GARACAACMAAAAGCTCSACCTCMAATCAGGKARGAATACCCGCTGAACT

TAAGCATATCAATAARCGGAGGAA

Plate 2.1 : Sanger Sequence Result for Organism Isolated from Grp B2(1)

(Source: Laboratory result, 2022)

ACKKGATTTGAAGGTCGAGCTTTTTGTTGTCTCGCAACACTCGCTCTCGG

CCGCCAAGCGTCCCTGAAAAAAAGTCTAGTTCGCTCGGCCAGCTTCGCTC

CCTTTCAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTCCGCTCCG

CTCCCCCAACTCTGCGCACGCGCAAGATGGAAACGACGCTCAAACAGGCA

TGCCCCCCCGGAATGCCGAGGGGCGCAATGTGCGTTCAAGAACTCGATGAT

TCACGATGGCTGCAATTCACACTAGGTATCGCATTTCGCTGCGCTCTTCA

TCGATGCGAGAACCAAGAGATCCGTTGTTGAAAGTTTTGTTTGTTTTTCS

TARATTTCTCTTGKCSACTATATGCTATATTCCACATTTTARGKGKTGKT

GKTTTCSTTCCGCTCACSCAGTGKAGTACTAAATCACAGKAATGATCCTT

CCGCAGGKTCACCTACSGAA

Plate 2.2 Sanger Sequence Result for Organism Isolated from Grp B2(3)

(Source: Laboratory result, 2022)

Table below represent the prediction of likely organism from genbank when blasted.The predicted organism showed a percentage of 100% similarity with genetic information of *Aspergillus flavus* present on genbank data base with reference number MT645322.

Table 4.6 Fungi Identification from Sanger Sequence for Sample B_1

Name of Sample	B_1
Percentage ID	100%
Predicted Organism	*Aspergillus flavus*
Genbank Accession	MT645322.1

(Source: Laboratory result, 2022)

Table below represent the prediction of likely organism from genbank when blasted.The predicted organism showed a percentage of 100% similarity with genetic information of *Pichia kudriavzevii* present on genbank data base with reference number MT071789.1

Table 4.7 Fungi Identification from Sanger Sequence for Sample B_2

Name of Sample	B_2
Percentage ID	100%
Predicted Organism	*Pichia kudriavzevii*
Genbank Accession	MT071789.1

(Source: Laboratory result, 2022)

The results from third set of eight samples of *solanum lycopersicum* with signs and symptoms of infection asigned to group C with 4 fruits samples from Cherry and Royal varieties each with one controls from each varieties were read as indicated below in Table of 4.8 From all the eight fruits of both varieties, no isolate were recorded from both varieties. The results from two consecutive conventional culture and sub-culture were as shown in Fig 4.11 and Fig 4.12

Fig 4.11 Freshly cultured Royal variety of *Solanum lycopesricum* on PDA for grp C1

Fig 4.12 First Cultured Royal Variety of *Solanum lycopesricum* on PDA for Group C1 and C2.

(Source: Laboratory result, 2022)

The figure 4.3.2 below represent tomato samples from group C which showed no substancial growth after some days at 28^0C.

Fig : 4.13 No Substantial Fungus Growth after Some Days

(Source: Laboratory result, 2022)

Table 4.4 below represent growth observed on direct culture plate method from cherry and royal varieties of the samples gotten from Obada market denoted as group C.From the 4 samples of each varieties, No growth were observed from all the 4 samples cultured.

Table 4. 8 Growth Distribution of Pure Isolates from the Cherry and Royal tomato Varieties of Market C, Royal variety C1(C_1) - C1($_4$) and C2, Cherry Variety (C2($_1$) – C2($_4$).

C_1	G	NG	C_1C	C_2	G	NG	C_2C
1	-	-	-	1	_	_	_
2	_	_		2	_	_	
3	_	_		3	_	_	
4	_	_		4	_	_	

(Source: Laboratory result, 2022)

Key note

C1 - Royal variety

C2- Cherry variety

C1C- Control for Cherry variety

C2C- Control for Royal variety

NG - No growth G- Growth

The results from fourth set of eight fruits of *solanum lycopersicum* with signs and symptoms of infection asigned to group D with four fruits from Cherry and Royal varieties each with one controls from each varieties were read as indicated Table 4.9 below. From all the eight fruits of both varieties, only two pure isolate were recorded from one variety. The results from conventional culture and sub -culture were as shown in Fig 4.14, 4.15, 4.16 and Fig 4.17 respectively. Result from sanger sequencing and Genbank identifiction were represented below with Plate 3, plate 3.1, plate 3.2, Table 4.9, Table 4.10 and Table 4.11 respectivey. The two pure isolates from the two varieties were identified as *Aspergillus niger* and *Penicillium citrinum* from Group D_1 and D_2 with frequency of 3 out of 4 of samples cultured.

The Figures 4.14 and 4.15 below represent tomato samples from group D, freshly cultred on potato dextrose agar.

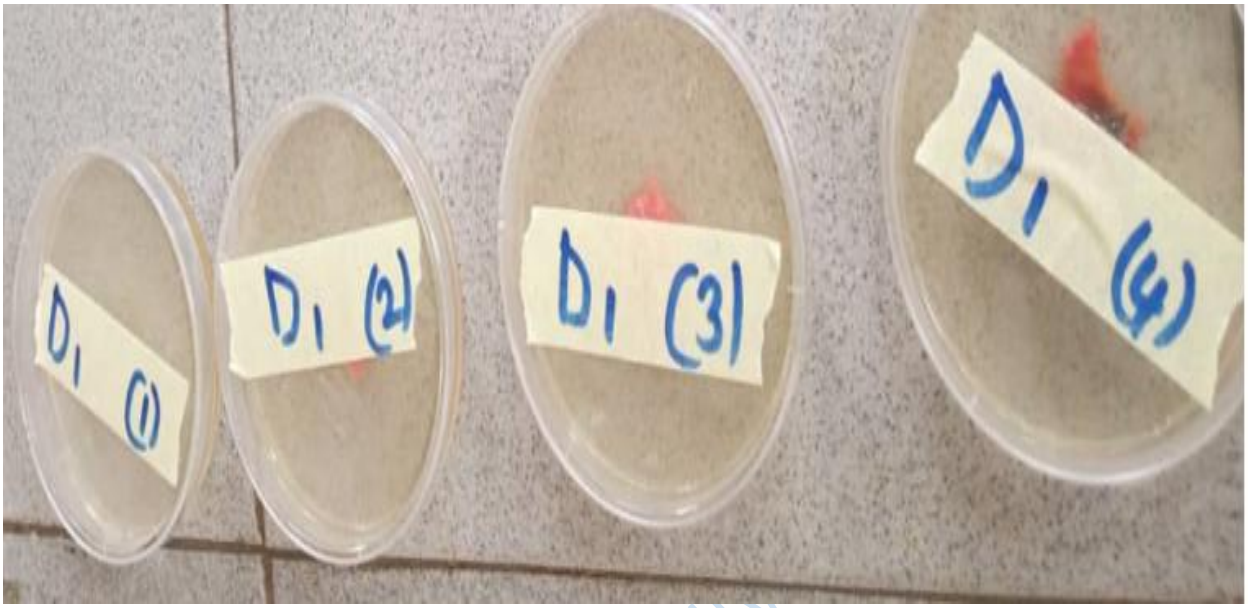


Fig 4.14 Freshly Cultured Royal Variety of *Solanum lycopesricum* on PDA for grp D1

Fig 4.15 Freshly Cultured Royal Variety of *Solanum lycopesricum* on PDA for grp D2.

(Source: Laboratory result, 2022)

The Figure 4.16 below represent growth observed from samples from group D after some days on potato dextrose agar

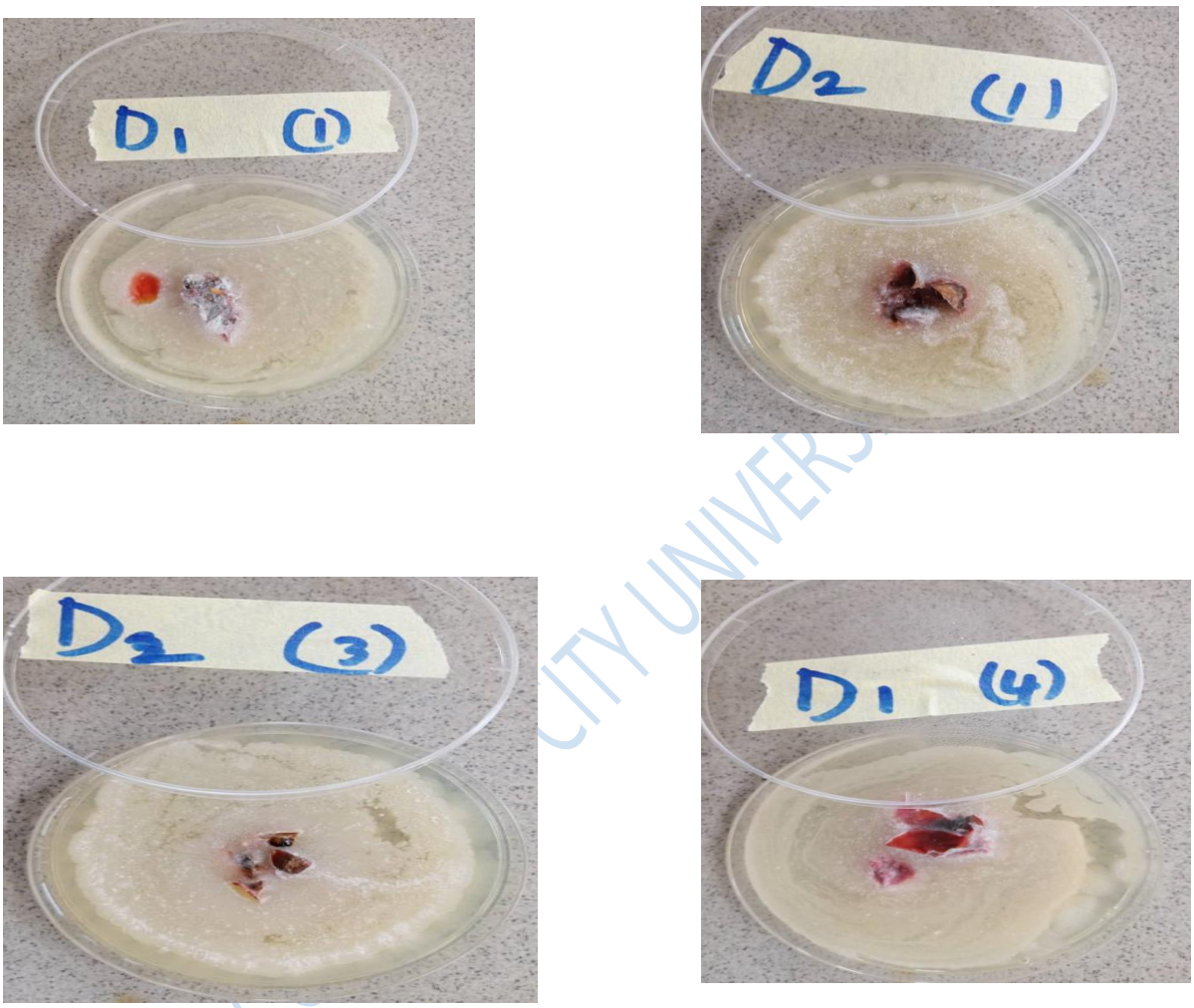


Fig 4.16 First Culture of Grp D1 and D2

(Source: Laboratory result, 2022)

The figure 4.4.2.1 below represent the growth observed from group D samples when sub-cultured for pure isolates.

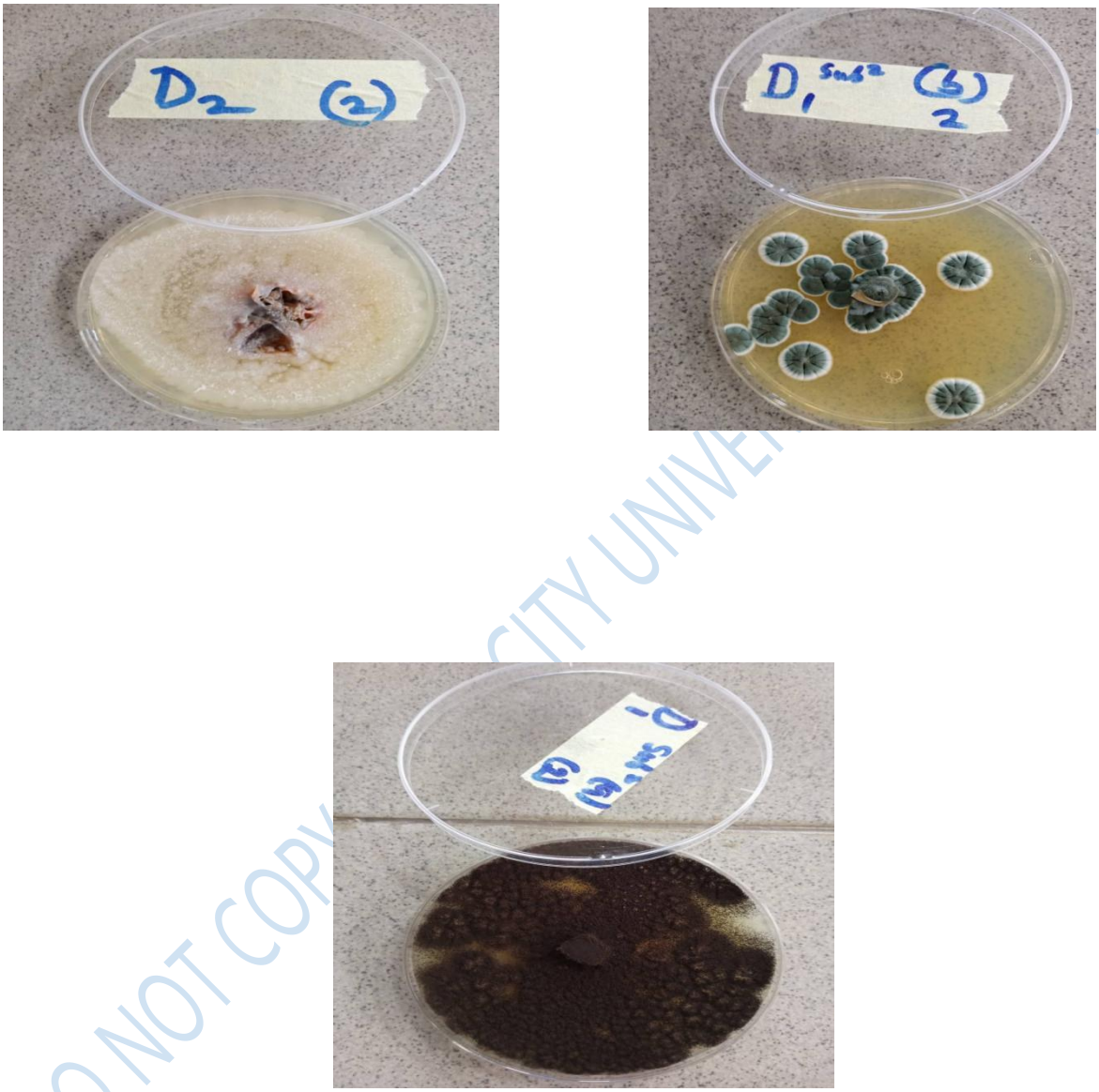


Fig 4.17 Sub- culture of Grp D1 and D2

(Source: Laboratory result, 2022)

Table below represent growth observed on direct culture plate method from cherry and royal varieties of the samples gotten from Oluwoo market denoted as group D. From the 4 samples of each varieties, growth were observed from 3 samples out of the 4 samples cultured from both varities.

Table 4.9 Growth Distribution of Pure Isolates from the Cherry and Royal tomato Varieties of Market D, Royal variety D1(1) - D1(4) and D2, Cherry Variety (D2(1) – D2(4).

D_1	G	NG	D_1C	D_2	G	NG	D_2C
1^3	+	-	-	1^3	+	_	-
2	+	_		2	+	_	
3	+	_		3	+	_	
4	_	_		4	+	_	

(Source: Laboratory result, 2022)

Key note

D1 - Royal variety , D2C- Control for Royal variety

D2- Cherry variety , 1^3- number of Royal and Cherry variety growth recorded

D1C- Control for Cherry variety G- Growth

NG - No growth

The Plates (Plates; 3, 3.1, 3.2 and 3.3) below Represent the Order of Nucleotides of Each Organism Isolated at the End of Sanger Sequencing Technique for Group D.

CCYKKGAATCCGGAGGTCACCTGGAAAAATGGTTGGAAAACGTCGGCAGG

CGCCGGCCAATCCTACAGAGCATGTGACAAAGCCCCATACGCTCGAGGAT

CGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAGAGGGGG

ACGGCGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGA

CAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTC

GATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCG

TTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTG

ATTGCATTCAATCAACTCAGACTGCACGCTTTCAGACAGTGTTCGTGTTG

GGGTCTCCGGCGGGCACGGGCCCGGGGGGCAAAGGCGCCCCCCCGGCGGC

CGACAAGCGGCGGGCCCGCCGAAGCAACAGGGTATAATAGACACGGATGG

GAGGTTGGGCCCAAAGGACCCGCACTCGGTAATGATCCTTCCGCAGGTTC

ACCTACGGAA.

Plate 3 : Sanger Sequence Result of Fungi Isolated from Grp_D1(3)

(Source: Laboratory result, 2022)

ACAGATCCGAGGTCAACCTGAGATAATTAAAGGTTGGGGGTCGGCTGGCG

CCGGCCGGGYCTACTAGAGCGGGTGACGAAGCCCCATACGCTCGAGGACC

GGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGCGGGGGGGA

CGGGGCCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACA

GGCATGCCCTCCGGAATACCAGAGGGCGCAATGTGCGTTCAAAGACTCGA

TGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTT

CTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTAAT

TTCGTTATAGGTCTCAGACTGCAACTTCAGACAGCGTTCAGGGGGGCCGT

CGGCGGGCGCGGGGCCCGCCGAGGCAACATAGGTTCGGGCAACACGGGTG

GGAGGTTGGGCCCCGAGGGGCCCGCACTCGGTAATGATCCTTCCGCAGGT

TCACCTACGGAA.

Plate 3.1 : Sanger Sequence Result for Organism Isolated from Grp D2(2)

(Source: Laboratory result, 2022)

GGSSGGGGTCTTTGGGGCCAACCTCCCATCCGTGTCTATTATACCCTGTT

GCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTTTGCCCC

CCGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGT

GCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATC

TCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGT

GAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCC

CCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAG

CCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCG

AAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTC

ACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTTT

TTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA

TCAATAAGCGGAGGAA

Plate 3.2 : Sanger Sequence Result of Fungi Isolated from Grp D1(4)

(Source: Laboratory result, 2022)

AGSGGGGGCCTCGGGGGCCAACCTCCCACCCGTGTTGCCCGAACCTATGT

TGCCTCGGCGGGCCCCGCGCCCGCCGACGGCCCCCCTGAACGCTGTCTGA

AGTTGCAGTCTGAGACCTATAACGAAATTAGTTAAAACTTTCAACAACGG

ATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAA

TGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCG

CCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTC

AAGCCCGGCTTGTGTGTTGGGCCCCGTCCCCCCCGCCGGGGGGACGGGCC

CGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCG

TCACCCGCTCTAGTAGGCCCGGCCGGCGCCAGCCGACCCCCAACCTTTAA

TTATCTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGC

ATATCAATAAGCGGAGG

Plate 3.3 : Sanger Sequence Result of Fungi Isolated from Grp D1(4)

(Source: Laboratory result, 2022)

Table below represent the prediction of likely organism from genbank when blasted.The predicted organism showed a percentage of 99.83% similarity with genetic information of *Aspergillus niger* present on genbank data base with reference number MG228418.1.

Table 4.10 Fungi Identification from Sanger Sequence for Sample D

Name of Sample	$D1_a$
Percdentage ID	99.83%
Predicted Organism	Aspergillus niger
Genbank Accession	MG228418.1

(Source: Laboratory result, 2022)

Table below represent the prediction of likely organism from genbank when blasted.The predicted organism showed a percentage of 99.82% similarity with genetic information of *Penicillium citrinum* present on genbank data base with reference number MN879404.1.

Table 4.11 Fungi Identification from Sanger Sequence for Sample D_{2b}

Name of Sample	$D2_b$
Percdentage ID	99.82%
Predicted Organism	Penicillium citrinum
Genbank Accession	MN879404.1

(Source: Laboratory result, 2022)

The results from control samples from both varieties showed no growth for days at 28^0C.

The figure 4.5 below represented culture from apparently clean samples used as control for cherry and Royal varieties with no visible growth of any organism.

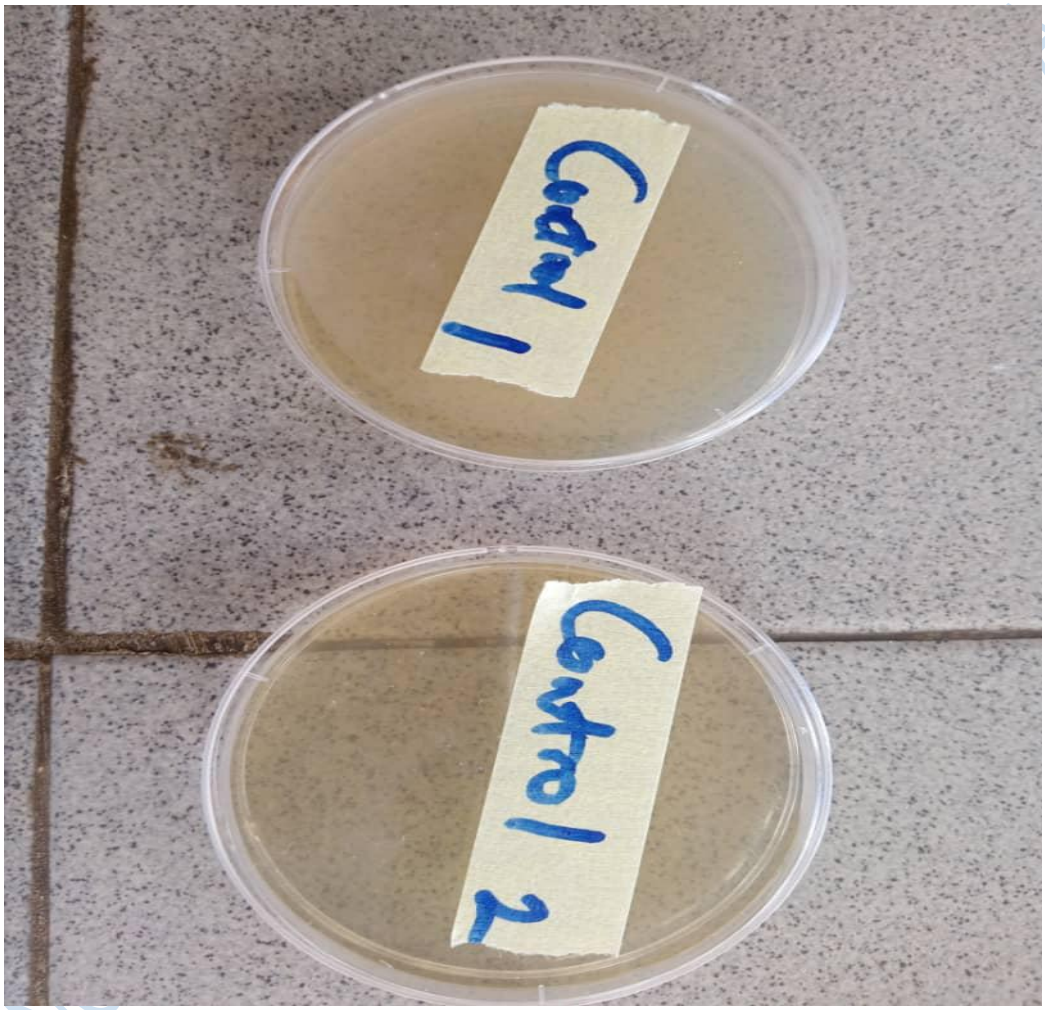
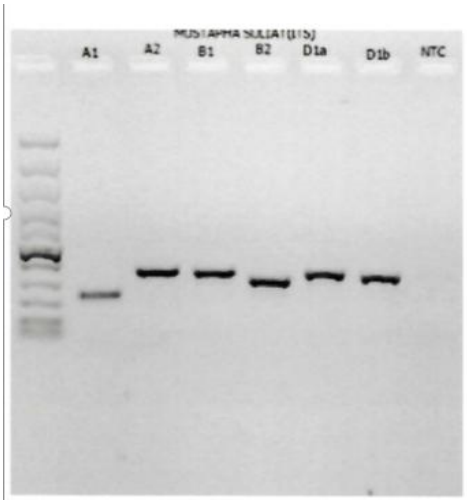


Fig 4.18 Apparently Clean Samples Showing no Growth of Fungus.

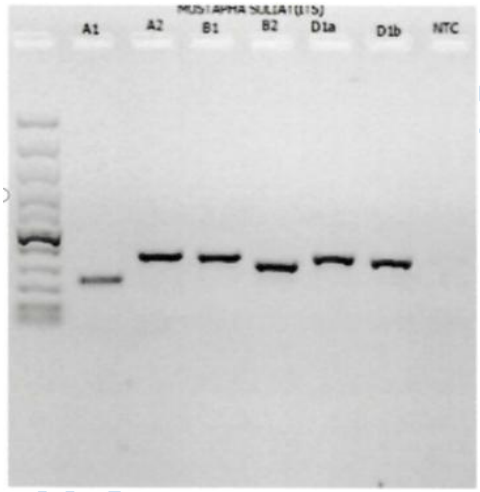
(Source: Laboratory result, 2022)

4.2 Photomicrograph Image of Internal Transcribed Spacer

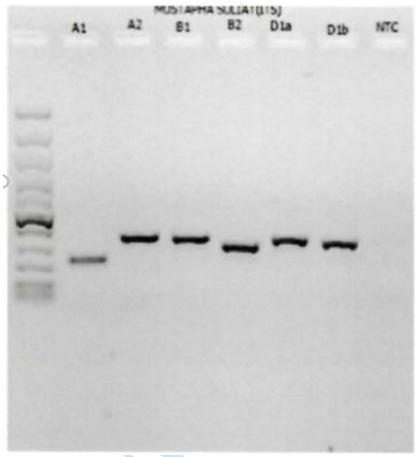
Figure 4.6 below represent Photomicrograph Images of Gel Electrophoresis for Each of the Organism Isolated from Each Sample and their Frequencies.



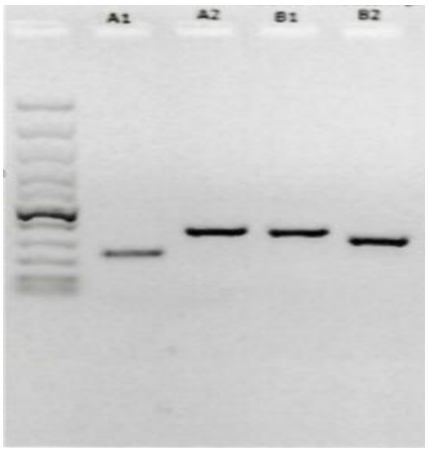
Frequeny 1



Frequency 2



Frequency 3



Frequency 4

Fig 4.19 A Photomicrograph Image of an Agarose Gel Indicating the Amplification of the ITS Target Region.

(Source: Laboratory result, 2022)

The table below represent the summary of organism isolated and identified to species level from this study and their locations.

Table 4.12 Conclusion of Blast Prediction of Each Isolate Analyzed

Name of organisms	Location
A1 *Geotrichum candidum*	Sasa Market
A2 *Rhizopus delemar*	Sasa market
B1 *Aspergillus flavus*	Eleekara market
B2 *Pichia kudriavzevii*	Eleekara market
$D1_a$ *Aspergillus niger*	Oluwoo market
$D2_b$ *Penicillium citrinum*	Oluwoo market

(Source: Laboratory result, 2022).

4.3 Discussion of Findings

Phytopathogenic fungal species have been traced and isolated from several researches as the cause of enormous loss in quantity and quality of tomato crop yields and this has been a major economic issue in the global agricultural sector. Precise and rapid detection and identification of plant infecting fungi are essential to facilitate effective management of disease. Molecular techniques have become most important methods for accurate plant disease diagnosis. Fungi have been found to be the most destruction pathogen in tomato fruits rots leading to economic loss of tomato in field, storage and markets. It has been reported that 21% of tomato produced in Nigeria were lost to rots in the field and 20% in storage and markets. Although, tomato fruits contain sugars and high level of nutritive elements with low pH value which make them target and susceptible to fungal pathogen infections.

The increasing world population and tomato fruits consumption necessitates well-organized plant disease management and control in agriculture to assure food security and safety of which an efficient and effective framework for early alert and quick response is a crucial element to combat phytopathogenic fungi. Diagnosis of fungal plant pathogen is of significance in the area of plant protection as it contributes to improving crop vigor and health. Therefore, fungal disease management requires accurate diagnosis of diseases which is chiefly based on the identification of causative agents. Moreover, it is very important to confirm fungal diseases even though the diagnosis based on the visible signs and symptoms may be very difficult to distinguish. The list that covers a known plant disease, its typical sign and symptoms, its known potential phytopathogen for a precise host is a perquisite for disease diagnosis before the advent of molecular method diagnosis.

Olden day's conventional methods include culturing, isolation, re-inoculation, microscopic techniques and biochemical tests which require knowledge and expertise in fungal plant

pathology and taxonomy. Molecular phytodiagnostic techniques need no sign and symptoms before detection and identification of diseases and causative organism can be established. It functions effectively on symptomless plant which can always give edge on prevention instead of curation or eradication.

The tomato fruits sample from Obada market of Osun state did not show any growth from both varieties. It shows that *Geotrichum candidum, Rhizopus delemar, Aspergillus flavus* and *Aspergillus niger* has highest frequencies of occurrence and percentage while *Pichia kudriavzevii, Penicillium citrinum* has lower frequency and percentage. The findings of this study can be linked to work of Nizamani S., Khaskheli A.M and Jiskani S.A from India where *Geotrichum candidum* was reported as one of the common fungal species responsible for tomato fruits rots which may be traced to level pH of tomato fruits[2]. It is in agreement with findings of another two researchers, Etaware and Oyetunji from department of botany and microbiology, University of Ibadan where *Aspergillus flavus* and *Aspergillus niger* were isolated and identified as most common fungi pathogenic species responsible for highest percentage in tomato fruits rots[3].

The result agreed with the work done at Ungwan Rimi, Kaduna State by Mohammed S.S.D and Kuhiyep C.Y where it was reported that *Aspergillus niger* had the highest rate of occurrence in the tomato fruits rot and the study concluded that the fungus may be the major organism responsible for the spoilage of tomato fruits. This study goes in line with work done on tomato fruit rot by Sobowale A.A and Oguntoye O, from Botany department, University of Ibadan and department of crop, soil and pest management technology, Rufus Giwa Polytechnic, Owo that *Geotrichum candidum* and *Fusarium oxysporum* were isolated and identified from major markets of two Southwestern state and one North central state of Nigeria. It was established that the two organism are frequently responsible for most of tomato fruits rots.

Hussain A, Khan S.W, Alli S, Faiz F, Hussain M, Alli A, Shams U.R and Qasim S from Karakoram International University concluded from their work on tomato fruits rots that *Aspergillus flavus* and *Aspergillus niger* were the dominating fungal species isolated and identified as the major cause of most tomato fruits rots in the district Gilgit, Balistan [4]. Tomato fruits rot caused by fungi is a major problem of second most important crop across the globe and the prevention, management and control must be of global action in order to reduce to minimum. Tomato fruits is said to have low pH and the high moisture content which make the crop a suitable medium for microbial growth especially fungi and some bacteria that dwell very well in acidic habitat or host. Although, some tomato fruits rots are caused by contamination through mishandling and unhygienic exposure when transporting the fruits to the vendors from farm and not always from farm soil or land. The fungi identified have no peculiarity to the geographical location of where they are isolated . The infection and the effect of the fungi on tomato fruits is not based on the geographical location as they can be found across the globe from the properties and characteristics of fungi identified . So, those isolated from tomato samples from Osun state markets has no peculiarity to Osun state and fungi isolated from Oyo state markets are not based on the location because those Phytopathogenic fungi causing tomato fruits rot can actually be isolated across the globe and that explains why tomato fruits rot is a major challenge across the world.

Endnotes

1. Bernal & Eduardo. "*Development of Tomato (S. Lycopersicum) Lines with Resistance to Xanthomonas Spp. And Use of Genetic Resources to Characterize Infection and Diversity in Pathogen Populations*." The Ohio State University, 2020.

2. Nizamani, S, A.A. Khaskheli, A.M. Jiskani, S.A. Khaskheli, A.J. Khaskheli, G.B. Poussio, H. Jamro, M.I. & Khaskheli: *Isolation and Identification of the Fungi Causing Tomato Fruit Rot Disease in the Vicinity of Tandojam, Sindh*: **Agricultural Science Digest**. 41: 2021, 186-190.DOI: 10.18805/ag.D-269

3. Hussain A, S. Wali Khan, S. Qasim, F. Faiz & A. Ali, *Geostatistical Analysis ofTtomato Fruits Rot and Diversity of Sssociated Fungal Species*, **Journal of Animal and Plant sciences**. 31(4), 2020.

4. Ávila M.K. & Romero H.M., *Plant Responses to Pathogen Attack: Molecular Basis of Qualitative Resistance*. **Review Facultad Nacional de Agronomía**. 70(2) 2017, 8225-8235

5. Franco D. A., Arango J.F, Hurtado-Salazar A & Ceballos-Aguirre N., *Development, Production, and Quality of "Chonto" Type Tomato Grafted on Cherry Tomato Introductions.* 2018, 65(2):150-157.

6. . S Pavan Kumar S, A Srinivasulu & K Raja Babu, *Symptomology of Major Fungal Diseases on Tomato and Its Management*: **Journal of Pharmacognosy and Phytochemistry**; 7(6): 2018, 1817-1821.

7. García-Enciso E.L., Benavides-Mendoza A., Flores-López M.L., Robledo-Olivo A., Juárez-Maldonado A & González-Morales S., *A Molecular Vision of the Interaction of Tomato Plants and Fusarium oxysporum* 10(577), 2017, 72-127.

8. Holland, JE, AE Bennett, AC Newton, PJ White, BM McKenzie, TS George, RJ Pakeman, JS Bailey, DA Fornara & RC Hayes. "*Liming Impacts on Soils, Crops and Biodiversity in the Uk: A Review."* **Science of the Total Environment**. 610, (2018): 316-332.

Chapter Five

Conclusion

5.1 Summary of Findings

Based on the findings of this research work, there are 6 different fungal species isolated and characterized molecularly using molecular tools like polymerase chain reaction, Sanger sequencing method and referenced on NCBI data base which identified the fungal species as *Aspergillus niger, Rhizopus delemar, Aspergillus flavus, Geotrichum candidum, Penicillium citrinum and Pichia kudriavzevii* that were found to be associated with tomato fruits rots in certain vegetable markets of Oyo and Osun state, South-west Nigeria. The fungi isolated from Eleekara and Sasa markets of Oyo state have highest frequency and percentage compared to fungi isolated from Oluwo market of Osun state.

5.2 Conclusion

Basic sanitary rules during plantation, harvesting, storage and handling during selling of fresh tomatoes should be employed to improve hygienic condition of the fresh tomato fruits consumed to avoid food- borne outbreak especially when eaten raw. Tomatoes fruits rots is higher in fresh ones compared to the sun-dried tomato fruits because the moisture content has been reduced to a level unfavourable for fungal growth. These fungi cause significant loss of tomato fruits to the farmers, companies that use it as raw materials for their finished products and vendors. There is a need in the future to come up with special preventive methods like resistant cultivar, basic sanitary rules, sun drying, organic preservative which is the best of preservation to minimize tomato fruits rots caused by fungal organism. Moreover, the storage of tomato should be done at a temperature and relative humidity that does not favour the growth of fungi.

5.3 Recommendations

i. There is a need in the future to come up with special preventive methods like planting resistant cultivar, sun drying of tomato fruits, organic preservative which is the best of preservation to minimize tomato fruits rots caused by fungal infection.

ii. The storage of tomato should be done at a temperature and relative humidity that does not favour the growth of fungi.

iii. Fungi attack on tomato fruits result in loss of economic resources as well as food poisoning from mycotoxin therefore, basic sanitary rules should be followed in order to reduce to the barest minimum the menace of tomato fruits rot.

iv. The fruits are usually transported from areas of production to areas of consumption in locally woven baskets and sacks under conditions that encourage the growth of fungi. These baskets and sacks should be sanitized before and after use or discarded after single use.

v. Frequent inspection of the fruits for sale by food inspectors is also recommended which will go a long way in preventing the consumption of contaminated tomato fruits thereby reducing the health hazards posed by the mycotoxin produced by fungi especially *Aspergillus flavus.*

5.4 Contribution to Knowledge

The prevalence of *Geotrichum candidum, Aspergillus flavus and Aspergillus niger* species were significantly higher among other fungal species identified in this study and previous studies. More emphasis should be on control, prevention and management on those fungal species and most essentially, tomato cultivars that will be resistant to these species of fungi should be focused more on. This will reduce having higher frequency and percentage of these fungi and in turn increases the quality and quantity of tomato yields.

5.5 Suggested Areas for Further Research

Further study should be carried out on larger sample size and more locations in other to capture more fungi that may be causing tomato fruits rots. More locations will generate more data that will help in planning of how to reduce the menace of fungi effect to barest minimum on *Solanum lycopersicum* fruits

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Bibliography

E-Books

Abhay K. Pandey, Abhishek Kumar, K.Dinesh, Richa Varshney & Pranab Dutta: *The Hunt for Beneficial Fungi for Tomato Crop Improvement – Advantages and Perspectives.* Plant –Stress 6.2022.

Alex S.J. , Kurdekar A., Zhao J., & Hewlett I.. "*Application of Nanotechnology in Biosensors for Enhancing Pathogen Detection." :* Nanomedicine and Nanobiotechnology. 10(5) 2018.

Alexandria K., Wisconsin. *Horticulture Division of Extension*, Madison Plant Pathology, 2017, XHT1250.

André V. C. , C. Castroverde D. M, Yang & S. He. *"Plant–Pathogenwarfare Under Changing Climate Conditions."* Current Biology *28*(10), 2018. 619-634.

Annio G., Jennings T. L., Tagit O., & Hildebrandt N.. *"Sensitivity Enhancement Of Forster Resonance Energy Transfer Immunoassays by Multiple Antibody Conjugation on Quantum Dots."* 29(6) ,2018, 2082-2089.

Arsenovic, M., Karanovic, M., Sladojevic, S., Anderla, A. & Stefanović, D. *Solving Current Limitations of Deep Learning Based Approaches for Plant Disease Detection. Symmetry* 11(12), 2019.

Ashraf U. & Tang X., *Yield and Quality Responses, Plant Metabolism and Metal Distribution Pattern in Aromatic Rice under Lead (Pb) Toxicity*. Chemosphere. 2017, 176: 141–155.

Ávila M.K. & Romero H.M., *Plant Responses to Pathogen Attack: Molecular Basis of Qualitative Resistance*. Review Facultad Nacional De Agronomía. 70(2) 2017, 8225-8235.

Eduardo B. "*Development Of Tomato (S. Lycopersicum) Lines with Resistance to Xanthomonas Spp. and Use of Genetic Resources to Characterize Infection and Diversity in Pathogen Populations."* The ohio State University, 2020.

Barbedo, J. G. A. *Factors Influencing the Use of Deep Learning for Plant Disease Recognition. Biosyst. Eng.* 172, 2018, 84–91.

Barbedo, J. G. A. *Impact of Dataset Size and Variety on the Effectiveness of Deep Learning and Transfer Learning for Plant Disease Classification*. Computer. Electron. Agric. 153, 2018, 46–53.

Batuman, Ozgur, Thomas A Turini, Michelle Lestrange, Scott Stoddard, Gene Miyao, Brenna J Aegerter, Li-Fang Chen, Neil Mcroberts, Diane E Ullman & Robert L Gilbertson. "*Development of an IPM Strategy for Thrips and Tomato Spotted Wilt Virus In Processing Tomatoes in the Central Valley of California*." *Pathogens* 9, No. 8 (2020): 636.

Baysal- Gurel F., *Pseudocercospora Fuligena (Black Leaf Mold). Invasive Species of Compendium, Wallingford, 2020, United Kingdom CABI.*

Bernal & Eduardo. "*Development of Tomato (S. Lycopersicum) Lines with Resistance to Xanthomonas Spp. and use of Genetic Resources To Characterize Infection And Diversity in Pathogen Populations*." The Ohio State University, 2020.

Bhimanagoud K., Mahmood R., Nagesha S.N, Nagaraja M.S, Prashant D.G, Kerima O.Z, Karosiya A. & Chavan M.. *"Field Application of Bacillus Subtilis Isolates for Controlling Late Blight Disease of Potato Caused by Phytophthora Infestans."* Biocatalysis And Agricultural Biotechnology(22), 2019, 101366

Bisegna O, C. P., Swami N.S & Caselli F.. *"Single-Cell Microfluidic Impedance Cytometry: from Raw Signals to Cell Phenotypes Using Data Analytics*." Lab On Achip . 21(1), 2021, 22-54.

Botero V., Hoyos-Carvajal L. & Marín J., *Detection Of Asymptomatic Plants of Solanum Lycopersicum L. Infected with Fusarium Oxysporum using VIS Reflectance Spectroscopy.* Ciencias Hortícolas 12(2), 2018, 436-446.

Brahimi, M., Arsenovic, M., Laraba, S., Sladojevic, S., Boukhalfa & K., Moussaoui, A "Deep Learning for Plant Diseases: Detection and Saliency Map Visualisation," in *Human and Machine Learning.* Eds. Zhou, J., Chen, F. (Cham, Switzerland: Springer International Publishing), 2018, 93–117.

Carmona S.L., D. Burbano-David, Gómez M., López W., Ceballos N., Castaño-Zapata J., Simbaqueba J. & Soto-Suárez M., *Characterization Of Pathogenic and Non-Pathogenic Fusarium Oxysporum Isolates Associated with Commercial Tomato Crops In the Andean Region of Colombia.* Pathogens. 9(70), 2020. 1-23.

Christian J Silva, Casper Van Den Abeele, Isabel Ortega- Salazar,Victor Papin, Jaclyn A Adaskaveg, Duoduo Wang, Clare L Casteel, Graham B Seymour, Barbara Blanco-Ulate, Journal Of Experimental Botany, 72(7), 2021, 2696-2709.

Concetta P. , Margherita L. , De Prisco R.,, Coppola E.,, Grilli E., Russo C. & Isidori M., *Tomato Plants (Solanum Lycopersicum L.) Grown in Experimental Contaminated Soil: Bio Concentration of Potentially Toxic Elements and Free Radical Scavenging Evaluation*. 13 (10), 2020, 1371.

Couto, D. & Zipfel, C. *Regulation of Pattern Recognition Receptor Signalling In Plants*. Nature Reviews Revision. 16(9), 2021, 537-552.

Deka, Bhabesh & Azariah Babu. "Chapter-3 Thrips (Scirtothrips Dorsalis, Hood): Vectors of Tospoviruses in Agricultural Crops." *Essentials Of*, (2020): 35.

Djangsou H., Francia E., Ronga D. & Matteo B., *"Blossom End-Rot In Tomato (Solanum Lycopersicum L.): a Multi-Disciplinary Overview of Inducing Factorsand Control Strategies."*Scientiahorticulturae 249, 2019, 49-58.

Domenico R., Da Lio D., Panattoni A., Salemi C., Cappellini G., Bartolini L. & Parrella G.. *"Rapid and Sensitive Detection of Tomato Brown Rugose Fruit Virus in Tomato and Pepper Seeds by Reverse Transcription Loop-Mediated Isothermal Amplification*

*Assays (Real Time and Visual)And Comparison with Rt-Pcr End-Point and Rt-Qpcr Methods."*Frontiers in Microbiology 12, 2021.

Edel-Hermann V, & C. Lecomte, *Current Status of Fusarium Oxysporum Formal Speciales Andraces* .Phytopathology. 109(4), 2019, 512-530.

Elham A. Kazerooni, Sajeewa S.N. Maharachchikumbura, Velazhahan Rethinasamy, Hamed Al-Mahrouqi & Abdullah M.Al-Sadi. *Fungi Diversity in Tomato Rhizosphere Soil under Conventional and Desert Farming Systems*. Frontires in Microbiology, 2017.01462.

Elena Z., A. Froehling, Schoenher C., Zunabovic-Pichler M., Schlueter O & Jaeger H.. *"Potential of Flow Cytometric Approaches for Rapid Microbial Detection And Characterization In The* Food *Industry—A Review."* Foods 10(12). 2021.

Eleonora S., Paltrinieri S. & A. Bertaccini. "*Phytoplasma Transmission by Seed." Inphytoplasmas: Plant Pathogenic Bacteria-Ii*, 131-147, Springer, 2019.

Elisabeth K. , Roberto S. , Gabriele C. & Wilfried S. , *Effect of Tomato Variety, Cultivation, Climate And Processing on Sola L 4, an Allergen from Solanum Lycopersicum* 14 (10), 2018, 1371.

Ellen C & Johnson B. N. "*Electrochemical Biosensors For Pathogen Detection*." Biosensors And Bioelectronics 159, 2020.

Evelyn Elizabeth Villanueva Gutierrezan Overview of Recent Studies of *Tomato (Solanum Lycopersicum Spp) From a Social, Biochemical and Genetic Perspective on Quality Parameters, Alnarp*-Sweden: Sveriges, Lantbruksuniversitet.3, 2018.

Farag M.F, *First Record of Melanospora Chionea as a Possible Cause of Pink Root Rot Disease on Tomato Plants in Egypt*. J Plant Pathol Microbiol. 11(500).2020. Doi: 10.35248/2157-7471.20.11.500.

Ferentinos, K. P. *Deep Learning Models for Plant Disease Detection and Diagnosis. Computer. Electron. Agric.* 145, 2018, 311–318.

Franco D.A., Arango J.F., Hurtado-Salazar A, & Ceballos-Aguirre N., *Development, Production, and Quality of "Chonto" Type of Tomato Grafted on Cherry Tomato Introduction* 65(2), 2018, 150-157.

Fuentes, A. F., Yoon, S., Lee, J. & Park, D. S. High-Performance Deep Neural Network-Based Tomato Plant Diseases and Pests Diagnosis System with Refinement Filter Bank. *Front. Plant Sci.* 9, 2018, 1162.

Fuentes, A. F., Yoon, S & Park, D. S. *Deep Learning-Based Phenotyping System with Global Description of Plant Anomalies and Symptoms*. Front. Plant Sci. 10, 2019, 1321.

Fuentes, A., Yoon, S., Kim, S. C. & Park, D. S. A. *Robust Deep-Learning-Based Detector for Real-Time Tomato Plant Diseases and Pests Recognitio*n. *Sensors* 17, 2022.

García-Enciso E.L., Benavides-Mendoza A., Flores-López M.L., Robledo-Olivo A., Juárez-Maldonado A & González-Morales S., *A Molecular Vision of The Interaction of Tomato Plants and Fusarium Oxysporum* 10(577), 2017, 72-127.

Geetharamani G. & Arun Pandian J., "Identification of Plant Leaf Diseases using a Nine-Layer Deep Convolutional Neural Network," *Computers & Electrical Engineering*, 76, 2019, 323–338.

Godinho D.P.., Serrano H.C., Da Silva A.B., Branquinho & Magalhaes C. S. *Effect Of Cadmium Accumulation on the Performance of Plants and of Herbivores that Cope Differently with Organic Defenses.* Frontier Plant Science. 2018; 9: 1–12.

Gordon T., *Fusarium Oxysporum and the Fusarium Wilt Syndrome.* Annual Review Phytopathology. 2017, 55:23-39.

Gupta S.K. & Gupta M .. *"Diseases Of Vegetables Under Protected Cultivation Conditions."* Plant Disease Research. *33*(1) 2018: 1-14.

Hagassou, Djangsou, Enrico Francia, Domenico Ronga & Matteo Buti. *"Blossom End-Rot in Tomato (Solanum Lycopersicum L.): A Multi-Disciplinary Overview of Inducing Factors and Control Strategies."* Scientia Horticulturae 249, 2019: 49-58.

Hashem H.A., Shouman A.I. & Hassanein R.A., *Physico–Biochemical Properties Of Tomato (Solanum Lycopersicum) Grown in Heavy–Metal Contaminated Soil*. Actaagr. 68(4), 2017, 334–341.

Hashemi, M, Dania T, Murilo S, Clara B.C, Jenifer S, Christian B Andersen & Laura J Grenville-Briggs. "*The Hunt for Sustainable Biocontrol of Oomycete Plant Pathogens, A Case Study of Phytophthora Infestans*." Fungal Biology Reviews, 2021.

Hermann E & Lecomte V. C. *Current Status of Fusarium Oxysporum Formed Species and Races.* Phytopathology. 109(4): 2019, 512-530.

Holland, JE, AE Bennett, AC Newton, PJ White, BM Mckenzie, TS George, RJ Pakeman, JS Bailey, DA Fornara & RC Hayes. "*Liming Impacts on Soils, Crops and Biodiversity In The Uk: a Review."* Science of the Total Environment 610, 2018: 316-332.

Hyder S., Gondal R., Ahmed S.T, Sahi A & Hannan A. *First Report of Charcoal Rot in Tomato Caused by Macrophomina Phaseolina from Pakistan.* 2018.

Jiang, P., Chen, Y., Liu, B., He, & Liang, C. *Real-Time Detection of Apple Leaf Diseases Using Deep Learning Approach Based on Improved Convolutional Neural Networks* , 7, 2019, 59069–59080.

Jong-Tar K., Chang L.L., Yuan Yen C., Hua Tsai T., Chi Chang Y., Tang Huang Y., & Chien Chung Y.. "*Development of Fluorescence in Situ Hybridization as a Rapid, Accurate Method Fordetecting Coliforms in Water Samples."*Biosensors 11(1), 2021, 8.

Joseph M. S. , Dennis P. B., Kuang Z., Pelton A. & Naik R. R. "*Creation of Stable Water-Free Antibody Based Protein Liquids*." Communications Materials 2(1), 2021. 1-11.

Joyati D & Mishra H. N.. "*Recent Advances in Sensors for Detecting Food Pathogens, Contaminants, and Toxins: a Review."*European Food Research and Technology, 2022, 1-24.

Kaashima G., *Tomato Rot Diseases: Causes, Types, Treatment and Prevention*, 15, 2021, 18:31.

Kannan R.A, A. Solaimalai, M. Jayakumar & U.Surendran. "*Advance Molecular Tools to Detect Plant Pathogens." in Biopesticides,* Elsevier, 2022.401-416:

Kang Q., Liu Q., Huang Y., Xia Y. & Zhang S.. *"Management of Bacterial Spot of Tomato Caused by Copper-Resistant Xanthomonas Perforans using a Small Molecule Compound Carvacrol."* Cropprotection *132,* 2020: 105114.

Kaur, S., Pandey, S & Goel, S. Plants Disease Identification and Classification through Leaf Images: a Survey. *Arch. Comput. Methods Eng.* 26, 2019. 507–530.

Kenneth O. , Ekong I., Markson I. O & Enwere K.. "*Fingerprint Biometric System Hygiene Andthe Risk of Covid-19 Transmission."*JMIR Biomedical Engineering. 5(1). 2020.

Khakimov A., Salakhutdinov I, Omolikov A & Utaganov S. "*Traditional And Current-Prospective Methods of Agricultural Plant Diseases Detection: a Review." in IOP Conference*series:Earth and Environmental Science, 951, 2022.

Khan M.A.H., Rao M. V& Li Q.. "*Recent Advances In Electrochemical Sensors for Detectingtoxic Gases: No(2), So(2) and H(2)S."Sensors (Basel)* 19 (4), 2019.

Khan N., Maymon M & Hirsch A.M., *Combating Fusarium Infection using Bacillus- Based Antimicrobials.* 5(4), 2017, 75.

King B.A., Tarkalson D.D, Sharma V.& Bjorneberg D.L. "*Thermal Crop Water Stress Index Baseline Temperatures for Sugarbeet in Arid Western Us."*Agricultural Water Management 243, 2021

Kisa D., *Expressions of Glutathione-Related Genes and Activities of Their Corresponding Enzymes in Leaves of Tomato Exposed to Heavy Metal. Russ*. *Journal of Plant Physiology*. 64(6), 2017, 876–882.

Kumar H., "*Biotechnology: Discoveries and their Applications in Societal Welfare*." *Biotechnology Business-Concept to Delivery.* Springer, 2020, 3-44.

Kumar, S Pavan, A Srinivasulu & KR Babu. *"Symptomology of Major Fungal Diseases on Tomato and Its Management."* Journal of Pharmacognosy and Phytochemistry 7 (6)2018: 1817-1821.

Kumbar, B, Riaz M, Nagesha S.N,Nagaraja M.S, Prashant D.G, Ondara Z. K, Arti Karosiya & Mohan Chavan. *"Field Application of Bacillus Subtilis Isolates for Controlling Late Blight Disease of Potato Caused by Phytophthora Infestans."* Biocatalysis and Agricultural Biotechnology 22, 2019: 101366.

Lavorgna M. , Orlo E.., Nugnes R. , Piscitelli C., Russo C. C., & Isidori M. *Capsaicin In Hot Chili Peppers: In Vitro Evaluation of Its Antiradical, Antiproliferative and Apoptotic Activities*. Plant Foods and Human Nutrition. 2019; 74(2): 164–170.

Leander D. Melomey, Agyemang Danquah,Samuel K. Offei, Kwadwo Ofori, Eric Danquah & Micheal Osei, Review on Tomato (*Solanum Lycopersicum,L)* Improvement Programmes in Ghana, 2019. Doi: 10.5772/Intechopen

Li J., Gao M., Gabriel D.W., Liang W & Song L., *Secretome-Wide Analysis of Lysine Acetylation in Fusarium Oxysporum F. Sp. Lycopersici Provides Novel Insights into Infection-Related Proteins.* Frontiers in Microbiology.11, 2020b, 559440.

Li, J., Fokkens L. Conneely, L.J & Rep, M, *Partial Pathogenicity Chromosomes in Fusarium Oxysporum are Sufficient to Cause Disease and can be Horizontally Transferred*. Environmental Microbiology, 22(12): 2020a, 4985-500.

Liu S., Shang Y., Jiao Y., & Li. N. "*DNA-Based Plasmonic Nanostructures and their Optical and biomedical Applications."* 32(40), 2021.

Liu, B., Zhang, Y., He, D. & Li, *Y. Identification of Apple Leaf Diseases Based on Deep Convolutional Neural Networks. Symmetry* 10(11), 2018.

Lookabaugh A., Thomas B., Shew B., Butler S.C & Louws F.J., *First Report of Black Leaf Mold of Tomato Caused by Pseudocercospora Fuligena in North Carolina,* American Phytophathological Society, 2017, 10.1094.

Martina Sinno, Marta Ranesi, Laura Gioia, Giada D' Errico & Sheridan Lois Woo. Endophytic Fungi of Tomato and Potential Application for Crop Improvement, Agriculture, 10(12), 2020, 587.

Ma J., Du K., Zheng F., Zhang L., Gong Z., & Z. Sun, *"A Recognition Method for Cucumber Diseases using Leaf Symptom Images Based on Deep Convolutional Neural Network," Computers and Electronics In Agriculture,* Vol. 154, Pp. 18–24, 2018.

Magaji B., Fai F., Yirankiyuki F.F & Simon S.Y..*Accumulation of Heavy Metals by Vegetables Grown in Kembu Farms, Gombe, Nigeria.* IRJPAC. 21(5): 2020 1–5.

Maikel S.B.F. , Scholten O. E & Van Kan J. A. "*Peeling the Onion: Towards a Better Understanding of Botrytis Diseases of Onion."*Phytopathology® 111(3), 2021, 464-473.

Majumdar & Dipanwita. "Application Of Microbes in Synthesis of Electrode Materials for Supercapacitors." in *Application of Microbes in Environmental and Microbial Biotechnology*, Springer, 2022. 39-92.

Mannai, Sabrine, Hayfa Jabnoun-Khiareddine, Bouzid Nasraoui & Mejda Daami-Remadi. *"Biocontrol of Pythium Damping-off on Pepper (Capsicum Annuum) With Selected Fungal and Rhizobacterial Agents*." International Journal Of Phytopathology 9(1), 2020: 29-42.

Manuel J.J., Ortega L, Cubillas J. J. & Feito F. R. *"Multispectral Mapping On 3d Models and Multi-Temporal Monitoring for Individual Characterization of Olive Trees*." Remote Sensing 12,(7) ,2020.

Martini, Marta, D. Delić, L. Liefting & H. Montano. *"Phytoplasmas Infecting Vegetable, Pulse and Oil Crops." in Phytoplasmas: Plant Pathogenic Bacteria-1,* Springer ,2018, 31-65.

Maryam H., Tabet D., Sandronm. I Benavent-Celma, C., Seematti J., C. Andersen B. & Grenville-Briggs L. J. *"The Hunt For Sustainable Biocontrol of Oomycete Plant Pathogens, a Case Study of Phytophthora Infestans*. Fungal Biology Reviews, 2021.

Massantini R. , Radicetti E. , Frangipane M.T. & Campiglia E., *Quality of Tomato (Solanum Lycopersicum L.) Changes under Different Cover Crops, Soil Tillage and Nitrogen Fertilization Management. Agriculture.*2021; 11(2):106.

Mcdougall, Sandra, Zenaida Gonzaga, Gordon Rodgers, Adam Goldwater, Lucy Borines, Reny Gerona, Moises Neil Seriño, Marina Labonite, Nelda Gonzaga & Ms Valeriana Justo. *"Project Integrated Crop Management (Icm) to Enhance Vegetable Profitability and Food Security In the Southern Philippines and Australia*." (2019).

Mehrunisa Sheikh, Samina Mehnaz, Muhammad Bila Sadiq, Prevalence of Fungi in Fresh Tomatoes and their Control by Chitosan and Sweet Orange (*Citrus Sinensis*) Peel Essential Oil Coating. Journal of the Science of Food and Agriculture, 101(15), 2021, 6248-6257.

Murillo-Gómez P., Hoyos R. & Chavarriaga P., *Organogenesis In-Vitro using Three Tissue Types of Tomato Plants* Agronomía Colombiana.35 (1), 2017, 5-11.

Muhammad Saqib Malik, Shabeer Haider, Abdul Rehman, Shafiq Ur Rehmam, Muhammad Jamil, Muhammad Anees, Prevalence of Fungi in Fresh Tomatoes and their Control by Chitosan and Sweet Orange (*Citrus Sinensis*) Peel Essential Oil Coating, Journal of Basic Microbiolgy, 62, 2022: 48-62.

Murugan L., Krishnan N., Venkataravanappa V., Saha S., Mishra A.K., Sharma B.K & Rai A.B, *Molecular Characterization and Race Identification of Fusarium Oxysporum F. Sp. Lycopersici Infecting Tomato in India.*Biotechnology.2020,10(11).

Nicola L.Ioos R & A.Santini. "*Fast And Reliable Molecular Methods to Detect Fungal Pathogensin Woody Plants."*Applied Microbiology and Biotechnology .104(6) 2020, 2453-2468.

Nicholas R.F. & E. Mitcham J. "*Validation and Demonstration of a Pericarp Disc System Forstudying Blossom-End Rot of Tomatoes."*Plant Methods 17(1), 2021: 1-10.

Nizamani, S, A.A. Khaskheli, A.M. Jiskani, S.A. Khaskheli, A.J. Khaskheli, G.B. Poussio, H. Jamro, M.I. & Khaskheli: *Isolation and Identification of the Fungi Causing Tomato Fruit Rot Disease in the Vicinity of Tandojam, Sindh*: Agricultural Science Digest. 41: 2021, 186-190.DOI: 10.18805/Ag.D-269

Ning Z., Yang G., Pan Y., Yang X., Chen L. & Zhao C.. "*A Review of Advanced Technologies and Development for Hyperspectral-Based Plant Disease Detection in the Past Threedecades*." *Remote Sensing* 12(19), 2020: 3188.

Ninkovic, Velemir, Dimitrije Markovic & Merlin Rensing. "*Plant Volatiles as Cues and Signals In Plant Communication."* Plant, Cell & Environment 44(4), 2021, 1030-1043.

Opoku B.A, Kwoseh C, Gyasi E &. Moses E, *Incidence and Severity of major Fungal Diseases on Tomato in Three Districts within the Forest and Forest-Savannah Agro-Ecological Zones of Ghana.* Ghana Jnl. Agric. Sci. 56 (2), 2021, 46 – 60.

Ogunsiji A.O., Ibrahim T.O & Odusanya F.A. "*Management Strategies of Forest Plantdiseases: a Review."International Journal of Plant & Soil Science*, 2020, 87-95.

Orawan H., Yoohat K., Danwisetkanjana K., Kumpoosiri M., Rukpratanporn S., Theppawong Y., Phuengwas S., Makornwattana M., Charlermroj R., & Karoonuthaisiri N.. "*Double Antibody Pairs Sandwich-Elisa (Daps-Elisa) Detects Acidovorax Citrulli Serotypes with Broad Coverage."* Plos One 15(8), 2020.

*Oxide And Hydrogen Sulfide: An Indispensable Combination for Plant Functioning."*Trends in Plantscience. 26(12) .2021 1270-1285.

Ozgur B., Turini T. A. Lestrange, M., Stoddard S., Miyao G., Aegerter B. J. Chen, L.F., Mcroberts N., D. Ullman E & Gilbertson R. L.. *"Development Of An Ipm Strategy For Thrips and Tomato Spotted Wilt Virus in Processing Tomatoes in the Central Valley of California*." Pathogens. 9(8). (2020), 636.

Pavan Kumar S, A Srinivasulu & K Raja Babu, *Symptomology of Major Fungal Diseases on Tomato and Its Management*: Journal of Pharmacognosy and Phytochemistry; 7(6): 2018, 1817-1821.

Paul, Rajesh, Emily Ostermann & Qingshan Wei. *"Advances in Point-Of-Care Nucleic Acid Extraction Technologies for Rapid Diagnosis of Human And Plant Diseases." Biosensors and* Bioelectronics 169, 2020.

Pavan S, Srinivasulu A & Babu K. R. *"Symptomology of Major Fungal Diseases on Tomato and Its Management." Journal of Pharmacognosy and Phytochemistry*7(6) 2018,1817-1821.

Pereira, Leonel, Kiril Bahcevandziev & Nilesh H Joshi. *Seaweeds as Plant Fertilizer,* Agricultural Biostimulants and Animal Fodder: CRC Press, 2019.

Prasanth M, A. J. Tamhankar, S. Leptihn & N. Ramesh. "*Pharmacological and Immunological Aspects of Phage Therapy."*Infectious Microbes & Diseases 1(2), 2019. 34-42.

Przybylska- Balcerek A.., Frankowski J., & Stuper- Szablewskak. *The Influence of Weather Conditions on Bioactive Compound Content in Sorghum Grain.* European. Food Research. Technology. 2020; 246: 13–22.

Qiao, Kang, Qingchun Liu, Yi Huang, Ye Xia & Shouan Zhang. "*Management Of Bacterial Spot of Tomato Caused by Copper-Resistant Xanthomonas Perforans Using a Small Molecule Compound Carvacrol*." Crop Protection 132, 2020: 105114.

Rabab Sanoubar, Lorenzo Barbanti, Fungal Diseases on Tomato Plant Under Greenhouse Condition, European Journal of Biological Research Review,2017,2449-8955,Article ISSN.

Rahul R, K. Soumyashree , R. Nandan & A. Jagarlapudi. *"Precisionagriculture And Unmanned Aerial Vehicles (Uavs)."* In *Unmanned Aerial Vehicle: Applications in Agriculture and Environment,* Springer, 2020. 7-23:

Ramcharan A., Baranowski K., Mccloskey P., Ahmed B., Legg J., & D. Hughes *"Deep Learning for Image-Based Cassava Disease Detection*," Frontiers in Lant Science, 8,2017, 1852.

Ramesh V.R., Masini L., Mcdougal R., Panda P., Zinger L. De, Brus-Szkalej M., Lankinen Å. & Grenville-Briggs L. J. "*The Presence of Phytophthora Infestans in the Rhizosphere of a wild Solanum Species May Contribute to off-Season Survival and Pathogenicity*." Applied Soil Ecology. 2020, 148.

Rampersad S, N., *Pathogenomics and Management of Fusarium Diseases in Plants*. Pathogens. 9(340) 2020, 21.

Rao G., Huang S., Ashraf U., Mo Z. , Duan M. & Pan S. , *Ultrasonic Seed Treatment Improved Cadmium (Cd) Tolerance in Brassica Napus L.* Ecotoxicology Environmental Safe. 2019; 185:1–6.

Raptis S., Gasparatos D. Economou-Eliopoulos, M. & Petridis A., *Chromium Uptake by Lettuce as Affectedb by the Application of Organic Matter and Cr (VI)-Irrigation Water: Implications to the Land Use and Water Management*. Chemosphere. 210, 2018, 597–606.

Ray L. D.. Ray D., Langham & Kimberly A. *"Fungi, Oomycetes, Bacteria, and Viruses Associated with Sesame (Sesamum Indicum L.).* Cochran ,2021.

Redmon, J & Farhadi, A.,YOLO9000: Better, Faster, Stronger. *IEEE Conference on Computer Vision and Pattern Recognition.* 2017, 6517–6525.

Reitz, Nicholas F & Elizabeth J Mitcham. "*Validation and Demonstration of a Pericarp Disc System for Studying Blossom-End Rot of Tomatoes*." Plant Methods. 17(1), 2021, 1-10.

Reitz, Stuart R, Yulin Gao, William DJ Kirk, Mark S Hoddle, Kirsten A Leiss & Joe E Funderburk. *"Invasion Biology, Ecology, and Management of Western Flower Thrips."* Annual Review Of Entomology. 65, 2020: 17-37.

Ren S., He K., Girshick R., & Sun J., "*Faster R-CNN: Towards Real-Time Object Detection with Region Proposal Networks,"IEEE Transactions on Pattern Analysis and Machine Intelligence,* 39(6), 2017, 1137–1149.

Riikka P., Barderas R., Benito-Peña E. & Moreno-Bondi M. C. "*Recombinant Antibodies and Theiruse for Food Immunoanalysis*." Analytical and Bioanalytical Chemistry, 2021, 1-25.

Rishi B.. "Exploring the Drivers of Xanthomonas Population Dynamics on Tomato and Pepper." (2019).

Riveron G., Javier T. & Aquino-Jarquin G.. "*Crispr/Cas13-Based Approaches for Ultrasensitive and Specific Detection of Micrornas."*Cells 10(7) 2021, 1655.

Rodríguez-Ortega W.M., Martínez V., Nieves M., Simón I., Lidón V., Fernández-Zapata J.C., Martínez N.J.J.,Cámara-Z J. & Garciasánchez F., *Agricultural and Physiological Responses of Tomato Plants Grown in Different Soil Less Culture Systems with Saline Water under Greenhouse Conditions.* Scientific Reports. 9(6733): 2019, 1-13.

Rutigliano, R. Marzaioli, S. De Crescenzo & M. Trifuoggi, *Human Health Risk Fromconsumption of Two Common Crops Grown in Polluted Soils*. Science Totalenvironment 2019; 691: 195–204.

Sandra M., Gonzaga Z., Rodgers G., Goldwater A., Borines L., Gerona R., Neil Seriño M., Labonite M., Gonzaga N., & Ms V. Justo. "*Project Integrated Crop Management (Icm) to Enhance Vegetable Profitability and Food Security in the southern Philippines and Australia*." 2019.

Satta, Eleonora, S. Paltrinieri, & A. Bertaccini, "*Phytoplasma Transmission By Seed." in Phytoplasmas: Plant Pathogenic Bacteria-Ii*,Springer. 2019, 131-147.

Sbartai H. , Sbartai I., Djebar M.R., & Berrebbah H., *Phytoremediation Of Contaminated Soils By Heavy Metals—*"*Case Tomato"*.ISHS Acta Hortic. 2017; 1159: 95–100.

Segorbe D., Di Pietro A., Pérez-Nadales E. & Turrà D., *Three Fusarium Oxysporum Mitogenactivated Protein Kinases (Mapks) have Distinct and Complementary Roles In Stress Adaptation and Crosskingdom Pathogenicity.* Molecular Plant Pathology. United Kingdom. 18(7) 2017, 912-924.

Sergi B.O., Abramova N., Uria N. & Bratov A.. *"Impedimetric Transducers Based on Interdigitated Electrode Arrays for Bacterial Detection–a Review*." Analytica Chimica Acta. 1088, 2019, 1-19.

Shaoqing,C. Ling P., Zhu H. & Keener H. M.. *"Plant Pest Detection Using an Artificial Nose System: A Review."* Sensors 18 (2) , 2018, 378.

Shenghui S., Zhou R., Yahao L , Liu Bo, Guoxiang P. , Q. Liu, Q. Xiong, X.Wang, X.Xia & J. Tu. "*Bacterium, Fungus, and Virus Microorganisms for Energy Storage and Conversion*." Small Methods 3 (12), 2019. 1900596.

Shpileuski. "*B-1, 3-Glucan Effect On The Photosynthetic Apparatus And Oxidative Stress Parameters of Tomato Leaves under Fusarium Wilt*." Functional Plant Biology 47(11) ,2020, 988-997.

Shuai W., Chelliah R., Rubab M., Hwan Oh D., Jalal Uddin M. & Ahn J.. "*Bacteriophages As Potential Tools for Detection and Control Of Salmonella Spp. in Food Systems."* Microorganisms7(11), 2019, 570.

Singh V.K, Singh H.B. & Upadhyay R.S., *Role of Fusaric Acid in the Development of 'Fusarium Wilt' Symptoms in Tomato: Physiological, Biochemical and Proteomic Perspectives.* Plant Physiology and Biochemistry. 2017, 118:320-332.

Stefano P., Matić S., Tiberini A., Giovanni Caruso A., Bella P., Torta L., Stassi R &Davino S.. *"Loop Mediated Isothermal Amplification: Principles and Applications in Plant Virology*." Plants 9(4), 2020, 461.

Stuart S.R.., Yulin G. , William K. D.J, Hoddle M. S., Leiss K. A. & Funderburk J. E., *"Invasion Biology, Ecology, and Management of Western Flower Thrips*." Annual Review Of Entomology65, 2020; 17-37.

Testen A.L, Chala A., Azerefegne F. & Miller S.A. *First Report of Corky Root Rot of Tomato Caused By Pathogen Pyrenochaeta Lycopersici in Africa* .2019.

Traversari S., Cacini S.,Galieni, A.; Nesi, B.; Nicastro, N.; Pane, C. Precision *Agriculture Digital Technologies for Sustainable Fungal Disease Management of Ornamental Plants*. Sustainability, 13, 2021, 3707.

Trieu N., Aaydha Chidambara V., Zoëga Andreasen S., Golabi M., Than Linh Q., Duong Bang D. & Wolff A.. *"Point-of-Care Devices For Pathogen Detections: the Three most Important Factors to Realize Towards Commercialization*." Tractrends in Analytical Chemistry, 2020.

Trong L.V., Tuong L.Q., Thinh B. B., N.T Khoi & V.T Trong, *Physiological and Biochemical Changesin Tomato Fruit (Solanum Lycopersicum L.) during Growth Ripening Cultivated in Vietnam*. Bioscience Research. 2019 16(2):1736-1744.

Van Der Does H.C., Constantin M.E., Houterman P.M., Takken F.L., Cornelissen B.J, Haring M.A, Van Der Burg H.A, & M. Rep, *Fusarium Oxysporum* Colonizes the Stem of Resistant Tomato *Plants, the Extent Varying with the R-Gene Present*. *European Journal of Plant Pathology*. 2019. 154:55-65.

Vijai S., Sharma N. & Singh S.. "*A Review of Imaging Techniques for Plant Disease Detection."*Artificial Intelligence in Agriculture, 2020.

Wang B., Yu H., Jia Y., Dong Q., Steinberg C., Alabouvette C., Edel-Hermann V., Kistler C., Ye K., Ma L.J, & Guo L.*, Chromosome-Scale Genome Assembly of Fusarium Oxysporum Strain Fo47, a Fungal Endophyte and Biocontrol Agent*. Molecular Plant Microbe Interaction. 2020. 33(9):1108-1111.

Wang, G., Sun, Y. & Wang, J. Automatic Image-Based Plant Disease Severity Estimation Using Deep Learning. *Comput. Intell. Neurosci.* 2017, 1–8.

Wenshu He, , Can B, Maria L. Gómez, Xin H, Derry Al, Changfu Z, Victoria A.N, Aamaya B. Perera, Pedro C.B & Saba-Mayoral A. *"Contributions of the International Plant Science Community to the Fight against Infectious Diseases in Humans—Part 2:*

Affordable Drugs in Edible Plants for Endemic and Re-Emerging Diseases." Plant Biotechnology Journal 19(10), 2021,1921-1936.

Yuan G., Zhou Y. & Chandrawati R.. "*Metal and Metal Oxide Nanoparticles to Enhance the Performance of Enzyme-Linked Immunosorbent Assay* (Elisa)." ACS Applied Nano Materials. 3(1),2019, 1-21.

Yuqing, WANG, Yaxian Zhang, GAO Zhipeng & YANG Wencai. "Breeding for Resistance to Tomato Bacterial Diseases in China: Challenges and Prospects." *Horticultural Plant Journal* 4(5), 2018: 193-207.

Zaizai D, C. Tang, Z. Zhang, W. Zhou, R. Zhao, L. Wang, J. Xu, Y. Wu, J. Wu & X. Zhang. "*Simultaneous Detection of Exosomal Membrane Protein and RNA by Highly Sensitive Aptamer Assisted Multiplex–Pcr."*ACS Applied Bio Materials 3(5), 2019, 2560-2567

Zwolak A., Sarzyńska M., Szpyrka E & Stawarczyk K., Sources *of Soil Pollution by Heavy Metals and their Accumulation in Vegetable - A Review.* Water Air Soil Pollution, 2019; 230:1–9.

Journals

Ogunsiji A.O. , T. O Ibrahim & F.A Odusanya. "*Management Strategies of Forest Plant Diseases: A Review." International Journal of Plant & Soil Science,* 2020, 87-95.

Barbedo, J. G. New Automatic Method for Disease Symptom Segmentation in Digital Photographs of Plant Leaves. *Eur. J. Plant Pathol.* 147 (2), (2017). 349–36.

Benn A.M.L., N. Heng C.K, Broadbent J.M. & Thomson W.M. "*Studying the Human Oralmicrobiome: Challenges and the Evolution of Solutions*." *Australian Dental Journal* 63(1) 2018, 14-24.

Masniza S., K. Shafie A., Ismail A.S., Mohd Said N.A., Masdor N.A., Salleh N. H.Md, Teik T.S., Bunawan S. N., Abdul Talib M.A. & Jaffar N.S.. *"Electrochemical Impedimetric Biosensor Based on Silicon-on-Insulator Nanogap for the Detection of Banana Blood Disease Bacterium*." *Malaysian Journal of Analytical Sciences* 25 (2), 2021.184-192.

Mouelhy E., Mansour A. T., Nasry S.A., O. El-Dahab A., Sabry D & Fawzy El-Sayed K.. *"In Vitro Evaluation of the Effect of the Electronic Cigarette Aerosol, Cannabis Smoke, and Conventional Cigarette Smoke on the Properties of Gingival Fibroblasts/Gingival Mesenchymal Stem Cells."Journal of Periodontal Research*, 2021.

Piscitelli C , Lavorgna M. , Isidori M., Russo C., De Prisco R. & Abbamondi G.R. , *Antioxidant And Antiproliferative Activities Of Different Cultivars of Tomatoes (Lycopersicon Esculentum) On Tumoral Cell Lines. Journal of Advance Biology*. 2017; 2(10):2061–2072

Sabrine M., Jabnoun-Khiareddine H., Nasraoui B & Daami-Remadi M.. "*Biocontrol Of Pythium Damping-Off On Pepper (Capsicum Annuum) with Selected Fungal and Rhizobacterial* Agents." *International Journal of Phytopathology* 9(1) .2020, 29-42.

Srinivas C., Nirmala D., Narasimha K. Murthy, C.D., Mohan, Lakshmeesha T.R., Singh B., N.K. Kalagatur, S.R. Niranjana, A. Hashem, A.A. Alqarawi, B. Tabassum, E.F Abd_Allah, S. Chandra Nayaka & R.K Srivastava, *.Fusarium Oxysporum F. Sp. Lycopersici Causal Agent of Vascular Wilt Disease of Tomato: Biology to Diversity– A Review. Saudi Journal of Biological Sciences* 2019. 26(7):1315-1324.

Tian, Y., Yang, G., Wang, Z., Li, E. & Liang, Z. Detection of Apple Lesions in Orchards Based On Deep Learning Methods of Cycle GAN and YOLOV3-Dense. *J. Sens.* 2019, 1–13.

Trebolazabala, J. M., Maguregui, H. Morillas, Z. García-Fernandez, A.De Diego & Madariaga J.M..*Uptake of Metals by Tomato Plants (Solanum Lycopersicum) and Distribution inside the Plant: Field Experiments in Biscay (Basque Country). Journal of Food Composition Analysis.* 59, 2017, 161–169.

Wooding M, E. R. Rohwer & Y. Naude. "*Determination of Endocrine Disrupting Chemicals And Antiretroviral Compounds In Surface Water: A Disposable Sorptive Sampler with Comprehensive Gas Chromatography - Time-of-Flight Mass Spectrometry and Large Volume Injection with Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry*." *Journal of Chromatography*. 1496, 2017, 122-132.

Zakawa N.N, Timon D, Yusuf CS, Tizhe TD, Bala UJ, Isa A, Waja S & Alphonsus G: *Isolation and Control of Fungal Rot Pathogen of Tomato Fruit using Aqueous Leaf Extracts of Azadirachta Indica in Mubi, Adamawa State.* Res J Plant Pathol. 2(1):2019, 09.

Zirui F., Cheng Y. Lu. & James J. L., "*Recent Advances in Biosensors for Nucleic Acid and* Exosome Detection." *Chonnam Medical Journal*. 55(2), 2019, 86-98.

Magazine Articles

Azad C. S. ,Rautela P., Gupta S. & Singh R.P, "*Major Diseases Of Chili and their Management." in diseases of Fruits and Vegetable Crops*, Apple Academic Press, 353-377: 2020.

Leonel P., Bahcevandziev K. & Joshi N. H. *Seaweeds As Plant Fertilizer, Agricultural Biostimulants And Animal Fodder:* CRC Press, 2019.

Pankaj, Rautela, Supriya Gupta, CS Azad & R.P Singh. "Diseases of Tomato Crops And Their Management." In *Diseases Of Fruits And Vegetable Crops*, 181-209: Apple Academic Press, 2020.

Rautela P., Gupta S., Azad C.S., And Singh R. P.. *"Diseases Of Tomato Crops And Their Management." in Diseases of Fruits and Vegetable Crops*, Apple Academic Press, 181-209:, 2020.

Stein K, Coulibaly D., Stenchly K., Goetze D., Porembski S., & Lindner A., *Bee Pollination Increases Yield Quantity and Quality of Cash Crops In Burkina Faso*, West Africa Science. Report. 7, 2017, 17691.

Textbooks

Bhabesh D & Babu A. "*Chapter-3 Thrips (Scirtothrips Dorsalis, Hood): Vectors of Tospoviruses in Agricultural Crops."* Essentials of Science, 35.2020.

Bulgari R., Franzoni G., Ferranti A., *Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions.* Agronomy.2019, 306(9): 1–3.

Claudia N., Noorlander M., M. Hubbell A.. *"Tomato Spotted Wilt Virus Of Tomato And Pepper*." (2019).

Fluorescence-Based Assays for High-Throughput Screening." Analytical Chemistry 91(1) .2018, 482-504.

V.C. Biju, L. Fokkens, P.M. Houterman & M. Rep, B.J.C. Cornelissen, *Multiple Evolutionary Trajectories have Led to the Emergence of Races in Fusarium* Oxysporum Applied and Environmental Microbiology. 83(4), 2017, 02548-16.

Website

Agronet. 2021. Information and Communication Network of the Colombian Agricultural Sector. Available Online: http

Food and Agriculture Organization of the United Nations. Plant Diseases and Pests. Available Online: Http://Www.Fao.Org/Emergencies/Emergency-Types/Plant-Pests-and-Diseases/En/.

Appendices

Appendix 1

Abbreviation	Meaning
PCR -	Polymerase Chain reaction
gDNA-	Genomic deoxyribonucleic acid
DNA-	Deoxyribonucleic acid
BLAST-	Basic Local Alignment Search Tool
NCBI-	National Centre for Biotechnology Information

Appendix 2

Rotting Cherry Tomato Samples Used

Appendix 3

Rotting Royal Tomato Samples Used

Appendix 4

Culturing of Rotting Tomato Samples at Microbiology Laboratory, Lead City University

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