VARIETIES OF TOMATO FRUIT, ABUNDANCE AND MOLECULAR BIODIVERSITY OF ALTERNARIA ALTERNATA (L) TOMATO FRUIT CANKER, FROM FARMERS FIELD IN NIGER STATE, NIGERIA

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MTech\SLS\2018\8292

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSTIY OF TECNOLOGY MINNA, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF TECNOLOGY (MTech) IN BOTANY (MYCOLOGY)

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ABSTRACT

Tomato (*Solanumlycopersicum L*) is an important vegetaive crop worldwide. However, the quality of fruit production is hindered by fruit canker caused by *Alternaria alterrnata.* Thereforethis research investigated the biodiversity of *Alternaria alternata* from farmers' tomato fieldsin Niger state. Three hundred samples of Tomato fruit with canker samples were collected from thirty (30) farms across all the three Agricultural Zones of Niger State, namely Bida, Lapai, Lemu, Kataeragi and Sabon-gida (zone 1), Garatu, Sabon-dag and Paiko (zone 2), Wushishi and Zungeru (zone 3). Isolation of fungal species was done using dilution of 10^4factor on PDA. Identification of tomato varieties were done following standard procedures. Molecular diversity were examined using Internal Transcribed Spacer (ITS) marker. Two hundred (200) isolates were obtained. The order of occurrence of highest to least was as follows. Samples from Bida (17.5 %) has the highest occurrence of canker followed by samples from Lemu (14 %) whiles samples from Garatu (5 %) were the least with occurrence of fruit canker.Varietal results shows that Ronita V.F (29.5 %) has the highest followed by Roma V.F (22 %) whiles variety Chico (9 %) recorded the least incidence of *Alternariaalternata* across selected study areas.The blast results obtained from this study showed that samples from Bida (OK175632), Lapai (OK175633), Sabon gida (OK175635) Kataeregi (OK175636) from Zone I. Garatu (OK175637), Paiko (OK175639) from Zone II. Wushsishi (OK175640) from Zone III, all had 100% presences of *Alternaria alternata*, while Lemu (OK175634) from Zone I and Sabon daga (OK175638) from Zone II, are 99.80%. Dendogram obtained from the molecular analysis grouped the fungal strains in to two (2) major clusters. *Alternaria alterrnata*were abundant in all the three zones under the study. Morphological and molecular studies showed that all the isolates were *Alternaria alterrnata* but of different strains. The biodiversity analysis shows that different strains of *Alternaria alternata* werepresent and responsible for tomato fruit canker in Niger state. Therefore, research on control of *Alternaria* species should be intensified to proffer solution to reduce economic loses.

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LIST OF ABBREVIATIONS

BOA	Bank of Agriculture
BOI	Bank of Industry
FAO	Food and Agricultural Organization
IAR	Institute for Agricultural Research

IFPRI-PBS	International Food Policy Research Institute-Programme for Biosafety Systems
MoFA	Ministry of Food and Agriculture
NEXIM	Nigerian Export Import Bank
PDA	Potato Dextrose Agar
Mm	millimeter
Mg	milligram
°C	Degree Celsius
%	Percentage

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Tomato (*Solanum lycopersicum L*) is an annual crop belonging to the family *solanaceae*. It is one of the most important vegetable crops cultivated around the worldwide (Singh *et al*., 2011).Tomato is an important vegetable which is widely consumed in various form in Nigeria with 25,000 tons of fresh tomato produced annually (FAO, 2019). Currently, about 40% of the domestic market in Nigeria sales of products comes from horticultural crops in which tomato is one of the most important crops grown in the country (Donkoh *et al*., 2013). While tomatoes are fruits, they are botanically classified as berriesand are commonly used as vegetable ingredient or side dish. In the human diet, it is an important source of micronutrients, certain minerals (notably potassium) and carboxylic acids, including ascorbic, citric, malic, fumaric and oxalic acids (Zoran *et al*., 2014).

Together with its derived products, tomato is one of the major food sources of carotenoids, providing an estimated 80% of daily intake of lycopene, and folate, ascorbic acid, flavonoids, a-tocopherol and potassium in the western diet (Daniela *et al*., 2013). With high content of lycopene, tomato is used in cancer treatment especially in prostate cancer (Singh *et al*., 2011), also reduces the risk of breast, neck and head cancer. Tomato is consumed in diverse ways; raw or cooked, in many dishes, sauces, salads and drinks. According to the National Cancer Institute, there is enough data to show that people who consume large amount of tomato products have significantly decreased risk of prostate, lung and stomach cancer and this is mainly because tomato contributes to the health of the immune system through the formation of blood vessels (Samuel and Oriji, 2015).

Numerous varieties of the tomato plant are widely grown across the world including Nigeria, with greenhouses, various irrigation system allowing for the production of tomatoes throughout all seasons of the year. Unfortunately, Nigeria is not able to export tomato and its products because of inadequate production and high demands of domestic consumers. The major constraints of tomato production in Nigeria are inadequate supply of good quality seeds, lack of proper diseases and pest management, inadequate storage facilities, and lack of sufficient processing facilities (Samuel and Oriji, 2015). To produce competitive products, proper machinery is required, and these machines are costly since most of them are imported. Raising business capital is difficult in Nigeria because of high cost of funding and few available venture capital companies and the fact that commercial banks require stiff collaterals (Robinson *et al*., 2010).

Despite all these constraints, lack or poor managements of diseases and pests are major concern leading to low production of this vegetable crop in the Northern part of the country especially in Niger State. Tomato is incited by taxonomic diverse groups of phytopathogens among which are fungi, bacteria, viruses and parasitic nematodes diseases (Eloff and Mc-Gaw, 2014). Tomato plants are susceptible to several fungi, bacteria and viruses. Fungi and bacteria cause foliar (leaf), fruit, stem or root diseases. (Eloff and Mc-Gaw 2014). Damage caused by diseases can result in considerable yield losses to farmers (Shankara *et al*., 2005). However, there are about 200 known diseases of tomato, of which 30 are economically important (Firas *et al*., 2017).

Like other pathogens, fungi caused more problems to this plant because most of the species are cosmopolitan in nature. They are only noticed when their disease severity is high and yield reduction increases. However, this plant is susceptible to fungal attack due to its thin skin (Shankara *et al*., 2005). *Alternaria alternata* is one of the most

popular fungal species of genus *Alternaria*. It can be found in soil, as a saprophyte, also in plants where it causes diseases and also as a pathogen (during its pathogenesis phase) where it causes animal diseases by producing various toxic substance many species of the genus *Alternaria* causes diseases in tomato, potato and eggplant in all the continents of the world. The fungi are frequently associated with fruit canker; leaf blight and stem blight of the *Solanaceae* plant family are *A. alternata*, *A. arborescens* and *A. tenuissima.* (Nabahat *et al*., 2014).

The study of diversity is a useful tool to understand the biology of pathogenic populations has suffices to determine the distribution of pathogens, epidemiologic issues, interactions between host and pathogenic species, and pathogenic variability. Such investigations could also lead to the identification of strategies for disease control (Leiminger *et al*., 2013) and currently, internal transcribed spacer ribosomal RNA (ITS rRNA) is a new, simple, and easy molecular tools used to in detecting pathogenic fungi at the species level.

1.2 Statement of the Research Problems

Despite the economic importance of tomatoes (*solanum lycopersicum*) as source of food supplements, its production throughout Nigeria, particularly by smallholder farmers is mostly affected by several constraints. These constraints include several fungal and viral disease, nematodes, foliar, soil and storage pests, and scarce source of improved varieties as well as limited knowledge in the production of the fruits. These constraints greatly reduce yields, quality and market value of tomatoes and discourage many farmers from growing the fruit, even in major production areas such as Niger state. (Lengai *et al*., 2017).

Numerous pathogens reduce the quality and quantity of tomato fruit, most especially fungal pathogen, among which is *Alternaria alternata* causing canker in fruit. *Alternaria alternata* is one of the major disease causing high economic losses to tomato in Nigeria (Lengai *et al*., 2017). And overtime, the control strategy has been the application of chemical as well as botanical fungicides, which has failed to yield the desired result as most of these species of this pathogen developed resistance through mutation. Therefore evaluating the bio-diversity of the different tomato varieties cultivated and incidence fruit canker pathogen *(Alternaria alternata)* in Niger state will increase the information needed for the management of the disease in the study area.

1.3 Aim and Objectives of the study

Aim of the study

The Aim of this study is to evaluate Varieties of Tomato Fruit, Abundance and Molecular Biodiversity of *Alternaria Alternata (L*) Tomato Fruit Canker in Niger State, Nigeria.

Objectives of the study

The Objectives of the study are to;

i. identify tomato species/varieties cultivated in Niger state.
ii. determine the abundance of fruit canker on the field.
iii. isolate and identify *Alternaria alternata*pathogen of tomato fruit canker
iv. determine the percentage frequency of *Alternaria alternata* associated with tomato fruit canker in Niger state
v. determine the molecular bio-diversity of *Alternaria alternata* isolated.

1.4 Justification for the Study

Tomato (*Solanium lycopersicum*) is one of the most important vegetable fruit grown in many parts of Nigeria of which Niger state is one of the producing states. However, despite the economic importance of this crop, its susceptibility to disease as a result of perishability (moisture) of this crop was seen as a great threat to the tomato farmers. However the most common disease that affecting tomato is fruit canker caused by *Alternaria alternata* which is often control by fungicides.

Overtime the development resistance by the pathogen has called for other alternative control method such as genetic mutation of the variety. Therefore evaluating the diversity of the pathogen *Alternaria alternata* in Niger state is important in developing a mutation variety of resistance

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of Tomato

Tomato plant (*Solanum lycopersicum L.)* is mostly self-pollinated but partly also cross pollinated (Shankara *et al*., 2005). The fruit is fleshy berry, globular to oblate in shape and 2-15 cm in diameter. The immature fruit is green and hairy. Ripe fruits range from yellow, orange to red. Stem growth habit ranges between erect and prostrate. It grows to a height of 2- 4 m. The leaves are spirally arranged and are between 15 - 50 cm long and 10 - 30 cm wide. Inflorescence is clustered and produces 6-12 flowers. Tomato has numerous seeds, which are kidney or pear shaped. They are hairy, light brown 3-5 mm long and 2-4 mm wide. The embryo is coiled up in the endosperm. Approximate weight of 1000 seeds range between 2.5 – 3.5 g (Shankara *et al*., 2005).

2.2 Scientific Classification of Tomato

Tomato belongs to

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family:*Solanaceae*

Genus:*Solanum*

Species*:lycopersicum*

Binomial name: *Solanum lycopersicum* L.

Synonyms: *Lycopersicon lycopersicum* (L.) H.Karst.

Lycopersicon esculentum Mill.

Source: Adepoju (2014)

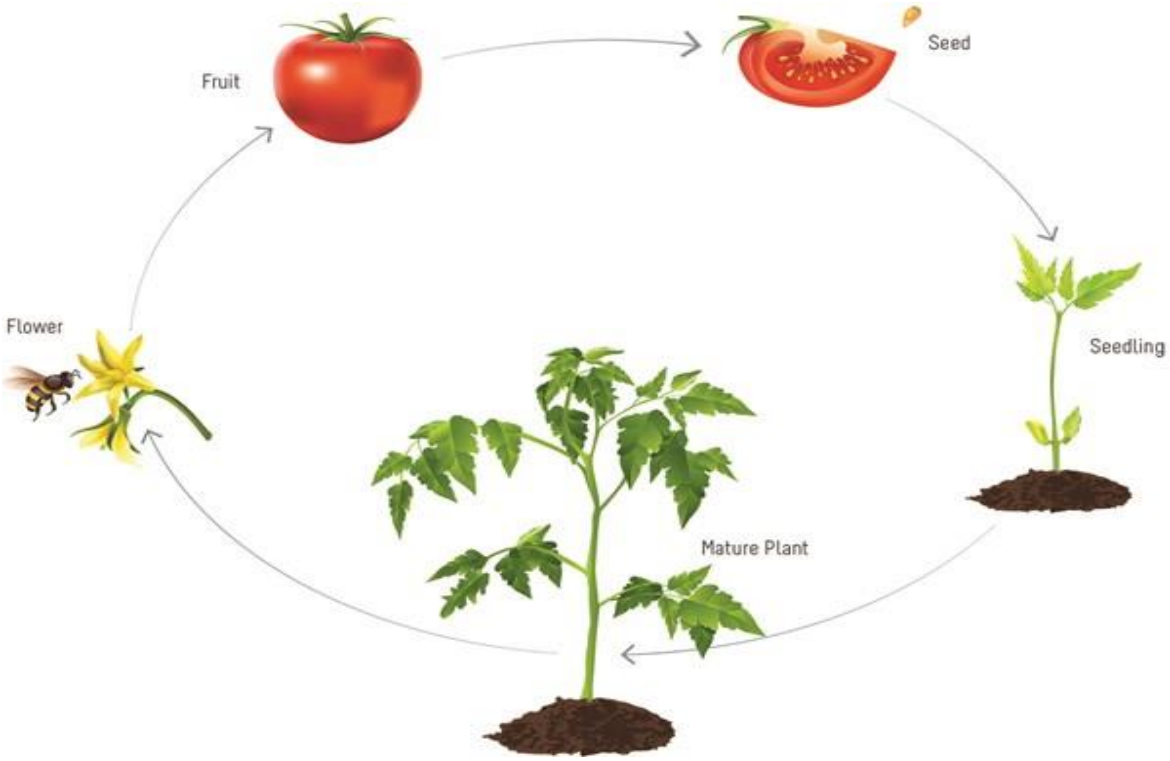


Figure 2.1 Tomato life cycle

Source: Jones (2012)

2.3 Origin and Cultivation of Tomato

Tomato was first domesticated in Mexico in mid-16th century (Paran *et al.*, 2007). It was widely distributed as a wild plant in the tropics and subtropics. The crop has become widely grown around the world because of its importance and value (Adepoju, 2014). Tomato is widely grown in the Central, East and West Africa particularly, in Ghana and Nigeria. Tomato is widely cultivated across Nigeria. Smallholder farmers planting on between 0.5 and 4 hectares of land account for 90% of production, with the balance contributed by commercial producers (PWC, 2018).

Tomatoes have been recognized as important components of human nutrition particularly in sub-Saharan Africa (SSA) where there is a serious problem of malnutrition (Kumar, 2017). Tomatoes are requirements of human diet, which nourish

growth, development and reproduction of human beings and highly medicinal (Kumar, 2017). These crop species are not only good sources of essential vitamins and minerals, they are widely used as the complement to starchy staple foods. Tomatoes are first raised in nurseries before transplanting to the field. The input supplies required include seeds, fertilizer, pesticides, nursery supplies, greenhouse, and ancillary equipment. Most of the inputs are not produced in Nigeria, making them a little more expensive than what the farmers can afford.

2.4 Varieties of Tomato

Various varieties of tomato (Norman, 1992) available worldwide have been classified, based on shape and color of the fruit, height of the tomato plant, days to maturity, disease resistance and other growth characteristics which are usually determinate or indeterminate. Some foreign varieties include big boy, beef master and Goliath, all from Canada. Tomato F1 No.7 and F1 Tyking 5 are from Vietnam. Red Cherry, Floradade, jam Roma and Royal sluis are from U S A. Starke Money maker and Starke Heinz 1370 are from South Africa. The available cultivars in West Africa include Pectomech EEC, Pectomech CEE, Burpee Roma VF and Ronita VF (Robinson *et al*., 2010). Clottey *et al*. (2009) reported that major tomato varieties in Vea. (Upper East region), Ghana, are Pectomech, Tropimech and Roma (Robinson *et al*., 2010) also cited two varieties of tomato grown in Ghana as Bolga and Ashanti. Robinson *et al*. (2010) mentioned varieties such as Rasta, Power, Power Rano, and Wosowoso, grown under rain-fed conditions with Power Rano often preferred due to its high tolerance and resistance to diseases, described the Power' variety as the predominant variety for cultivation Ghana.

2.4.1 Varieties of tomato in Nigeria

In Nigeria, the elite tomato varieties developed in the 1970’s for the industries, by the Institute for Agricultural Research (IAR), Ahmadu Bello University, are still in use.

(Samuel and Oriji, 2015) highlights the different varieties of tomatoes available in Nigeria. However, there are few varieties that were recommended by the International Institute for Tropical Agriculture (IITA) because of their high yield. The fruit have been classified into five botanical varieties namely commune (common tomato), cercisiforme (cherry tomato), periforme (pear tomato), garandiforme (potato leave tomato) and validum (upright tomato), (Tsado, 2016).

Table 2.1 IITA Recommended Varieties of Tomatoes for High Yield.

S/N	Variety	Description
1	Ife No.1	High yielding determinate bushy plant.
2	Ronita	Plum-shaped high quality fruits. Moderately resistant to root-knot nematode.
3	Local Cultivars	Fairly resistant to virus, round and irregular shaped fruits, softand prone to cracking. Plants are intermittently tall and produce fruits for longer period.
4	H9 - 1-6	Resistance to foliage disease,high yielding with firm fruits

(Source: IITA, Ibadan.)

2.5 Uses and Nutritional Value of Tomato

Tomato can be used as vegetable served with rice and salads. Its principal use in Nigeria is in soups and stews (Tambo and Gbemu, 2010). They abound with essential nutrients such as vitamins and minerals. Tomatoes are important not only because of the large amount consumed, but also because of their high health and nutritional contributions to humans. The tomato processing industry has made tremendous advances, developing many forms of tomato-based foods, such as sauces, catsup (ketchup), puree, pastes, soups, juices and juice blends, and canned tomatoes either whole or in diced, sliced, quartered or stewed form (Preedy and Waltson 2008).

The tomato's attractive color and flavor have made it a dietary staple in many parts of the world. Nutritional considerations also bring the tomato to the forefront. In the human diet, it is an important source of micronutrients, certain minerals (notably potassium) and carboxylic acids, including oxalic, citric, and ascorbic, malic, fumaric acids (Hernandez-Suarez *et al*., 2007).

2.6 Economic Importance of Tomato

Nigeria is the fourteenth largest producer of tomatoes in the world and the second largest producer in Africa and the third largest importer of processed tomato commodities (Eloff and Mc-Gaw, 2014). Tomato is cultivated continuously throughout the year in Nigeria. About 1.8 million metric tons of fresh fruits are produced for domestic consumption, (Donkoh *et al*., 2013) with national demand of about 2-3 million metric tons annually; this leaves a demand gap of about 500,000 metric tons (FAO, 2020). Other factors that make tomato important so much in this country is that it is a constituent in almost all diets. The fruit is eaten either raw or cooked in the form of soup, jam, ketch-up and salad, among others (Asare-Bediako *et al*., 2007). It is a good source of vitamins and minerals which help to make a balanced diet for children and pregnant women (Preedy *et al*., 2008). The leaf is used for medicinal purposes for example to cure ring-worm; a fungal disease that affects the skin (Preedy *et al*., 2008).Tomatoes are grown both in most home gardens and commercially as one of the words most popular vegetables.

In Africa, vegetables constitute the fourth largest group of commodities produced for various uses. Among the vegetables, tomato is highly appreciated and consumed in African countries especially in Nigeria for the purpose of diet and healthcare (Kumar, 2017). Tomato is grown for its fruit and is used in varieties of ways for the production of puree pastes, juices, and canned fruit or mixed in chilli sauces. Tomato fruit is found

to have high amount of vitamin C. The seed contains 22- 29% crude fat; 15 – 28% crude fibre; 5 – 10% ash content and 23 – 34% crude protein (Tsado, 2016).

2.7 Production of Tomato

Globally, tomato is the second most widely consumed vegetable after potato. To produce a fruit, the tomato plant requires between 90 and 150 days (Behzadian *et al*., 2015; FAO, 2020). Global tomato production was 182.26 million tonne in 2018, China accounting for the largest 33.81 % (FAO, 2019). The modern cultivated varieties of tomato are thought to have originated from the wild tomato with common name such as vine-like herb of the nightshade family (Pardee, 2008). It is native to the Andean regions of the coastal strip of western and southern America (Cox, 2000). Nigeria has the largest area harvested for fresh tomato in Africa with 541,800 ha followed by Egypt with 214,016 ha (FAO, 2019).The difficulty in accessing inputs and technology makes it impossible for farmers to maximize production in most part of the world (Daniela *et al*., 2013)

In Nigeria, most of the farmers have very small holdings, making commercial production impossible Most of the tomato producers are peasants or small-scale farmers (60%), although there are a few medium scale farmers (30%) and large-scale farmers (10 %).Smallholder farmers planting on between 0.5 and 4 hectares of land account for 90% of production, with the balance contributed by commercial producers (PWC, 2018). Land preparation for small-scale farmers is done with manual tools (hoe). Medium scale farmers use a combination of hoes and tractor operated ploughs. Large-scale farmers use ploughs for land preparation and other preparation activities (transplanting, weeding, fertilizer application, etc.). Harvesting is done manually for all farmer categories.

Tomato fruit are commonly red in color and it comes in different shapes based on the variety. The plum tomatoes are oblong tomatoes bred to make excellent tomato sauce. Plum tomatoes are also called processing or paste tomatoes. These tomatoes are grown to be cooked, roast and blend. The santom tomatoes are small,snappy bite-sized tomatoes. This tomatoes type is reminiscent of the wild berries in south America. They can be incredibly juicy, popping at the slightest pressure Preedy and Waltson (2008).

2.7.1 Climatic requirements for tomato production

Tomato is a warm-season plant. It requires a relatively cool, dry climate for high yield. However, it is adapted to a wide range of climatic conditions from temperate to hot and humid tropical. The optimum temperature for most varieties is between 21 and 24 °C. The plant tissues are damaged below 10 °C and above 38 °C (Singh *et al*., 2011). Light intensity affects the color of the leaves, fruit set and fruit color. Water stress and long dry periods will cause buds and flowers to drop off, and the fruits to split. However, if rains are too heavy and humidity is too high, the growth of molds will increase and the fruit will rot (Shankara *et al*., 2005).

2.7.2 Soil requirements for tomato production

Tomatoes can be cultivated in a variety of soils, provided such soils are warm, have high water holding capacity, good aeration and are free of salt. The soils must contain a readily available supply of plant food and a pH of 5.5 to 6.8 (Singh *et al*., 2011). In general, sandy loam soils prepared in deep friable condition is important for the development of a strong root system (Shankara *et al.*, 2005).

2.7.3 Constraints of tomato production

Tomato production is adversely affected by several factors, which include limited and high cost of labor, climatic factors, inadequate marketing, storage systems and diseases

(Srinivasan, 2010).It has been estimated that an average of over 40% of the yield of tomato is lost annually due to diseases (Srinivasan, 2010). Disease pathogens do not only reduce the yield of tomato in terms of quantity, but also reduce quality (Abubakar *et al*., 2004).

Tomato is prone to infections by many diseases right from the seedling stage to harvest. Several fungi, insects, viruses, bacteria and nematodes frequently attack tomato plants as well as fruits (Youdeowei, 2002).Some of the constraints faced by components of the value chain for tomato can be solved by adequate Research and Development (R&D). Most research centers lack equipment and laboratories to do cutting edge research. In addition, when they do research and come up with good results, commercialization is a major problem. Areas that require research intervention include: improved planting materials; cultural practices; disease management; technologies for harvesting, handling, storage, processing and transportation. Closely related to R&D is the issue of relevant skills required to use modern technology in all aspects.

2.8 Fruit Quality

Tomato production is challenged by several problems in the world including the scarcity of water resources, soil salinization other antibiotic stress (Fahad *et al.*,2017). Tomato fruit quality for fresh consumption is determined by appearance (color,size, shape freedom from physiological disorders, and decay),firmness, texture,dry matter, and organoleptic (flavor) and nutraceutic (health benefit) properties. The quality of tomato is mainly attributed to its aroma volatiles,sugar and acid content, while the nutraceutical quality is defined by its mineral,vitamins,carotenoid, and flavonoid content. Postharvest durability and fruit safety are also very important quality criteria for product distribution

and marketing. Tomato fruit quality are affected by growing conditions. (Diouf *et al.*, 2018).

2.9 Fungal Diseases of Tomatoes, their Characteristics and Management

Plant diseases constitute major constraint to tomato crop production. This often result in a great degree of crop losses and may range from slight to 70 % loss (Srinivasan *et al*., 2010). Tomato plants are subjected to attack by numerous diseases wherever the crop is grown. Fungal diseases are responsible for significant economic losses sustained by tomato producers each year. Fungal infection is often caused by fungal spores that land on leaves, germinate there and penetrate the plant tissue through its stomata, wounds, or sometimes even directly through the plant's skin (Shankara *et al*., 2005). The filaments develop at an increasing rate in the affected plant tissue, from which they extract nutrients and into which they may excrete substances that are toxic to the plant (Agrios, 2005).

The affected plant tissue usually dies off. Fungal pathogens such as *A. alternata* causing fruit canker (Ellis *et al*., 2006), *A. solani* the causal agent of early blight disease (Fahad *et al.*, 2017) and *Fusarium oxysporum* f. sp. lycopersici, the causal agent of wilt disease are considered as major agents of yield reduction to the crop (Stone and White 2006). Early blight disease is a three -phase disease, which produces leaf spots, stem canker and fruit canker. The harmful effects of the fungus are usually limited to the affected area, but there are some types of fungi (such as *Fusarium* and *Verticillium* spp.) that invade the plant's vascular tissues (xylem) and thus, spread throughout the plant (Agrios, 2005).

Plate I: Infested tomato fruit by *Alternaria alternata*

Source: Adapted from Marie iannotti

2.9.1 Post-harvest pathogens

A variety of rots and decay caused by fungi or bacteria can be found on stored products. These post-harvest diseases can start before or after harvesting. Plants or fruits infected in the field may not develop symptoms until stored, and the presence of high temperatures and high moisture during storage can stimulate the infections to continue to develop on fruits and vegetables. Penetration can occur through natural openings, but most post-harvest pathogens need wounds, cuts, or bruises caused during harvesting to infect the host (Eloff and Mc-Gaw, 2014). Post-harvest losses of fruit and vegetables can reach up to 25 % of the total production in industrialized countries and even more than 50 % in developing countries (Nunes, 2012).

The most obvious symptoms of fungal diseases are leaf spots. These spots are normally round or oval, but they can also be polygonal or spindle-shaped with pointed ends. In an early stage of fungal infections, moist areas may be noticeable on the leaves, where the leaf will later die off. At a later stage of some infections, the leaf spots have a dead

brown center and are surrounded by a light or dark-colored halo. Concentric rings of different shades of brown or grey may form around the center (Sobia *et al*., 2016).

2.9.2 Fruit canker of tomato and its management

Fruit canker of tomato caused by *A. alternata* is one of the most common and destructive diseases of tomato in areas of heavy dew, rainfall and relative humidity (Nunes, 2012). *Alternaria* sporulates best at about 27 ºC when abundant moisture is present. The fungus survives in infected plant debris in or on the soil for at least one and perhaps several years. It can also be seed-borne. The spores can be transported by water, wind, insects, other animals including man, and machinery. The fungus can cause disease on foliage (leaf blight), stem (collar rot / stem cankers), seedling (damping-off), and fruit (fruit canker). It can result in severe damage during all stages of plant development. The leaf blight phase, commonly referred to as early blight, is the most important phase of the disease and can result in complete loss of the crop when incidence is severe. Under irrigated conditions, susceptible hybrids can be severely damaged by early blight incurring a loss of 50 to 80 % (Singh *et al*., 2011).

The control of tomato early blight disease has been almost exclusively based on the application of chemical fungicides. Several effective fungicides have been recommended for use against this pathogen, but they are not considered to be long term solutions, due to concerns of expense, exposure risks, fungicide residues and other health and environmental hazards. Use of clean seed saved from disease-free plants, practicing three-year crop rotation with non-susceptible crops and the use of resistant or tolerant varieties are possible control measures. (Singh *et al*., 2011).

2.10 Importance of *Alternaria alternata*

Alternaria alternata's presence in many regions of the world, this fungus has been found to be responsible for different diseases during the postharvest shelf-life of many different horticultural products including stem-end rot of mangoes (Amin *et al.,* 2011), black rot in cherry tomatoes (Wang *et al.,* 2011), core browning and moldy core of apples (Gao *et al.,* 2013), fruit spot on apples (Harteveld *et al.,* 2013), moldy core and core rot in apples (Shtienberg, 2012), black rot of mandarins, black rot of kiwi fruit (Kwon *et al.,* 2011). *Alternaria* late blight of pistachios and *Alternaria alternata* (Black Rot, Black Spot), brown spot on the hybrids of tangerine × grapefruit as well as on grapefruit (Peever *et al.,*2002). *.A. alternata* is a fungus that can infect many fruits, however, this fungus has been reported to infect other parts of plants like seeds (Szopinska *et al.,* 2012), leaves (Harteveld *et al.,* 2013), stems and flowers reducing the agricultural production either directly by infecting the fruit or indirectly by impairing the physiology of plant photosynthesis (Samuel and Oriji, 2015).

Among all the fungi that affect tomato *Alternaria alternata* is one of the fungus that has been related to food poisoning due to the production of mycotoxins which include alternariol, altenuene, alternariol monomethyl ether, altertoxins and L-tenuazonic acid (Amin *et al.,* 2011). Some of these are dangerous and, indeed, alternariol and alternariol methyl ester can increase the rate of breaks in the DNA of human carcinoma cells by inhibiting DNA relaxation and stimulating the cleavage of DNA by topoisomerases I, IIα and IIβ (Fahad *et al.,* 2017). This species is also known to cause fungal keratitis in humans (Xu *et al.,* 2013). There are several causes of fruit and vegetables postharvest losses; however, one of the most important is fungal attack (Xu *et al.,* 2013). This is of paramount importance because fungi can sporulate and resist the postharvest treatments

aimed at eliminating the microorganisms present (Fatima *et al.*, 2009: Harteveld *et al.*, 2013).

According to Adachi *et al.* (1993), an analysis of the DNA intergenic space region of 322 *A. alternata* isolates was carried out by restriction fragment length polymorphism using a DNA region containing two entire copies of the nuclear rDNA as a probe. The isolates of *A. alternata* were obtained from 13 areas of Japan. The analysis found only eight different genotypes with different frequencies depending upon the sampled area. However, by using a DNA region of low repetitive DNA as a probe, the presence of 46 genotypes within 104 isolates from one region was recorded. Furthermore, analysis of 68 isolates of one of the groups created by using the ribosomal DNA was able to find 24 different genotypes.

Analysis of the variability of 56 *A. alternata* strains isolated from pistachio, obtained from four different regions of California, was carried out using the same approach as the experiment described before. Furthermore, the probe utilized was the one including two entire copies of the nuclear rDNA. In this case, the presence of 34 genotypes was recorded, suggesting a much larger variability (Aradhya *et al.*, 2011). Analysis of *A. alternata* strains isolated from tomato using random amplified length polymorphism with 29 primers was carried out. In this experiment, it was possible to differentiate 65 genotypes within 69 different isolates analysed, suggesting again that there is a large variation within the *A. alternata* species (Morris *et al.*, 2000). In agreement with this work, analysis with random amplified length polymorphism using five primers was able to find 30 different genotypes within 30 isolates of *A. alternata* (Weir *et al.*, 1998). In another experiment, 112 strains of *A. alternata* isolated from mature pines (Pinus tabulaeformis) were analyzed by random amplified microsatellites including the next two triplets at the 3′ end: CCA and CGA. The phylogenetic analysis clearly showed 105

different genotypes within the 112 strains suggesting that there is a large variation within the species (Guo *et al*., 2014).

In one study, 14 strains of *Alternaria* found in several hosts in Washington and California were studied using several analyses. The physiological analysis of growth morphology was carried out in several media, including potato dextrose agar, dichloran rose bengal yeast extract sucrose agar and weak potato dextrose agar. The amplification and sequences of the rDNA ITS region, a region of the gene encoding the glyceraldehyde-3-phosphate dehydrogenase and a region of the gene encoding the plasma membrane ATPase was carried out. Using the ITS, ATPase and the glyceraldehyde3-phosphate dehydrogenase sequences, the same result was found, that is, it was not possible to differentiate *A. tenuissima* and *A. alternata* (Lawrence *et al.*, 2013).

2.10.1 Reproduction and life cycle *Alternaria alternata*

When grown in culture, *A. alternata* has been shown to develop and grow as elongated chains with conidiophores that are dark brown in color. Spores, referred to as conidia are produced (growing as buds from the conidiophores) from the conidiophores asexually, when viewed under the microscope, these spores appear larger in size with a dark appearance. Compared to the spores produced by A. *solani,* the conidia of A. *alternata* have been shown to have shorter beaks and fine longer septa (Simmons, 2007)

Depending on the environment, the conidia are dispersed differently. For instance, some of the conidia may be dispersed by being spread by wind or transported by water. Once they land on a suitable substrate or parts of a plant (leaves, fruit, seed) the spores begin to germinate in the presence of moisture and ideal temperature range. Here, they again grow as elongate chains and with continued favorable conditions, start producing spores

from the tip of their hyphae for the cycle to continue. (Guo *et al*., 2014). The following are some of the characteristics of A. *alternata:* A pale or dark brown conidiophore that may be straight or flexuous in appearance, Brownish conidia with a short beak or no beak at all. Conidia with a smooth surface (or a little warty), Pathogenesis Brown and Black Spots on Plant Leaves

Once dispersed, these spores can land on plant leaves where they start germinating. As they continue to germinate on leaves, particularly the edge of young leaves; they cause necrosis and chlorosis as they develop while utilizing nutrients from the leaves. These lesions can also be seen in the fruits of a plant. As the fungi continue to grow and reproduce, it can spread to the leaves of other plants, affecting all plants in the area. With such plants as tomatoes, the plants have been shown to be prone to *Alternaria* rot, where the organism penetrates the fruit through the lenticels and spreads within the fruit. This can spread to other fruits causing damage (darkening of the fruit's interior) and can greatly cost farmers with fruits in particular, *Alternaria* grows rapidly given that fruits provide favorable conditions for growth; moisture and nutrients required for growth and reproduction (Escrivá *et al*., 2017).

2.10.2 Biology of *Alternaria alternata* infection process

Initial events in the infection process caused by fungi in general, as well as by *A. alternata*, are spore adhesion to the cuticle and directed growth of the germ tube on the plant surface. Germ tubes and hyphae elongate by apical deposition of wall glycoproteins and polysaccharides such as chitin and glucans. (Peever *et al.*, 2002). During extension of the fungal apex, these components are assembled into microfibrils as a result of hydrogen bonding and cross-linking of adjacent polysaccharide chains. Another minor component of *Alternaria's* hyphae is melanin (Chen *et al.*, 2012). This

initial, not metabolically demanding (passive) adhesion is followed by a second stage involving secretion of a film unsheathing the germ tube and parts of the cuticle in the vicinity of the hyphae (Jones, 1994).

These fungal sheaths, which are associated with the germ tube of many fungi, are assumed to mediate adhesion and preparation of the infection court. Once conidia germinate on the surface of the host tissue, a germ tube and an appressorium are formed (Yamagishi *et al.,* 2006). The germ tube is a specialized structure distinct from the fungal mycelium, often growing only a very short distance before it differentiates into an appressorium. From the appressorium, a specialized narrow hyphal strand, called the penetration peg, arises *and* advances into and through the cuticle and cell wall. Penetration of the plant takes place only if melanin (dark pigment) accumulates in the appressorial cell wall. It appears that melanin produces a rigid structural layer and, by trapping solutes inside the appressorium, causes water to be absorbed. This increases the turgor pressure in the appressorium and, in this way, it induces the physical penetration of the plant by the penetration pegs (Chen *et al*., 2012).

Alternaria alternata is generally considered as a weak and opportunistic pathogen that follows different routes for penetrating plant tissue, like wounds (Ahmed *et al.,* 2016) natural openings such as lenticels, stem ends, and pedicels (Prusky *et al.,* 2013) and by direct breaching of the host cuticle (Mersha *et al*., 2012), which enables the pathogen to enter the unripe tissue and remain quiescent for weeks until the fruit ripens. Typically, tissues weakened by different stresses, senescence, or wounding are more susceptible to *Alternaria* infections than healthy tissues (Mmbaga *et al*., 2011).Although it is known that there is a quiescent phase in the life cycle of *Alternaria*, it is not entirely clear whether the ungerminated or the germinated appressorium represents the quiescent

stage. Experimental evidences indicate that appressoria germinate to produce infection hyphae prior to the onset of quiescence.

The fungus ceases growth soon after appressorium formation and remains in a quiescent state until fruit ripens. (Prusky *et al*., 2002) reported that *A. alternata* can remain quiescent on cuticles of unripe mango and avocado fruits although it can colonize the mesocarp of the same peeled fruits, suggesting that specific changes during fruit ripening affect the transition into an active infection. It has been reported that the activation of quiescent infection in *A. alternata* is facilitated by large families of genes encoding cell-wall degrading enzymes, such as glucanases (Eshel *et al.*, 2002).

The most common path for fruit infection used by *A. alternaria* is through wounds produced by mechanical damage, sunburn or chilling injury, before, during or after harvest, as observed on tomatoes (Nabahat *et al*., 2014), apples (Shtienberg, 2012), persimmon and mango (Barkai-Golan *et al.*, 2008).In addition, *Alternaria* infection via natural openings has been observed in table grapes, mandarin, and tangelo, among others. In table grapes, it was observed that *Alternaria* hyphae penetrated the berries through stomata, lenticels and micro cracks in the epidermis (Ansari *et al*., 2012). The formation of appressoria at the tips of germ tubes, hyphal tips, and on short side branches was observed. According to the authors, the adhesion of appressoria was very prominent, and some appressoria were completely embedded in cuticular wax. The fungus did not form an extensive network of intercellular hyphae within the living tissue after entry via natural openings. Rather, hyphae remained localized in the substomatal cavities or lenticels and, although the fungus was able to grow inside epidermal cells adjacent to microcracks in the epidermis, or those surrounding wounds, it would appear that this process is a slow one. *Alternaria* species have been reported to be the most frequent fungal in infected plants and in agricultural commodities. Alternariol (AOH),

alternariol monomrthyl ether (AME), and tenuazonic acid (TA) are some of the main *Alternaria* mycotoxins that can be found as contaminants of food (Fernandez *et al.*, 2010).

Alternaria has over 50 pathogenic and nonpathogenic species that are morphologically similar to each other. The identification of these species at the species level by means of the traditional and morphological methods is time-consuming, difficult, and in most cases, not sufficiently precise (Loganathan *et al*., 2016). With recent advances in molecular biology, researchers have started using DNA-based molecular techniques and sequencing of genetic regions for the quick and accurate detection of fungi at the species level. Moreover, the first step in the study of plant and human pathogens is the identification of fungal species and investigation of their genetic diversity. Therefore, the accurate identification of fungal species provides scientists with useful information about the genetic diversity of the fungal population.

2.10.3 Weather and climatic condition of *Alternaria*

Alternaria species is a hardy fungus and can live in extreme conditions. *A. alternata* can pass the winter in the soil, seed, infected crop debris or perennial host tissue, such as bark, nodes, and scaly leaves, as mycelia and/or conidia. Some strains can produce survival structures to resist the unfavorable conditions. While some *Alternaria* species need stimuli to initiate conidiophore formation and sporulation, *A. alternata* can sporulate easily without triggering. The spores of A. *alternata* can be propelled into the air by a shift from wetness to dryness, a rapid increase in humidity or exposure to red light. Sajad *et al.* (2017) reported that conidia of *A. alternata* germinate quickly if moisture is present and begin to produce toxin even before they penetrate the tissue. *Alternaria* brown spot infection is most associated with environmental conditions

(Canihos *et al.*, 1999) and leaf or fruit maturity of tangerines. In addition, it has been reported that, in these fruits, the fungus sporulates most prolifically on necrotic lesions of mature or senescent leaves when the substrate is lightly moistened or at high relative humidity. Conidia are also released after rainfall and/or sudden changes in humidity and dispersed by wind (Timmer *et al.*, 2000).

2.10.4 *Alternaria* taxonomy

Alternaria has long been mainly based on conidial morphology and sporulation pattern. (Woudenberg *et al*., 2013) analyzed by using a statistical method based on the size of conidia and concluded that *Alternaria* isolates producing small spores and they are known as "collective species" or *alternata*. Similar types of findings were reported by various researchers (Tsuge, 2003). Furthermore, (Simmons, 2007) introduced 3-dimensional sporulation pattern as a mean of scoring small-spore species to facilitate their segregation and identification. More recently, small-spore forming *Alternaria* species were grouped into the *alternata* section and it comprises almost 60 *Alternaria* species (Woudenberg *et al*., 2013). The molecular variation within *alternata* section is low and these species were mainly differentiated on the behalf of phenotypic variation. This it is also well recognized that pathogenic populations (pathotypes) with narrow host range exist within the *alternata* section. *A. arborescens* responsible for the tomato stem canker constitutes a typical example (Simmons, 2007).

2.10.5 Factors favoring *Alternaria alternata* infection

Fruit fungal infection may occur, in addition to the growing season, during harvesting, handling, transport, postharvest storage and marketing conditions, or after purchase by the consumer. Fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly susceptible to fungal decay (Jia *et al*., 2013).

The variation in weather and climate has led to a lot of devastating consequences and effects in various parts of the country (Odjugo, 2010). These include flooding, deforestation, desertification, erosion, drought, sea level rise, heat stress, pests and diseases, erratic rainfall patterns, and land degradation. More specifically, the South-south geopolitical zone is mainly affected by sea level rise and deforestation- induced changes; South-East by erosion, flooding and land degradation: North-Central by changes due to de-vegetation and over-grazing; North- East by drought, desertification and heat stress, and North-West also by drought, desertification and heat stress (Ozor, 2009).

When temperature exceeds the optimal level for biological processes, crops often respond negatively with a steep drop in net growth and yield. Khanal (2009), stated that heat stress might affect the whole physiological development, maturation and finally reduces the yield of cultivated crop. The negative effect on agricultural yields will be exacerbated by more frequent weather event high air temperatures reduce tillering, causes stunting (Futakuchi, 2005) and accelerate the developmental rate of Tomato thereby leading to shortened growing cycles. Thus higher temperatures induced by climate change may lead to substantial adverse effects on Tomat oproduction.One of the most serious long-term challenges to achieve sustainable growth in Tomato production is Climate change (Wassmann *et al*., 2007). Tomato productivity and sustainability are threatened by biotic and abiotic stresses, and the effects of these stresses can be further aggravated by dramatic changes in global climate. Drought and flood already cause widespread Tomato yield losses across the globe and the expected increase in drought and flood occurrence due to climate change would further add to Tomato production losses in the future. Thus the major challenge is the potential adverse effect of changing climate on Tomato production and being the factor limiting increase in annual yield.

Droughts can also affect irrigated Tomato fields with poor water control. Water stress has been identified as one of the most important production constraint in Africa.

2.11 Weather and climate of Niger state

Climate change is a global crisis. It is estimated, by the inter-grommet panel on climate change, that there will be increase in global mean temperature of about 1^{o}C above the present value by the year 2025 and 3before the end of next Century (Lawrence *et al.*, 2013). Agriculture also had its effect on climate change just as climate had on agriculture. According to (Nunes, 2012).Agriculture sources such as animal husbandry/ manure management and agricultural soils account for about 52% of global nitrous oxide (N_2O)emissions. In the past, deforestation and intensive agriculture (cultivating grassland) have contributed significantly to the increase in atmosphere from agricultural activities.

Tomatoes are warm season crop and are sensitive to high humidity / rain. Thus, increases in yield are experienced in well drained, sandy loam, and rich in humus soils. The planting season is between August and September. However, where irrigation farming is practiced, the best time for planting is during the dry season. Location, climate and vegetation area is located in the North central Nigeria. The study area has an alternating wet and dry season. The rainy season spans between May and October with total annual rainfall of about 1324mm (NIMET, 2010) while the dry season stats from November to April with harmattan occurring between December and February. The average annual temperature ranges in the area is about 30^{0}C (NIMET, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Samples

Tomato fruit showing symptoms of cankers were collected from tomato farms across the Agricultural zones of Niger State. A total of hundred (300) of different samples of tomato fruits verities were collected from thirty (30) farms across all the three Agricultural Zones of Niger State. Zone I comprises of Bida, Lapai, Lemu, Kataeregi, and Sabon Gida villages; Zone II are Garatu, Paiko and Sabon-daga while Zone III comprises of Wushishi and Zungeru. The global positioning systems (GPS) of the sample locations were taken. The sample was collected in a sterile and well tagged polythene bags based on the production rate of the farm and were transported to Laboratory of the Department of Plant Biology, Federal University of Technology Minna, Niger State, Nigeria.

3.2 Identification of Variety of Tomato in each Location

Varieties of tomato collected were first assigned identification no (ID) before proper identification using the common/local names used in each location Table 3.1 below:

3.3 Preparation of Media

3.3.1 Potato dextrose agar (PDA)

Thirty nine (39) gram of PDA (Hi-media) was suspended in 1000 ml distilled water and heated to dissolve the powder completely; the medium was sterilized by autoclaving at 121^{o}C for 15 minutes (Manufacture's guide).

Table 3.1 GPS of the Sample Site of Canker Infected Tomato Fruit in Niger state.

S/N	Sample ID	Sample Site Collection	Zone	Latitude N	Longitude E
1	BTS	Bida	I	9.10^{O}.36’	6.00 ^{O} .56’
2	LPTS	Lapai	I	9.04^{O}.53’	6.57 ^{O} .03’
3	LTS	Lemu	I	9.39 ^{O} 36’	6.03 ^{O} .62’
4	KTS	Kataeragi	I	9.35 ^{O} 33’	6.28 ^{O} 31’
5	SGTS	Sabon Gida	I	9.24 ^{O} 96’	6.17 ^{O} .92’
6	GTS	Garatu	II	9.48 ^{O} 32’	6.43 ^{O} .94’
7	STS	Sabon-daga	II	9.42 ^{O} 55’	6.38 ^{O} .56”
8	PTS	Paiko	II	9.43 ^{O} 54’	6.63 ^{O} .44
9	WTS	Wushishi	III	9.72 ^{O} 21’	6.11 ^{O} .3”
10	ZTS	Zungeru	III	9.80 ^{O} 97’	6.15 ^{O} .53’

Key: TS- Tomato sample

3.4 Isolation of Fungi

Serial dilution technique was used to isolate fungi associated with infected tomato fruits. One(1g) of each tomato sample was aseptically cut and put inside a test tube containing 9ml of sterile distilled water, vortexed mix to allow even distribution of organism present in the sample. A dilution factor 10^4 was used as stock solution. Thereafter, 1ml of the dilution was aseptically taken from the suspension and transferred into sterile Petri dish 10ml of potato dextrose agar and 1ml of chlorophenicol were added and poured into the Petri dish. The plates were swirled gently to allow even distribution of the sample. Incubation was done at room temperature (28 ± 2 ^{o}C) for 72 hours. (Adebola *et al*., 2012). The percentage frequency of occurrence of the friut was obtained using the formula: (Daniela *et al.*, 2013).

$$\% \text{ frequency} = \frac{\text{tomato containing fungi}}{\text{total number of tomato assessed}} \times 100$$

3.5 Characterization and Identification of the Isolates

The pure cultures of the fungus was identified on the basis of their colony growth pattern, conidial morphology and pigmentation using the slide culture technique and microscopic examination. The identity of the fungus was confirmed with the aid of a mycological atlas (Adebola 2014). Stock cultured was preserved in PDA slant and refrigerated at 4 ^{o}C. The purification of the isolates was carried out using the single-spore method.

3.6 Pathogenicity Test of the Isolate

The test was carried out according to the method developed by Ali *et al*. (2014), on healthy tomatoes.Tomato fruits without symptoms of Alternaria fruit canker were surface sterilized with 70% ethanol. Four (4)mm cylindrical plug tissues were cut out from the fruits with a sterilized cork borer. Seven day old fungus culture was placed in the hole under a sterile condition, then the hole was covered and sealed. Three replicates and control were prepared.The inoculated tomato fruits and the control were placed in sterile plastic with cover (3 fruit per plastic). The decay rate of each tomato fruit was observed at interval of 24hrs, 3days and 5days for characteristic symptoms of *Alternaria alternata*. The fungi was later re-isolated from the inoculated tomato fruits with canker symptomsand compared with the initial isolates used for inoculation.

3.7 Molecular Analysis of *Alternaria alternata*

3.7.1 Polymerase chain reaction (PCR Amplification), the DNA Amplification was carried out at the Bioscience center, of (IITA) Ibadan.

3.7.2 DNA extraction

The DNA of the *Alternaria alternata* strains were extracted using protocol described by Wang *et al.,* (2011)**.** The test fungal *Alternaria alternata* was grown in potato dextrose broth on a shaker and 1 ml of the culture suspension was transfer into a sterile crysogenic storage tube containing 200 ul of sterile glycerol and store at -70 ^{o}C. The fungal tissues (~2.5 mg) from the isolates which it was inoculated into a sterile 1.5 ml micro centrifuge tube and 400 ul of API buffer was added (DNeasy Tissue kit). Freeze cycle was applied to lyse the fungal cells using crushed ethanol and a boiling water bath; the cycle will be repeated seven times and was also boiled in a water bath after the last cycle of freeze cycle. One (1) ml micropipette tip was used to grind any visible tissue in the tubes for 5secs. The tubes were centrifuge at 10,000 rpm for 10 minutes at 4 °C and the supernatant was collected in 1.5 ml centrifuge tube. Fifty (50) µl of TE buffer was added to dissolve the DNA. The DNA was eluted in 50 µl buffer and stored at −20 °C for PCR amplification. The DNA was quantified using Nanodrop Spectrophotometer and diluted to 10 µl for Polymorphic Chain Reaction (PCR) analysis. The samples were screen using primers.

3.7.3 Fungi polymerase chain reaction (PCR) analysis

To use the ITS gene for characterization of fungi, ITS universal primer set which flank the ITS1, 5.8S and ITS2 region was used; PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each ITS 1. 5’ TCC GTA GGT GAA CCT GCG G 3’and - ITS 4: 5’ TCC TCC GCT TAT TGA TAT GC. 3’primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl

DNA template. PCR was carried out in a Gene Amp 9700 PCR System. Thermal cycler (Applied Biosystem Inc., USA) with a PCR condition include a cycle of initial denaturation at 94^{o}C for 5 minutes, followed by 35cycles of each cycle comprised of 30secs denaturation at 94 ^{o}C, 30 seconds annealing of primer at 55^{o}C, 1.5 minutes extension at 72 ^{o}C and a final extension for 7 minutes at 72 ^{o}C.

3.8 Gel Amplification

The amplified gene fragments were checked on a 1.5% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

3.9 Data Analysis

The morphological data were analyzed by Evolutionary analysis inferred by using the Maximum Likelihood method (Eloff and McGaw., 2014).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Varieties of tomato cultured in Niger state

(Table 4.1) showed the five (5) varieties of tomato cultivated at sampling sites in Niger state with their local names. Plum tomato 1 (chico) was collected at Garatu and Sabon daga. Plum tomato 2 (Roma V.F) was collected from Bida, Kataeragi and Wushishi. Samtom-12 (Ronita) was collected from Lapai and Zungeru. Plum tomato 3 (San Marzano) was collected from Bida, Gartu and Sabon daga. Samtom -3 (Piac enza 0164) was collected at Lemu and Sabon Gida (Plate III A – E). These are areas where tomato mostly cultivated across the state (Table 4.1).

Table 4.1: Varieties of Tomatoes Cultivated in Niger State

S/N	**Varietalname**	**Commo nname**	**Local name in Niger State**
1	Plum Tomato 1	Chico	Na Guwari
2	Plum Tomato 2	Roma V.F	Kurugi
3	Samtom-12	Ronita	Mai dariya
4	Plum Tomato 3	San Marzano	Dan erka
5	Samtom-3	Piac enza 0164	Lasomoni

Plate III: Tomato Varieties Grown by Farmers in Niger State.

A. Samtom -3 B. Plum tomato 3 C. Samtom -3 D. Plum tomato 2E. Plum tomato

4.1.2 Varieties of tomato cultivated at sample sites in Niger state

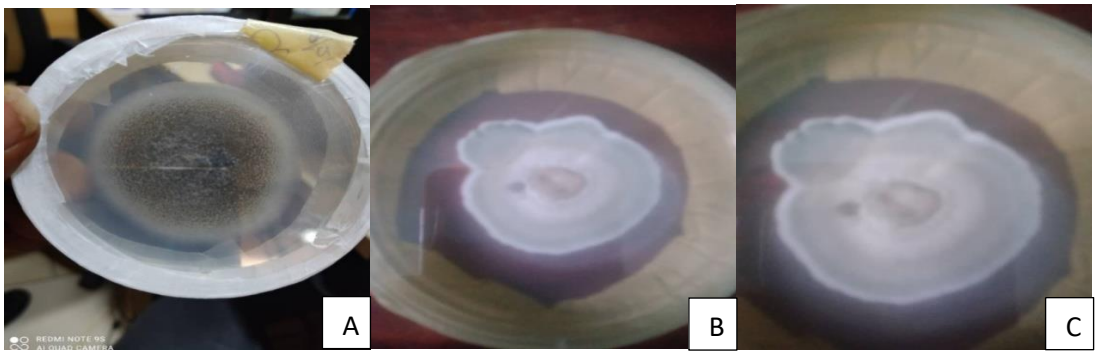
The varieties of tomato cultivated at sampling sites in Niger State with their local names are shown in table 4.2. The five varieties of tomato samples were collected across the states from places where they are mostly cultivated. The Plum tomato 2 (Roma V.F) was collected from Bida, Paiko, Kataeragi and Wushishi, Samtom-12 (Ronita) was collected from Wushishi, Lemu, Lapai and Zungeru, Plum tomato 3 (San Marzano) was collected from Bida, Garatu, Kataeragi, Sabon Gida and Sabon Daga while Samtom -3 (Piac enza 0164) was collected from Lemu and Sabon Gida.

Table 4.2: Local and Varietal Names of Tomato Samples Collected from Farmers Fields in Niger State

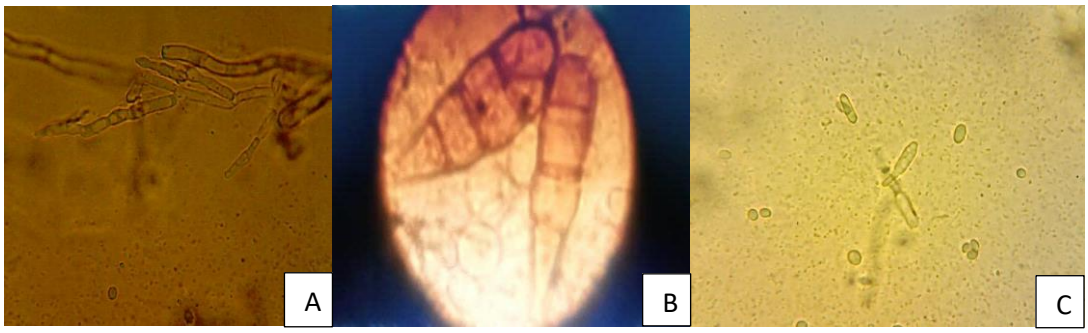
Zone	location	Tomato variety collected	Local Name of variety
Zone I	Bida	San Marzano and Roma V.F	Dan Erka and Kurugi
	Lapai	Ronita V.F	Maidariya
	Lemu	Ronita V.F and Piac enza 0164	Maidariya and Lasomoni
	Kateerigi	San Marzano and Roma V.F	Dan erka and Kurugi
	Sabon Gida	San Marzano and Piac enza 0164	Dan erka and Lasomoni
Zone II	Paiko	Roma V.F	Kurugi
	Garatu	San Marzano and Chico	Dan erka and Na gwari
	Sabon Daga	San Marzano and Chico	Dan erka and Na gwari
Zone III	Wushishi	Ronita V.F and Roma V.F	Maidariya and Kurugi
	Zungeru	Ronita V.F	Maidariya

4.1.3 The morphological features

The morphological features of culture of the isolated and identified *Alternaria alternate* (Plate I and II) shows typical colonies of lettuce-green to olive green in color and often have a prominent (2–5mm) white margin on potato dextrose agar. Isolates produced colonies that was over 70mm in diameter after 7–10 days based on the sporulation habit of single-spore colonies.



Plates I: *Alternaria alternata* morphology on Potato Dextrose Agar. Culture at 7dayS. (A) -Front View of *A.alternata* at 7days of incubation (B) - Rear View of *A. alternate* at 7days of incubation (C) Rear View of *A. alternata* at 10days of incubation.



Plates II Photormicrograph of *Alternaria alternata*.
A. Conidia at ×40. B. Conidial chains and chain branching of *A. alternata*. C. Conidia at ×100. (Photographs).

4.1.4 Percentage frequency of tomato fruits infected with canker.

The percentage occurrence of fruit with canker from all the varieties of tomatoes commonly cultivated in Niger State (Table 4.3).From the results showed that the highest percentage of canker infected tomato fruits was recorded in Ronita V.F (29.5%) variety, followed by San marzano (25%), and Chico (9 %) had the least. The high percentage incidence obtained in variety Ronita VF, San Marzano and Roma VF may be due to the nutritional constituent, temperature of the area as well as output yield of the varieties as farmers in the area choose these varieties despite it susceptibility to the pathogen.

Table 4.3: Percentage of Infected Fruits with Canker from Tomato Fruits Collected from Farmers Fields in Niger State

Varieties	Total fruits collected	% infected fruits with canker
Roma V.F	44	22
Chico	18	9
San Marzano	50	25
Piac enza 0164	29	14.5
Ronita V.F	59	29.5

4.1.5 Frequency of *Alternaria alternata* in tomato varieties collected at different locations

The percentage frequency of *A. alternata* isolated from infected tomato fruits across the zones (Table 4.4) show that samples from Bida (17.5%) recorded the highest frequency of *Alternaria alternata* followed by Lemu (14%), Sabon gida and Zungeru (11%) which are significantly higher than other samples with the least frequency observed in samples from Garatu (5 %). The differences observed across the study area might be due to climatic conditions especially in semi warm and warm areas. The warm and semi-warm regions with high relative humidity have favorable environmental conditions for the disease cycle. If the necessary measures are not taken to control the disease. Diseases constitute a serious limiting factor to tomato production in Nigeria, it can be suggested that the intake of spoilt tomatoes could be dangerous, since these organisms produce spores and toxins that could cause severe food poisoning that will result in fatal outcome. This will make a difference in the lives of tomato farmers.

Table 4.4: Percentage Frequency of *A. alternata* isolated from Sample Location across the Zones

Zones	Location	Variety	No. of Tomato samples collected	No. infectedwithCanker	Percentageincidenceofcanker (%)
I	Bida	Roma V.F	15	15	17.5
		San Marzano	35	20	
	Lapai	Ronita V.F	30	15	7.5
	Lemu	Ronita V.F	15	12	14
		Piac enza 0164	25	16	
	Kataerigi	Roma V.F	15	11	8.5
		San Marzano	20	6	
	Sabon Gida	San Marzano	20	9	11
		Piac enza 0164	10	13	
	Paiko	Roma V.F	20	10	9
	Garatu	Chico	15	12	5
II		San Marzano	20	6	
	Sabon Daga	Chico	15	6	7.5
		San Marzano	25	9	
III	Wushishi	Ronita V.F	20	10	9
		Roma V.F	15	8	
	Zungeru	Ronita V.F	35	22	11

4.1.6 Pathogenicity test

Three samples of the five different varieties of tomato fruits where used for pathogenicity test to confirm if *Alternariaalternata* is actually one of the causative organisms of canker in tomato fruits. The samples were punctured at the middle using 2mm cork borer and inoculated at the same spot with *Altrnariaalaternata* inoculum. They were left for observation for three and seven days after inoculation. The results showed (Plate V) severe decay in the tomato fruit samples, on infected portions, fungal growth was clearly visible. These symptoms resemble those found under natural conditions. Tomato Fruits inoculated with distilled water showed no sign of infection. Initial symptoms were observed after three and seven days of inoculation

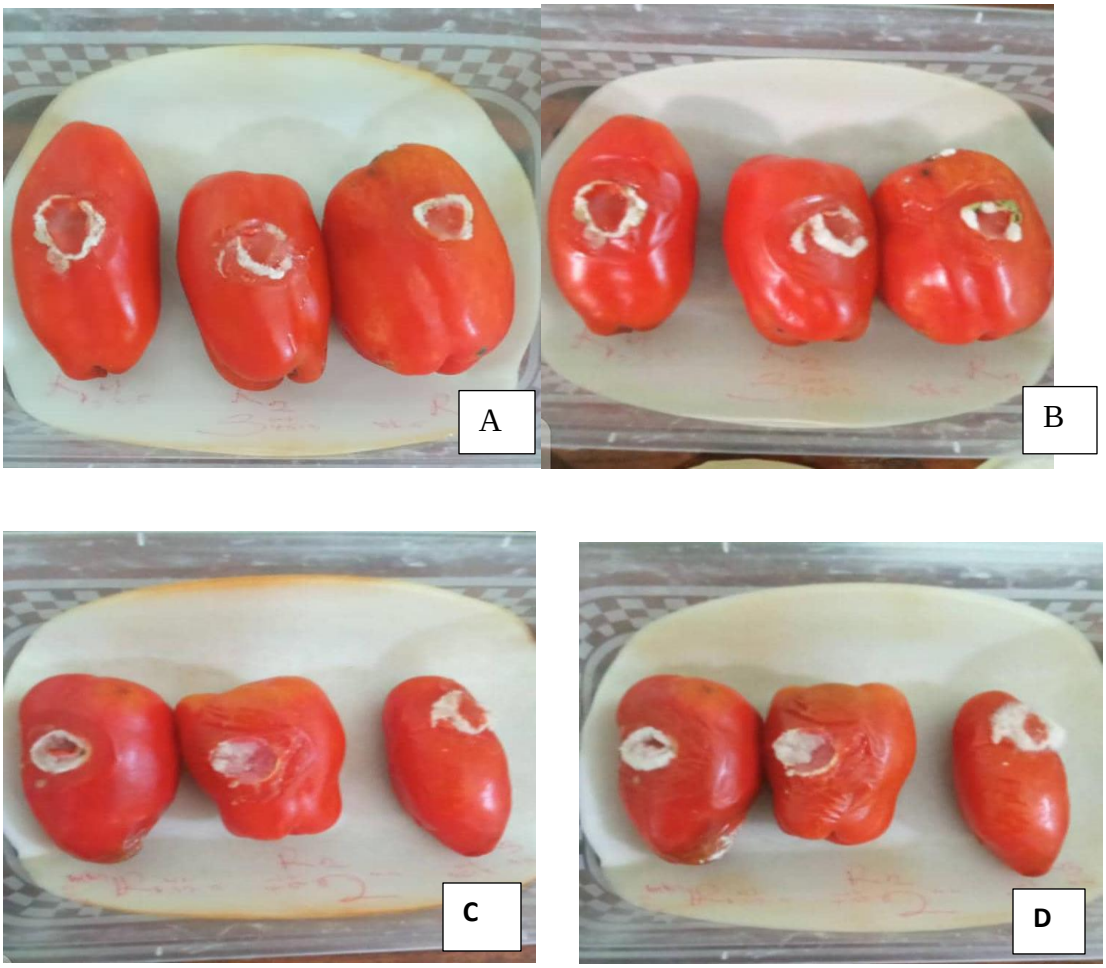


Plate V Pathogenicity Test: Tomato fruit samplesinocuoated with *Alternaria alternata* showing infected portions at 3 and 7 days after innoculation.(A and B). Plum 3 tomato fruits, (C and D) Santom 3 tomatofruits.

4.1.7 Molecular identification of *Alternaria* isolates using the sequencing of ITS1 region

The sequencing of ITS1 led to the identification of *Alternaria* species that are morphologically similar.

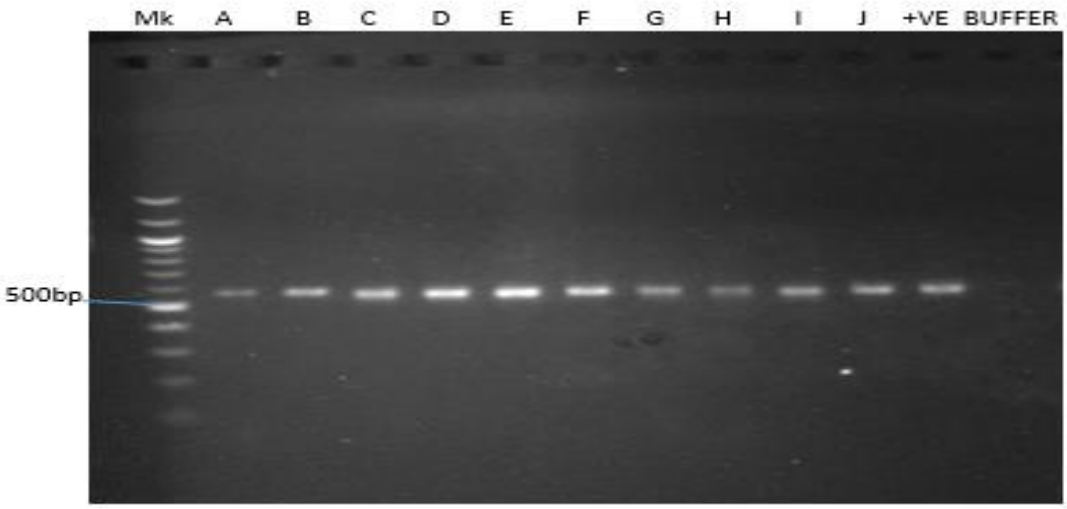


Plate VI: Gel Electrophlorograph using ITS

Table 4.5: **Similarity Index**

Sample ID	Scientific name	Max score	Total score	Query cover	E value	Identity (%)	Accession
TSA	*Alternaria alternate*	905	905	99	0	100.00	OK175632
TSB	*Alternaria alternate*	878	878	100	0	99.80	OK175633
TSC	*Alternaria alternate*	898	898	100	0	99.00	OK175634
TSD	*Alternaria alternate*	904	904	100	0	100.00	OK175635
TSE	*Alternaria alternate*	900	900	100	0	100.00	OK175636
TSF	*Alternaria alternate*	961	961	100	0	100.00	OK175637
TSG	*Alternaria alternate*	898	898	100	0	99.80	OK175638
TSH	*Alternaria alternate*	898	898	99	0	99.70	OK175639
TSI	*Alternaria alternate*	902	902	99	0	99.80	OK175640
TSJ	*Alternaria alternate*	915	915	100	0	100.00	OK175641

4.1.8 Edited sequence using blast tool

Table 4.5 Shows the Identity of the Edited Sequence using Blast Tool as Provided by NCBI Database. The tested isolates were edited in order to generate a consensus sequence from forward and reverse sequence in the amplification using sequence assembly software (DNA BASER). A consensus sequence was analyzed by NCBI BLAST database for fungal identities. The ITS3-ITS4 region of identified fungi at > 98% similarity was compared with 100% similarity of the 10 region identified fungi at 99 to 100% matched with *alternaria alternata*. The results showed that the sequence of the extracted isolates had the highest similarity of 99-100% with *A. alternata* species.

4.1.9: The phylogenetic relationship of *Alternaria alternata*

The phylogenetic relationship of the isolated *A. alternata* (figure 4.1) is showed in a cluster analysis dendrogram obtained from the molecular analysis grouped the fungal strains into two (2) major clusters. The first cluster, which is KAA and SGAA and the second cluster which is GAA and ZAA, which means they all have genetic relationship, while BAA, WAA, LPAA, SAA, LAA and PAA are all clustered together, shows they are closely related by indicating their genetic relatedness but having different strains. This is further classifies into 8 different subclusters which are BAA, KAA, LAA, LPAA, SGAA, ZAA and WAA. This study suggests that perhaps there is a large variation within the species of *A. alternata* isolated. The phylogenetic analysis clearly showed different genotypes within the strains suggesting that there is a large variation within the species.

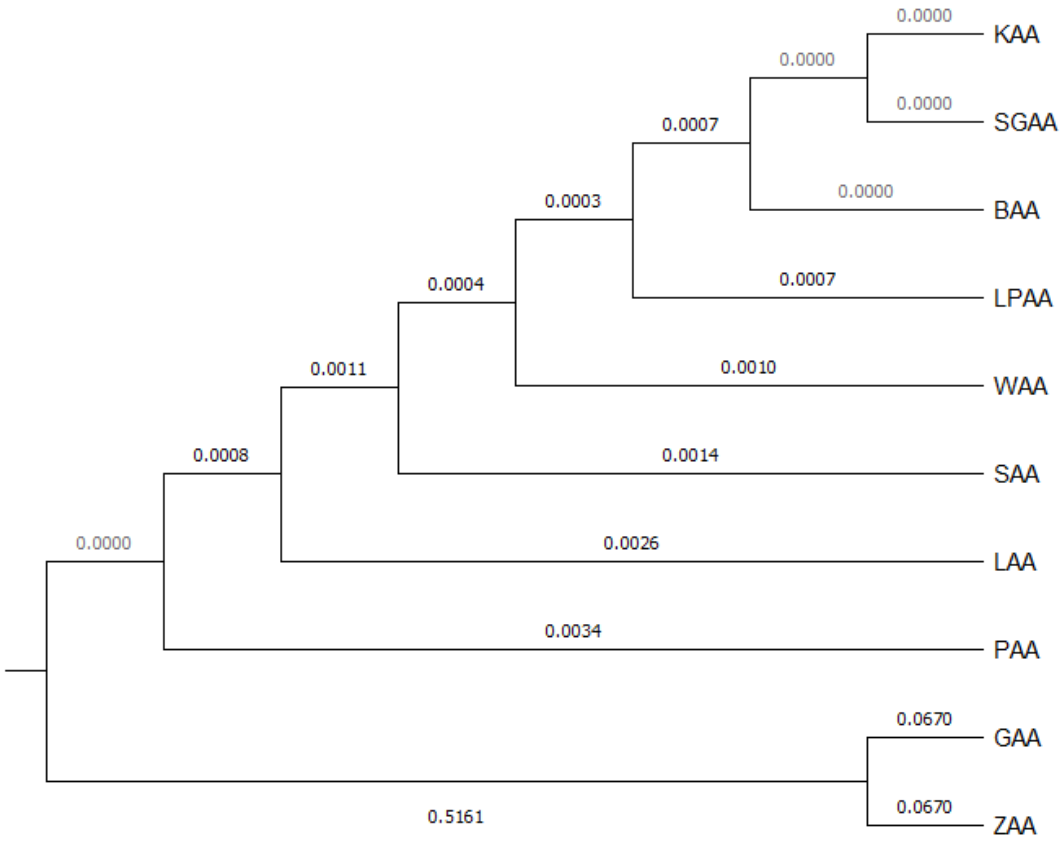


Figure 4.1: Dendrogram of (*Alternaria alternata)* Isolated from Different Tomato from Fields.

4.1.10 Pairwise genetic distance

Pairwise genetic distance (Table 4.6) shows the sequence differences between any two fungi isolate which indicated that the higher the value the higher the dissimilarity. Comparing BAA AND GAA this shows that there is a high difference of 0.520, LPAA and GAA shows the difference of 0.526 while in GAA and ZAA shows that it recorded the highest genetic similarity which was 0.000 and 0.000, this shows that the *Alternaria* are of the same species but different strains.

Table 4.6: Pairwise Genetic Distance Showing the Sequence Difference Between any two Fungi Isolated Sequence

	BAA	LPAA	LAA	KAA	SGAA	GAA	SAA	PAA	WAA
BAA									
LPAA	0.004								
LAA	0.008	0.006							
KAA	0.006	0.006	0.010						
SGAA	0.002	0.004	0.010	0.002					
GAA	0.520	0.526	0.004	0.517	0.521				
SAA	0.006	0.004	0.004	0.010	0.008	0.522			
PAA	0.010	0.008	0.004	0.014	0.012	0.006	0.008		
WAA	0.004	0.002	0.004	0.008	0.006	0.010	0.004	0.008	
ZAA	0.481	0.489	0.006	0.483	0.486	0.002	0.012	0.006	0.012

4.1.11: **ISSR: Inter simple sequence repeat, PIC: polymorphic information content of ten (10) *A. alternata***

>ISSR and PIC strain from Bida

ATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGC
TGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCC
ACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAA
TTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGC
GTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGC
TGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAG
CACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTT
CAACTTA

>ISSR and PIC strain from Lapai

ACATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGCT
GAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCA
CCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATT
AATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTG
GAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCA
CAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCC

>ISSR and PIC strain from Lamu

ATCCTTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGCT
GAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCA
CCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATT
AATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTG
GAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCA
CAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCACTAAGCCTTTTTTTCAA
CTT

>ISSR and PIC strain from Kataeragi

ATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGC
TGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCC
ACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAA
TTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGC
GTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGC
TGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAG
CACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTC
AACTT

>ISSR and PIC strain from Sabon gida

CATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGCTG
AATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCAC
CACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATT
AATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTG
GAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCA
CAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTCAA
CTT

>ISSR and PIC strain from Garatu

ATCATTACCGAGTGCGGGTCCTTTGGGCCCAACCTCCCATCCGTGTCTATTGTA
CCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGC
CCCCCGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGT
GCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTT
GGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGC
AGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCC
GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTT
GGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACC
GCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTGGCCG
GCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTT

>ISSR and PIC strain from Sabon daga

ATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGC
TGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCC
ACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAA
TTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCCGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGC
GTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGC
TGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAG
CACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTC
AACTT

>ISSR and PIC strain from Paiko

AAAATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGC
TGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCC
ACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAA
TTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGC
GTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGC
TGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAG
CACAAGTCGCACCCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTC
AACTT

>ISSR and PIC Strain from Wushishi

TTAATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTT
GCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGC
CCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACA
AATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCG
AATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGA
GCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTT
GCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGC
AGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTT
CAACTT

>ISSR and PIC strain from Zungeru

ATCATTACCGAGTGCTGGGTCCTTCGGGGCCCAACCTCCCACCCGTGCTTACCG
TACCCTGTTGCTTCGGCGGGCCCGCCTTCGGGCGGCCCGGGGCCTGCCCCCGGG
ACCGCGCCCGCCGGAGACCCCAATGGAACACTGTCTGAAAGCGTGCAGTCTGA
GTTGATTGATACCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGC
ATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAG
TGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCAT
GCCTGTCCGAGCGTCATTTCTCCCCTCCAGCCCCGCTGGTTGTTGGGCCGCGCC
CCCCGGGGGCGGGCCTCGAGAGAAACGGCGGCACCGTCCGGTCCTCGAGCGTA
TGGGGCTCTGTCACCCGCTCTATGGGCCCGGCCGGGGCTTGCCTCGACCCCCAA
TCTTCTCAGATT

Key: ISSR: inter simple sequence repeat, PIC: polymorphic information content

4.2 Discussion

4.2.1 Varieties of tomato cultivated at sample sites in Niger state

The common varieties of tomato studied in work corroborates the findings Kumar (2017) who reported similar findings, also Ugonna *et al*. (2015) who reported that the Tomato varieties available in Nigeria are (Samtom- 1, Samtom- 2, Samtom-3 Piacenza 0164, Samtom– 4, Samtom– 5 CHICO, Samtom-a6**,** Samtom-7 Roma- VF, Samtom-8, Samtom--9, and Samtom-10) respectively. However, the difference in one variety over another could be due it diversity as to geographical richness as reported by Ugonna *et al.* (2015), that there are about 7500 tomato varieties worldwide. The diversity of plum tomatoes over others may be due to it being popular among home gardeners and organic producers, because they produce more interesting and flavorful fruits as reported by Allen *et al.* (2014).

4.2.2 Percentage frequency of tomato fruits infected with canker.

The percentage occurrence of tomato fruits infected with canker in this present research reveals that, from all the tomato varieties commonly cultivated in Niger state, Ronita V.F, variety had 29.5%, which was followed by San marzano with 25%, and the Chico variety which had a lower infection rate of 9%. The high percentage incidence obtained in variety Ronita VF, San Marzano and Roma VF may be due to the nutritional constituent, temperature of the area as well as yield of the varieties or even consumer preference, as farmers in these areas choose these varieties despite it susceptibility to the pathogen. This result corroborated with the findings of several researchers as presented by (Nabahat *et al*., 2014) who reported high frequency of occurrences in tomato fruits canker to be 16.51%. Sajad *et al.* (2017) reported Percentage frequency of occurrences of canker in tomato fruits

at maximium level 12.51%. High percentage incidence of canker in tomato fruits was also recorded by the researchers.

4.2.3 Frequency of *Alternaria alternata* in tomato varieties collected at different locations

The percentage frequency of *A. alternata* isolated from infected tomato fruits across the zones as seen in Table 4.4 shows that samples collected from Bida recorded 17.5% of *Alternaria alternata* infection rate which was higher than what was recorded for Lemu (14%), Sabon gida and Zungeru had both incidence to be 11%, samples from Garatu 5%. This is in line with the results of (Kumar 2017) who reported that the maximum disease incidence of 39.81% was observed in *Alternaria alternata*, tomato production in Niger State has been reduced by diseases like *Alternarai alternanta* whose relationships with host crop were unknown to the farmers.

Similarly, Shankara *et al*. (2005) reported that these diseases were found more frequently in tomato fruits, stating that the infections are declining the importance of tomato cultivation in the major growing areas. Moreso, in 2017 Sajad *et al.* (2017) reported that tomatoes were found to be infected with *A. alternata*, and with the highest frequency of occurrences occurred at 16.51% in India. The data from this present research shows apparently that different kinds of tomato diseases had different percentage incidence. Bright *et al*. (2012) reported that tomatoes were found to be infected with *A. alternata* in all the districts with the highest incidence of 63.9 % occurring in Agogo, followed by 43.5 % in Offinso-North Districts Ghana.

4.2.4 Pathogenicity of *Alternaria alternata*

Tomato fruits inoculated with the pathogen *Alternaria alternata* under laboratory conditions showed severe decay with the infected portions showing clearly visible fungal growth. These symptoms resemble those found under natural conditions. Fruits inoculated with distilled water had no visible sign of infection. This result is in agreements with the findings of Bright *et al*. (2012); Abdelnasser and Seifollah (2019) who reported similar findings that the isolated pathogen from infected portions showed a similar kind of colony growth and conidial morphology at day 3, and at day 8 the symptoms of the disease appeared fully on the tomatoes except the control. Hence fungal isolates were capable of causing diseases if proper conditions were given.

However, Sajad *et al*. (2017) reported that many studies carried out with respect to occurrences, causal organisms, severity, losses and pathogenicity. The present study showed that many fungal pathogens are associated with tomato diseases. *Alternaria alternata* are found to be major disease causing organisms which also appeared to be most active of all the pathogens that result losses of economic resources. Temperature and Humidity are important factor, which affect the vegetable fruits and provides medium for the growth of fungal pathogens.

4.2.5 Molecular identification of *Alternaria* isolates using the sequencing of ITS1 region

The Gel Electrophotograph showed above is used for the identification of fungi extraction at a particular region, it is a dominate marker showing the amplification of all the selected 10 isolates based on the band size using the ITS universal primers. This shows that the PCR product of 550 bp was amplified efficiently for all fungal isolates, indicates a positive

amplification of the isolates. Regarding this, the investigation of the genetic diversity of *A. alternata* species using the ISSR marker would facilitate the identification of suitable and effective strategies for controlling the fungal and mycotoxin contamination of human nutrition. This result is in collaboration with the reports of Abdelnasser and Seifollah (2019). The sequencing of ITS1 led to the identification of *Alternaria* species that are morphologically similar. The production of various mycotoxins by *A. Alternata* species leads to the contamination of livestock and human food.

4.2.6 Edited sequence using blast tool

A consensus sequence was analyzed by NCBI BLAST database for fungal identities. The ITS3-ITS4 region of identified fungi at > 98% similarity was compared with 100% similarity of the 10 region identified fungi at 99 to 100% matched with *alternaria alternata*. The results showed that the sequence of the extracted isolates had the highest similarity of 99-100% with *A. alternata* species

This result is in agreement with the work (Leiminger, *et al.,* 2013). Sequences of the ITS-rDNA region of the isolates were compared with those of the NCBI site using the BLAST software. The results showed that the sequence of the extracted isolates had the highest similarity of 99-100% with *A. alternata* species.

4.2.7 The phylogenetic relationship of *Alternaria alternata*

The dendrogram obtained from the cluster group of the species was drawn using ISSR on the basis of the Dice similarity coefficient) Figure 4.1). The correlation coefficient was obtained, and similarity range was estimated at 48-74%, based on which eight major groups This level of genetic diversity can be obtained as a result of a series of evolutionary

processes, including mutation, recombination, and migration. Grouping isolates with a distant position can be due to the transfer of the fungus from point to point.

This result corroborated with the findings of several researchers as presented by (pozzi *et al*. (2019) that, the dendrogram obtained from the cluster group of the species was drawn using ISSR based on the Dice similarity coefficient). The correlation coefficient was obtained as 0.66, and similarity range was estimated at 48-74%, based on which six major groups (i.e., A-F) were achieved by cutting the dendrogram into a similarity level of 60%. The obtained groups included Group A (four members), Group B (two members with a similarity of 100%), Group C (one member), Group D (two members), Group E (three members), and Group F (four members)**.**

4.2.8 Pairwise genetic distance

The Genetic diversity and polymorphic information below in Figure 4.6is showing the strains which are indicating the result of the conducted Sequence analysis by showing the difference in the strains. Location sample GAA AND ZAA which is having the genetic similarity of 0.000, while KAA and SGAA are also having the genetic similarity of 0.000 and are both indicating that they were found under the same cluster in the dedorogram,This clearly shows the different genotypes within the sequencing strains suggesting that there is variation within the species, they are all genetically *alternaria alternata* but are having a little difference in the strains which may have been due to environmental factor. This result is in agreement with the work of (Pozzi *et al.* 2019). The results of this study showed the suitability of ISSR marker for genetic variation studies in *Alternaria* species. This marker has been used by other researchers to study the genetic diversity of *Alternaria* species

4.2.9 ISSR: Inter simple sequence repeat, PIC: polymorphic information content of ten (10) *A. alternata*

Name and number of scored bands and number of polymorphic bands and polymorphic inter simple sequence repeat primers used in 10 isolates of *Alternaria alternenta*

The amplification of the ITS1 region in PCR was accomplished using two primers, namely ITS1 and ITS4. This result is related with the work of (Leiminger, *et al.* 2013). Who used ITS1 region in PCR and was accomplished using two primers namely ITS1 (5'TCCGTAGGTGAACCTGCGG-3') and ITS4 (TCCT CCGCTTATTGATATGC-3'). The PCR reaction was performed in a Biorad thermocycler (S 1000TM).

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATION AND CONTRIBUTION TO KNOWLEDGE

5.1 Conclusion

The results from this study indicates that Bida sample tomato has the highest incidence of fruit canker while Ronita V.F has the highest incidence of *Alternariaalternata* found in tomatoes samples across selected areas of Niger State

The bio diversity analysis shows that different strains of *Alternaria alternata* werepresent and responsible for tomato fruit canker in Niger state.

5.2 Recommendations

Pubic enlightenments should be carried out to educate producers and consumers on the effects of consuming tomato fruit infested with canker diseases

Research on control of *Alternaria* species of tomatoes pathogen should be intensified so as to proffer solution to the farmers in the State to reduce economic losses caused by fungal pathogen, so as to proffer solution to the farmers, thereby improving the production of tomato in the state.

5.3 Contribution to Knowledge

The thesis established that *Alternaria alternata* is present in farmers' field in Niger state. Samples of tomatoes from Bida had the highest occurrence of canker (17.5 %); this was followed by tomato samples from Lemu (14 %) while the least was obtained from Garatu (5 %). Varietal results of tomatoes showed that Ronita V.F had the higest percentage of

frequeancy of *A. alternata* (29.5 %) while San Marzano, Roma vf,Piac enza 0164 and chico had 25.0%,22.0%,14.5% and 9..0% respectively Molecular characterization (blasting) showed that samples from Bida, Lapai, Sabon gida, Kataeregi, Garatu, Paiko, Wushsishi, and Lemu had 100% presences of *Alternaria alternata. The* Molecular characterization further grouped the fungal strains into two (2) major clusters and eight (8) sub-clusters, indicating that they are of different strains of the same species.

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