

ASSESSMENT OF STORAGE METHODS ON THE SHELF LIFE, NUTRITIONAL
COMPOSITION AND FUNGAL SPOILAGE OF TOMATO (*SOLANUM LYCOPERSICUM* L.)
FRUITS

BY

GARUBA, TAOFEEQ

MATRIC NUMBER 08/68ER003

B.Sc. (SOKOTO), M.Sc. (ILORIN)

A THESIS SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY, FACULTY OF
LIFE SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
AWARD OF DOCTOR OF PHILOSOPHY (Ph.D.) DEGREE IN PLANT BIOLOGY,
UNIVERSITY OF ILORIN, NIGERIA.

FEBRUARY, 2018

CERTIFICATION

I certify that the studies reported in this thesis were conducted under the supervision of Prof. O. T. Mustapha and Prof. P. O. Oladele in the Department of Plant Biology and Department of Microbiology respectively, University of Ilorin, Ilorin, Nigeria. The Thesis has been read and accepted as meeting the requirements of the Department and the University for the award of the Ph.D. degree in Plant Biology (Plant Pathology).

Professor O. T. Mustapha

(Supervisor)

Professor P. O. Oyeyiola

(Co-supervisor)

Dr. A. A. AbdulRahaman

Head of Department

Examined this on -----day of ----- 2018

External Examiner-----

--

DEDICATION

This research work is dedicated to my parents.

ACKNOWLEDGEMENTS

“Alhamdulilah”. I give thanks to Almighty Allah, the most Beneficent and the most Merciful, for giving me knowledge and strength to complete this work. May the peace and blessings of Ar-Rahaman be upon the soul of Prophet Muhammed (SAW).

My gratitude goes to my supervisors, Profs. O. T. Mustapha and G. P. Oyeyiola, for their efforts, patients and understanding in mentoring me throughout the programme. I say “Jazzakumu lahu Khaira”. My special thanks also go to the Head of the Department, Dr A. A. Abdulrahman for his encouragements and advice that finally translated to this success. The supports and useful contributions of Dr. B. U. Olayinka, Dr. A. A. Lateef, Dr. (Mrs) K. A. Abdulkareem, Mr. G. S. Olahan and Mr. S. B. Adeyemi are appreciated. My appreciations are extended to Profs. E. O. Etejere, J. A. Morakinyo and P. O. Fatoba and Drs K. S. Olorunmaiye, C. O. Ogunkunle, D. A. Animashaun and S. O. Oyedeji. Also included are Mr. F. O. Egbede, Departmental Technologists and all non-academic staff of the Department of Plant Biology for their moral supports and series of encouragements.

My acknowledgement goes to my parents, Late Alhaji and Alhaja Garuba Ameen lah, for their care, love, prayer and support. May Allah forgive my father and spare the life of my mother with sound health. I profoundly appreciate endurance, perseverance, patience and spiritual support of my wife during the programme. May Allah ease her affairs. It is unforgettable to recognize my children, Taofeeqoh, Abubakar and Ahmad, for their prayers. My siblings, Toyyibah, Risqot, Yusuf, Maryam, Sa’ad, Sheriff deen and Ridwannullah, are special. Many thanks to the entire family and may He strengthen the bond.

Lastly, I must recognize the contribution of my project students (2015/2016 and 2016/2017 Session). They helped meaningfully during the field and laboratory stages of the work. My gratifications are extended to my friends and those who assisted in one way or the other that led to my success. “Jazzakumu lahu Khaira” to you all.

ABSTRACT

Tomato (*Solanum lycopersicum* L.) fruit is an important component of daily diet. The fruit is perishable and commonly attacked by fungi during storage. Hence, it becomes imperative to enhance its shelf life and minimize its spoilage. The objectives of this study were to evaluate the efficacy of storage structures and botanicals in prolonging the shelf life of four varieties of tomato fruits under different storage conditions, determine the effects of storage conditions on nutritional composition of four varieties of tomato fruits, evaluate the efficacy of botanicals on fungal load during storage, isolate and identify fungi associated with deteriorated tomato fruits using both morphological and molecular tools and determine the degree of virulence of fungal isolates and their biomass in different carbon and nitrogen-rich media.

Four varieties of tomato were used in this study: two local varieties (Hausa and Yoruba varieties) and two improved varieties (Tropimech and Roma VF varieties). Storage structures used were plastic crate, raffia basket and pot in pot refrigerator, while botanicals (wood ash of *Vitellaria paradoxa*, sawdust of *Khaya ivonresis* and *Oryza sativa* straw) were preservatives. Each botanical and sampled fruits from each variety were mixed in ratio 1:2 and stored accordingly. The shelf life was studied and lycopene content, proximate and mineral composition and fungal load were determined. The isolated fungi were identified using macromorphological and micromorphological features. Internal Transcribed Spacer (ITS) regions of the ribosomal DNA (rDNA) of fungi was amplified and sequenced. Pathogenicity test and frequency of occurrence for each isolate were carried out. Biomass of the isolates in response to carbon and nitrogen sources were determined. All data were analyzed using a statistical software called Statistical Package for Social Science (SPSS), version 16.00. One-way Analysis of Variance (ANOVA) was used to determine the differences within the variety. means were separated using Dunca

Multiple Range Test (DMRT). Univariate analysis of variation (under General Linear Model) was used to determine the interactions among the fixed factors (variety, storage and botanicals). Statistical software Origin 7.0 was used to plot the line graphs as well as bar charts.

The findings from the study revealed that the shelf life of all the varieties was prolonged in pot in pot refrigerator for up to 20 days and storage structures had no significant effect on the loss of firmness at $p \leq 0.05$. Proximate analysis showed that moisture was highest (95.92%), followed by carbohydrate (9.04%) in all varieties irrespective of storage structure and botanicals. Mineral composition of all stored tomato fruits was significantly influenced by interactions between variety, storage and botanical at $p \leq 0.05$. Sawdust had higher antifungal potential than rice straw and ash by reducing the fungal loads in the tomato fruits. *Aspergillus japonicus*, *Rhizopus oryzae*, *Curvularia geniculata*, *Fusarium proliferatum* and *Fusarium oxysporum* were associated with spoilt tomato fruits of all the varieties. Sequencing of the ITS regions of rDNA of the isolates confirmed their identities. All the fungal isolates were pathogenic to different degrees with the local tomato varieties more susceptible than improved varieties. *Curvularia geniculata* occurred less frequently than other isolates. Biomass of each isolate was dependent on carbon and nitrogen

sources in the media.

The study concluded that pot in pot refrigerator was the only suitable structure in elongating the shelf life of tomato fruits. Sawdust was very efficacious to reduce incidence of fungal spoilage in tomato fruits, followed by rice straw. Pot in pot refrigerator and sawdust are recommended to minimize postharvest losses of tomato fruits.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Tomato (*Solanum lycopersicum* L.) belongs to Solanaceae (night shade) family whose colour can be red, yellow or green depending on the variety and degree of ripeness. Other members of Solanaceae include *Solanum melogena*, *S. tuberosum*, *Capsicum annum*, *C. frutescence* and *Nicotiana tabacum*. It is native to South America, especially Peru and Galapagos Island (Matthew, 2011). Peralta and Spooner (2007) reported that the origin of tomato was traced to Peru and Mexico. Tomato is the second most important vegetable worldwide due to its nutritional values. Environmental factors such as temperature, the nature of the soil, rainy weather and frost can have a detrimental effect on the quality and shelf life of fruits and vegetables (Bachmann and Earles, 2000).

Solanum lycopersicum is grown in many parts of Nigeria both as rainy and dry season crops. Wilts caused by *Fusarium oxysporum*, *Sclerotium rolfsii* and *Pseudomonas solanacearum* are the important diseases of tomato in the Savanna and Forest zones of Nigeria. Decays caused by pathogens have been variously reported to constitute up to 50% of losses in fresh tomato produced in African countries (FAO, 2008a). High moisture content makes tomato and other succulent fruits to be vulnerable to post harvest loss. Poor handling of agricultural produce also accounts for the losses (Adeoye *et al.*, 2009). Fungi, viruses, bacteria and nematodes are implicated to cause damage to agricultural produce both at pre-harvest and post-harvest stages.

Tomatoes fruits are harvested at various stage of ripeness and this determines the type of storage conditions to be employed. Fully ripe tomato fruits are stored at 2-5°C for few days to avoid

chilling injury (Passam *et al.*, 2007). The unpleasant aroma of fruit stored at 5°C is caused by the loss of the principal volatile compounds that was detected by gas chromatography (Maul *et al.*, 2000). Enzymatic change during ripening also determines the changes in the flavour and aroma constituents of the fruits (Krumbein *et al.*, 2004). However, ripening and shelf life of tomato fruit can be delayed by enclosure of tomato fruits in polyethylene or other form of plastic packaging materials (Srinivasa *et al.* 2006). Bailén *et al.* (2006) reported that the fruit quality of tomato can be improved by inclusion of an ethylene adsorbent (granular activated carbon) within polypropylene bags in which tomatoes at the turning stage are stored at 8°C. This reduced ethylene level and consequently reduced weight loss, softening and colour change. High Ethylene synthesis and respiration are characteristics of climacteric fruits like tomato and these physiological activities are hampered by exposing mature green fruits to atmospheres containing ethanol from 2.5 to 25 ml per 2.5 kg fruit (Thakur *et al.* 2000).

Tomato fruit is highly nutritional as it is very rich in vitamin A and ascorbic acid. The fruits have a considerable amount vitamin K and calcium. Both of these nutrients are very indispensable in strengthening and performing minor repairs on the bones and bone tissue. Interestingly, tomatoes decrease the amount of hazard posed by smoking cigarettes. The fruits contain chlorogenic acid and coumaric acid that protect the body from carcinogens that are generated produced from cigarette smoke (Bhowmik *et al.*, 2012). Tomatoes are known to contain lycopene which is a form of carotene (Sesso *et al.*, 2003) and its content varies significantly depending on variety, environment and ripening (Brandt *et al.*, 2006). Lycopene is an antioxidant and can help prevent the development of many forms of cancer such as breast cancer, neck cancer and prostate cancer (Jian *et al.*, 2007). Cooked or raw tomatoes are considered as the best and reliable source of this antioxidant. The quantity of carotenes and their antioxidant potentials are determined by tomato

variety and maturity (Arias *et al.*, 2000). The role of lycopene is more than prevention of cancer development. It protects the skin against harmful ultraviolet rays and this make it to be useful in cosmetic industries (Stahl and Sies, 2004). Tomato has organoleptic quality and this can be experienced when it reaches the full red colour stage but before excessive softening. Red colour of tomato is achieved due to chlorophyll degradation, lycopene synthesis and other carotenoids as chloroplasts are transformed to chromoplasts (Radzevičius *et al.*, 2009).

The most important causes of spoilage in fruits and vegetables after harvest are certain fungi and bacteria. Viruses and nematodes play a minor role in post-harvest losses. About 20 different fungal species are associated with postharvest loss of tomatoes. Important disease-causing fungi include identified *Aspergillus niger*, *Rhizopus nigricans*, *Rhizopus stolonifer* and *Mucor spp* which had been recognized as the principal rot-causing agents of tomato (Matthew, 2011). A common bread-mold fungi (*Rhizopus stolonifera*), grows very aggressively even on refrigerated fruits. On tomato, *Rhizopus* rot is characterized by water-soaked and may exude a clear liquid. Also, sour rot pathogen (*Geotrichum candidum*) turns the flavor taste of tomato to sour. Gray mold disease of tomato caused by *Botrytis cinerea* is more severe in greenhouses with optimum temperatures, high humidity, and stagnant air (Gleason and Edmunds, 2006). Anthracnose is a common disease of tomato, occasionally attack pepper and other solanaceous fruits. The causal agent of the disease is *Colletotrichum coccodes*.

Disease development in tomato is of varying degree. Most common and obvious symptoms of deterioration result from the activities of fungi (Matthew, 2011). During post-harvest activities, wounds or physical injuries sustained by tomato fruits serve as an entrance for the pathogens especially passive invaders and such fruits are liable to microbial infection (Matthew, 2011).

Temperature is one of the major factors that determines postharvest quality of fruits. The ethylene production that induces ripening and respiration are affected by storage temperature, age of the fruit and cultivar (Oyewole, 2012). The storage life of climacteric fruits can be improved by low temperature, 90% humidity, removal of ethylene production, storing in 5% carbon IV oxide, use of chemicals and irradiation (Shaun and Ferris, 1997). One way of prolonging shelf life of tomato in tropics is the use of moist sawdust, in which the evaporation cools the fruits and decreases the rate of respiration (Johnson and Hodari-Okoe, 1999). The sawdust from different wood species has different potential to absorb and retain water and this is due to differences in the compositions. There may also be differences in the microorganisms which would infect stored tomato treated with the preservatives (Johnson and Hodari-Okoe, 1999). Wood ash possesses insecticidal and antifungal properties (Aborisade, 2003). Earthenware pot had been used for the storage of fruits and vegetables (Akomolafe and Aborisade, 2007).

1.2 Statement of the Problem

Postharvest loss is a collective loss along agricultural value chain, from handling and harvest (in the field) to storage, processing, packaging and transportation. Once fruit is harvested and removed from the orchard, the postharvest fruit rot phase commences and this can occur in storage, in the market (wholesale and retailed stores), in the household refrigerator, or in customer's fruit display basket (Michailides *et al.*, 2010). In most of developing countries, postharvest loss is considerably higher as a result of poor postharvest techniques (Babalola *et al.*, 2010). The quality and nutritional composition of fresh produce like tomato, is affected by postharvest handling and storage condition (Sablani *et al.*, 2006).

The postharvest loss can be caused by biological, chemical, mechanical, biochemical reactions, physical, physiological, psychological and microbiological factors (Arah *et al.*, 2015). Diseases of fruits may be caused by pathogens such as bacteria, nematodes and viruses and fungi or environmental factors like unfavourable temperature, light, relative humidity and unavailability of oxygen (Agrios, 2005). Postharvest diseases of fresh produce (fruit and vegetables) and mycotoxin contamination of some susceptible crops contribute to major losses to growers, processors, marketers and consumers. Postharvest diseases are the result of latent infections that occur in the field during the growing season and infections from wounding during harvest and handling operations (Michailides and Manganaris, 2009).

One of the major challenges especially in developing countries is how to enhance food security and ensure its long term sustainable development (Kiaya, 2014). Despite high demand of tomato, postharvest spoilage has been of serious concern. Postharvest diseases start from the field but the symptoms on infected fruits may remain latent until they get to the store. Stored fruits are liable to different rots caused by pathogens such as bacteria and fungi. Postharvest loss prevents people to have access to sufficient and adequate agricultural produce especially perishable fruits such as tomato. Most of these fruits are perishable due to their physiological nature as they have high moisture content, high respiration rate as well as the succulent texture. These properties not only promote physiological damages but also support microbial invasion and colonization of the fruits (Karim and Hawlader, 2005).

There are different fungi responsible for fruit rots of tomato during storage. Many of these achlorophyllous organisms are apparent and grow on the surface of the infected hosts with different macromorphological features. The extent and severity of damage caused by fungi depends on their nature and type. Fungal identification is difficult and can definitely result to

diagnostic error (Sangoi *et al.*, 2009). In addition, many fungi share similar macromorphological and micromorphological features and this makes their identification a tedious task. The use of morphological approaches in fungal systematics may not be enough for lower-level (species) classification and become enigma without molecular tools.

1.3 Justification for the Study

Fruits and vegetables are nutritious, valuable foods full of flavour. In a hungry and increasingly competitive world, reducing postharvest food losses is a major agricultural goal. Many factors are responsible for postharvest losses in fresh fruits. These include environmental conditions such as heat or drought, mechanical damage during harvesting and handling, improper postharvest sanitation, and pathological and physiological factors. Micro-organisms, especially fungi have been reported to cause extensive deterioration of fruits and vegetables. Attempts had been made to reduce postharvest losses of tomato by modifying ambient temperature such as refrigeration. Due to epileptic power supply and for the benefit of the rural dwellers that do not have access to electricity, there is need to consider Pot in Pot technology as well as some botanical preservatives as substitutes and to test how effectiveness they are in fruit preservation.

Fungal spoilage of tomato fruits has contributed immensely to the postharvest losses making unbalance system between demand and supply of these vegetable fruits. Fungi, among other microorganisms are responsible for the spoilage. It becomes necessary to identify these organisms with high degree of precision to reduce the risk of not only loss but also contamination of the fruits. Biology and physiology of fungi are dissimilar and this makes their control cumbersome. However, detection, isolation as well as identification become imperative to proffer appropriate management techniques and control strategies.

1.4 Aim of the Study

This research work aimed at assessing the effects of different storage methods on the shelf life, nutritional composition and fungal spoilage of four varieties of tomato fruits.

1.5 Objectives of the Study

The objectives of this study were to:

- evaluate the efficacy of storage structures and botanicals in prolonging the shelf life of four varieties of tomato fruits under different storage conditions
- determine the effects of storage conditions on nutritional composition of four varieties of tomato fruits
- evaluate the efficacy of botanicals on fungal load during storage
- isolate and identify fungi associated with deteriorated tomato fruits using both morphological and molecular tools
- determine the degree of virulence of fungal isolates and their biomass in different carbon and nitrogen-rich media.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cultivation of tomato

Tomato is one of the most the cultivated vegetable in the world. China is the world's top tomato grower responsible for about one-quarter of the world's tomato acreage. Nigeria and Turkey are also in the rank of major producers of the tomato (FAO, 2008b). Tomato is usually propagated from seed and the soil should be well-prepared, loose and in good tilth. The seeds are usually broadcasted on the bed and maintain for three to four weeks before transplanting on moist soil. The plants need moderately high requirement of nitrogen to promote better growth, flowering and fruit set. Tomato also need high quantity of potassium and this enhances colour taste, firmness, sugar and acids. Compost, poultry manure and farmyard manure are forms of organic manure that contains both micro- and macronutrients.

Tomato requires dry but relatively cool climate for better yield and fruit quality. The plant can survive in wide range of environmental conditions but optimum temperature lies between 21 and 24°C. The plant tissues are adversely affected when temperature is below 10°C and above 38°C (Naika *et al.*, 2005). During land preparation, ploughing is very important because it enhances the soil structure as well as its water holding capacity. Besides, it also reduces soil borne pathogens by exposing the soil to the sun. watering is very important because tomato is not resistant to drought. The quantity of water is required lies on the type of soil and weather (Naika *et al.*, 2005).

2.2 Storage of tomato Fruits

The majority of the fruits and vegetables are stored to meet the demands of the fresh market and food industries. The two classes of agricultural produce decay during prolonged storage and this may be as a result of biochemical activities, physiological aging and pathogenic attack. The decay or deterioration of fruits is characterized by degradation of appearance, textural quality and aroma (Ahvenainen, 1996; Watada *et al.*, 1996). A proper postharvest handling especially storage is very indispensable because most of the losses incurred after are usually as a result of poor handling and improper storage. Losses in tomato fruit is highly alarming due to the fact that it faces several problems during transportation, storage and marketing. This makes the fruit not only lose its potential quality but also encounter a substantial postharvest loss (Nasrin *et al.*, 2008). The fruit is nutritional but the nutrients decrease as the storage period increases (Idah *et al.*, 2010).

A modified atmosphere alters the normal storage conditions to provide optimum atmosphere for increasing the storage length and quality of produce (Phillips, 1996). Some varieties of tomatoes were recommended to be kept at 10°C or higher to prevent a condition known as chilling injury (Roberts *et al.*, 2002). This condition is a symptomatic condition characterized by irregular color development, excessive softening, surface pitting, and increased decay (Cantwell, 2008). However, low oxygen (1-5%) and high carbon dioxide reduces the respiration rate of fruits and vegetables and inhibit ethylene production, thus preventing ripening (Lee *et al.*, 1996; Sargent and Moretti, 2004). Packing materials used in storage play various roles including insulation against unstable temperatures, moisture retention, and minimization of disease transmission. Clean straw, dry leaves, corn stalks, hay and sawdust are usually used for insulation. The

materials can be recycled and turned to mulch. Moisture retention of produce is usually achieved with moistened sand, sawdust or peat moss (Anonymous, 2015).

2.3 Shelf life of tomato Fruits

According to Rao *et al.* (2011), shelf life of fruit can be determining by considering the duration of the fruit in the storage without losing its acceptability and marketability. When more than 50% of fruits display undesirable symptoms such as shrinkage or decay due to pathogens and chilling injury, lot of fruits was considered to have attained end of shelf life (Tsegay *et al.*, 2013). Moreover, deterioration of tomato fruits requires involvement of modern technologies their minimize their spoilage and elongate their storage periods (Gonzalez-Aguilar *et al.* 2009). Extending the storage life of tomato fruit has become necessary for domestic and export markets. Tomato has a short shelf life at a normal atmospheric condition. The perishability of this vegetable fruit may be as a result of its moisture content. Perez *et al.* (2003) reported that the quality of most fruits especially tomato is influenced by water loss during storage and this is determined by relative humidity and temperature conditions. Spoilage of tomato during storage makes the fruit scanty or unavailable during off season. Postharvest loss has considerable effects on the income of the farmer (Davoodia *et al.*, 2007). The shelf life of tomato fruits at room temperature is up to seven (7) days (Požrl *et al.*, 2010). Tomato and cherry are usually stayed up to 18 days at 2-5°C especially when used as components on fresh-cut vegetables trays(Akbudak *et al.*, 2007; Ilic and Fallik, 2007). The shelf life of tomato fruit can be extended up to 2 to weeks (Shahnawaz *et al.*, 2012). Castro *et al.* (2005) reported that storage at low temperature (10-15°C) and relative humidity between 85-95% have tendency to prolong the shelf life of tomato and in addition to that, both ripening rate and chilling injury are insignificant at this condition. High

temperature and low humidity usually favour fruits decay and accompanying with degradation of texture and nutritional qualities (Žnidarčič and Požrl 2006).

The atmospheric conditions affect the shelf life of tomato and as a result of this, the need for developing postharvest technology to extend the storage life of this fruit becomes imperative. According to Shahnawaz *et al.* (2012), packaging materials polyethylene bag and newspapers were identified as a factor that contribute to extension of shelf life of fruits. This may be achieved through inhibition of physiological deterioration as well as minimization of water loss. Besides, storage materials can modify the ambient condition and create gas atmospheres around the fruit which may probably slow down the respiratory activity of tomato which will eventually retard the ripening process.

Opiyo *et al.*, (2005) opined that the storage life of tomato can be improved by harvesting the fruit at optimum stage of maturity (mature green). Ripe (fully red) tomato may be required by instant consumer but undesirable if the fruits are planned to transport to a very long distance. Preservation of tomato is cumbersome in the tropics due to poor transportation networks and high atmospheric temperature which encourage decay of the produce (Ajayi and Oderinde, 2013).

2.4 Preservation of Tomato Fruits

Tomato fruits are highly perishable. One of the sanitary measures employed to extend the storage life is washing system. This system ensures removal of dirt, soil, insect and other foreign materials and this may eventually translate to reduction in microbial contamination in the fruits (Seymour, 2003). Vigorous washing and decontamination of fruits and fresh leafy herbs may be difficult because of their fragility and succulence.

There are number of techniques used in fruit preservation. Control of storage temperature is an important factor controlling biochemical reactions, enzymatic activities, transpiration and microbial growth ((Wiley, 1994). Temperature management during storage can inactivate or prevent deteriorative physiological defects (De Roever, 1998). Immediately after harvest, the fruits are stored in raffia baskets for transit. Matthew (2011) reported that about 40% of the content of such baskets is lost to rotting and decay which is a severe financial loss to growers, middlemen and retailers. This is an indication that raffia basket without any preservative is not good for transporting such perishable agricultural produce.

Disinfectants are used to surface sterilize fresh produce. Chlorine is commonly and widely used, it is relatively affordable and effective. This disinfectant comes in different forms like sodium hypochlorite, calcium hypochlorite and gaseous chlorine (Garret, 1992). A concentration of 100ppm is allowed as industrial standard for disinfestation of fruits.

2.5 Lycopene Content of Tomato Fruits

The basic properties that influence consumer purchase decision are the textural quality, shape, size, shape, freshness, organoleptic (flavour) as well as the colour of the fruits especially tomato (Požrl *et al.*, 2010). The primary colour of many varieties of tomato is redness and this is determined by lycopene content (Frusciante *et al.*, 2007; Khairia *et al.*, 2015). Lycopene is a bright red carotenoid pigment present in tomato and some other fruits that assume similar colour such as papayas, watermelon and red carrot. This form of carotenoid is the most abundant and efficient free radical scavenger with potential found to be more than twice that of β - carotene (Nour *et al.*, 2013).

The form of lycopene that is predominantly present in tomato and all its products is trans-lycopene amounting to about 35-96% of the total lycopene and low levels of cis-lycopene

ranging from 1% to 22% of the total lycopene content (Schierle *et al.*, 1997). They are health stimulating fruits due to the antioxidants present in them. Presently, the demand for antioxidant rich fruits is becoming interesting due to the realization of their health benefits. The antioxidants components of fruit include but not limited to lycopene, phenolic, flavonoids compounds and vitamins (Sánchez-Moreno *et al.*, 2003). Antioxidant agents hamper oxidation of molecules by preventing initiation of oxidizing chain reactions (Radzevičius *et al.*, 2009). Fruits are a good source of natural antioxidants containing different components that protect against harmful-free radicals and have associated with reduction in mortality rates caused by cardiovascular diseases and cancers (Shui and Leong, 2006).

Free radicals are thought to be responsible for damaging the body's cells and their genetic makeup leading to some chronic diseases. Clinton (1998) identified the most important antioxidants as carotenes. Lycopene is the predominate carotene in tomato fruits and its concentration varies depending on ripening, environment and variety (Brandt *et al.*, 2006). Colour in tomato is the most important external characteristic to check ripeness and postharvest life and is a major factor in the consumer's purchase decision. Red colour due to the degradation of chlorophyll and synthesis of lycopene and other carotenoids, as chloroplasts are converted into chromoplasts (Radzevičius *et al.*, 2009). The health benefits of lycopenes are unimaginable. High levels of lycopene stave off cancers such as cervical cancer, prostate cancer, rectal cancer, colon cancer and cancers of the mouth, stomach, pharynx and esophagus (Bhowmik *et al.*, 2012).

2.6 Nutritional Importance of Tomato Fruits

Fruits and vegetables are very essential in our diets. They are sources of minerals, vitamins, fibers, and antioxidants, which are essential for a healthy and well-balanced diet. Consumers like

going for fruits of high quality based on their appearance (color), sensory quality (texture and taste), and nutritive values. Storage may change the nutritional quality of the fruits, sometimes negatively (Gil *et al.*, 2006). During storage, there are certain biosynthetic pathways that contribute to the development of organoleptic qualities in fruits. An ideal quality development in fruit is as a result of the degradation of starch into sugars and that of cell wall, as well as the biosynthesis of several secondary metabolites, which provide color and flavor to the fruits. The developments of ideal color, flavor, sugar levels, and optimal firmness are key parameters that provide satisfactory fruit quality (Sharma *et al.*, 2008).

Generally, fruits are a natural source of basic nutritional elements. Water is the most dominant element in fruits accounting for about 90% of the total mass (Vincent *et al.*, 2009). Tomatoes fruits are a good source of protein containing about 19 amino acids principally glutamic acid (Matthew, 2011). Many minerals support body cells and structures while others regulate body processes (Dahl and Turner, 2015). Tomato contains many vitamins and minerals that ensure good health. It is an excellence source of vitamins B6, ascorbic acid, and niacin and minerals that function as cofactors in enzymatic activities (Nour *et al.*, 2013; Luthria *et al.*, 2006). Tomato fruits are very good sources of potassium. The element is an electrolyte that is very indispensable for the human body to function normally. Potassium regulates movement of nutrients and wastes into and out of the cells (Psota, 2014). Olaniyi *et al.* (2010) reported that tomato is very rich in calcium, magnesium, phosphorous, iron, zinc and copper. Calcium gives strength to the bones and iron in the diet is necessary for formation of hemoglobin, that carries oxygen in the blood, and myoglobin, which transports oxygen to muscle tissue. The element also plays a vital role in muscle contraction and relaxation. Magnesium helps in functionality of enzymes, muscle contraction as well as energy transport. The health of nerves and formation of blood cells are

being maintained by copper. Zinc boosts Immunity, enhances growth and development, hastens wound healing and helps synthesis of proteins and DNA.

2.7 Postharvest Losses of Tomato

Tomato plants are prone to be attacked by pathogens such as fungi, bacteria and viruses. As virus infection affect the growth and yield of the plant, fungi and bacteria cause leaf or foliar, stem as well as fruit diseases (Naika *et al.*, 2005). Post-harvest losses are described as any change in the availability, edibility, wholesomeness and quality of agricultural products which begin from the field during harvesting. Estimates of the post-harvest losses of food grains in the developing world from mishandling, spoilage and pest infestation are put to 25% meaning that one-quarter of what is produced is unavailable to the consumer. The effort and money expended to raise such produce are lost forever (FAO, 1989).

Postharvest technology plays vital roles in preventing qualitative and quantitative losses in fruits and vegetables and the losses are among the challenges faced by developing countries (Babitha and Kiranmayi, 2010). Biological processes such as ripening and respiration continue even after harvest till its energy reserves can sustain it (Padmini, 2006). Tomatoes are susceptible to tremendous biochemical changes until spoilage finally sets in after harvest due to attack from bacteria, yeast, mould and viruses. Fungi were implicated to cause spoilage in tomatoes samples rather than bacteria. *Aspergillus niger* and *Fusarium* were found with highest frequency of occurrence in spoiled samples with a few samples containing *Penicillium sp* (Ghosh, 2009). The fungi that are commonly caused postharvest in the fruits are *Aspergillus phoenicis*, *A. niger*, *Absidia* spp, *Trichoderma* spp, *Fusarium oxysporum*, *F. moniliformis*, *Mucor* spp, *Rhizopus stolonifer*, *Penicillium* spp, *Alternaria alternata*, and *Geotrichum* spp (Etebu *et al.*, 2013). The rots caused by these fungi may be named after the specific etiological agent (*Alternaria* rot,

Fusarium rot and *Rhizopus* rot) or base on the symptoms such as soft rot, sour rot, grey mould, stem end rot and watery rot (Ramaswamy, 2015). Also, *Bacillus megaterium*, *Listeria monocytogens*, *Bacillus laterosporus* and *Morganella morganii* were bacteria isolated from tomato samples (Ibrahim *et al.*, 2011a). Tomato contains about 90% water of fresh weight and high percentage of water makes the fruit perishable (Babitha and Kiranmayi, 2010). Weight loss during storage may be as a result of water loss which may depend on temperature and relative humidity conditions (Perez *et al.* 2003).

The production of fruits and vegetables has been an essential sector in the total world agricultural output. Both pre-harvest and post-harvest losses are threats to availability of agricultural produce. Post-harvest losses are described as any change in the availability, edibility, wholesomeness and quality of agricultural products which begin from the field during harvesting. Estimates of the post-harvest losses of food grains in the developing world from mishandling, spoilage and pest infestation are put to 25% meaning that one-quarter of what is produced is unavailable to the consumer. The effort and money required to produce such produce are lost forever (FAO, 1989).

Immediately after harvest, the obtainment of nutrient by fruit from the mother plant ceases. Therefore, fruits have limited postharvest life. Postharvest losses are caused by physiological, mechanical and pathological factors (Arah *et al.*, 2015). Individual or overall effects are influenced by external conditions such as temperature, relative humidity and light. Mechanical factors include any physical injury as a result of poor handling, harvesting, transporting and storage. The injuries may cause by unsuitable field or marketing containers with sharp edges and poor nailing, over packing, dropping, throwing or walking on produce and poor harvesting practices. Physiology of stored fruits is majorly centered on three activities: respiration,

transpiration and ripening. Ripening of fruits and vegetables is often characterized by complex physiological and biochemical changes. The physiological changes that occur during ripening of tomatoes include degradation of starch and reduction of glucose and fructose, degradation of chlorophyll, synthesis of pigments such as β -carotene and lycopene (yellow colouration), enhanced activity of cell wall-degrading enzymes, evolution of aroma compounds, and an increase in respiration and energy production, occur during the progression of ripening (Paliyath and Murr, 2006). Ripening instigates the catabolic breakdown of starch releasing sugars, and organic acids are converted back to Sugars through a process called gluconeogenesis (Sharma *et al.*, 2008).

Spoilage of tomato fruit is an undesirable change in the quality of the fruit caused by abiotic and biological agents (Samuel and Orji, 2015). The spoilage poses a serious threat to the consumer as it affects taste, smell and texture of the fruit. These fungi produce mycotoxins that are liable to cause mycotoxicoses in man after ingestion or inhalation (Baker, 2006). These harmful secondary metabolites are not limited to area of infection but mix with water and diffuse across all parts of the fruits (Samuel and Orji, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Planting of Tomato Seeds

Four (4) varieties of tomato were used in this study: two local (Hausa and Yoruba) and two improved (Tropimech and Roma VF) varieties. The seeds of local varieties were procured from Kwara State Ministry of Agriculture while improved varieties were obtained from National Horticultural Research Institute (NIHORT), Ibadan. The seeds of each variety were planted in a farm located at Lasaju along Ilorin-Ogbomosho Express Road. At maturity, fruits of each variety were manually harvested and packed into raffia baskets sterilized with 70% ethanol. The baskets containing the harvested fruits were transported in an open van to the Plant Biology Department (Laboratory Unit), University of Ilorin, Ilorin. Each variety was designated as V1 = Hausa variety, V2 = Yoruba variety, V3 = Tropimech variety and V4 = Roma VF variety

3.2 Preparation and Modification of Storage Materials

Freshly harvested fruit samples of each variety were sorted to eliminate bruised, punctured and damaged ones. The fruits were surface sterilized with 70% ethanol and rinsed three times with sterile distilled water. Three (3) storage structures were employed viz: plastic crate, raffia basket and pot in pot refrigerator.

Pot in pot refrigerator was prepared by purchasing two clay pots of different sizes. The smaller pots were put inside the larger pot leaving the space of 2-3cm between the pots. The larger pot was previously filled with sand to a height that placed the smaller pot in even height with the larger pot. The space between the pots was also filled with sand leaving a small gap at the top.

Water was poured to the sand until it was completely saturated and unable to take more water. Soaked white cloth was placed over the top of inner pot to cover the opening completely.

3.3 Storage of Tomato Fruits with Preservatives

Three botanical preservatives used were wood ash of *Vitellaria paradoxa*, sawdust (shaving) of *Khaya ivonresis* and rice (*Oryza sativa*) straw. The methods of Baoua *et al.* (2012) and Johnson and Hodari-Okae (1999) were used with modifications. Each botanical and sampled fruits from each variety were mixed in ratio of 1:2 which is equivalent to 1.75 kg of botanical to 3.5 kg of fruits and in each storage device. The experimental setups were described below: VPCA (Variety + Plastic crate + Ash), VPCR (Variety + Plastic crate + Rice Straw), VPCS (Variety + Plastic crate + Sawdust), VPCC (Variety + Plastic crate + Control), VPPA (Variety + Pot in Pot + Ash), VPPR (Variety + Pot in Pot + Rice Straw), VPPS (Variety + Pot in Pot + Sawdust), VPPC (Variety + Pot in Pot + Control), VRBA (Variety + Raffia Basket + Ash), VRBR (Variety + Raffia Basket + Rice Straw), VRBS (Variety + Raffia Basket + Sawdust) and VRBC (Yoruba Variety + Raffia Basket + Control). Observations were made every day

3.4 Determination of Effects of Storage Methods on Shelf Life and Physical Properties of the Tomato Fruits

All samples were stored under different conditions as described in Section 3.3. Shelf life of fruits was evaluated using the method of Rao *et al.* (2011). When more than 50% of fruits showed symptom of shrinkage or spoilage (fruit rot), such fruits were considered to have reached their shelf life.

At an interval of 4 days, the weights of the samples were checked. The following mathematical representation was used to determine weight loss.

Percentage weight loss = $(W1 - W2 \times 100) / W2$

Where

W1 = Final weight

W2 = Initial weight

Firmness was determined as described by Daulagala and Daundasekera (2015) using textural hand feel and this was scored on a scale of 1- 4 (where 1 = not firm, 2 = averagely firm, 3 = firm and 4 = very firm).

Colour was assessed on a scale of 1-5 (AMS, 1991), where 1 = Large green, 2 = Breaker, 3 = Pink, 4 = Light Red and 5 = Red-ripe.

3.5 Determination of Lycopene in Stored Tomato Fruits

Lycopene was extracted and estimated using the method adopted by Ordoñez-Santos and Ledezma-Realpe (2013). For the determination, 0.1 g of each fruit sample was weighed in a tube, and then 7 mL of 4:3 ethanol/hexane was added, the tube was capped, covered with aluminum foil. The tube was then placed on shaker for 1 h, after which 1 mL of sterile distilled water was added and shaking was continued for a further 5 min. A sample of the organic (hexane) phase was read at 503 nm compared with hexane in a Genesys 10 UV-Vis spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI, USA).

The lycopene content in the hexane extracts was then calculated using the formula stated below:

$$\text{Lycopene } (\mu\text{g/g}) = (A_{503} \times 537 \times 2.7) / (0.1 \times 172)$$

where 537 g/mole is the molecular weight of lycopene, 2.7 is the volume (mL) of the hexane layer, 0.1 g is the weight of sample added, and 172 mM⁻¹ is the extinction coefficient for lycopene in hexane.

3.6 Determination of Effects of Storage on Proximate Composition of the Fruits

Proximate analysis was carried out and the procedures for determinations of moisture, ash, protein, fibre, crude fat and carbohydrate were described as follows.

3.6.1 Determination of Percentage Moisture

The percentage of moisture was determined using the method of AOAC (2000). The crucibles with the samples were placed in hot air oven and maintained at 60°C and dried for 2 hours. Crucibles were removed, cooled in the desiccator and weighed. The process of heating, cooling and weighing was repeated until constant weight attained. The percentage of moisture was determined by the following formula:

$$\text{Moisture content} = (M1 - M2) / (M1 - M) \times 100$$

Where M = Weight of the crucible in gm

M1 = Weight of crucible with sample before drying in gm

M2 = Weight of crucible with sample after drying in gm

3.6.2 Determination of Percentage Ash

Ash content was determined using the method of Okalebo *et al.* (2002). Five grams of each sample was weighed and heated using muffle furnace at 600°C for 3 hours until a light-grey ash was produced. Percentage ash content was estimated using the following calculation.

3.6.3 Determination of Percentage Protein

The Micro Kjeldahl Method of AOAC (2000) was used. It involved digestion, distillation and titration. Two grams of each of the sample was added to 10ml of concentrated H₂SO₄ in a heating tube. The catalyst (one tablet of selenium) was added. The mixture was transferred to the fume cupboard and heated. The digest was diluted with distilled water. For distillation, 10ml of the digest mixed with equal volume of 45% NaOH solution and transferred into a Kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 4% boric acid solution containing 3 drops of methyl red indicator. The distillate was titrated against 0.1N HCl.

The percentage nitrogen was estimated using the following formula:

$$\text{Nitrogen (\%)} = \frac{(100 \times 14 \times VF \times T)}{(100 \times Va)} \times 100$$

Where

N = Normality of the titrate (0.1N)

VF = Total volume of digest = 100ml

T = Titre value

Va = Aliquot volume distilled

The percentage crude protein was determined by:

$$\% \text{Crude protein} = \% \text{Nitrogen} \times 6.25$$

3.6.4 Determination of Crude Fat

Crude fat determination was achieved by extraction method (AOAC, 2000). Five grams of each sample was extracted with 150 ml petroleum ether in a Soxhlet Extractor at a boiling point of 80°C. The extraction was done for 6 hours with moderate boiling using electrothermal heater. The conical flask containing the crude fat and solvent was connected to a condenser to remove the solvent for 1 hour. The percentage crude fat content was estimated using the calculation below:

$$\text{Crude fat (\%)} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

3.6.5 Determination of Crude Fibre

This was determined using the method adopted by Shahnawaz et al. (2009). Each of the defatted sample was transferred into a beaker and 200ml of pre-heated 1.25% H_2SO_4 was added. The solution was boiled for 30minutes and maintaining constant volume by addition of hot water. The buckener flask funnel fitted with Whattman filter paper was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was filtered through the funnel under sufficient suction. The residue was washed severally with boiling water (until the residue was neutral to litmus paper) and transferred back to the beaker. Then, 200ml of pre-heated 1.25 Na_2SO_4 was added and boiled for another 30minutes. It was filtered under suction and washed thoroughly with hot water and twice with ethanol. The residue was dried at 65°C for about 24hours and weighed. The residue was transferred into a crucible and placed in a muffle furnace (400-600°C) and ashed for 4hours. This was cooled in a dessicator and weighed.

Crude Fibre (%)=(Dried weight of residue before ashing – weight of residue after ashing)/(
Weight of sample) ×100

3.6.6 Determination of Percentage Carbohydrate

Carbohydrate content of each sample was determined by difference. This was done by subtracting the sum of moisture, ash, protein, crude fat and crude fibre percentage from one hundred. That is;

Carbohydrate (%) = 100 – (% moisture + % ash + % protein + % crude fat + % crude fibre)

3.7 Determination of Minerals in Stored Tomato Fruits

In determining the minerals present in the samples, 2g of each sample was ashed in a muffle furnace. Fifteen milliliters of 20% (v/v) of nitric acid solution was added to the ash in the crucible to break up the ash. This was boiled, filtered and acid-washed through Whatman paper. The residue and the paper were washed 3 times with deionized water. Atomic absorption spectrophotometer (Pye Unicam sp. 9 AAS) was used for determination of phosphorous (P), potassium (K), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe) and calcium (Ca).

3.8 Determination of Fungal Population in Stored Tomato

This was done using the method of Rompre et al. (2002). Potato Dextrose agar (PDA) was poured to petri dishes and allowed to solidified. One millilitre of homogenized 10⁻³ dilution sample was inoculated into the plates and incubated at 25±2°C. After 72hrs of incubation, the number of mold colonies was counted by colony counter.

3.9 Collection of Spoilt Tomato Fruits

The deteriorated fruits of each variety were aseptically collected into labeled sterilized plastic crates and taken to the laboratory for pathological examinations.

3.10 Preparation of Culture Medium

Potato Dextrose Agar (PDA) was used to isolate fungi. This nutrient medium was commercially obtained in dehydrated form. In preparing the medium, 39g of powdery PDA was suspended in 1L of distilled water. The powder was dissolved by heating with frequent agitation until complete dissolution was observed. This was sterilized in an autoclave at 121°C for 15 minutes. After autoclaving, Chloramphenicol (30mg/L) was added to inhibit the bacterial growth (Amadi *et al.*, 2014). The prepared medium was stored at 8-15°C when not in use.

3.11 Isolation of Fungi from Deteriorated Tomato Fruits

The samples were surface sterilized with 70% ethanol by swabbing method for 2 minutes, then rinsed with several changes of sterile distilled water and was blotted dry with sterile filter papers. The sterilized fruits were put in a desiccator containing cotton wool moistened with distilled water to create a micro-humidity chamber of 100% relative humidity which was measured by direct air reading hygrometer. This was done so as to induce the growth of fungi (Arekemase, 2008). After series of sub-culturing, pure cultures of each isolate were obtained from emerging mycelial colonies and maintained on PDA slant in Mc Cartney bottles (Oladiran and Iwu, 1993). The bottles were stored in a refrigerator.

3.12 Morphological Identification of Fungal Isolates from Deteriorated Fruits

The isolates were identified based on macroscopic and microscopic characteristics of the pure culture of each fungal isolate. The macroscopic properties observed were size of colony (whether filamentous or colonial), surface colour of colony and colour of the reverse side of the plate. Microscopically, the features observed were the nature of the hyphal wall (thick or thin and shape), presence or absence of spores, colour of the hyphae, presence or absence of septa, type and nature of reproductive apparatus (sporangiophore or conidiophore), shape and colour of the spore (Fawole and Oso, 2007).

3.13 Molecular Identification of Fungal Isolates from Deteriorated Tomato Fruits

Molecular identification of fungal isolates involved three major stages viz: DNA Extraction, Polymerase Chain Reaction and DNA Sequence.

3.13.1 DNA Extraction and Purification

Pure culture of each fungal isolate (7-day old) was used. Genomic DNA of each isolate was extracted and purified using Zymo Research DNA kit (The Epigenetics Company, USA) and guided by the attached protocol.

From the pure culture of each isolate, the pure mycelia were scrapped and transferred into micro-centrifuge tube. Then, 750µl Lysis solution was added to the tube and shake for about 10minutes using voltex mixer to suspend the cells. 100mg (wet weight) fungal that have been re-suspended was added to 200µl of water. The ZR Bashing Bead™ Lysis tube was centrifuge at 5,000xg for 2 minutes after which 400µl supernatant was transferred to a Zymo- spin™ IV Spin

filter in a collection tube and centrifuge at 5,000xg for 2 minutes. 1200µl of fungal DNA binding buffer was added to the filtrate in the collection tube.

Furthermore, 800µl of the mixture was then transferred from the filtrate to a zymo-spinTM11C column³ in a collection tube and was centrifuge at 5,000xg for 2 minutes. The flow through from the collection tube was then discarded and the last step was repeated. 200µl DNA pre-wash Buffer was added to the Zymo-spinTM 11C column in a new collection tube and centrifuge at 5,000xg for 2 minutes. Then 500µl fungal DNA wash buffer to the Zymo-spinTM11C column and centrifuge at 5,000xg for 2 minutes. The Zymo-spinTM11C column was then transferred to a clean 1.5ml micro-centrifuge tube and 100µl DNA Elution Buffer was added directly to the column matrix and centrifuge at 5,000xg for 1 minute to elute the DNA. Ultra-pure DNA was then gotten.

3.13.2 Polymerase Chain Reaction

This was done in the Central Research Laboratory, University of Ilorin. The Internal Transcribed Spacer (ITS) region of each isolate was amplified using universal primer pairs: ITS1 (5'-TCCGTAGGTGAACCTGCGG3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Liu *et al.*, 2007). The PCRs were done in a 25µl reaction volume containing DNA templates as summarized in Table 1. Amplification was carried out on a thermal cycler PCR machine and programmed (Table 2).

3.13.3 DNA Sequence

Sanger sequencing method was used to determine the chains of nucleotides in the DNA templates. The proliferated DNAs were sent for sequencing. This was done in Inqaba Biotech-africa's Genomics Company, Pretoria, South Africa.

3.14 Sequencing Alignment and Phylogenetic Analysis

The consensus DNA sequence for individual isolate was generated using Seqtrace software (Seqtrace-win-0.9.0). The ITS nucleotide sequences for each fungal isolate were compared with those in the open access database of National Centre for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool for Nucleotides (BLASTN) Sequences. Alignment of ITS DNA sequences was done using AliView software (AliView version 1.17.1). Phylogenetic trees were generated between query sequence and subject sequences downloaded from NCBI using Molecular Evolutionary Genetics Analysis (MEGA version 6.0 programme).

3.15 Determination of Frequency of Occurrence for Fungal Isolates

Frequency of occurrence of fungi species was determined as described by Adebola *et al.* (2014) and Mostafa and Kazem (2011) using the following formula:

$$FO (\%) = (ns \times 100) / N$$

where FO is frequency of occurrence, ns is the number of plates that fungi species appeared, N is the number of plates incubated for sample area.

3.16 Pathogenicity Test of the Fungal Isolates

Pathogenicity or decay test was carried out to verify if the fungal isolates were really responsible for the decay and spoilage of the studied fruits. Healthy fruits were surface sterilized with 70% ethanol. Cylindrical plug tissues were cut from the fruits using a sterilized 5mm sized cork borer. Agar disc containing 7-day old fungal cultures of each isolate were aseptically placed in these holes, then covered with the plug and sealed with petroleum jelly. The control samples were not inoculated with any fungal isolate. Each sample was put in a transparent plastic container, above

filter paper moistened with distilled water to create a micro-humidity environment and incubated at $27\pm 2^{\circ}\text{C}$. The points of inoculation of each type of fungi were constantly examined. The diameter of rotten portion of the samples was measured with calibrated ruler and recorded accordingly. The fungi were later re-isolated from inoculated samples and compared with initial isolates (Tafinta *et al.*, 2013).

3.17 Physiological Studies of the Fungal Isolates

The growth rate of each fungal isolate was studied following the method of Ibrahim *et al.* (2011b). From the 7-day old pure culture of each fungal isolate, 4mm agar discs were taken and aseptically placed in the centre of sterile petri dishes containing PDA medium. The inoculated plates were incubated at 25°C . Three replicates were done for each isolate. The linear growth of each isolate was measured using calibrated ruler and the mean values were recorded.

Physiological responses of each fungal isolate to carbon sources was carried out using the method of Suleiman and Akaajime (2010). The basal medium consisted of: 1.0g, KCl; 0.5g, Mg $\text{SO}_4 \cdot 7\text{H}_2\text{O}$; 3.0g, Ca $(\text{NO}_3)_2$; 1.0g, K_2HPO_4 ; 0.01g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The carbon sources were glucose, starch and fructose. Then, 10g of each carbon molecule was dissolved into 100ml sterile distilled water in sterile conical flasks. Ten milliliter of each sugar solutions was separately added to 20ml sterile basal medium in the flasks. Each flask was inoculated with 5mm diameter disc of 7-day old fungus culture growing on PDA such that the mycelia mats were uppermost and floated on the medium. Three replicate flasks were used for each carbon source.

Table 1: Polymerase Chain Reaction setup

Component	25µl Reaction
2X Master Mix with	12.5µl
Standard Buffer	
10µM Forward Primer	0.5µl
10µM Reverse Primer	0.5µl
DNA Template	1µl
Nuclease- Free Water	to 25µl

Table 2: Programming of thermal cycler Polymerase Chain Reaction for 35 cycles

Step	Temperature	Time
Initial Denaturation	94°C	30seconds
Denaturation	94°C	30 seconds
Annealing	55 °C	60 seconds
Extension	72 °C	1 minute
Final Extension	72 °C	5 minutes
Hold	4 °C	α

3.18 Data Analysis

All data were analyzed using a statistical software called Statistical Package for Social Science (SPSS), version 16.00. One-way Analysis of Variance (ANOVA) was used to determine the differences within the variety. The level of significance used in F ratio was $p < 0.05$. Where F ratio was significant, means were separated using Dunca Multiple Range Test (DMRT). Univariate analysis of variation (under General Linear Model) was used to determine the interactions among the fixed factors (variety, storage and botanicals). Statistical software Origin 7.0 was used to plot the line graphs as well as bar charts.

CHAPTER FOUR

4.0 RESULTS

4.1 Shelf life of Tomato Fruit

Temperature changes were observed in each storage structure. Pot in Pot refrigerator had lowest temperature. The temperature ranges in plastic crates and raffia baskets were 25-27.5°C and 23-25°C respectively (Table 3). The maximum shelf life of Hausa variety of tomato fruits stored in plastic crate was observed in V1PCR to be 17 days and the minimum was in V1PCA to be 15 days. Both V1PCS and V1PCC lasted for 16 days. In pot in pot refrigerator, V1PPC had the longest shelf life of 20 days and the least in that storage structure was V1PPA with 16 days. For the raffia basket, all treated fruits with control lasted for 16 days except V1RBA that was 15 days. Generally, pot in pot refrigerator was the best and in particular, V1PPC had the longest shelf life. The results were summarized in Figure 1.

The maximum storage period of Yoruba variety (19 days) was observed in V2PPC. The fruits in V2RBA had the shortest shelf life (12 days), followed by V2RBR and V2PPA (14 days). Yoruba variety in plastic crates did not exceed 15 days in storage. However, pot in pot refrigerator was the best storage structure to extend the shelf life of this variety. The results were summarized in Figure 2.

In Tropimech variety, V3PCA had the lowest storage life of 10 days. The shelf life of V3PCR and V3PCS were extended by 3 days when compared to V3PCA. In control, V3PCC, the fruits could not sustain storage period beyond 11 days. The overall assessment within this variety revealed that pot in pot refrigerator performed better in extending the shelf life than the other two storage structures. However, the least storage life in pot in pot refrigerator was recorded in V3PPA (12 days). V3PPS was observed to perform best and had highest storage life of 17 days.

Table 3: Temperature Changes within the Structures Containing Tomato Fruits during Storage

	Storage Period (Days)	Minimum Temperature (°C)	Maximum Temperature (°C)	Average Temperature (°C)
Plastic crate	2 nd	23.00	27.00	25.00
	4 th	25.00	26.00	25.50
	6 th	24.00	26.00	25.00
	8 th	25.00	28.00	26.50
	10 th	25.00	27.00	26.00
	12 th	25.00	26.00	25.50
	14 th	26.00	28.00	27.00
	16 th	25.00	28.00	26.50
	18 th	25.00	27.00	26.00
	20 th	26.00	28.00	27.00
Pot in Pot	2 nd	20.00	23.00	21.50
	4 th	20.00	22.00	21.00
	6 th	19.00	21.00	20.00
	8 th	19.00	20.00	19.50
	10 th	17.00	20.00	18.50
	12 th	17.00	19.00	18.00
	14 th	18.00	20.00	19.00
	16 th	17.00	19.00	18.00
	18 th	19.00	21.00	20.00
	20 th	18.00	20.00	19.00
Raffia Basket	2 nd	22.00	24.00	23.00
	4 th	23.00	25.00	24.00
	6 th	22.00	24.00	23.00
	8 th	23.00	26.00	24.50
	10 th	22.00	25.00	23.50
	12 th	22.00	25.00	23.50
	14 th	23.00	25.00	24.00
	16 th	24.00	26.00	25.00
	18 th	23.00	26.00	24.50
	20 th	23.00	25.00	24.00

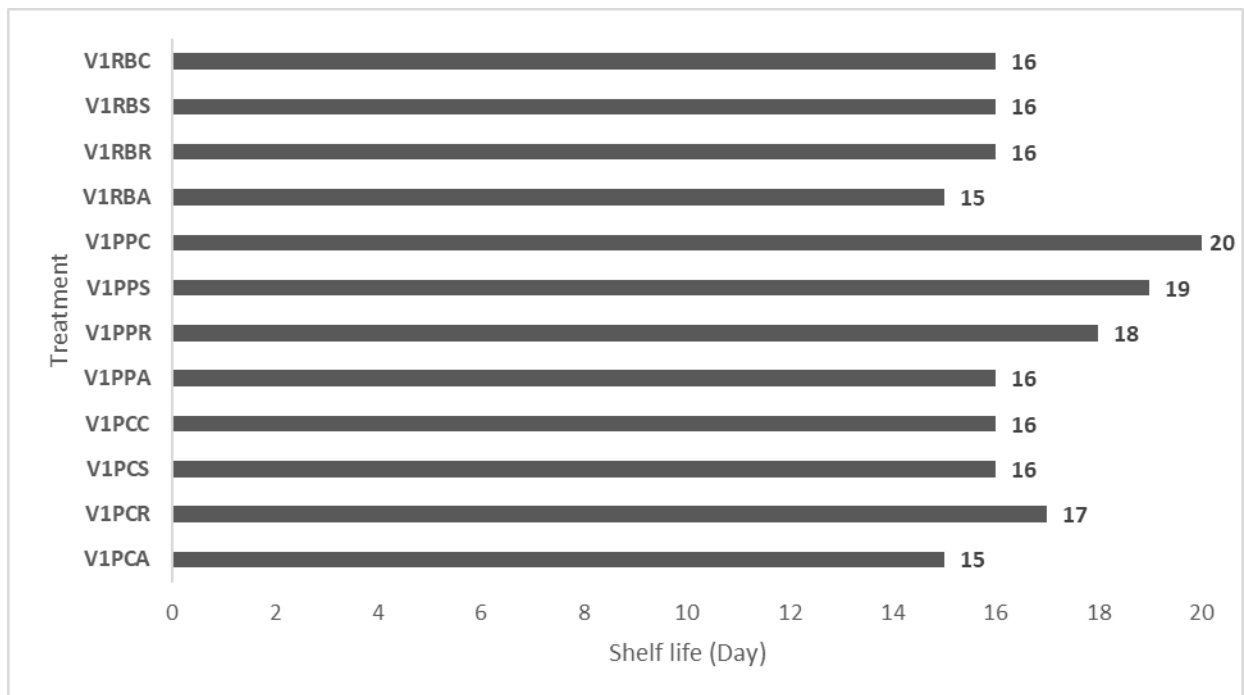


Figure 1: The shelf life of Hausa variety as influenced by storage with botanicals

V1PCA = Hausa Variety + Plastic crate + Ash

V1PCR = Hausa Variety + Plastic crate + Rice Straw

V1PCS = Hausa Variety + Plastic crate + Sawdust

V1PCC = Hausa Variety + Plastic crate + Control

V1PPA = Hausa Variety + Pot in Pot + Ash

V1PPR = Hausa Variety + Pot in Pot + Rice Straw

V1PPS = Hausa Variety + Pot in Pot + Sawdust

V1PPC = Hausa Variety + Pot in Pot + Control

V1RBA = Hausa Variety + Raffia Basket + Ash

V1RBR = Hausa Variety + Raffia Basket + Rice Straw

V1RBS = Hausa Variety + Raffia Basket + Sawdust

V1RBC = Yoruba Variety + Raffia Basket + Control

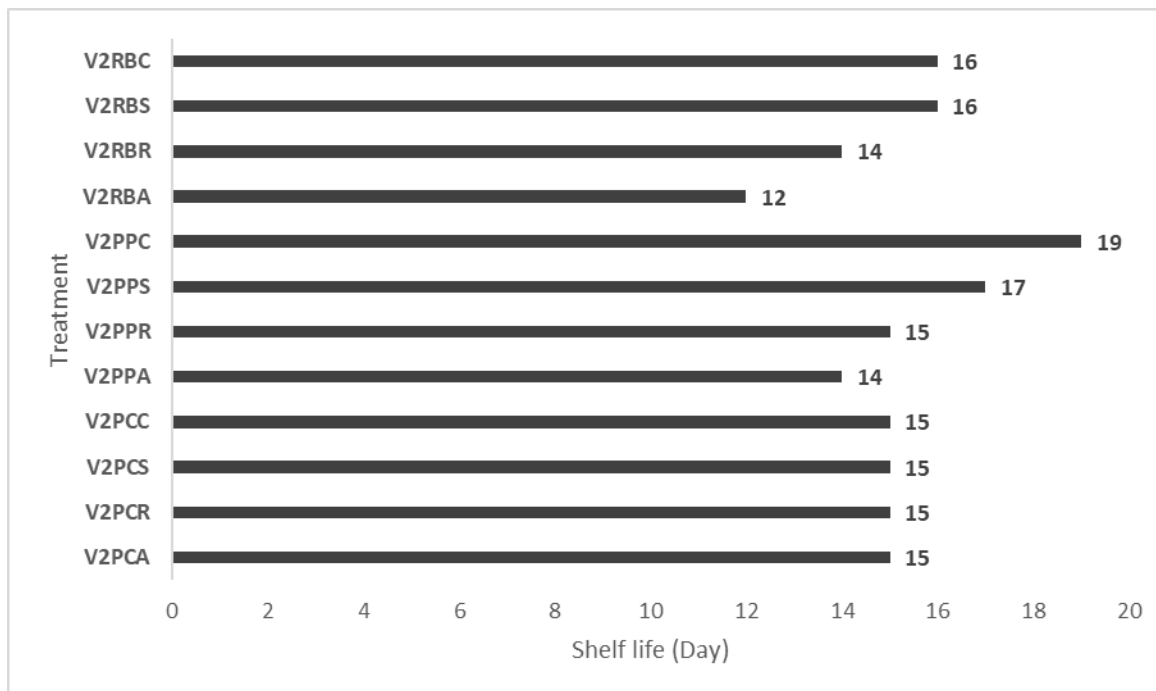


Figure 2: The shelf life of Yoruba variety as influenced by storage with botanicals

V2PCA = Yoruba + Plastic crate + Ash

V2PCR = Yoruba Variety + Plastic crate + Rice Straw

V2PCS = Yoruba Variety + Plastic crate + Sawdust

V2PCC = Yoruba Variety + Plastic crate + Control

V2PPA = Yoruba Variety + Pot in Pot + Ash

V2PPR = Yoruba Variety + Pot in Pot + Rice Straw

V2PPS = Yoruba Variety + Pot in Pot + Sawdust

V2PPC = Yoruba Variety + Pot in Pot + Control

V2RBA = Yoruba Variety + Raffia Basket + Ash

V2RBR = Yoruba Variety + Raffia Basket + Rice Straw

V2RBS = Yoruba Variety + Raffia Basket + Sawdust

V2RBC = Yoruba Variety + Raffia Basket + Control

The shelf life of V3PPR and V3PPC could not be extended beyond 15 days. V3RBA and V3RBC had 11 days apiece as their storage lives while V3RBR had 13 days. In raffia baskets, V3RBS was able to attain 14 days and happened to be the highest shelf life (Figure 3).

In the plastic crates, V4PCA and V4PCC had shelf lives of 10 days and 11 days respectively whereas 12 days was observed in both V4PCR and V4PCS. As observed in other three varieties, pot in pot refrigerator was the best storage method for the enhancement shelf life. V4PPS and V4PPC had the highest storage lives of 16 days. The shelf lives of V4PPA and V4PPR were shorter and had 13 days and 14 days respectively. In raffia baskets, V4RBA was quickest to attain shelf life (10 days). The shelf lives of V4RBR and V4RBC were 12 days. The storage life obtained in V4RBS was highest (13 days) (Figure 4).

4.2 Change in Firmness of Tomato during Storage

The fruits of all the varieties had physical quality of firmness at Day 1. The fruits began to lose their firmness as the storage progressed. The loss of firmness depended on the storage structure. All the varieties in PCA, PCR and PCC lost the firmness completely at Day 20 and the fruits became pulpy. In V1 and V2, the lowest score for firmness was recorded at Day 16 (Table 4).

All the varieties in PPA could no longer retain their firmness at Day 20. In PPR, the two local varieties, V1 and V2, were scored 1.00 each at Day 20 but the improved varieties (V3 and V4) were able to retain their firmness insignificantly and they scored 1.33 (V3) and 1.50 (V4) respectively. Only V1 in PPS had a score of 1.00 at Day 20 while the firmness of the remaining varieties was ranged from 1.17 to 1.33. In control, PCC, all the varieties looked firmer than treated varieties in the same storage structure (Table 5). V1 and V2 lost their firmness in RBA at Day 16. All the varieties in raffia baskets with botanicals and control became very soft and score 1.00 at Day 20 except V4RBA (Table 6).

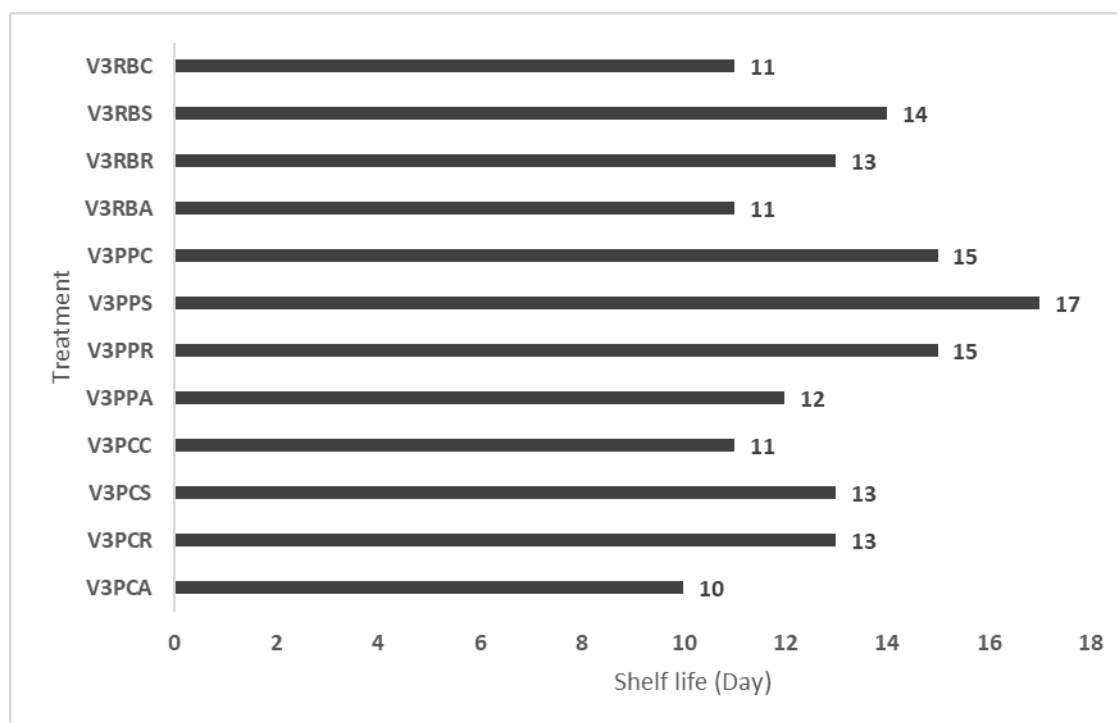


Figure 3: The shelf life of Tropimech variety as influenced by storage with botanicals

V3PCA = Tropimech Variety + Plastic crate + Ash

V3PCR = Tropimech Variety + Plastic crate + Rice Straw

V3PCS = Tropimech Variety + Plastic crate + Sawdust

V3PCC = Tropimech Variety + Plastic crate + Control

V3PPA = Tropimech Variety + Pot in Pot + Ash

V3PPR = Tropimech Variety + Pot in Pot + Rice Straw

V3PPS = Tropimech Variety + Pot in Pot + Sawdust

V3PPC = Tropimech Variety + Pot in Pot + Control

V3RBA = Tropimech Variety + Raffia Basket + Ash

V3RBR = Tropimech Variety + Raffia Basket + Rice Straw

V3RBS = Tropimech Variety + Raffia Basket + Sawdust

V3RBC = Tropimech Variety + Raffia Basket + Control

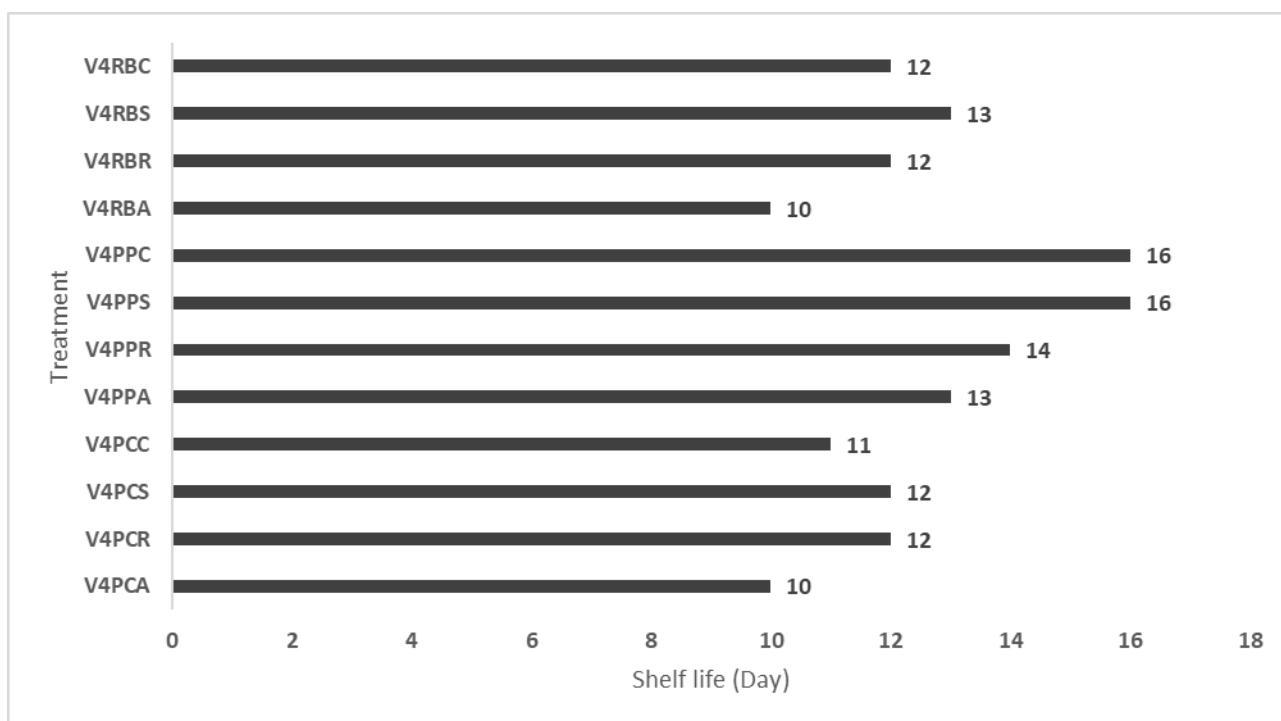


Figure 4: The shelf life of Roma VF variety as influenced by storage with botanicals

V4PCA = Roma VF + Plastic crate + Ash

V4PCR = Roma VF + Plastic crate + Rice Straw

V4PCS = Roma VF + Plastic crate + Sawdust

V4PCC = Roma VF + Plastic crate + Control

V4PPA = Roma VF + Pot in Pot + Ash

V4PPR = Roma VF + Pot in Pot + Rice Straw

V4PPS = Roma VF + Pot in Pot + Sawdust

V4PPC = Roma VF + Pot in Pot + Control

V4RBA = Roma VF + Raffia Basket + Ash

V4RBR = Roma VF + Raffia Basket + Rice Straw

V4RBS = Roma VF + Raffia Basket + Sawdust

V4RBC = Tropimech Variety + Raffia Basket + Control

Table 4: Influence of Plastic crate on the firmness of tomato during storage

	DAYS	V1	V2	V3	V4
PCA	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	2.83±0.31b	3.33±0.21b	2.67±0.33b	3.50±0.22a
	8	2.17±0.17c	1.50±0.22c	2.50±0.22bc	2.33±0.21b
	12	1.33±0.21d	1.33±0.21cd	2.00±0.00c	1.67±0.21c
	16	1.00±0.00d	1.00±0.00d	1.33±0.21d	1.33±0.21cd
	20	1.00±0.00d	1.00±0.00d	1.00±0.00d	1.00±0.00d
PCR	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	3.17±0.31b	3.12±0.17b	3.17±0.17b	3.66±0.21a
	8	2.00±0.26c	2.33±0.21c	2.33±0.21c	2.83±0.31b
	12	1.83±0.31c	1.83±0.40c	2.17±0.17c	1.83±0.40c
	16	1.00±0.00d	1.00±0.00d	1.50±0.22d	1.33±0.21cd
	20	1.00±0.00d	1.00±0.00d	1.00±0.00e	1.00±0.00d
PCS	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	3.17±0.17b	3.00±0.00b	3.33±0.21b	3.50±0.22a
	8	2.00±0.26c	2.17±0.17c	2.00±0.26c	2.83±0.17b
	12	1.67±0.21c	1.33±0.21d	2.17±0.17cd	1.83±0.31c
	16	1.00±0.00d	1.00±0.00e	1.50±0.22de	1.33±0.21cd
	20	1.00±0.00d	1.00±0.00e	1.00±0.00e	1.00±0.00d
PCC	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	2.67±0.21b	2.50±0.22b	2.67±0.21b	2.67±0.33b
	8	1.83±0.31c	2.00±0.26c	2.33±0.21bc	2.33±0.33bc
	12	1.83±0.17c	1.67±0.21c	2.00±0.00c	1.83±0.31cd
	16	1.00±0.00d	1.00±0.00d	1.50±0.22d	1.50±0.22de
	20	1.00±0.00d	1.00±0.00d	1.00±0.00e	1.00±0.00e

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF

PCA = Plastic Crate + Ash

PCR = Plastic Crate + Rice Straw

PCS = Plastic Crate + Sawdust

PCC = Plastic Crate + Control

Table 5: Influence of Pot in Pot Refrigerator on the firmness of tomato during storage

	DAYS	V1	V2	V3	V4
PPA	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	2.67±0.21b	2.67±0.21b	2.67±0.21b	2.67±0.22b
	8	2.50±0.22b	2.83±0.17b	2.67±0.21b	2.50±0.21b
	12	1.83±0.17c	1.83±0.17c	2.00±0.00c	1.83±0.17c
	16	1.00±0.00d	1.00±0.00d	1.33±0.21d	1.33±0.21d
	20	1.00±0.00d	1.00±0.00d	1.00±0.00d	1.00±0.00d
PPR	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	2.83±0.17b	2.67±0.21b	2.67±0.21b	2.83±0.30b
	8	2.33±0.21c	2.67±0.21b	2.67±0.21b	2.67±0.21b
	12	1.67±0.21d	1.67±0.21c	2.17±0.17bc	1.50±0.22c
	16	1.50±0.22d	1.50±0.22cd	1.67±0.21cd	1.50±0.22c
	20	1.00±0.00e	1.00±0.00d	1.33±0.21d	1.50±0.22c
PPS	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	3.17±0.17b	3.00±0.26b	3.17±0.31b	3.17±0.38b
	8	2.67±0.21c	2.50±0.22b	2.83±0.17b	2.67±0.21b
	12	2.00±0.00d	1.67±0.21c	1.83±0.17c	1.67±0.33c
	16	1.50±0.22e	1.50±0.22c	1.50±0.22c	1.33±0.21c
	20	1.00±0.00f	1.17±0.17c	1.33±0.21c	1.33±0.21c
PPC	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	3.33±0.21b	3.33±0.21b	3.17±0.17b	3.33±0.21b
	8	2.83±0.17b	2.67±0.21c	2.50±0.22c	2.67±0.21c
	12	2.17±0.17c	2.33±0.21c	1.83±0.17d	2.17±0.31c
	16	1.33±0.21d	1.50±0.22d	1.17±0.17de	1.50±0.22d
	20	1.33±0.21d	1.17±0.17d	1.50±0.22e	1.50±0.22d

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF

PPA = Pot in Pot + Ash

PPR = Pot in Pot + Rice Straw

PPS = Pot in Pot + Sawdust

PPC = Pot in Pot + Control

Table 6: Influence of Raffia Basket on the firmness of tomato during storage

	DAYS	V1	V2	V3	V4
RBA	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	2.5±0.22b	2.50±0.22b	4.00±0.00a	4.00±0.00a
	8	1.83±0.17c	1.83±0.17c	2.50±0.22b	2.67±0.33b
	12	1.50±0.22c	1.50±0.22d	2.33±0.21b	1.67±0.21c
	16	1.00±0.00d	1.00±0.00e	1.67±0.21c	1.50±0.22cd
	20	1.00±0.00d	1.00±0.00e	1.00±0.00d	1.33±0.21cd
RBR	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	2.67±0.21b	2.83±0.17b	3.00±0.00b	2.83±0.31b
	8	2.00±0.26c	2.33±0.21c	2.50±0.22b	2.17±0.31c
	12	1.67±0.21c	1.67±0.21d	1.67±0.21c	2.00±0.26c
	16	1.33±0.21de	1.17±0.17e	1.17±0.17cd	1.33±0.21d
	20	1.00±0.00e	1.00±0.00e	1.00±0.00d	1.00±0.00d
RBS	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	2.67±0.21b	2.67±0.21b	2.83±0.17b	2.83±0.31b
	8	2.17±0.17c	2.00±0.26c	2.33±0.33bc	1.83±0.31c
	12	1.83±0.17c	1.67±0.21cd	2.00±0.26c	1.67±0.21c
	16	1.17±0.17d	1.17±0.17de	1.33±0.21d	1.33±0.21cd
	20	1.00±0.00d	1.00±0.00e	1.00±0.00d	1.00±0.00d
RBC	1	4.00±0.00a	4.00±0.00a	4.00±0.00a	4.00±0.00a
	4	2.50±0.22b	2.33±0.21b	2.50±0.22b	3.17±0.31b
	8	2.00±0.26c	2.00±0.26bc	2.33±0.21b	2.17±0.31c
	12	1.83±0.17c	1.67±0.21c	1.67±0.21c	2.00±0.37cd
	16	1.00±0.00d	1.00±0.00d	1.33±0.21cd	1.33±0.21de
	20	1.00±0.00d	1.00±0.00d	1.00±0.00a	1.00±0.00e

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF

RBA = Raffia Basket + Ash

RBR = Raffia Basket + Rice Straw

RBS = Raffia Basket + Sawdust

RBC = Raffia Basket + Control

4.2.1 Influence of Varieties, Storage and Botanicals on the Firmness of Tomato Fruits

The firmness in Roma VF was significantly higher than the two local varieties. The storage structures had no significant effect on the firmness of stored tomato fruits. Botanical treatments had insignificant influence on the firmness. No significant difference was observed in interactions of two fixed factors except variety by storage. Significant difference was also observed in interactions of all three fixed factors (Table 7).

4.3 Colour Change in Tomato Fruits during Storage

The fruits of all the varieties were harvested at mature green stage (unripe at Day 1). The ripening was advancing as the storage period was increasing. Both V2PCA and V4PCA, at Day 16, were completely ripe. Also, in PCR and PCS, none of the varieties attained full ripeness at Day 16. All varieties in PCA were absolutely ripe at Day 20. In PCR, PCS and PCC, all the varieties were fully ripped except V3 which had scores of 4.83, 4.67, and 4.67 respectively. It was observed in PCC that only V3 had score that is less than 5.00 (Table 8).

The colour change in V3 was conspicuously distinguishable up to the final day in pot in pot refrigerator. The V3 had the lowest score for colour change meaning that the fruits were not perfectly red. All the varieties in PPA experienced absolute colour change to redness at Day 20 except V3. It was also noted that none of the variety in PPR, PPS and PPC reached 100% ripeness till the last day of the observation. Comparatively, this storage structure retarded the rate of ripening process in all the varieties (Table 9).

V3 was observed very closely in raffia basket. The variety was unable to have the highest score throughout the experimental period. RBA favoured the colour change to the point that full red

Table7: Firmness in stored tomato fruits as influenced by variety, storage and botanicals

Factor	Level of Factors	Firmness
Variety (V)	Hausa	1.03b
	Yoruba	1.03b
	Tropimech	1.06ab
	Roma VF	1.11a
SE		0.03
Storage (S)	Plastic crate	1.07a
	Pot in Pot	1.05a
	Raffia basket	1.05a
SE		0.02
Treatment (T)	Ash	1.00b
	Rice straw	1.06ab
	Sawdust	1.06ab
	Control	1.11a
SE		0.03
$V \times S$		<i>S</i>
$V \times T$		<i>NS</i>
$S \times T$		<i>NS</i>
$V \times S \times T$		<i>S</i>

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

S = Significant difference

NS = No significant difference

Table 8: Influence of Plastic crate on the colour of tomato

	DAYS	V1	V2	V3	V4
PCA	1	1.00±0.00c	1.00±0.00d	1.00±0.00d	1.00±0.00d
	4	1.33±0.21c	1.83±0.17c	1.33±0.21d	1.83±0.31c
	8	3.00±0.26b	3.67±0.33b	2.83±0.31c	4.17±0.40b
	12	3.50±0.22b	4.00±0.37b	4.00±0.26b	4.83±0.17a
	16	4.50±0.22a	5.00±0.00a	4.67±0.21a	5.00±0.00a
	20	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a
PCR	1	1.00±0.00c	1.00±0.00e	1.00±0.00d	1.00±0.00d
	4	1.50±0.22c	1.67±0.21d	1.50±0.22d	1.83±0.31c
	8	3.33±0.33b	4.00±0.26bc	2.33±0.21c	4.00±0.37b
	12	3.83±0.17b	4.17±0.17bc	3.83±0.31b	4.67±0.21a
	16	4.67±0.21a	4.67±0.21ab	4.33±0.21ab	4.83±0.17a
	20	5.00±0.00a	5.00±0.00a	4.83±0.17a	5.00±0.00a
PCS	1	1.00±0.00c	1.00±0.00c	1.00±0.00	1.00±0.00c
	4	1.33±0.21c	1.33±0.21c	1.17±0.17d	1.50±0.22c
	8	3.00±0.26b	3.67±0.33b	2.00±0.26cd	3.33±0.33b
	12	3.50±0.22b	3.50±0.34b	3.50±0.22b	3.50±0.22b
	16	4.50±0.22a	4.83±0.17a	4.33±0.21a	4.67±0.21a
	20	5.00±0.00a	5.00±0.00a	4.67±0.21a	5.00±0.00a
PCC	1	1.00±0.00d	1.00±0.00d	1.00±0.00d	1.00±0.00d
	4	1.83±0.31c	1.67±0.33c	1.50±0.22d	2.00±0.37c
	8	3.83±0.31b	3.83±0.31b	2.50±0.22c	4.17±0.31b
	12	4.50±0.22a	4.17±0.17bc	3.67±0.33b	4.67±0.21b
	16	5.00±0.00a	5.00±0.00a	4.33±0.21a	5.00±0.00a
	20	5.00±0.00a	5.00±0.00a	4.67±0.21a	5.00±0.00a

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF

PCA = Plastic Crate + Ash

PCR = Plastic Crate + Rice Straw

PCS = Plastic Crate + Sawdust

PCC = Plastic Crate + Control

Table 9: Influence of Pot in Pot Refrigerator on the colour of tomato of Tomato Fruits

	DAY	V1	V2	V3	V4
PPA	1	1.00±0.00d	1.00±0.00e	1.00±0.00c	1.00±0.00d
	4	1.33±0.21d	1.50±0.22d	1.33±0.21c	1.50±0.22d
	8	2.83±0.17c	2.67±0.21c	2.33±0.20b	3.33±0.21c
	12	4.17±0.31b	4.33±0.21b	2.67±0.33b	4.33±0.33b
	16	5.00±0.00a	5.00±0.00a	3.67±0.21a	5.00±0.00a
	20	5.00±0.00a	5.00±0.00a	4.17±0.31a	5.00±0.00a
PPR	1	1.00±0.00d	1.00±0.00d	1.00±0.00c	1.00±0.00c
	4	1.33±0.21d	1.33±0.21d	1.17±0.17c	1.33±0.00c
	8	2.33±0.21c	2.67±0.21c	1.67±0.33c	1.50±0.21c
	12	2.83±0.31c	2.97±0.22c	2.50±0.34b	2.67±0.21b
	16	3.67±0.21b	3.83±0.31b	3.50±0.22a	4.17±0.31a
	20	4.67±0.21a	4.83±0.17a	4.00±0.63a	4.17±0.31a
PPS	1	1.00±0.00d	1.00±0.00c	1.00±0.00e	1.00±0.00d
	4	1.17±0.17d	1.17±0.17c	1.17±0.17de	1.33±0.21d
	8	2.67±0.21c	2.33±0.21b	1.83±0.40cd	2.50±0.22c
	12	3.33±0.21b	2.83±0.17b	2.50±0.22c	3.33±0.21b
	16	4.33±0.21a	4.33±0.21a	3.33±0.21b	4.50±0.22a
	20	4.50±0.22a	4.50±0.22a	4.50±0.22a	4.50±0.22a
PPC	1	1.00±0.00c	1.00±0.00c	1.00±0.00e	1.00±0.00e
	4	1.50±0.22c	1.50±0.22c	1.17±0.17de	2.67±0.21d
	8	3.00±0.26b	3.00±0.25b	1.67±0.21d	2.67±0.17c
	12	3.50±0.34b	3.33±0.21b	2.33±0.21c	3.67±0.21b
	16	4.33±0.21a	4.33±0.21a	3.83±0.31b	4.33±0.21a
	20	4.67±0.21a	4.67±0.21a	4.50±0.22a	4.67±0.21a

Means followed by the same letters along the same column are not significantly different at $p \leq 0.05$

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF

PPA = Pot in Pot + Ash

PPR = Pot in Pot + Rice Straw

PPS = Pot in Pot + Sawdust

PPC = Pot in Pot + Control

had been recorded at Day 16. Drastic colour change was noticed in V4 from Day 4 to Day 8. Similarly, V1, V2 and V4 were able to get full score (5.00) at Day 20 irrespective of the present or absent of botanical treatments (Table 10)

4.3.1 Influence of Variety, Storage and Botanicals on the Colour of Tomato Fruits

No significant difference was observed in the colour of all the varieties except Tropimech. The colour change observed in tomato fruits stored in pot in pot refrigerator was significantly different from those of plastic crates as well as raffia baskets. The influence of ash on the colour was significantly higher than that of sawdust but was insignificantly different from rice straw and control. Significant difference was observed in variety by storage interaction as variety by treatment and storage by treatment displayed no significant difference. The interactions among the three fixed factors showed a significant difference (Table 11).

4.4 WEIGHT LOSS IN STORED TOMATO FRUITS

4.4.1 Weight Loss in Stored Hausa Variety of Tomato Fruits

In Hausa Variety, weight loss was observed in all the stored fruits. The weight loss was increasing as the storage period was progressing. In V1PCA, the percentage weight loss at Day 20 was 37.56% which was greater than the one recorded in V1PCR (27.38%) and V1PCS (27.38%) but behind the value obtained from V1PCC (37.77%) as summarized in Figure 5. The percentage weight loss in V1PPA at Day 4 showed no significant difference from the value recorded at Day 8. The highest weight loss was recorded at Day 20 (21.33%) and this was significantly different from that of Day 16 (13.89%). In V1PPR, the weight loss was as low as 2.03% at Day 4 but significantly inclined to 16.20% at Day 20. At Day 20 in V1PPS, 19.12% of weight was loss but showed no significant difference from that of Day 16 (14.96%).

Table 10: Influence of Raffia Basket on the colour of tomato

	DAY	V1	V2	V3	V4
RBA	1	1.00±0.00e	1.00±0.00e	1.00±0.00e	1.00±0.00d
	4	1.50±0.22d	1.50±0.22d	1.67±0.21d	2.00±0.26c
	8	3.67±0.21c	3.50±0.22c	3.67±0.21c	4.17±0.31b
	12	4.50±0.22b	4.50±0.22b	4.17±0.30bc	4.83±0.17a
	16	5.00±0.00a	5.00±0.00a	4.67±0.19ab	5.00±0.00a
	20	5.00±0.00a	5.00±0.00a	4.83±0.17a	5.00±0.00a
RBR	1	1.00±0.00d	1.00±0.00d	1.00±0.00d	1.00±0.00c
	4	1.33±0.21d	1.33±0.21d	1.33±0.21d	1.83±0.31b
	8	3.00±0.26c	3.83±0.31c	3.00±0.26c	4.33±0.33a
	12	3.67±0.21b	4.12±0.31bc	3.83±0.40b	4.50±0.22a
	16	4.67±0.21a	4.67±0.21ab	4.50±0.22ab	4.67±0.21a
	20	5.00±0.00a	5.00±0.00a	4.67±0.21a	5.00±0.00a
RBS	1	1.00±0.00c	1.00±0.00d	1.00±0.00d	1.00±0.00d
	4	1.50±0.34c	1.50±0.34d	1.33±0.21d	1.50±0.34d
	8	3.33±0.21b	2.67±0.33c	2.83±0.31c	3.00±0.26c
	12	3.67±0.21b	3.50±0.22b	3.50±0.22b	3.83±0.31b
	16	4.67±0.21a	4.67±0.21a	4.00±0.26ab	4.67±0.21a
	20	5.00±0.00a	5.00±0.00a	4.33±0.21a	5.00±0.00a
RBC	1	1.00±0.00c	1.00±0.00c	1.00±0.00c	1.00±0.00d
	4	1.67±0.42c	1.50±0.34c	1.50±0.22c	2.17±0.31c
	8	3.33±0.33b	3.33±0.21b	3.33±0.33b	3.83±0.31b
	12	3.83±0.17b	3.67±0.21b	4.33±0.33a	4.67±0.21a
	16	4.83±0.17a	4.67±0.21a	4.67±0.21a	5.00±0.00a
	20	5.00±0.00a	5.00±0.00a	4.67±0.21a	5.00±0.00a

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF

RBA = Raffia Basket + Ash

RBR = Raffia Basket + Rice Straw

RBS = Raffia Basket + Sawdust

RBC = Raffia Basket + Control

Table 11: Colour change of stored tomato as influenced by variety, storage and botanicals

Factor	Level of Factors	Colour
Variety (V)	Hausa	4.90a
	Yoruba	4.92a
	Tropimech	4.57b
	Roma VF	4.90a
SE		0.04
Storage (S)	Plastic crate	4.84ab
	Pot in Pot	4.75b
	Raffia basket	4.87a
SE		0.04
Treatment (T)	Ash	4.92a
	Rice straw	4.81ab
	Sawdust	4.75b
	Control	4.81ab
SE		0.04
$V \times S$		*
$V \times T$		NS
$S \times T$		NS
$V \times S \times T$		*

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

* = Significant difference

NS = No significant difference

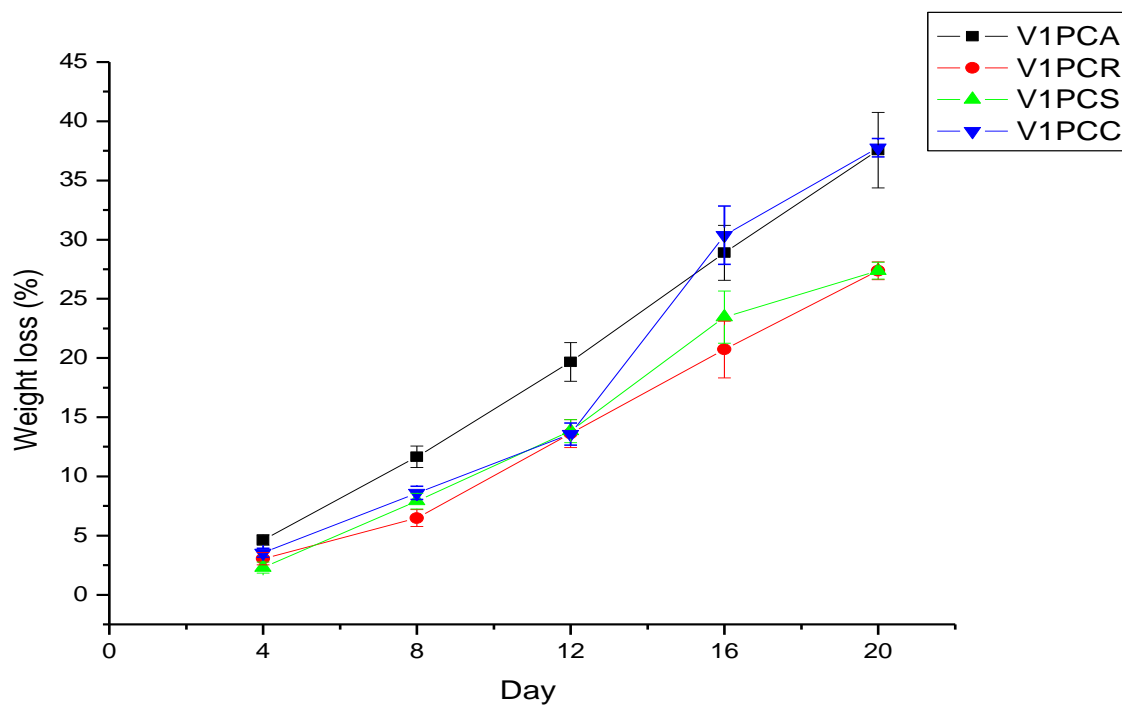


Figure 5: Weight loss of tomato (Hausa variety) stored in Plastic crate amended with botanicals

V1PCA = Hausa Variety + Plastic crate + Ash

V1PCR = Hausa Variety + Plastic crate + Rice Straw

V1PCS = Hausa Variety + Plastic crate + Sawdust

V1PCC = Hausa Variety + Plastic crate + Control

Also, at Day 4, V1PPC had 1.33% weight loss but significantly increased to 10.22% at Day 16 and insignificantly raised to 14.35% at day 20 (Figure 6).

V1RBA suffered 36.42% weight loss at Day 20 and this was observed to be the lowest in all V1 fruits stored in raffia basket. Contrarily, the highest weight loss in V1RB was 46.68%, recorded in V1RBR at Day 20. In V1RBS, 4.18% was the weight loss at Day 4 and significantly inclined to 20.95% and 39.10% at Days 12 and 16 respectively. The percentage weight loss in V1RBC from Day 4 to Day 12 showed no significant difference from one another but were significantly different from the values obtained at Days 16 and 20 (Figure 7).

4.4.2 Weight Loss in Stored Yoruba Variety of Tomato Fruits

As observed in Hausa variety, the Yoruba variety fruits were likewise losing weight and the loss was proportional to storage period. In V2PCA, the weight losses were significantly different from one another up to Day 20. The weight loss recorded at Day 20 (44.48%) was the highest not only in all the fruits stored in the plastic crates but also in other two structures within the variety. The weight loss of V2PCR at Day 20 was 28.40% and was significantly different from 25.51% that was observed at Day 16. The lowest weight loss (23.62%) was recorded in V2PCS at Day 20 across the plastic crate within the variety (Figure 8).

V2PPA had the weight loss 0.33% at Day 4 in storage this was significantly inclined to 12.47% at Day 20. The weight loss of V2PPR at Day 20 was 13.07% and was higher than that of V2PPS (12.44%). the control (V2PPC) has the lowest weight loss of 8.01% in pot in pot refrigerator and the highest value was observed in V2PPR (13.07%) as summarized in Figure 9.

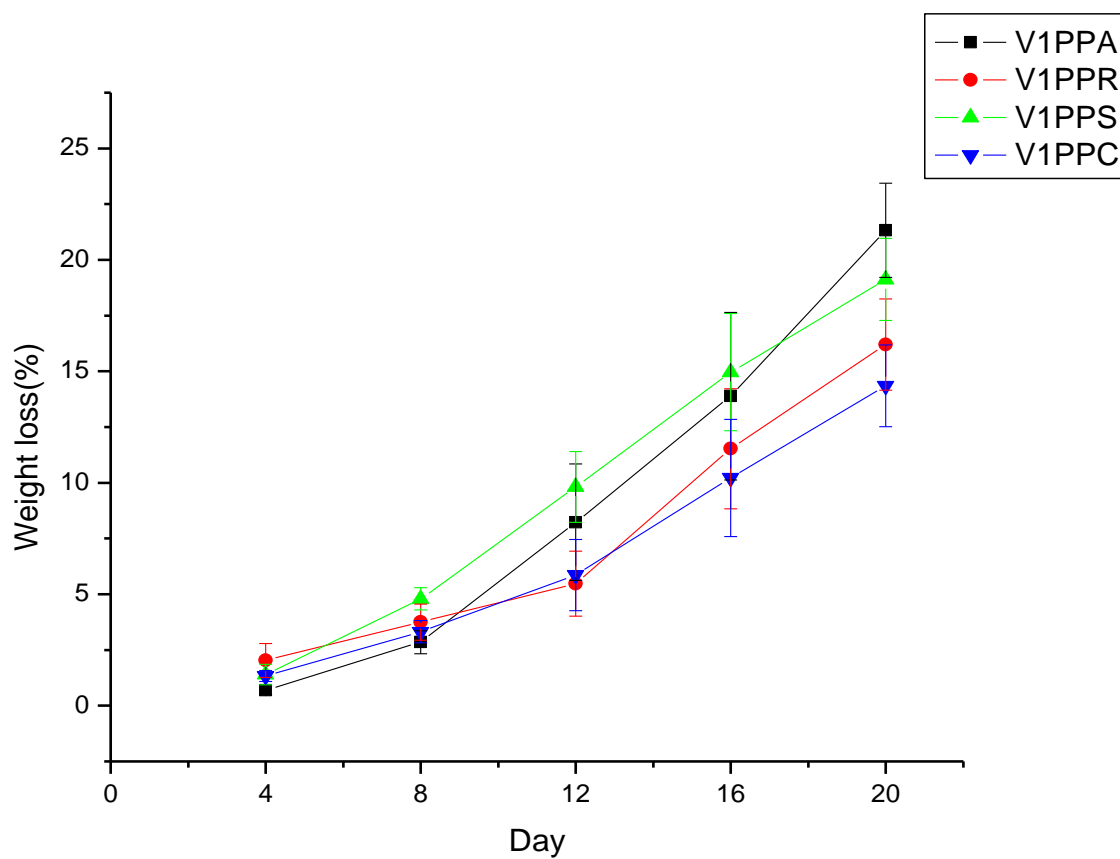


Figure 6: Weight loss of tomato (Hausa variety) stored in Pot in Pot refrigerator amended with botanicals

V1PPA Hausa Variety + Pot in Pot + Ash

V1PPR = Hausa Variety + Pot in Pot + Rice Straw

V1PPS = Hausa Variety + Pot in Pot + Sawdust

V1PPC Hausa Variety + Pot in Pot + Control

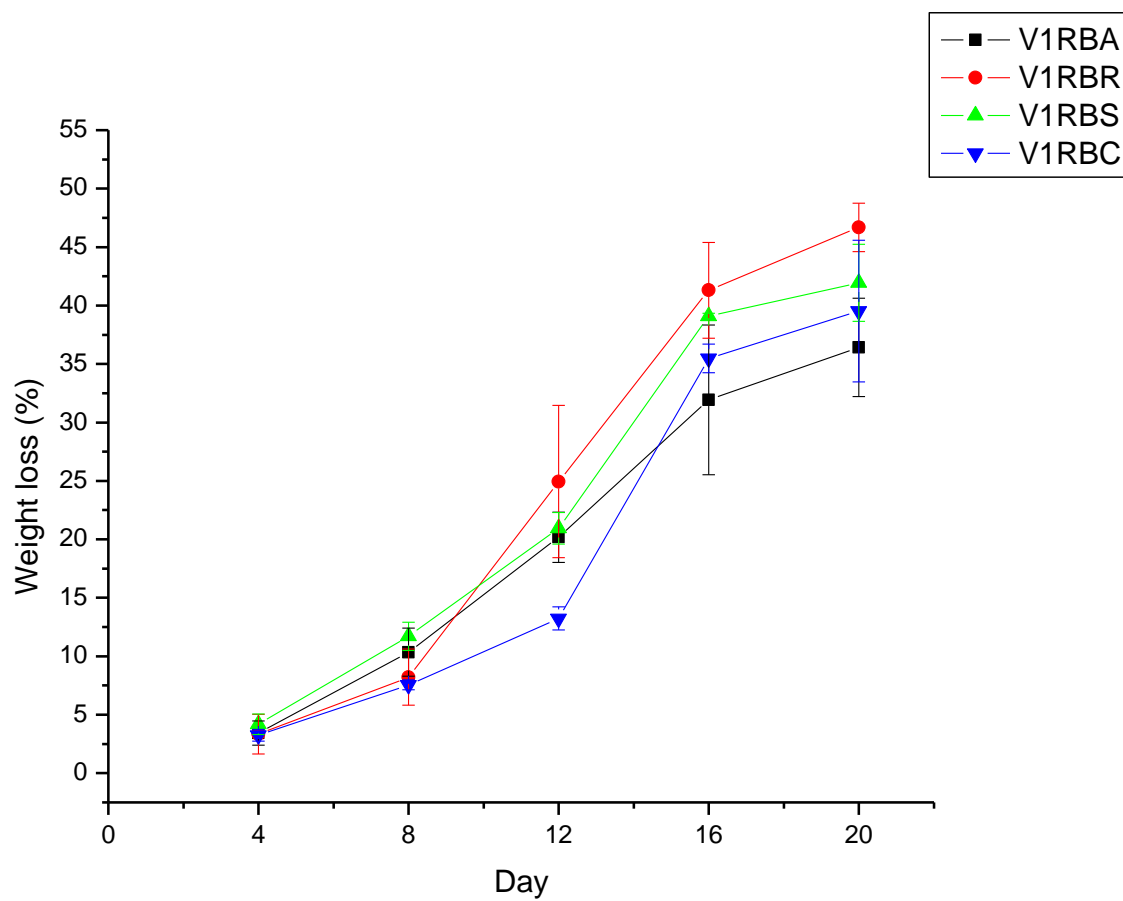


Figure 7: Weight loss of tomato (Hausa variety) stored in Raffia Basket amended with botanicals

V1RBA = Hausa Variety + Raffia Basket + Ash

V1RBR = Hausa Variety + Raffia Basket + Rice Straw

V1RBS = Hausa Variety + Raffia Basket + Sawdust

V1RBC = Hausa Variety + Raffia Basket + Control

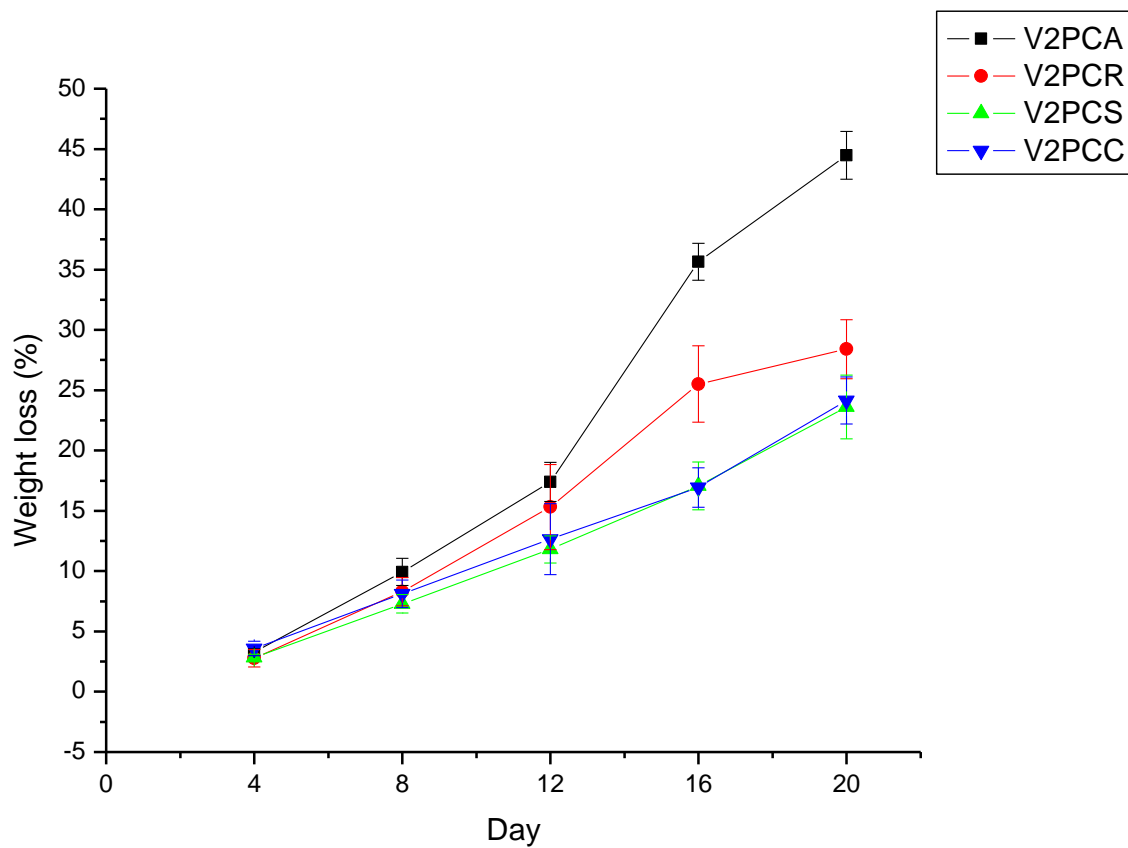


Figure 8: Weight loss of tomato (Yoruba variety) stored in Plastic crate amended with botanicals

V2PCA = Yoruba + Plastic crate + Ash

V2PCR = Yoruba Variety + Plastic crate + Rice Straw

V2PCS = Yoruba Variety + Plastic crate + Sawdust

V2PC = Yoruba Variety + Plastic crate + Control

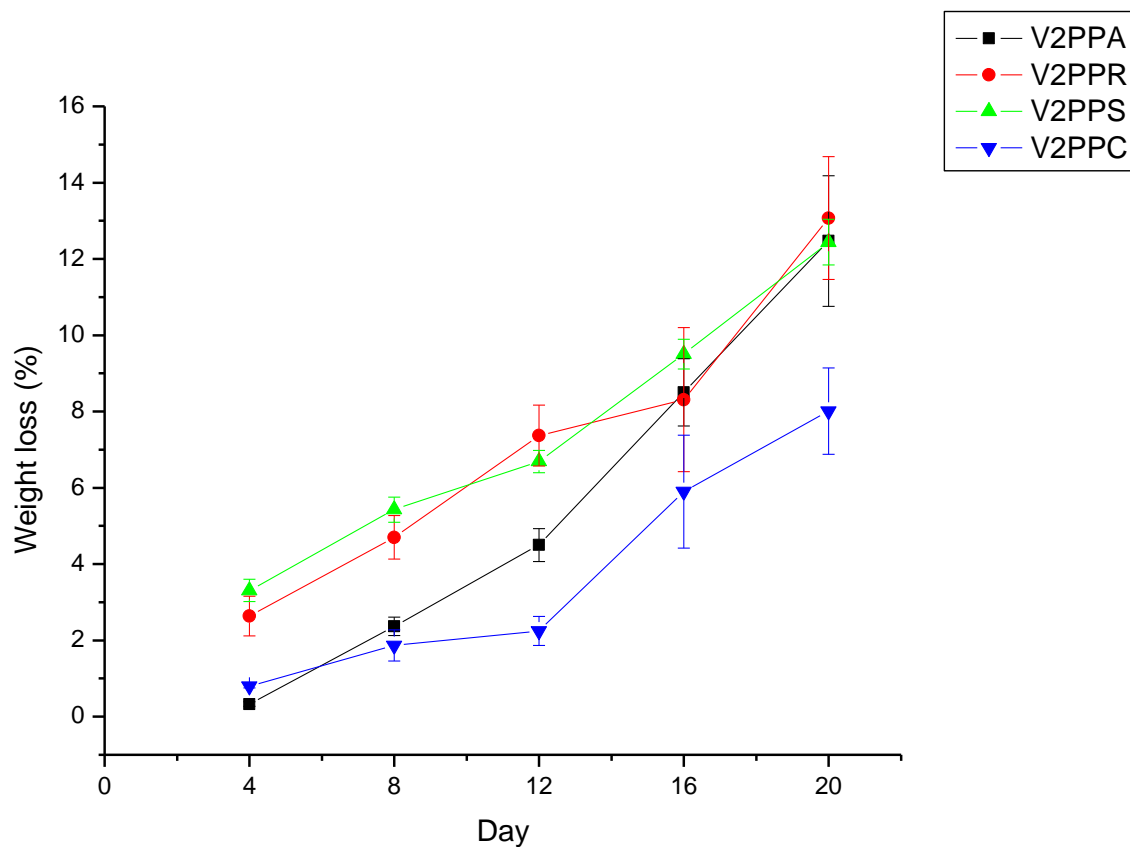


Figure 9: Weight loss of tomato (Yoruba variety) stored in Pot in pot refrigerator amended with botanicals

V2PPA Yoruba Variety + Pot in Pot + Ash

V2PPR = Yoruba Variety + Pot in Pot + Rice Straw

V2PPS = Yoruba Variety + Pot in Pot + Sawdust

V2PPC Yoruba Variety + Pot in Pot + Control

The weight loss of V2RBA was significantly increasing until Day 20 where the highest weight loss was observed in V2 fruits stored in raffia baskets. A significant change in weight loss was noticed in V2RBR at Day 8 (8.21%) and Day 12 (23.53%). There was no significant difference in the weight losses observed in Days 16 and 20. V2RBS had 36.62% weight loss at Day 20 which was significantly different from the one recorded at Day 16 (34.93%). V2RBC was with weight loss of 21.46% at Day 20 (Figure 10).

4.4.3 Weight Loss in Stored Tropimech Variety of Tomato Fruits

The weight loss of V3PCA at Day 4 was 2.01% with no significant difference from the one observed at Day 8. It had weight loss of 25.81% at Day 20 but showed no significant difference with the value recorded at Day 16. In V3PCR, 24.95% of weight was lost at Day 20, less than that of V3PCA. However, the lowest weight loss in plastic crate at Day 4, was recorded in V3PCS and increased to 25.27% at Day 20. In the control, V3PCC, the weight loss at the final day of the experiment was 25.14% which showed no significant difference from that of Day 16 (Figure 11).

The weight loss in V3PPA (0.24%) was very low at Day 4 compared to others and significantly increased to 13.59% at Day 20. V3PPR had 13.84% as its weight loss at Day 20 which was observed to be lowest in that category. Moreover, the highest weight loss (15.65%) was observed in V3PCS at Day 20 although it showed no significant difference with that of Day 16 (13.54%). The weight loss of V3PPC at Day 4 was 1.18% and 10.53% was observed at Day 20 (Figure 12).

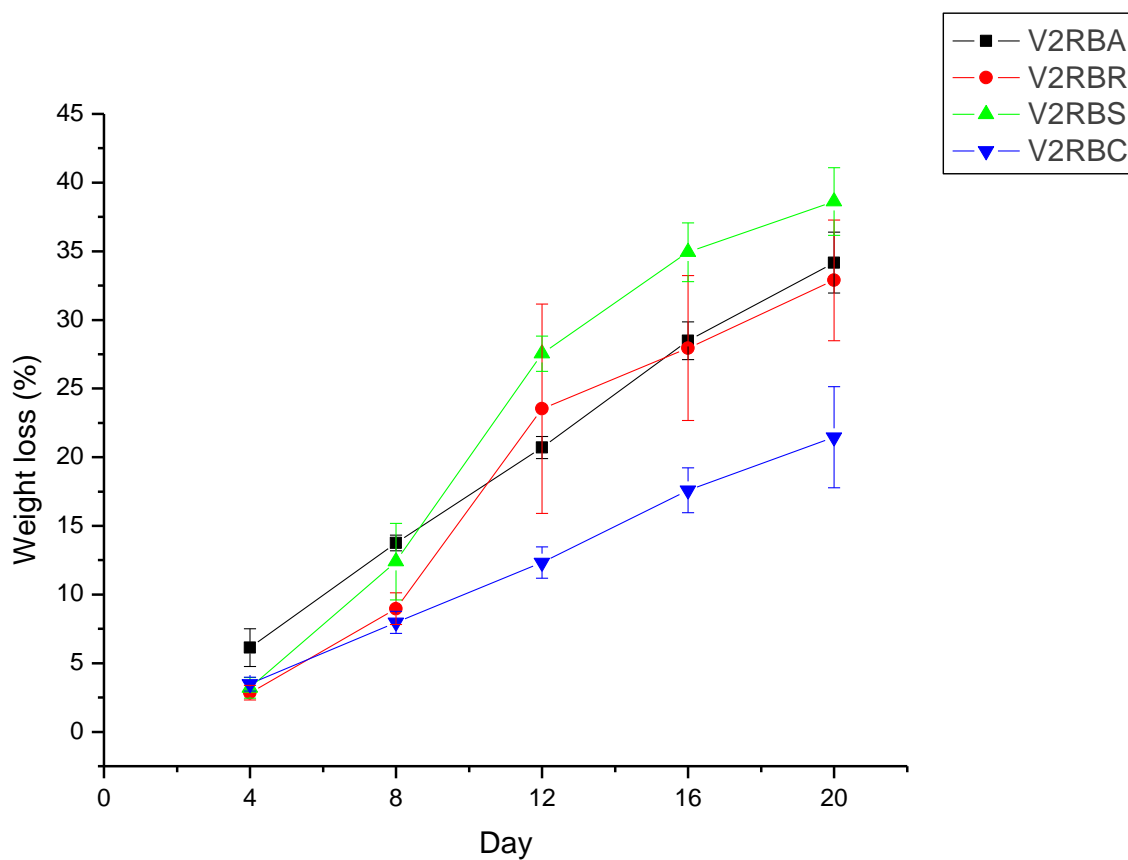


Figure 10: Weight loss of tomato (Yoruba variety) stored in Raffia Basket amended with botanicals

V2RBA = Yoruba Variety + Raffia Basket + Ash

V2RBR = Yoruba Variety + Raffia Basket + Rice Straw

V2RBS = Yoruba Variety + Raffia Basket + Sawdust

V2RBC = Yoruba Variety + Raffia Basket + Control

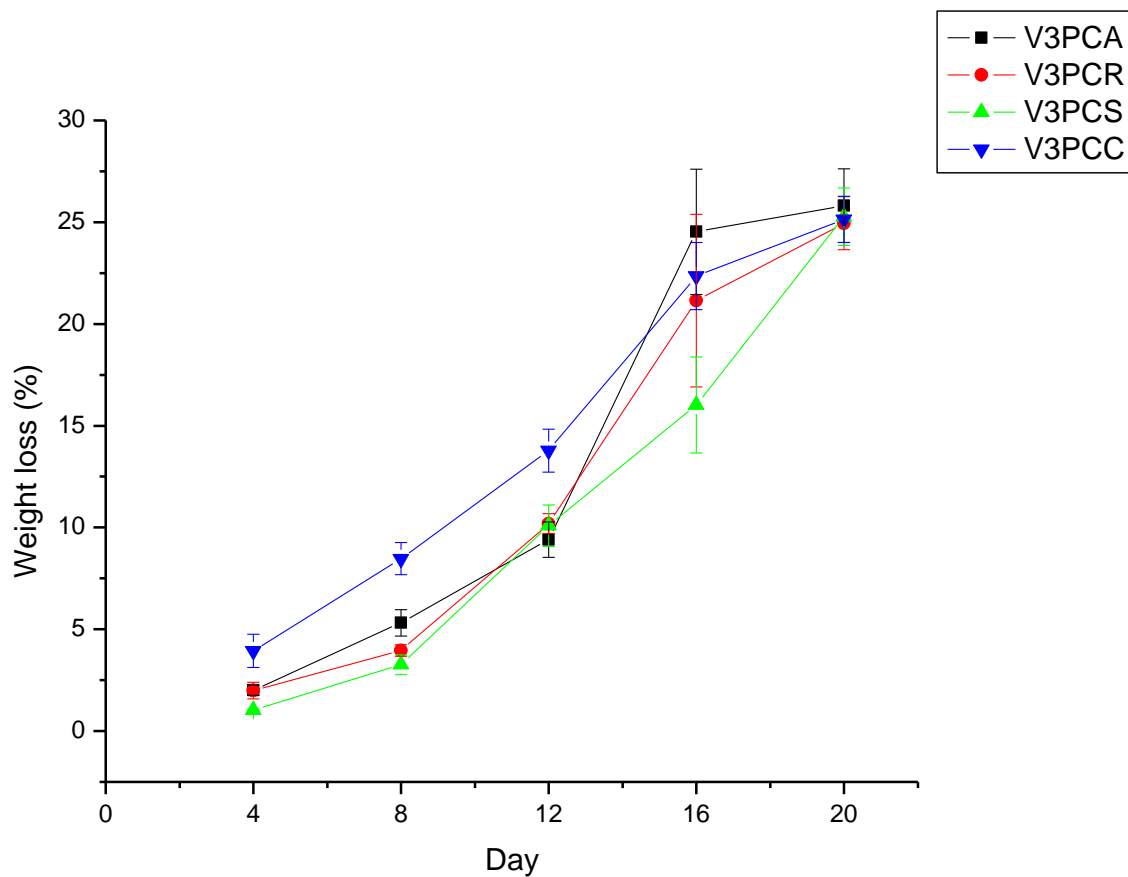


Figure 11: Weight loss of tomato (Tropimech variety) stored in plastic crate amended with botanicals

V3PCA = Tropimech Variety + Plastic crate + Ash

V3PCR = Tropimech Variety + Plastic crate + Rice Straw

V3PCS = Tropimech Variety + Plastic crate + Sawdust

V3PCC = Tropimech Variety + Plastic crate + Control

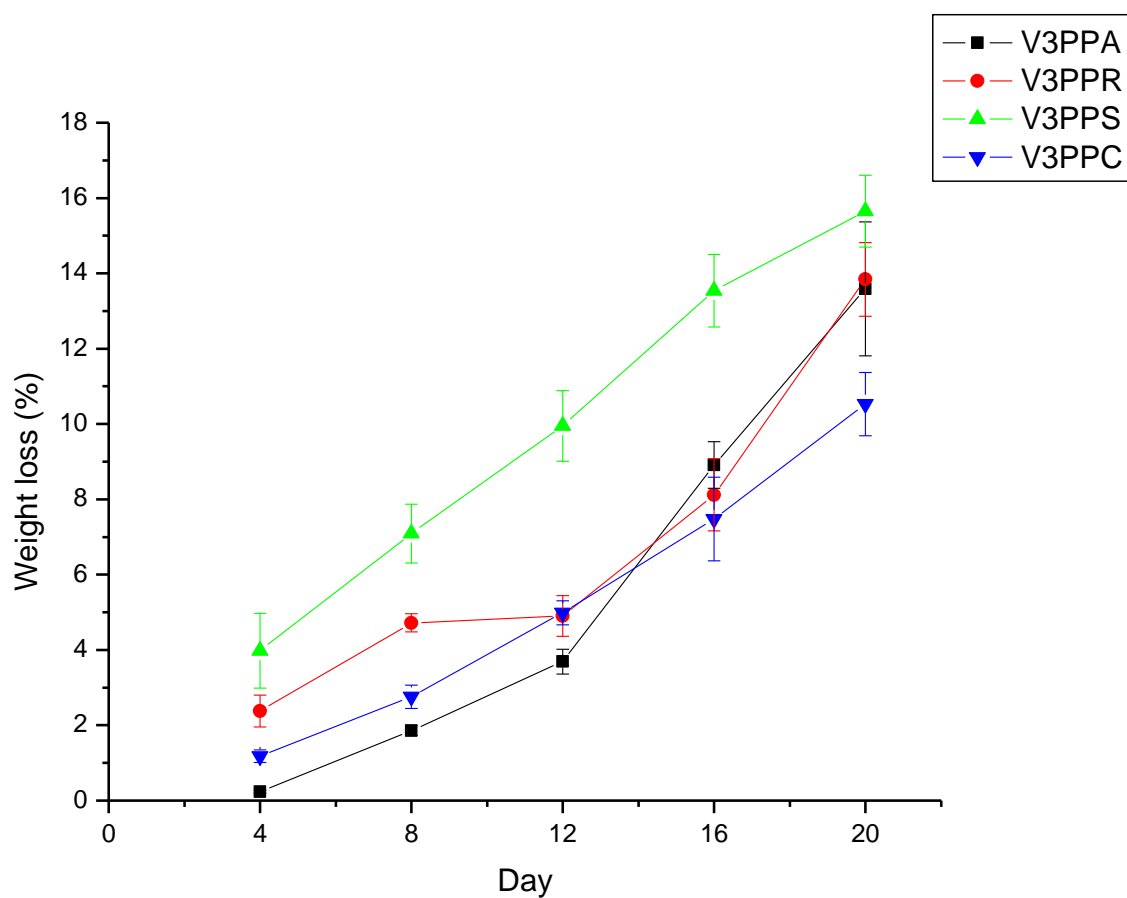


Figure 12: Weight loss of tomato (Tropimech variety) stored in pot in pot refrigerator amended with botanicals

V3PPA = Tropimech Variety + Pot in Pot + Ash

V3PPR = Tropimech Variety + Pot in Pot + Rice Straw

V3PPS = Tropimech Variety + Pot in Pot + Sawdust

V3PPC = Tropimech Variety + Pot in Pot + Control

The V3 Fruits stored in raffia baskets lost more weight than the fruits in both plastic crate as well as pot in pot refrigerator. The highest weight loss was observed in V3RBA (32.30%) at Day 20. In V3RBR, the weight loss at Day 4 was 2.04% and significantly increased to 29.63% at Day 20. There was an insignificant increase in weight loss of V3RBS from 28.31% at Day 16 to 31.82% at Day 20. In the control, the total weight loss in the last day was 30.57% (Figure 13).

4.4.4 Weight Loss in Stored Roma VF Variety

The weight loss in V4PCA at Day 16 was 13.97% and was significantly inclined to 22.76% at Day 20. Similar observation was noticed in V4PCR, V4PCS and V4PCC. Within the plastic crate, the highest weight loss at Day 20 was observed in V4PCC and the lowest was in V4PCS (Figure 14).

The weight loss in V4PPA was significantly increased from 0.56% at Day 4 to 13.77% at Day 20. V4PPR had lower percentage weight loss compared to what was observed in V4PPA at the same day. V4PPS lost weight of 11.70% at Day 20 and it was lowest recorded in this variety. The highest weight loss within pot in pot refrigerator was observed in V4PCC at Day 20 (Figure 15).

The weight loss of V4RBA at Day 16 was 15.17% and this was significantly increased to 22.94% at Day 20. The weight loss of V4RBR at Day 20 was also lower than that of V4RBA. The lowest weight loss at Day 20 within raffia basket was recorded in V4RBS. V4RBC had weight loss of 14.95% at Day 16 and was significantly increased to 24.38% at Day 20 and this was the highest in this structure (Figure 16).

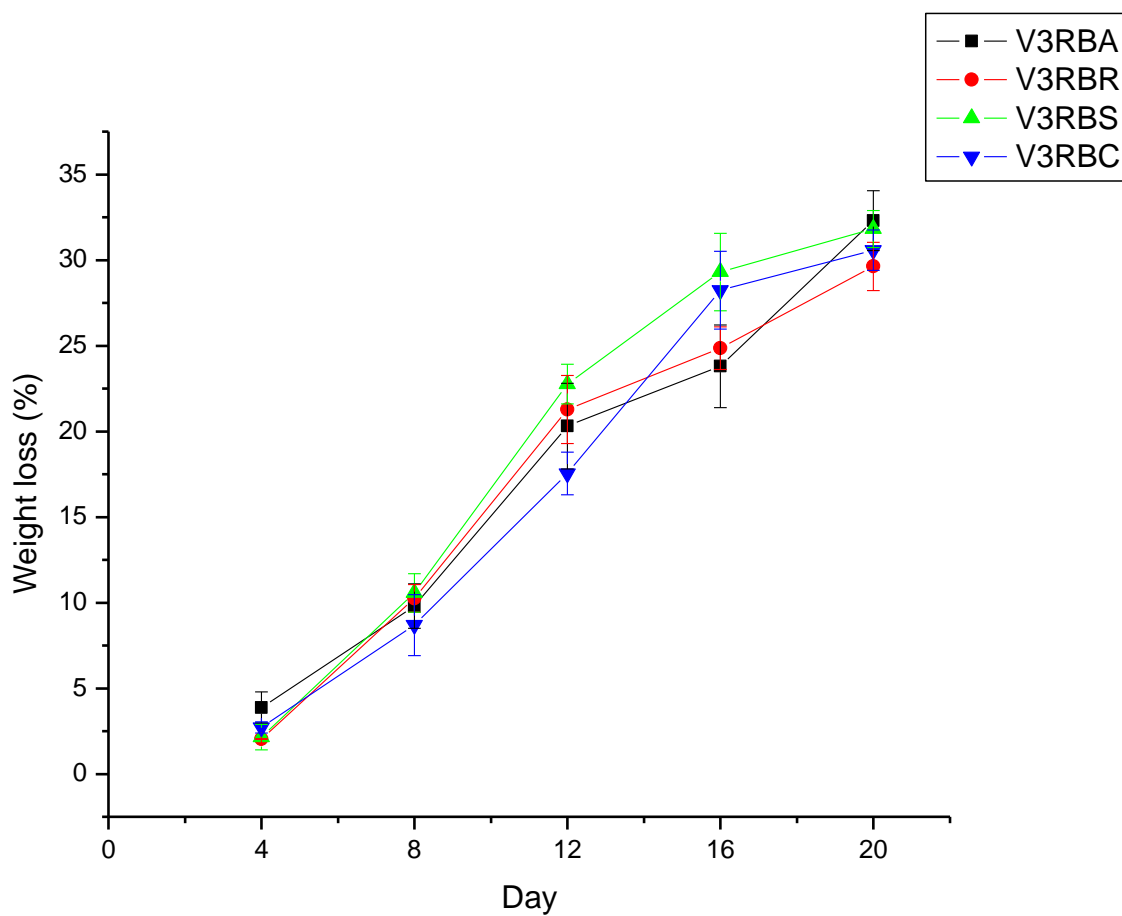


Figure13: Weight loss of tomato (Tropimech variety) stored in Raffia Basket amended with botanicals

V3RBA = Tropimech Variety + Raffia Basket + Ash

V3RBR = Tropimech Variety + Raffia Basket + Rice Straw

V3RBS = Tropimech Variety + Raffia Basket + Sawdust

V3RBC = Tropimech Variety + Raffia Basket + Control

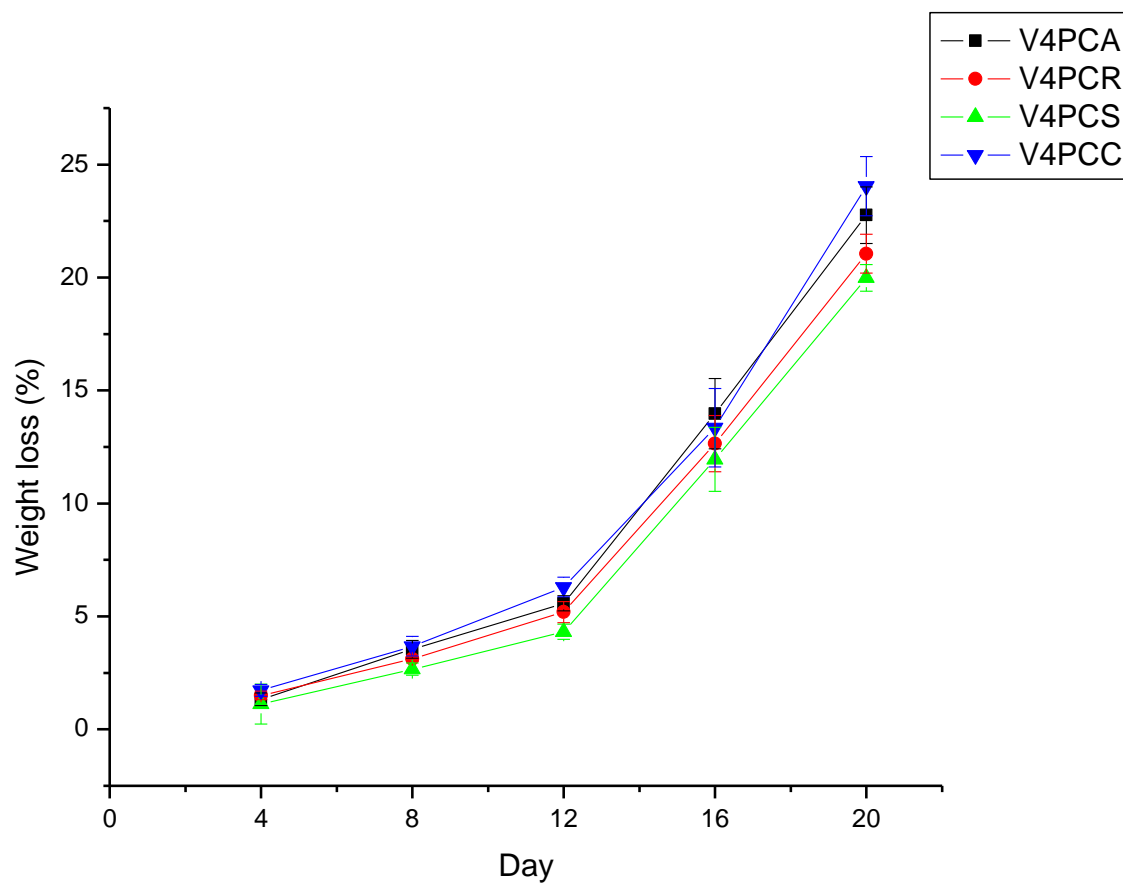


Figure 14: Weight loss of tomato (Roma VF) stored in plastic crate amended with botanicals

V4PCA = Roma VF Variety + Plastic crate + Ash

V4PCR = Roma VF Variety + Plastic crate + Rice Straw

V4PCS = Roma VF Variety + Plastic crate + Sawdust

V4PCC = Roma VF Variety + Plastic crate + Control

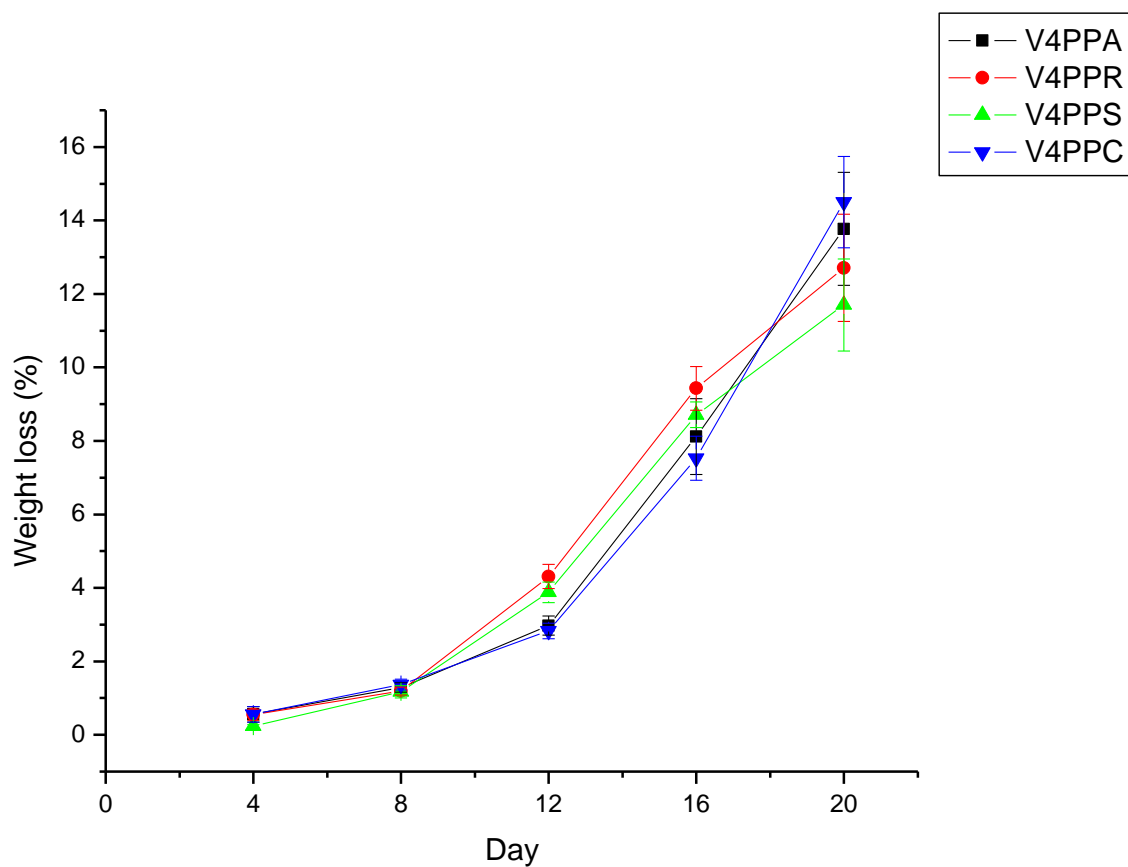


Figure 15: Weight loss of tomato (Roma VF variety) stored in pot in pot refrigerator amended with botanicals

V4PPA = Roma VF Variety + Pot in Pot + Ash

V4PPR = Roma VF Variety + Pot in Pot + Rice Straw

V4PPS = Roma VF Variety + Pot in Pot + Sawdust

V4PPC = Roma VF Variety + Pot in Pot + Control

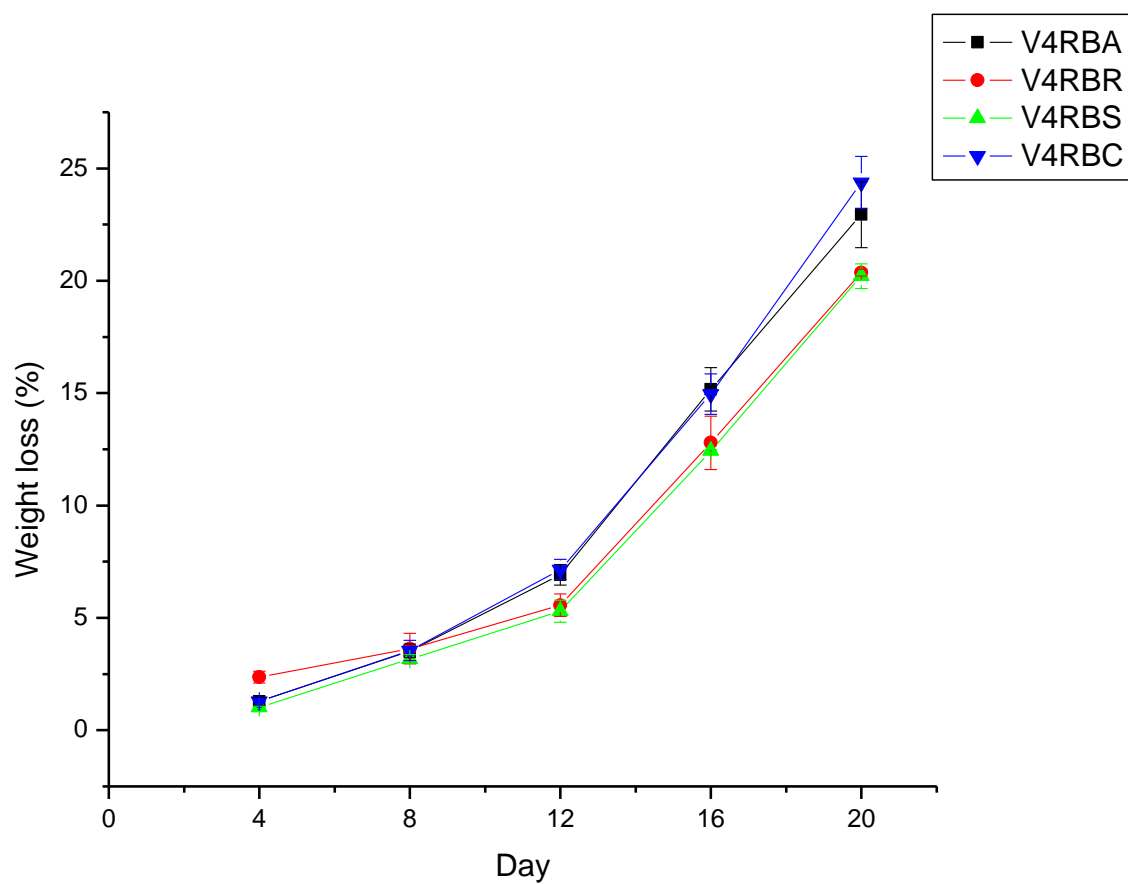


Figure 16: Weight loss of tomato (Roma VF variety) stored in Raffia Basket amended with botanicals

V4RBA = Roma VF Variety + Raffia Basket + Ash

V4RBR = Roma VF Variety + Raffia Basket + Rice Straw

V4RBS = Roma VF Variety + Raffia Basket + Sawdust

V4RBC = Roma VF Variety + Raffia Basket + Control

4.4.5 Influence of Variety, Storage and Botanicals on the Weight Loss of Tomato Fruits

Hausa variety had highest percentage weight loss and significantly different from other varieties. Also, raffia baskets supported weight loss more than both plastic crates and pot in pot refrigerator. With reference to botanical treatments, no significant difference was observed in weight loss except for ash. All interactions of factors were significant (Table 12).

4.5 LYCOPENE CONTENTS IN THE STORED TOMATO FRUITS

4.5.1 Lycopene Contents of Hausa Variety of Tomato Fruits

It was generally observed that the lycopene (a form of carotenoid pigment) determined the redness of tomato because as the lycopene in all the varieties of tomato fruits was increasing, the redness became more conspicuous until the fruits were fully ripe. The lycopene content in V1PCA was 11.42 $\mu\text{g/g}$ at Day 4 and it was increased to 102.94 $\mu\text{g/g}$ at Day 20. This red pigment carotenoid was estimated to be 98.16 $\mu\text{g/g}$ in V1PCR at Day 20 and this value was significantly different from the quantity estimated at Day 16 (90.07 $\mu\text{g/g}$). Also, 90.44 $\mu\text{g/g}$ of lycopene was present in V1PCS at Day 20. The highest lycopene in the fruits stored in plastic crates for this variety was recorded in V1PCC at Day 20 (106.04 $\mu\text{g/g}$) while the lowest was in V1PCS (90.44 $\mu\text{g/g}$) (Figure 17).

It was noticed that the lycopene in V1 fruits stored in pot in pot refrigerator was not as much as the quantity obtained in other storage structures. In V1PPA, there was a significant increase in lycopene from 85.22 $\mu\text{g/g}$ (Day 16) to 89.62 $\mu\text{g/g}$ (Day 20). This red pigment in V1PPR was 83.11 $\mu\text{g/g}$ at Day 20 which was significantly different from that of Day 16 (81.19 $\mu\text{g/g}$). V1PPC had lycopene of 90.22 $\mu\text{g/g}$ and this was highest estimated in this storage structure (Figure 18).

Table12: Weight loss in tomato fruits as influenced by variety, storage structure and botanicals

Factor	Level of Factors	Weight loss (%)
Variety (V)	Hausa	30.47a
	Yoruba	24.48b
	Tropimech	23.26b
	Roma VF	19.04c
SE		0.58
Storage (S)	Plastic crate	27.49b
	Pot in Pot	13.96c
	Raffia basket	31.50a
SE		0.50
Treatment (T)	Ash	26.47a
	Rice straw	23.93b
	Sawdust	23.98b
	Control	22.87b
SE		0.58
$V \times S$		*
$V \times T$		*
$S \times T$		*
$V \times S \times T$		*

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

* = Significant difference

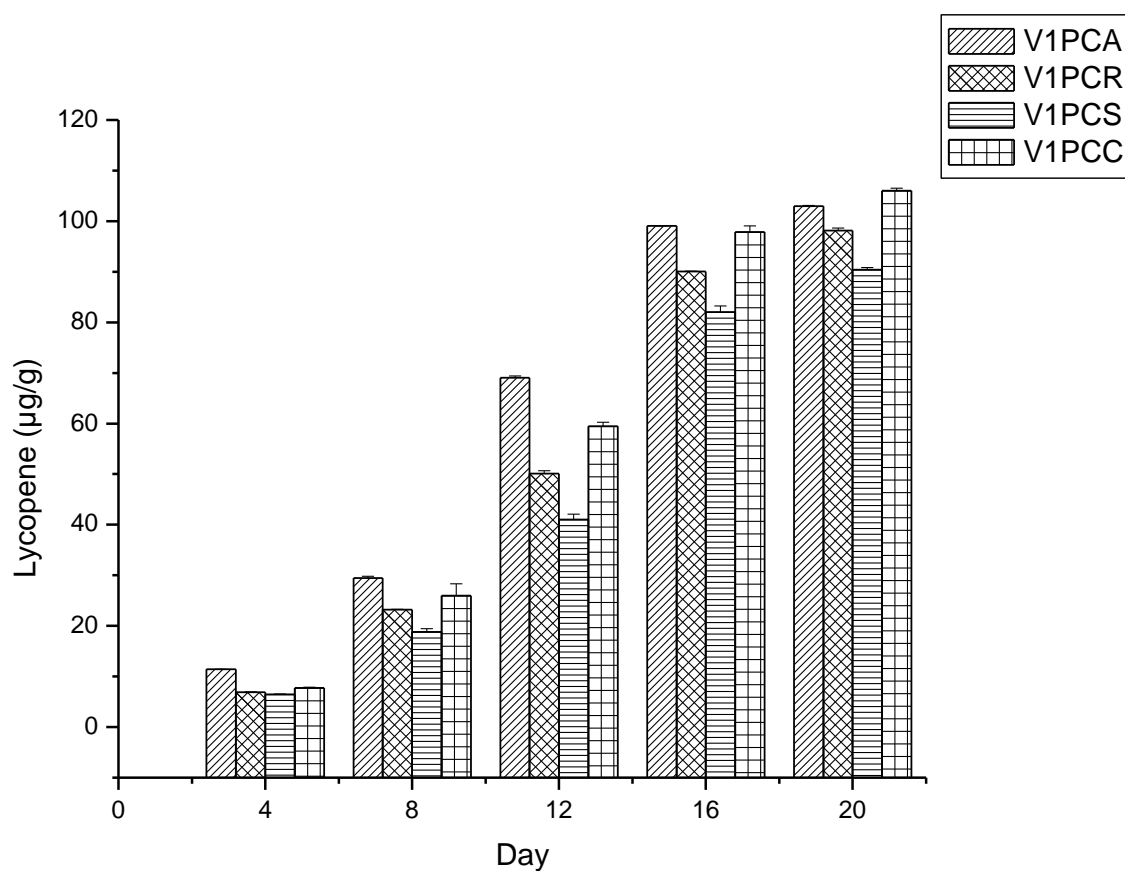


Figure 17: Lycopene content of tomato (Hausa variety) stored in plastic crates with botanicals

V1PCA = Hausa Variety + Plastic crate + Ash

V1PCR = Hausa Variety + Plastic crate + Rice Straw

V1PCS = Hausa Variety + Plastic crate + Sawdust

V1PCC = Hausa Variety + Plastic crate + Control

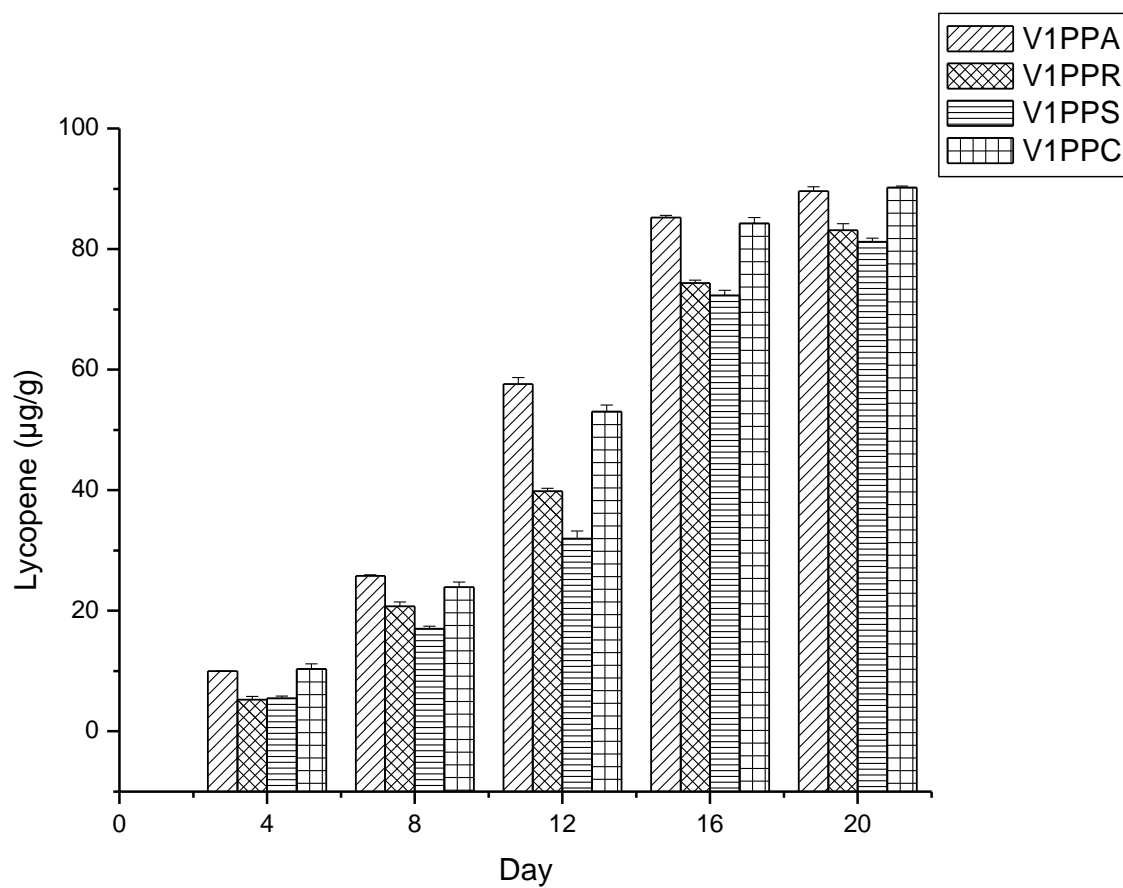


Figure 18: Lycopene content of tomato (Hausa variety) stored in pot in pot refrigerator with botanicals

V1PPA = Hausa Variety + Pot in Pot + Ash

V1PPR = Hausa Variety + Pot in Pot + Ricestraw

V1PPS = Hausa Variety + Pot in Pot + Sawdust

V1PPC = Hausa Variety + Pot in Pot + Control

It was observed that the highest value of lycopene ($130.51\mu\text{g/g}$) was recorded in V1RBA at Day 20 this was significantly different from the one obtained at Day 16 ($121.64\mu\text{g/g}$). In V1RBR, no significant difference was observed in the quantity of lycopene estimated at Days 12 ($55.25\mu\text{g/g}$) and 16 ($94.11\mu\text{g/g}$) but the two were significantly different from that of Day 20 ($105.91\mu\text{g/g}$). the lycopene in V1RBS at Day 20 was $104.05\mu\text{g/g}$ and was significantly lower than that of V1RBC which was quantify to be $115.9\mu\text{g/g}$ (Figure 19).

4.5.2 Lycopene Contents of Yoruba Variety of Tomato Fruits

The amount of lycopene in V2PCA at Day 20 was $108.70\mu\text{g/g}$ which was significantly higher than that of Day 16 ($105.93\mu\text{g/g}$). The quantity estimated in V2PCR at Day 4 was $9.06\mu\text{g/g}$ and there was a significant increase to $23.45\mu\text{g/g}$ at Day 8. At Day 20, the lycopene was estimated to be $100.35\mu\text{g/g}$. Similar trend was observed in V2PCS but its lycopene was $93.84\mu\text{g/g}$ at Day 20. In V2PCC, this carotenoid pigment was $114.50\mu\text{g/g}$, significantly higher than the value obtained at Day 16 ($104.36\mu\text{g/g}$) as shown in Figure 20

The lycopene of V2 fruits in pot in pot refrigerator was comparatively lower than other two storage structures. At Day 20 in V2PPA, $95.17\mu\text{g/g}$ was the value of lycopene recorded. More so, $82.72\mu\text{g/g}$ of it was present in V2PPR at Day 20. In V2PPS, as observed in other treatments, the lycopene was significantly increasing up to Day 20 and $81.43\mu\text{g/g}$ was recorded on the final day (Figure 21).

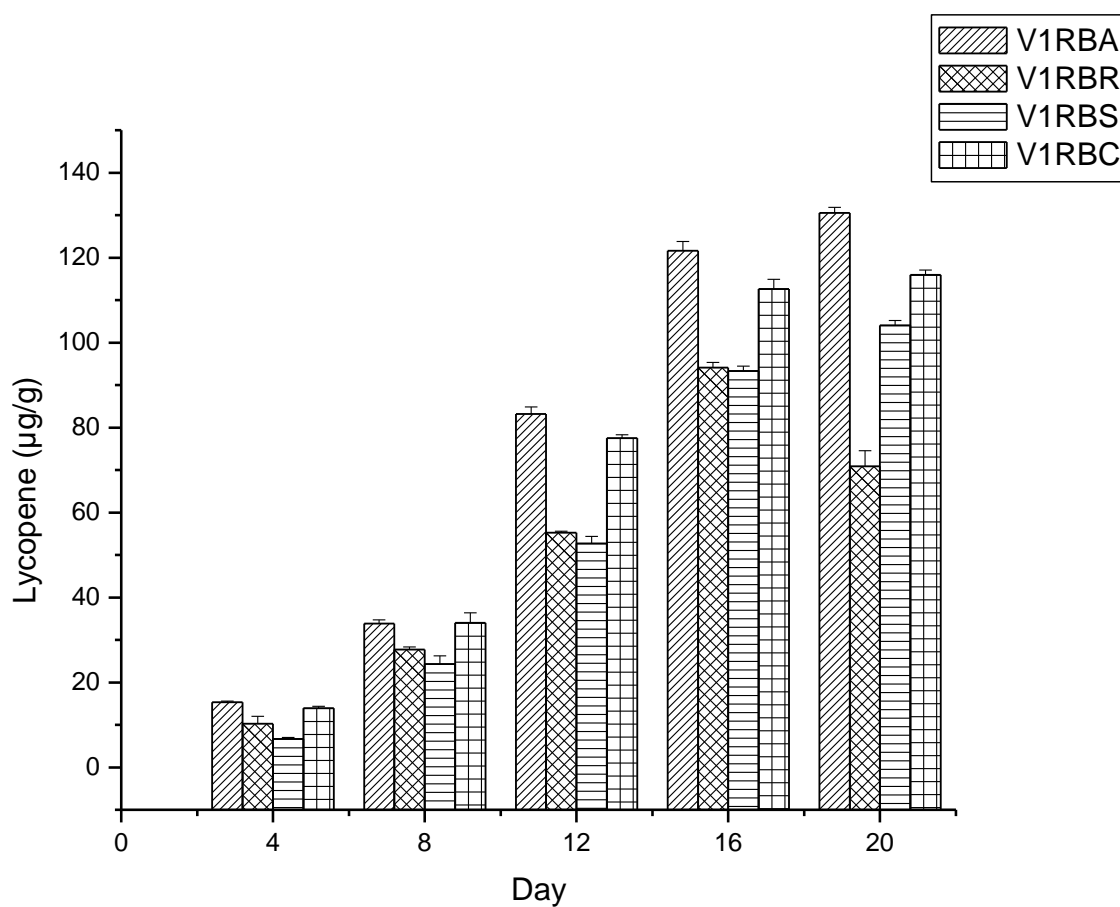


Figure 19: Lycopene content of tomato (Hausa variety) stored in raffia basket with botanicals

V1RBA = Hausa Variety + Raffia Basket + Ash

V1RBR = Hausa Variety + Raffia Basket + Rice Straw

V1RBS = Hausa Variety + Raffia Basket + Sawdust

V1RBC = Hausa Variety + Raffia Basket + Control

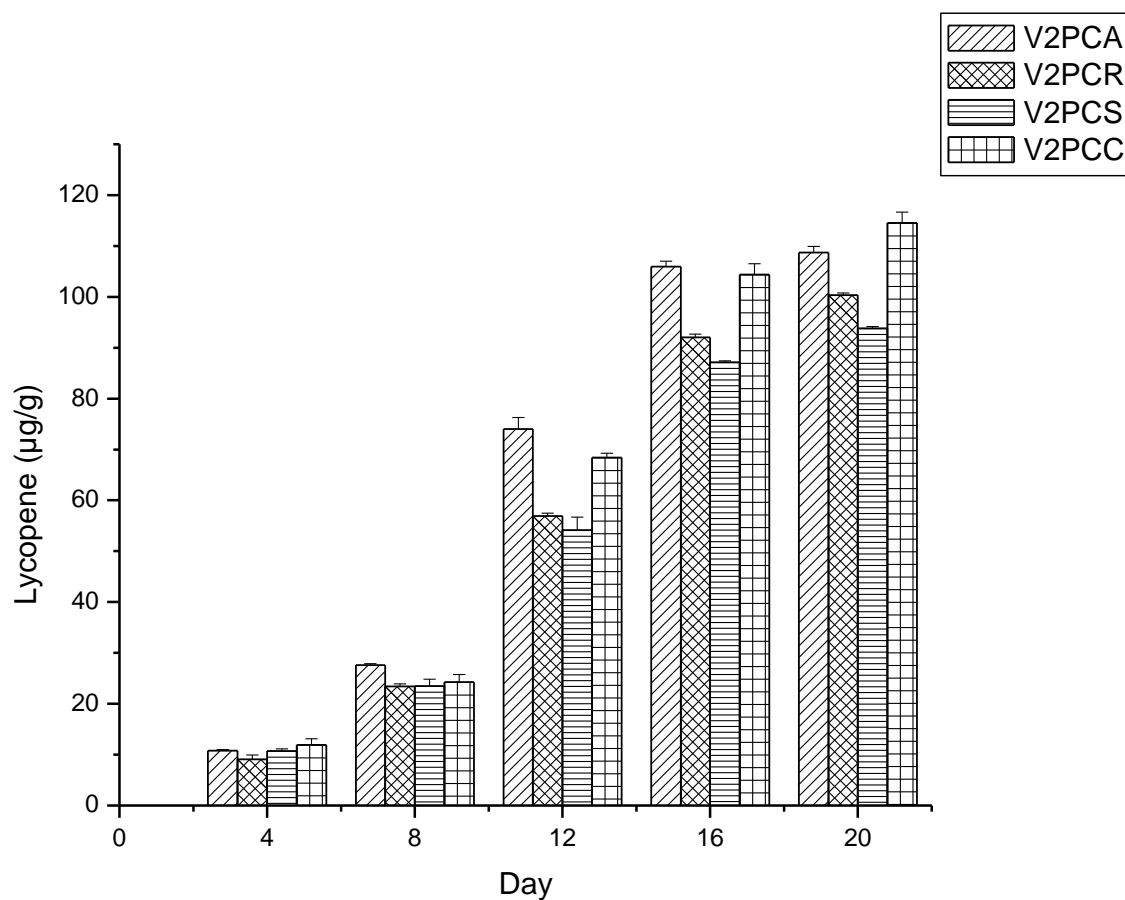


Figure 20: Lycopene content of tomato fruits (Yoruba variety) stored in plastic crates with botanicals

V2PCA = Yoruba Variety + Plastic crate + Ash

V2PCR = Yoruba Variety + Plastic crate + Rice Straw

V2PCS = Yoruba Variety + Plastic crate + Sawdust

V2PC = Yoruba Variety + Plastic crate + Control

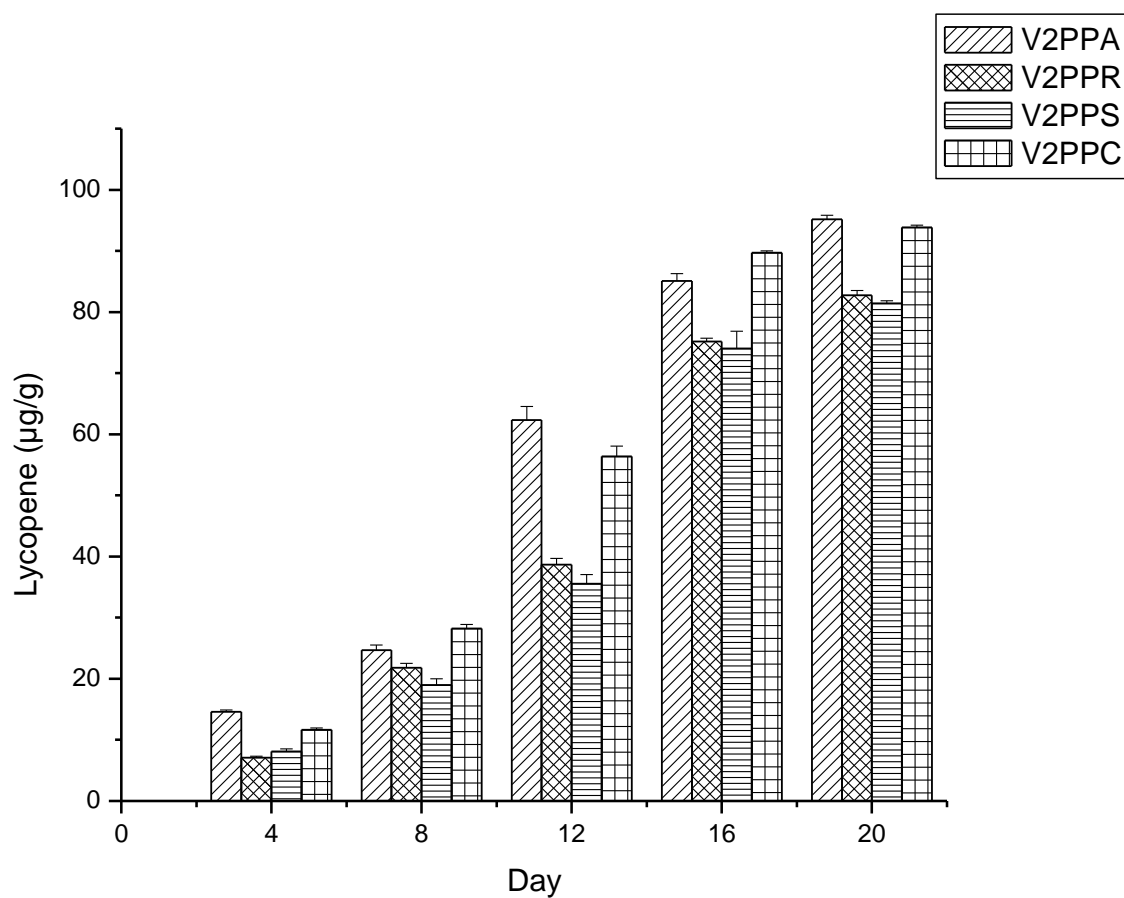


Figure 21: Lycopene content of tomato fruits (Yoruba variety) stored in pot in pot refrigerator with botanicals

V2PPA = Yoruba Variety + Pot in Pot + Ash

V2PPR = Yoruba Variety + Pot in Pot + Rice Straw

V2PPS = Yoruba Variety + Pot in Pot + Sawdust

V2PPC = Yoruba Variety + Pot in Pot + Control

V2RBA experienced significant increase in lycopene at Days 4 (17.30 $\mu\text{g/g}$), 8 (38.12 $\mu\text{g/g}$), 12 (87.56 $\mu\text{g/g}$), 16 (123.24 $\mu\text{g/g}$) and 20 (126.93 $\mu\text{g/g}$). All of them were significantly different from one another except those of Days 16 and 20. Similar trend was observed in V2RBR but its lycopene was 95.48 $\mu\text{g/g}$ at Day 20. However, V2RBS had 92.23 $\mu\text{g/g}$ at Day 16 which was significantly different from the value recorded at Day 20. Besides, 127.63 $\mu\text{g/g}$ of lycopene was observed in V2RBC at Day 20 and happened to be the highest value in this variety (both treated and untreated) (Figure 22).

4.5.3 Lycopene Contents of Tropimech Variety of Tomato Fruits

The mature green fruits of V3 had lycopene of 2.42 $\mu\text{g/g}$ (at Day 1). The quantity of this pigment was 106.65 $\mu\text{g/g}$ at Day 20 and was insignificantly different from the one obtained at Day 16 (98.00 $\mu\text{g/g}$). In V3PCR, 98.97 $\mu\text{g/g}$ was estimated as the amount of lycopene present at Day 20 less than the value obtained in V3PCA at the same period. At Day 4 in V3PCS, 7.52 $\mu\text{g/g}$ of lycopene was recorded and this was significantly increased to 19.37 $\mu\text{g/g}$ and 43.22 $\mu\text{g/g}$ at Days 8 and 12 respectively. At Day 20, 89.70 $\mu\text{g/g}$ was observed. In V3PCC, the amount of lycopene recorded at Day 20 (113.75 $\mu\text{g/g}$) showed a significant difference from the obtained at Day 16 (101.66 $\mu\text{g/g}$) (Figure 23).

The lycopene in V3PPA at Day 20 was 92.79 $\mu\text{g/g}$ and showed no significant difference from that of Day 16 (82.07 $\mu\text{g/g}$). Moreover, there was an insignificant increase in this red pigment at Day 4 (9.31 $\mu\text{g/g}$) when compared it to the value obtained at Day 1 (2.42 $\mu\text{g/g}$) in V3PCR and at Day 20, 95.61 was recorded. Similar trend was observed in V3PPS but unlike V3PPR, the lycopene at Day 20 (87.20 $\mu\text{g/g}$) had no significant difference from the value at Day 16 (81.81 $\mu\text{g/g}$). In V3PPC, the amount of lycopene was 91.4 $\mu\text{g/g}$ but significantly increased to 100.21 $\mu\text{g/g}$ at Day 20 (Figure 24).

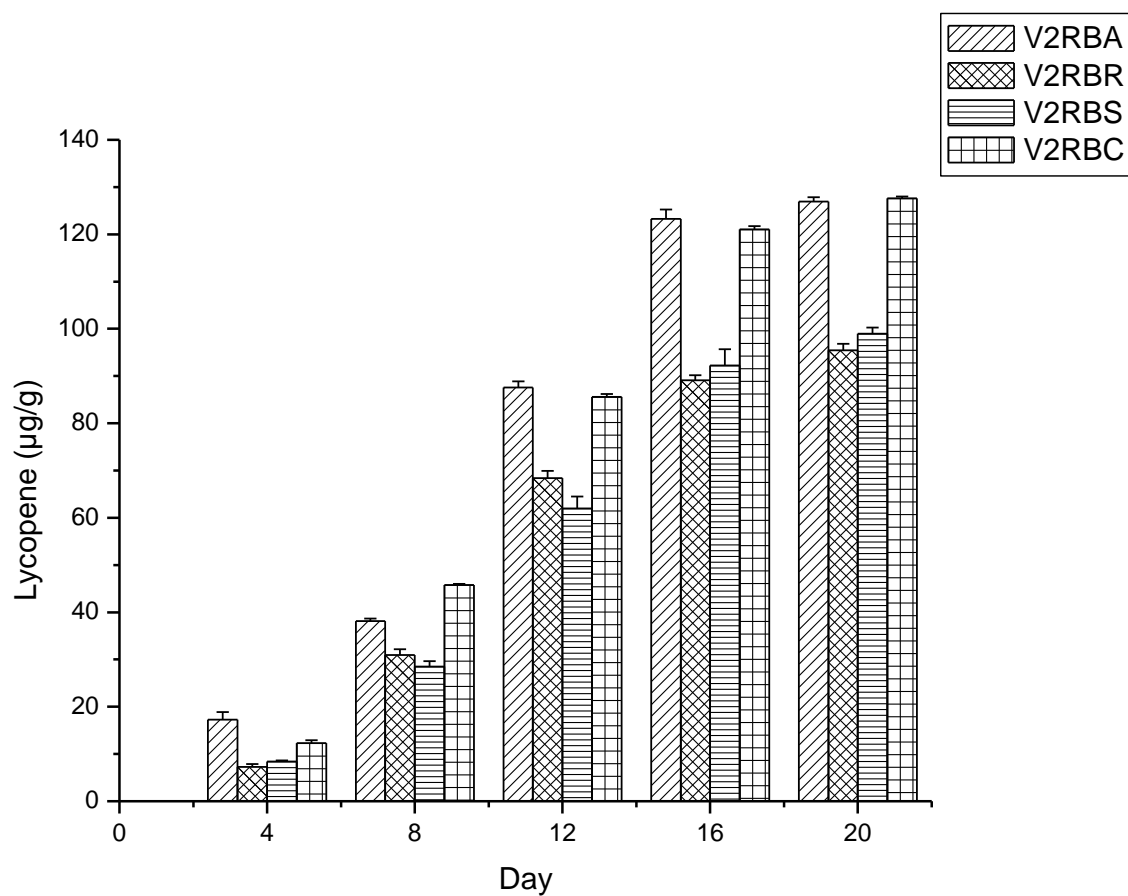


Figure 22: Lycopene content of tomato fruits (Yoruba variety) stored in raffia basket with botanicals

V2RBA = Yoruba Variety + Raffia Basket + Ash

V2RBR = Yoruba Variety + Raffia Basket + Rice Straw

V2RBS = Yoruba Variety + Raffia Basket + Sawdust

V2RBC = Yoruba Variety + Raffia Basket + Control

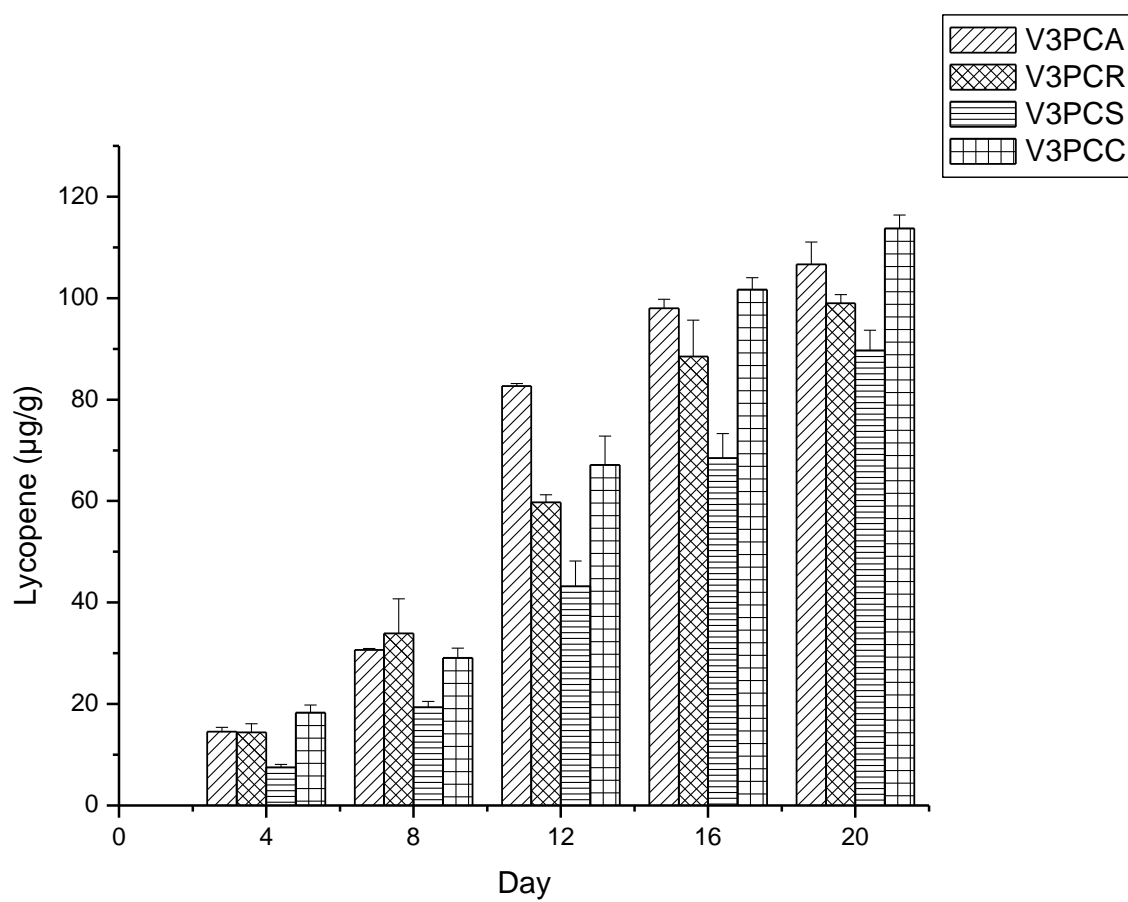


Figure 23: Lycopene content of tomato fruits (Tropimech variety) stored in plastic crate with botanicals

V3PCA = Tropimech Variety + Plastic crate + Ash

V3PCR = Tropimech Variety + Plastic crate + Rice Straw

V3PCS = Tropimech Variety + Plastic crate + Sawdust

V3PCC = Tropimech Variety + Plastic crate + Control

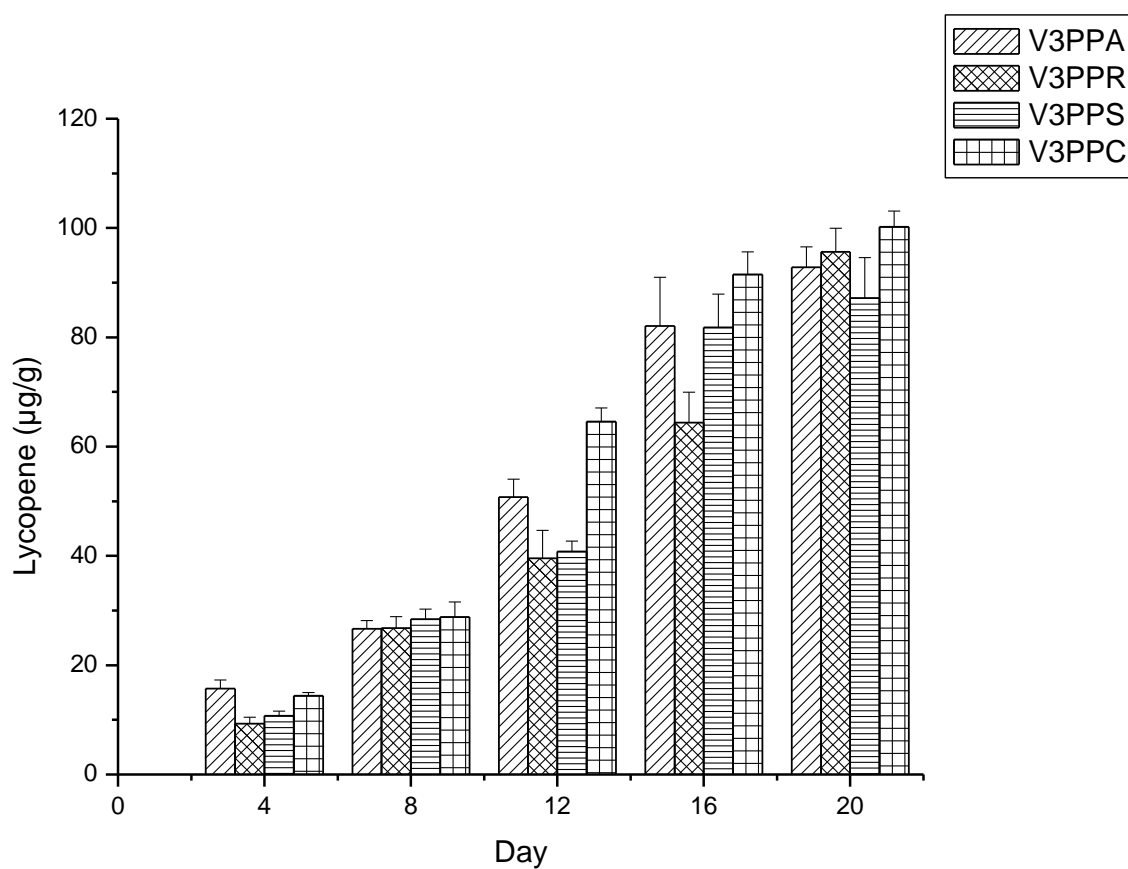


Figure 24: Lycopene content of tomato (Tropimech variety) stored in pot in pot refrigerator with botanicals

V3PPA = Tropimech Variety + Pot in Pot + Ash

V3PPR = Tropimech Variety + Pot in Pot + Rice Straw

V3PPS = Tropimech Variety + Pot in Pot + Sawdust

V3PPC = Tropimech Variety + Pot in Pot + Control

Lycopene determination in V3RBA showed that the amount of lycopene observed at Day 20 (107.91 $\mu\text{g/g}$) was significantly different from 95.74 $\mu\text{g/g}$ obtained at Day 16. That of V3RBR at Day 20 was 102.01 $\mu\text{g/g}$ and lesser than the values obtained in both V3RBA (107.91 $\mu\text{g/g}$) and V3RBS (102.96 $\mu\text{g/g}$) at the same day. The amount of lycopene observed at Day 20 in V3RBC (115.75 $\mu\text{g/g}$) was the highest in V3 stored in raffia baskets (Figure 25).

4.5.4 Lycopene Contents in Roma VF Variety

In V4, a significant increase in lycopene content was observed as the storage period increased. At Day 20, the lycopene content of V4PCA was 108.18 $\mu\text{g/g}$. Also, 104.05 $\mu\text{g/g}$ of lycopene was observed in V4PCR which was lower than the one estimated in V4PCA. In V4PCS, no significant difference was observed in the amounts of lycopene recorded at Days 16 (98.22 $\mu\text{g/g}$) and 20 (99.42 $\mu\text{g/g}$). it was generally observed that the V4 stored in plastic crates had highest lycopene of 109.81 $\mu\text{g/g}$ and this was specifically observed in V4PCC at Day 20 (Figure 26).

The lycopene content of V4PPA at Day 20 was 96.83 $\mu\text{g/g}$ and this was observed to be highest in all V4 stored in pot in pot refrigerator. V4PPR and V4PPS had lycopene contents of 90.99 $\mu\text{g/g}$ and 90.81 $\mu\text{g/g}$ respectively. The lycopene estimated in V4PPC (94.86 $\mu\text{g/g}$) was higher than those obtained in V4PPR and V4PPS (Figure 27).

It was noticed that the highest lycopene content in V4 was observed in V4RBA at Day 20. In V4RBR, the lycopene content at Day 8 was 26.59 $\mu\text{g/g}$ and was significantly increased to 82.02 at Day 16 and 104.81 $\mu\text{g/g}$ at Day 20. V4RBS had 104.82 $\mu\text{g/g}$ of lycopene at Day 20 and it was very close to the value obtained in V4RBR. The lycopene in V4RBC was 105.58 $\mu\text{g/g}$ at Day 20 and was second to V4RBA in this storage material (Figure 28).

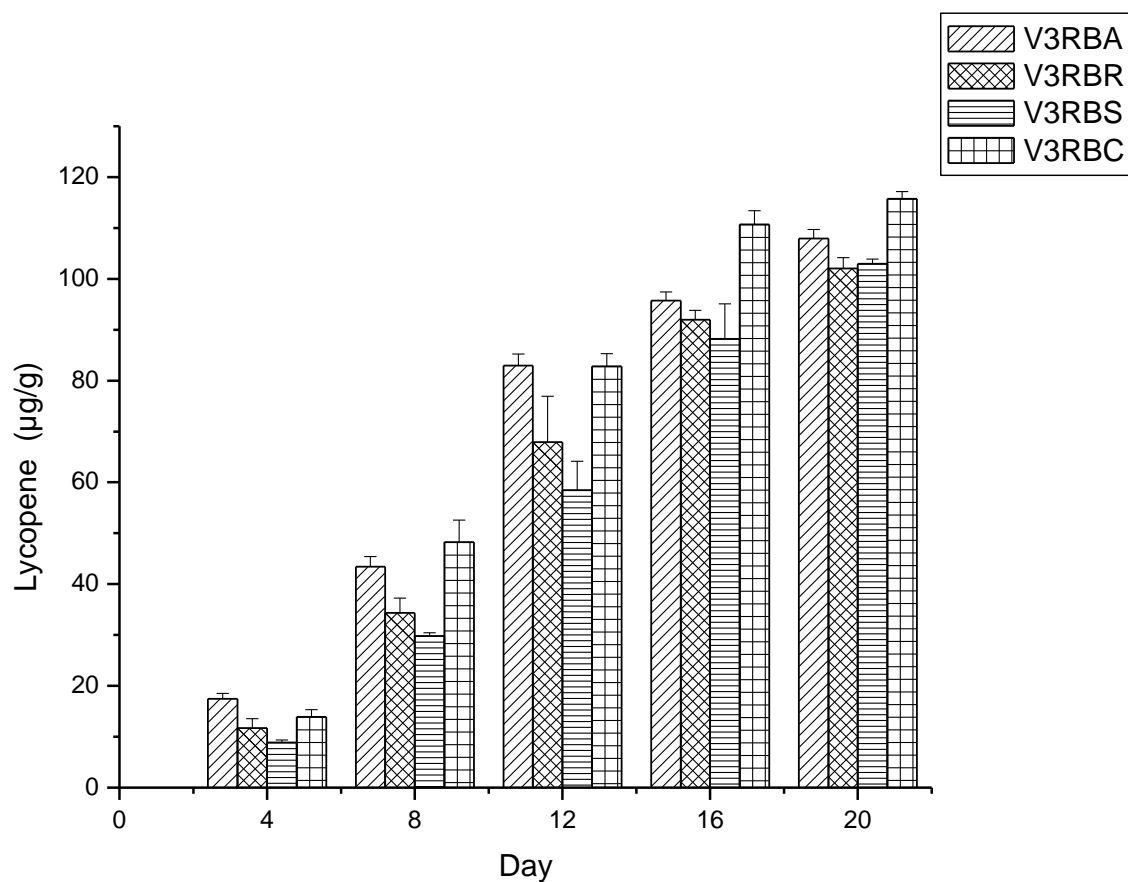


Figure 25: Lycopene content of tomato fruits (Tropimech variety) stored in raffia basket with botanicals

V3RBA = Tropimech Variety + Raffia Basket + Ash

V3RBR = Tropimech Variety + Raffia Basket + Rice Straw

V3RBS = Tropimech Variety + Raffia Basket + Sawdust

V3RBC = Tropimech Variety + Raffia Basket + Control

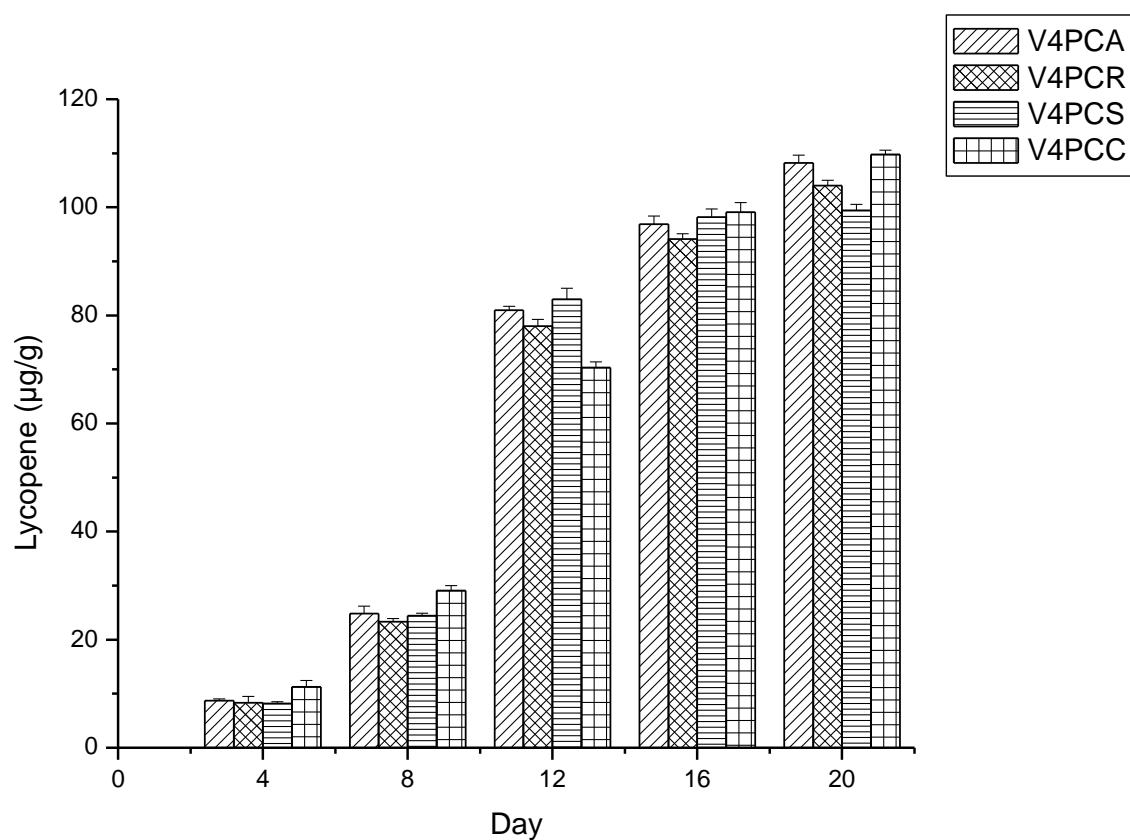


Figure 26: Lycopene content of tomato (Roma VF variety) stored in plastic crate with botanicals

V4PCA = Roma VF Variety + Plastic crate + Ash

V4PCR = Roma VF Variety + Plastic crate + Rice Straw

V4PCS = Roma VF Variety + Plastic crate + Sawdust

V4PCC = Roma VF Variety + Plastic crate + Control

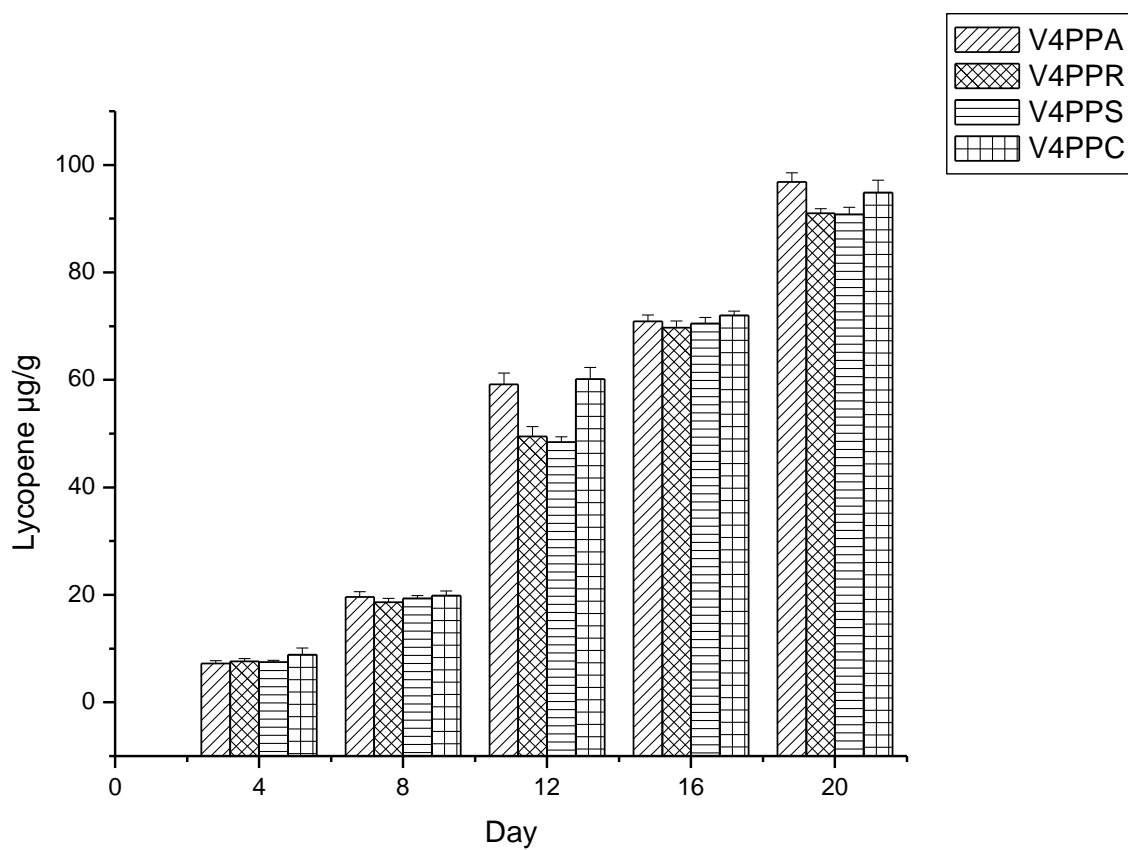


Figure 27: Lycopene content of tomato (Roma VF variety) stored in pot in pot refrigerator with botanicals

V4PPA = Roma VF Variety + Pot in Pot + Ash

V4PPR = Roma VF Variety + Pot in Pot + Rice Straw

V4PPS = Roma VF Variety + Pot in Pot + Sawdust

V4PPC = Roma VF Variety + Pot in Pot + Control

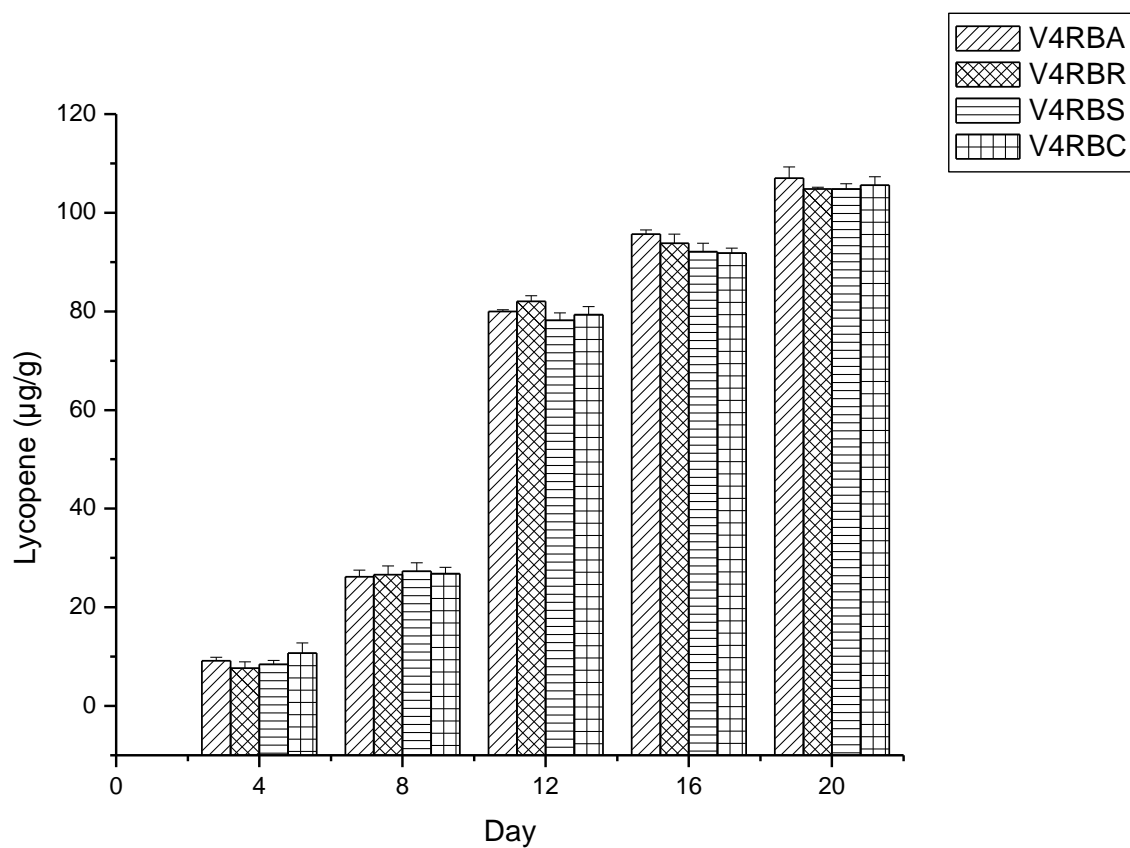


Figure 28: Lycopene content of tomato (Roma VF variety) stored in raffia baskets with botanicals

V4RBA = Roma VF Variety + Raffia Basket + Ash

V4RBR = Roma VF Variety + Raffia Basket + Rice Straw

V4RBS = Roma VF Variety + Raffia Basket + Sawdust

V4RBC = Roma VF Variety + Raffia Basket + Control

4.5.5 Influence of Variety, Storage and Botanicals on lycopene of Tomato Fruits

It was observed that Yoruba variety had highest lycopene content although there was no significant difference in the lycopene contents of all the varieties. The lycopene in tomato stored in raffia baskets was highest and significantly different from other two storage structures. The influence of ash on the amount of lycopene in tomato fruits was significantly higher than both rice straw and sawdust but insignificantly lower than the value recorded in the control. All forms of interactions between the fixed factors were significant (Table 13).

4.6 PROXIMATE ANALYSES OF STORED TOMATO FRUITS

4.6.1 Proximate Analysis of Hausa Variety of Tomato Fruits

The results of proximate analysis of all the varieties revealed that the moisture contents, among all the proximate elements, had the highest quantity irrespective of the treatments as well as the storage structures. The moisture content of V1RBC (95.71%) was highest in V1 and significantly higher than 94.00% of V1PCC but insignificantly different from 94.77% of V1PPC. V1PCA had moisture of 93.28% and this was significantly different from 93.81% of V1PPA. The lowest moisture contents were observed in sawdust-treated V1. No significant difference was observed in 92.04%, 92.69% and 92.82% of V1PCS, V1PPS and V1RBS respectively (Table 14).

The ash content of V1PCA was 0.38%, significantly higher than both treated and non-treated V1. The lowest ash (0.15%) was observed in V1RBC, although it was not significantly different from V1PCS (0.20%), V1PCC (0.19%), V1PPS (0.21%), V1PPC (0.18%), V1RBA (0.16%), V1RBR (0.19%) and V1RBS (0.16%) (Table 11). No significant difference was observed in protein contents of V1 (treated and control) except V1PCA with 0.22% of protein. V1PCC, V1RBR and V1RBS had fibre contents of 0.17%. The lowest fibre was recorded in V1PPA (0.08%).

Table 13: Lycopene composition of stored tomato fruits as influenced by variety, storage structures and botanicals

Factor	Level of Factors	Lycopene ($\mu\text{g/g}$)
Variety (V)	Hausa	99.85a
	Yoruba	101.63a
	Tropimech	101.13a
	Roma VF	101.43a
SE		0.58
Storage (S)	Plastic crate	102.84b
	Pot in Pot	90.41c
	Raffia basket	109.77a
SE		0.51
Treatment (T)	Ash	106.10a
	Rice straw	96.85b
	Sawdust	93.73c
	Control	107.35a
SE		0.58
$V \times S$		*
$V \times T$		*
$S \times T$		*
$V \times S \times T$		*

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

*= Significant difference

Table 14: Effects of storage methods on the proximate composition of tomato fruits(Hausa Variety)

Treatment	Moisture	Ash	Protein	Fibre	Lipid	Carbohydrate
V1PCA	93.28±0.16de	0.38±0.00a	0.22±0.00a	0.21±0.01a	0.12±0.00ab	5.78±0.15bcd
V1PCR	93.55±0.38cde	0.25±0.02bc	0.11±0.01b	0.15±0.01bc	0.13±0.01a	5.81±0.62bcd
V1PCS	92.04±0.41f	0.20±0.01cde	0.12±0.01b	0.13±0.01bcd	0.09±0.01bc	7.42±0.41a
V1PCC	94.00±0.49bcd	0.19±0.01cde	0.12±0.02b	0.17±0.02ab	0.09±0.01abc	5.42±0.51cd
V1PPA	93.81±0.39bcde	0.24±0.02bcd	0.12±0.01b	0.08±0.01e	0.10±0.00abc	5.64±0.40bcd
V1PPR	93.73±0.26bcde	0.28±0.03b	0.10±0.03b	0.09±0.01de	0.09±0.02bc	5.70±0.25bcd
V1PPS	92.69±0.24ef	0.21±0.05cde	0.12±0.04b	0.12±0.03cde	0.09±0.01abc	6.77±0.27ab
V1PPC	94.77±0.34ab	0.18±0.02de	0.08±0.00b	0.14±0.02bcd	0.08±0.01c	4.75±0.34de
V1RBA	94.60±0.35bc	0.16±0.03e	0.09±0.01b	0.15±0.01bc	0.08±0.02c	4.91±0.35cde
V1RBR	93.43±0.48cde	0.19±0.00cde	0.09±0.02b	0.17±0.00ab	0.10±0.02abc	6.02±0.47bc
V1RBS	92.82±0.39def	0.16±0.01e	0.11±0.01b	0.17±0.01ab	0.10±0.01abc	6.63±0.36ab
V1RBC	95.71±0.24a	0.15±0.01e	0.07±0.02b	0.15±0.01bc	0.09±0.01bc	3.83±0.25e

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V1PCA = Hausa variety + Plastic crate + Ash

V1PCR = Hausa variety + Plastic crate + Rice straw

V1PCS = Hausa variety + Plastic crate + Sawdust

V1PPC = Hausa variety + Plastic crate + Control

V1PPA = Hausa variety + Pot in Pot + Ash

V1PPR = Hausa variety + Pot in Pot + Rice straw

V1PPS = Hausa variety + Pot in Pot + Sawdust

V1PPC = Hausa variety + Pot in Pot + Control

V1RBA = Hausa variety + Raffia basket + Ash

V1RBR = Hausa variety + Raffia basket + Rice straw

V1RBS = Hausa variety + Raffia basket + Sawdust

V1RBC = Hausa variety + Raffia basket + Control

The amount of fat in V1 was comparatively low. No significant difference was observed in V1PCA (0.12%) and V1PPA (0.10%). The two were significantly different from V1RBA (0.08%) (Table 14)

The fat content of V1PCS, V1PCC, V1PPR, V1PPS and V1RBRC was 0.09%. The carbohydrate content was high, second to the moisture (Table 11). V1PCS was the richest in Carbohydrate (7.42%) and was insignificantly higher than V1PPS (6.77%) and V1RBS (6.63%). The least amount was recorded in V1RBC (3.83%) and showed no significant difference from V1RBA (4.91%) and V1PPC (4.75%) (Table 14).

4.6.2 Proximate Analysis of Yoruba Variety of Tomato Fruits

In this variety, moisture content took the lead with varying quantity in all the samples. V2RBC had 95.69% moisture and was insignificantly ranked above V2PCC (95.73%) and V2PCC (94.48%). In V2PCA, 95.14% of moisture was observed and insignificantly different from V2PPA (94.04%) and V2RBA (94.80). V2PCR had moisture content of 93.67% which was higher but insignificantly different from 93.57% and 93.25% of V2PPR and V2RBR respectively. The lowest moisture (92.77%) was observed in V2RBS (Table 15).

The ash content of V2RBR was the least (0.11%). The value for ash obtained in V2PCS (0.20%) was significantly higher than 0.12% of V2PPS and 0.13% of V2RBS. Those recorded in both V2PCC (0.16%) and V2PPC (0.20%) had no significant difference from each other (Table 15). For the protein content, no significant difference was observed in all treated samples as well as control except V2RBS that had the content of 0.19% (Table 15).

The fibre content varied considerably as V2PCC had the highest amount (0.21%). V2PCA had the fibre content of 0.12% and this was significantly different from that of V2PPA (0.20%) and

Table 15: Effects of storage methods on the proximate composition of tomato fruits (Yoruba Variety)

	Moisture	Ash	Protein	Fibre	Lipid	Carbohydrate
V2PCA	95.14±0.92ab	0.19±0.01abcd	0.09±0.01b	0.12±0.01bcd	0.10±0.01ab	4.34±0.91efg
V2PCR	93.67±0.27cde	0.14±0.04de	0.10±0.01b	0.15±0.23abc	0.14±0.01ab	5.80±0.28abcd
V2PCS	94.40±0.61bcd	0.20±0.01abc	0.09±0.01b	0.20±0.01a	0.14±0.02ab	4.97±0.61cdef
V2PCC	94.48±0.26abcd	0.16±0.01cde	0.08±0.01b	0.21±0.01a	0.11±0.01ab	4.97±0.23cdef
V2PPA	94.04±0.18bcde	0.23±0.03ab	0.09±0.01b	0.20±0.03a	0.11±0.01ab	5.33±0.19bcde
V2PPR	93.57±0.32cde	0.23±0.01a	0.08±0.02b	0.11±0.02bcd	0.09±0.01b	5.92±0.36abc
V2PPS	93.12±0.23e	0.12±0.02e	0.19±0.03b	0.10±0.01cd	0.12±0.02ab	6.36±0.21ab
V2PPC	95.73±0.33a	0.20±0.02abc	0.09±0.01b	0.07±0.01d	0.13±0.24ab	3.59±0.16g
V2RBA	94.80±0.15abc	0.17±0.01bcde	0.10±0.02b	0.18±0.02ab	0.10±0.01ab	4.64±0.15defg
V2RBR	93.25±0.26de	0.13±0.01de	0.08±0.01b	0.11±0.04bcd	0.08±0.00b	6.35±0.37ab
V2RBS	92.77±0.18e	0.13±0.01de	0.19±0.01a	0.08±0.01cd	0.09±0.01ab	6.82±0.17a
V2RBC	95.69±0.17a	0.11±0.00e	0.07±0.01b	0.15±0.01abc	0.11±0.02ab	3.88±0.18fg

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V2PCA = Yoruba variety + Plastic crate + Ash

V2PCR = Yoruba variety + Plastic crate + Rice straw

V2PCS = Yoruba variety + Plastic crate + Sawdust

V2PPC = Yoruba variety + Plastic crate + Control

V2PPA = Yoruba variety + Pot in Pot + Ash

V2PPR = Yoruba variety + Pot in Pot + Rice straw

V2PPS = Yoruba variety + Pot in Pot + Sawdust

V2PPC = Yoruba variety + Pot in Pot + Control

V2RBA = Yoruba variety + Raffia basket + Ash

V2RBR = Yoruba variety + Raffia basket + Rice straw

V2RBS = Yoruba variety + Raffia basket + Sawdust

V2RBC = Yoruba variety + Raffia basket + Control

V2RBA (0.18%). All rice straw-treated samples were not significantly different from one another in this variety unlike sawdust treated samples. The fibre in both V2PPS (0.10%) and V2RBS (0.08%) was significantly lower than that obtained in V2PCS (0.20%) (Table 15).

The highest lipid contents were observed in V2PCR and V2PCS (0.14%) while V2RBR had the lowest amount (0.08%). The value of lipid recorded in V2PPC (0.13%) was significantly different from 0.09% of V2PPR (Table 15). The amounts of carbohydrate in ash-treated samples were not significantly different from one another and similar trend was observed in rice straw-treated samples. The amounts of carbohydrate in this variety was ranged from 3.55% (V2PPC) to 6.82% (V2RBS) (Table 15).

4.6.3 Proximate Analyses of Tropimech Variety of Tomato Fruits

As observed in V2, the maximum moisture content was recorded in V3RBC (95.92%), followed by that of V3RBA (95.34%) with no significant difference. The moisture content in V3PCA (94.32%) and V3PPA (93.69%) showed no significant difference but the two were significantly different from 95.34% of V3RBA. V3PCR had water content of 93.97% but no significant difference was observed among all rice straw treated sample. Similarly, V3PCS had moisture content of 93.60% which was significantly different from both V3PPS (93.34%) and V3RBS (93.87) (Table 16).

Ash content in V3 was very low and no significant difference was observed in all the treated and control samples across the storage structures. However, the quantity of ash ranged from 0.04% (V3RBC) to 0.42% (V3PCC). The protein content in V3PPS (0.92%) was the highest in this category and it was significantly different from 0.11% of V3RBS but insignificantly different from 0.90% of V3PCS (Table 16).

Table16: Effects of storage on the proximate composition of tomato (Tropimech Variety)

	Moisture	Ash	Protein	Fibre	Lipid	Carbohydrate
V3PCA	94.32±0.00bcde	0.09±0.00a	0.23±0.00de	0.62±0.00c	0.12±0.00a	4.78±1.20ab
V3PCR	93.97±0.37cde	0.10±0.01a	0.23±0.05de	0.32±0.11d	0.05±0.02a	5.33±0.26a
V3PCS	93.60±0.24e	0.15±0.26a	0.90±0.05a	1.17±0.13b	0.09±0.14a	3.22±0.05bcd
V3PCC	95.03±0.63abc	0.42±0.34a	0.84±0.00ab	1.23±0.08b	0.07±0.01a	2.40±0.38d
V3PPA	93.69±0.42de	0.80±0.02a	0.54±0.11bcd	1.26±0.10b	0.05±0.01a	4.38±0.42abc
V3PPR	94.08±0.51bcde	0.09±0.00a	0.62±0.20abc	1.40±0.02ab	0.07±0.00a	3.74±0.37abcd
V3PPS	93.34±0.15e	0.36±0.28a	0.92±0.01a	1.27±0.03b	0.06±0.02a	4.05±0.18abcd
V3PPC	94.94±0.70abcd	0.05±0.01a	0.39±0.05cde	1.55±0.04a	0.07±0.01a	2.99±0.63cd
V3RBA	95.34±0.17ab	0.10±0.02a	0.77±0.08ab	1.23±0.08b	0.06±0.01a	2.50±0.23d
V3RBR	94.67±0.24abcde	0.12±0.04a	0.69±0.05abc	1.18±0.08b	0.09±0.01a	3.26±0.31bcd
V3RBS	93.87±0.47cde	0.06±0.01a	0.11±0.01e	1.20±0.07b	0.06±0.02a	4.69±0.53ab
V3RBC	95.92±0.20a	0.04±0.01a	0.39±0.25cde	1.19±0.02b	0.05±0.01a	2.42±0.42d

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V3PCA = Tropimech Variety + Plastic crate + Ash

V3PCR = Tropimech Variety + Plastic crate + Rice straw

V3PCS = Tropimech Variety + Plastic crate + Sawdust

V3PPC = Tropimech Variety + Plastic crate + Control

V3PPA = Tropimech Variety + Pot in Pot + Ash

V3PPR = Tropimech Variety + Pot in Pot + Rice straw

V3PPS = Tropimech Variety + Pot in Pot + Sawdust

V3PPC = Tropimech Variety + Pot in Pot + Control

V3RBA = Tropimech Variety + Raffia basket + Ash

V3RBR = Tropimech Variety + Raffia basket + Rice straw

V3RBS = Tropimech Variety + Raffia basket + Sawdust

V3RBC = Tropimech Variety + Raffia basket + Control

All V3 stored in raffia baskets had fibre contents that showed no significant difference from one another. The fibre content in V3PPA (1.26%) was insignificantly higher than 1.23% of V3RBA but significantly lower than 0.62% of V3PCA. The fibre in sawdust treated samples of V3 was not significantly different from one another (Table 16). The lipid content of V3 was comparatively infinitesimal and no significant difference was observed among them. The lowest carbohydrate content was estimated in V3PCR quantified to be 5.33% and this was significantly different from 3.26% of V3RBR. The amount of carbohydrate in all V3 controls showed no significant difference from one another (Table 16).

4.6.4 Proximate Analyses of Roma VF Variety

The highest moisture in Roma VF was observed in V4RBA (93.13%) and was significantly different from V4PCA (91.03%). The percentage moisture in V4PCR (90.01%) was the lowest and significantly different from both V4PPR (92.22%) and V4RBR (92.41%). In V4RBS, 92.99% moisture was recorded and significantly higher than V4PPS (91.56%) and V4PCS (90.56%) (Table 17).

The ash in V4 ranged from 0.58% (V4RBA) to 0.28% (V4PPR). The lowest and the highest protein contents were observed in V4PPA (0.07%) and V4PCR (0.25%) respectively. No significant difference was observed in all the botanically treated V4 stored in plastic crates. Similar observation was observed in V4 stored in pot in pot refrigerator except V4PPR (Table 17). The fibre contents in V4PCR, V4PCS and V4PPS were the same (0.19%). The highest lipid content was observed in V4PCA and was significantly higher than the amounts obtained in other treated V4 irrespective of the storage structures except V4RBA and V4RBS. V4PCR had 9.04% of carbohydrate and was significantly higher than both V4PPR (7.10%) and V4RBR (6.80%). Also, no significant difference was observed between V4PCS and V4PPS (Table 17).

Table 17: Effects of storage methods on the proximate composition of tomato fruits (Roma VF Variety)

	Moisture	Ash	Protein	Fibre	Lipid	Carbohydrate
V4PCA	91.03±0.41bcd	0.54±0.05ab	0.21±0.01ab	0.20±0.01abc	0.17±0.04a	7.84±0.45abc
V4PCR	90.01±0.47d	0.42±0.05cd	0.25±0.02a	0.19±0.02abc	0.09±0.01c	9.04±0.52a
V4PCS	90.56±0.04cd	0.47±0.01bc	0.24±0.03a	0.19±0.03abc	0.06±0.00c	8.47±0.06ab
V4PCC	92.98±0.51a	0.50±0.01abc	0.18±0.02bc	0.24±0.02a	0.07±0.01c	6.03±0.49d
V4PPA	91.56±0.29abcd	0.49±0.01abc	0.07±0.02e	0.17±0.01bc	0.08±0.01c	7.63±0.26abcd
V4PPR	92.22±0.50abc	0.28±0.06e	0.18±0.25bc	0.15±0.02cd	0.07±0.00c	7.10±0.46bcd
V4PPS	91.11±0.10bcd	0.33±0.01de	0.11±0.00de	0.19±0.01abc	0.08±0.01c	8.18±0.12ab
V4PPC	91.86±0.25abc	0.47±0.04bc	0.09±0.15e	0.23±0.03ab	0.06±0.01c	7.28±0.32bcd
V4RBA	93.13±0.22a	0.58±0.34a	0.09±0.00e	0.08±0.01e	0.10±0.02bc	6.01±0.21d
V4RBR	92.41±0.25ab	0.42±0.02cd	0.11±0.01de	0.10±0.02de	0.16±0.02ab	6.80±0.20bcd
V4RBS	92.99±1.43a	0.50±0.00abc	0.15±0.01cd	0.06±0.00e	0.10±0.03bc	6.20±1.40cd
V4RBC	93.01±0.01a	0.51±0.02abc	0.08±0.02e	0.11±0.00de	0.12±0.02abc	6.17±0.03cd

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V4PCA = Roma VF + Plastic crate + Ash

V4PCR = Roma VF + Plastic crate + Rice Straw

V4PCS = Roma VF + Plastic crate + Sawdust

V4PCC = Roma VF + Plastic crate + Control

V4PPA = Roma VF + Pot in Pot + Ash

V4PPR = Roma VF + Pot in Pot + Rice Straw

V4PPS = Roma VF + Pot in Pot + Sawdust

V4PPC = Roma VF + Pot in Pot + Control

V4RBA = Roma VF + Raffia Basket + Ash

V4RBR = Roma VF + Raffia Basket + Rice Straw

V4RBS = Roma VF + Raffia Basket + Sawdust

V4RBC = Tropimech Variety + Raffia Basket + Control

4.6.5 Influence of Varieties, Storage and Botanicals on the Proximate Composition of Tomato Fruits

Significant differences were observed in proximate composition due to variety, storage and botanicals. Tropimech and Roma VF showed higher values of all the proximate composition when compared to the two local varieties (Hausa and Yoruba). Roma VF had significantly higher values of ash, fibre, lipid and carbohydrate while Tropimech showed higher values of moisture and protein. The results of storage indicated that the use of raffia basket promoted higher values of moisture and lipid with concomitant reduction in all other proximate composition (Table 18)

With respect to botanicals, significant differences were recorded in all the proximate composition except for ash. Sawdust was significantly enhanced the lipid protein and carbohydrate of tomato over the other botanicals and the control. The control however showed significantly higher value of moisture and fibre contents when compared to other botanicals (Table 18)

The interactions between factors were also significant except for ash under variety by storage interaction; Moisture, ash, protein and carbohydrate under variety and treatment; and moisture and ash under storage and treatment interaction. Interaction between all the three factors (V x S x T) showed significant differences in all the proximate composition except for ash (Table 18).

Table 18: Proximate composition of stored tomato fruits as influenced by variety, storage structures and botanicals

Factor	Level of Factors	Moisture (%)	Ash (%)	Protein (%)	Fibre (%)	Lipid (%)	Carbohydrate (%)
Variety (V)	Hausa	93.70b	0.22b	0.11bc	0.15b	0.10b	5.72b
	Yoruba	94.22a	0.17bc	0.10c	0.14b	0.13ab	5.23c
	Tropimech	94.40a	0.14c	0.55a	1.14a	0.14a	3.65d
	Roma VF	91.91c	0.46a	0.15b	0.16b	0.10b	7.23a
SE		0.12	0.02	0.02	0.12		0.13
Storage (S)	Plastic crate	93.25b	0.28a	0.25a	0.35c	0.16a	5.73a
	Pot in Pot	93.39b	0.24ab	0.24a	0.45a	0.10b	5.59a
	Raffia basket	94.03a	0.22b	0.19b	0.40b	0.09b	5.07b
SE		0.11	0.02	0.01	0.01	0.01	0.11
Treatment (T)	Ash	93.73b	0.27a	0.22ab	0.38bc	0.10b	5.32b
	Rice straw	93.21c	0.22a	0.22ab	0.35c	0.10b	5.91a
	Sawdust	92.78d	0.24a	0.26a	0.41b	0.16a	6.15a
	Control	94.51a	0.25a	0.21b	0.45a	0.10b	4.48c
SE		0.12	0.02	0.02	0.01	0.01	0.13
V × S		*	NS	*	*	*	*
V × T		NS	NS	NS	*	*	NS
S × T		NS	NS	*	*	*	*
V × S × T		*	NS	*	*	*	*

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

*= Significant difference

NS = No significant difference

4.7 MINERAL ANALYSES OF STORED TOMATO

4.7.1 Mineral analysis of Hausa Variety of Tomato Fruits

The highest phosphorous (P) content was found in V1PPS although no significant difference was observed in all saw dust treated samples in Hausa variety. V1PPC had the lowest P content (Table 19). The amount of potassium (K) in V1PCA and V1RBA were significantly higher than the value recorded in V1PPA but all rice straw treated samples showed no significant difference (Table 19).

The values of magnesium (Mg) and copper (Cu) were comparatively low in this variety. All the treated fruits in both plastic crate as well as raffia baskets had higher Mg value than control but in pot in pot refrigerator, only V1PPR and V1PPS manifested such. The amount of Cu in all the samples was less than other minerals analyzed (Table 19). The different values of iron (Fe) in all ash and rice straw treated samples were significantly different at $p \leq 0.05$. V1PCS was observed with highest zinc (Zn) content and this was insignificantly higher than those of V1PPS and V1RBS. The amount of calcium (Ca) ranged from 5.45mg/L (V1PCA) to 3.45mg/L (V1PPS) (Table 19).

4.7.2 Mineral analyses of Yoruba Variety of Tomato Fruits

The amount of P in V2PCA showed no significant difference from the value recorded in V2PCS. The highest P was recorded in V2RBC. The K contents of fruits stored in pot in pot refrigerator were comparatively lower and no significant difference was observed in all treated samples within that storage structure. The amount of Mg in V2RBA was significantly higher than all other fruits stored within raffia baskets. No significant difference was observed within the pot in pot refrigerator except V2PPC. Cu had the lowest content among all minerals analyzed. In which the highest amount was quantified in V2RBC (Table 20)

Table 19: Effects of storage methods on the mineral composition of tomato fruits (Hausa Variety)

	P	K	Mg	Cu	Fe	Zn	Ca
V1PCA	7.50±0.04ab	6.53±0.07a	2.58±0.22de	0.23±0.00bc	5.93±0.04ab	5.17±0.38	5.45±0.45a
V1PCR	7.02±0.98abc	5.70±0.20bcd	3.25±0.38ab	0.18±0.02cd	5.99±0.06a	6.60±0.20	4.66±0.22b
V1PCS	7.87±0.37ab	4.49±0.13e	3.11±0.07abc	0.13±0.01d	2.12±0.07g	6.99±0.24	4.63±0.19b
V1PCC	5.52±0.05d	6.23±0.14ab	2.34±0.01ef	0.21±0.55bc	5.25±0.14abc	6.12±0.05	4.97±0.11ab
V1PPA	7.45±0.14ab	5.09±0.51de	3.22±0.00ab	0.19±0.02cd	2.39±0.01g	5.50±0.04	4.81±0.25ab
V1PPR	7.20±0.10abc	6.10±0.24ab	2.70±0.01cde	0.22±0.01bc	4.87±0.21cde	5.80±0.25	3.78±0.23d
V1PPS	8.05±0.32a	6.42±0.00a	3.30±0.10ab	0.13±0.01d	5.11±0.13bcd	6.07±0.05	3.45±0.03d
V1PPC	6.77±0.12bc	5.35±0.14cd	2.91±0.00bcd	0.16±0.29cd	4.98±0.20cde	4.49±0.25	4.56±0.14bc
V1RBA	5.32±0.20d	6.08±0.33abc	3.44±0.06a	0.27±0.03ab	4.29±0.32def	6.66±0.24	5.04±0.04ab
V1RBR	6.17±0.25cd	5.72±0.10bcd	1.41±0.13g	0.32±0.01a	4.00±0.01f	6.52±0.15	4.00±0.01cd
V1RBS	7.34±0.03ab	5.99±0.00abc	1.99±0.01f	0.16±0.03cd	4.20±0.14ef	6.86±0.13	3.95±0.03d
V1RBC	7.36±0.03ab	4.67±0.34e	1.18±0.07g	0.31±0.02a	3.56±0.79f	6.39±0.05	5.00±0.22ab

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V1PCA = Hausa variety + Plastic crate + Ash

V1PCR = Hausa variety + Plastic crate + Rice straw

V1PCS = Hausa variety + Plastic crate + Sawdust

V1PPC = Hausa variety + Plastic crate + Control

V1PPA = Hausa variety + Pot in Pot + Ash

V1PPR = Hausa variety + Pot in Pot + Rice straw

V1PPS = Hausa variety + Pot in Pot + Sawdust

V1PPC = Hausa variety + Pot in Pot + Control

V1RBA = Hausa variety + Raffia basket + Ash

V1RBR = Hausa variety + Raffia basket + Rice straw

V1RBS = Hausa variety + Raffia basket + Sawdust

V1RBC = Hausa variety + Raffia basket + Control

Table 20: Effects of storage methods on the mineral composition of tomato fruits(Yoruba Variety)

	P	K	Mg	Cu	Fe	Zn	Ca
V2PCA	6.52±0.25a	5.16±0.04cd	2.59±0.01bc	0.20±0.01bcd	6.07±0.04a	6.09±0.07ab	4.28±0.01abc
V2PCR	5.30±0.003cd	6.00±0.18ab	3.64±0.25a	0.25±0.04bc	5.25±0.14ab	5.45±0.22bc	3.21±0.00de
V2PCS	6.74±0.32a	5.36±0.15bc	2.39±0.13bc	0.31±0.03b	2.15±0.10fg	6.60±0.16a	4.54±0.01bc
V2PCC	4.92±0.14cde	5.37±0.09bc	2.13±0.10bcd	0.23±0.01bcd	2.26±0.17fg	4.63±0.34d	3.07±0.11de
V2PPA	4.75±0.28cde	2.74±0.25ef	1.89±0.29cde	0.14±0.03cd	4.23±0.60cd	2.64±0.32f	4.01±0.35bc
V2PPR	4.17±0.36e	2.95±0.34ef	1.43±0.62def	0.14±0.02cd	1.98±0.34g	2.57±0.39f	2.74±0.45e
V2PPS	2.80±0.25f	2.55±0.38f	1.35±0.17ef	0.10±0.01d	2.91±0.20ef	4.35±0.16d	2.90±0.29e
V2PPC	5.55±0.42bc	3.42±0.03e	2.36±0.11bc	0.17±0.01cd	4.42±0.22bc	3.44±0.19e	4.20±0.10abc
V2RBA	6.44±0.39ab	4.48±0.27d	2.84±0.26b	0.16±0.03cd	4.69±0.43bc	5.93±0.18ab	4.63±0.01ab
V2RBR	4.91±0.39cde	6.18±0.15a	1.03±0.06f	0.13±0.01cd	3.26±0.26e	4.70±0.43cd	3.76±0.23cd
V2RBS	4.54±0.40de	3.40±0.47e	1.03±0.08f	0.33±0.01b	1.82±0.12g	5.74±0.27b	3.03±0.43de
V2RBC	6.78±0.09a	5.30±0.04bc	1.17±0.09ef	1.07±0.13a	3.42±0.29de	5.47±0.10bc	4.81±0.07a

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V2PCA = Yoruba variety + Plastic crate + Ash

V2PCR = Yoruba variety + Plastic crate + Rice straw

V2PCS = Yoruba variety + Plastic crate + Sawdust

V2PPC = Yoruba variety + Plastic crate + Control

V2PPA = Yoruba variety + Pot in Pot + Ash

V2PPR = Yoruba variety + Pot in Pot + Rice straw

V2PPS = Yoruba variety + Pot in Pot + Sawdust

V2PPC = Yoruba variety + Pot in Pot + Control

V2RBA = Yoruba variety + Raffia basket + Ash

V2RBR = Yoruba variety + Raffia basket + Rice straw

V2RBS = Yoruba variety + Raffia basket + Sawdust

V2RBC = Yoruba variety + Raffia basket + Control

No significant difference was observed in the amount of Fe in V2PPA and V2RBA but the two were significantly different from V2PCA. The quantity of Fe in all rice straw treated tomato fruits were significant (Table 20). The Zn in V2PCS was highest and even significantly different from other sawdust treated samples. The difference in Ca content of all ash treated samples was insignificant. Ca in V2PCR was insignificantly different from V2PPR and V2RBR (Table 20).

4.7.3 Mineral analysis of Tropimech Variety of Tomato Fruits

The amount of P within the pot in pot refrigerator showed no significant difference. Similar observation was noticed in plastic crate except V3PCR. The values of K showed no significant difference within each storage structure except in pot in pot refrigerator where V3PPC stood out (Table 21). For the Mg, little significant difference was observed among all treated samples. As observed in other varieties, the values of Cu were lesser than all other minerals analyzed. No significant difference was observed in the amounts of Fe, Zn and Ca in all samples irrespective of the treatments and storage structure except the value of Zn in V3RBR and Ca in V3PCR (Table 21).

Table 21: Effects of storage methods on the mineral composition of tomato fruits(Tropimech Variety)

	P	K	Mg	Cu	Fe	Zn	Ca
V3PCA	10.73±0.40a	5.47±0.23a	4.16±0.44a	0.18±0.00a	5.03±0.42a	8.78±0.09a	7.91±0.42a
V3PCR	9.89±0.10b	5.19±0.08ab	3.40±0.14ab	0.17±0.01ab	4.46±0.20a	8.61±0.05ab	7.15±0.11b
V3PCS	9.98±0.32ab	5.19±0.03ab	3.62±0.19ab	0.16±0.01abc	4.47±0.15a	8.60±0.05ab	7.46±0.20ab
V3PCC	10.33±0.04ab	5.48±0.22a	3.75±0.10ab	0.14±0.02bcd	4.84±0.07a	8.87±0.12a	7.66±0.07ab
V3PPA	10.35±0.19ab	5.48±0.48a	3.59±0.31ab	0.13±0.01cd	4.70±0.04a	8.78±0.09a	7.94±0.30a
V3PPR	10.45±0.32ab	5.45±0.21a	3.46±0.26ab	0.12±0.02d	4.54±0.05a	8.76±0.11ab	7.44±0.12ab
V3PPS	10.54±0.32ab	5.41±0.18a	3.08±0.28b	0.15±0.01abcd	4.46±0.06a	8.72±0.29ab	7.43±0.07ab
V3PPC	10.36±0.27ab	4.48±0.10b	3.41±0.32ab	0.12±0.01d	4.63±0.04a	8.730.12ab	7.48±0.10ab
V3RBA	10.70±0.16a	5.55±0.23a	3.25±0.10b	0.15±0.00abcd	5.03±0.44a	8.34±0.10ab	7.66±0.20ab
V3RBR	9.83±0.07b	5.08±0.17ab	3.22±0.06b	0.14±0.02bcd	4.65±0.53a	8.24±0.22b	7.39±0.15ab
V3RBS	9.77±0.07b	5.21±0.23ab	3.24±0.13b	0.14±0.01bcd	4.77±0.53a	8.41±0.30ab	7.44±0.14ab
V3RBC	10.68±0.24a	5.48±0.25a	3.19±0.08b	0.12±0.00d	4.89±0.37a	8.37±0.06ab	7.68±0.16ab

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V3PCA = Tropimech Variety + Plastic crate + Ash

V3PCR = Tropimech Variety + Plastic crate + Rice straw

V3PCS = Tropimech Variety + Plastic crate + Sawdust

V3PPC = Tropimech Variety + Plastic crate + Control

V3PPA = Tropimech Variety + Pot in Pot + Ash

V3PPR = Tropimech Variety + Pot in Pot + Rice straw

V3PPS = Tropimech Variety + Pot in Pot + Sawdust

V3PPC = Tropimech Variety + Pot in Pot + Control

V3RBA = Tropimech Variety + Raffia basket + Ash

V3RBR = Tropimech Variety + Raffia basket + Rice straw

V3RBS = Tropimech Variety + Raffia basket + Sawdust

V3RBC = Tropimech Variety + Raffia basket + Control

4.7.4 Mineral analysis of Roma VF Variety of Tomato Fruits

The quantity of K in V4PCA was insignificantly higher than V4PPA and V4RBA. The effects of rice straw and sawdust on K content were insignificant except in V4PPR and V4PPS respectively. The botanicals and storage structures had no significant effects on the levels of K in the stored fruits. For the Mg, the mineral content varied considerably but no significant difference was recorded within the storage structure (Table 22).

The highest amount of Cu was observed in V4PCS, although it showed no significant difference within the plastic crate. Also, the values of Fe in all the samples in plastic crate and pot in pot refrigerator were insignificantly different except V4PPC (Table 22). The highest concentration of Zn was observed in V4PCA and V4PCC and the values of Ca ranged from 6.58mg/L (V4PPS) to 4.52mg/L (V4RBR) (Table 22).

4.7.5 Influence of Varieties, Storage and Botanicals on the Mineral Composition of Tomato Fruits

Significant differences were observed in the mineral compositions of the stored tomato fruits of all the varieties used. The P, Mg and Ca contents of the improved varieties (Tropimech and Roma VF) were significantly higher than those of local varieties (Hausa and Yoruba). Roma VF had the highest K and significantly followed by Hausa variety. The Cu content of Yoruba variety was highest and significantly different from all other varieties (Table 23).

For the storage, plastic crate supported highest significant contents of P, K, Mg, Fe, Zn and Ca in stored tomato. The tomato fruits stored in pot in pot refrigerator had the lowest value of K, Cu and Zn (Table 23). The mineral compositions of stored tomato as influenced by botanicals were significantly different from one another. Ash promoted higher values of P, Mg, Fe and Ca (Table 23).

The interaction between the fixed factors (V x S, V x T, S x T and V x S x T) had p value of < 0.05, showing that they are significant (Table 23).

Table 22: Effects of storage methods on the mineral composition of tomato fruits(Roma VF Variety)

	P	K	Mg	Cu	Fe	Zn	Ca
	mg/L						
V4PCA	16.11±0.23a	7.77±0.29a	3.36±0.05a	0.21±0.01abcd	5.40±0.14a	8.40±0.11a	6.39±0.14ab
V4PCR	14.53±0.15cd	7.41±0.12a	3.05±0.24ab	0.21±0.02abcd	5.52±0.18a	7.67±0.17b	5.96±0.09bc
V4PCS	14.36±0.14d	7.18±0.10a	3.26±0.16a	0.25±0.01a	5.52±0.19a	7.64±0.08b	5.63±0.11c
V4PCC	15.74±0.24ab	7.69±0.41a	3.20±0.09a	0.24±0.01ab	5.18±0.05abc	8.40±0.10a	6.37±0.03ab
V4PPA	15.98±0.34a	7.80±0.34a	3.33±0.00a	0.23±0.03abc	5.60±0.36a	8.35±0.19a	6.40±0.20ab
V4PPR	15.68±0.43ab	7.18±0.12a	3.10±0.15ab	0.20±0.01cd	5.54±0.27a	8.32±0.84a	6.57±0.16a
V4PPS	15.60±0.21ab	7.14±0.33a	3.12±0.07ab	0.18±0.00de	5.43±0.28a	8.33±0.78a	6.58±0.15a
V4PPC	15.72±0.75ab	7.27±0.16a	2.77±0.34ab	0.15±0.02e	4.66±0.19c	6.91±0.22c	5.71±0.16c
V4RBA	15.45±0.19abc	7.44±0.49a	3.05±0.08ab	0.20±0.01bcd	4.91±0.14abc	7.00±0.26c	6.00±0.23bc
V4RBR	14.46±0.11d	7.26±0.64a	2.40±0.37b	0.17±0.00de	4.52±0.22c	6.33±0.21d	4.52±0.23d
V4RBS	14.48±0.07d	7.19±0.06a	2.46±0.40b	0.19±0.01cd	4.55±0.07c	6.34±0.17d	4.95±0.16d
V4RBC	14.95±0.16bcd	7.47±0.27a	3.07±0.14ab	0.22±0.01abc	4.73±0.28bc	6.72±0.16cd	5.83±0.09c

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V4PCA = Roma VF + Plastic crate + Ash

V4PCR = Roma VF + Plastic crate + Rice Straw

V4PCS = Roma VF + Plastic crate + Sawdust

V4PCC = Roma VF + Plastic crate + Control

V4PPA = Roma VF + Pot in Pot + Ash

V4PPR = Roma VF + Pot in Pot + Rice Straw

V4PPS = Roma VF + Pot in Pot + Sawdust

V4PPC = Roma VF + Pot in Pot + Control

V4RBA = Roma VF + Raffia Basket + Ash

V4RBR = Roma VF + Raffia Basket + Rice Straw

V4RBS = Roma VF + Raffia Basket + Sawdust

V4RBC = Tropimech Variety + Raffia Basket + Control

Table 23: Mineral composition of stored tomato as influenced by variety, storage structures and botanicals

Factor	Level of Factors	P	K	Mg	Cu	Fe	Zn	Ca
mg/L								
Variety (V)	Hausa	6.97c	5.70b	2.62c	0.21b	4.39c	6.18c	4.53c
	Yoruba	5.29d	4.41d	1.99d	0.27a	3.54d	4.80d	3.76d
	Tropimech	10.30b	5.29c	3.45a	0.14c	4.71b	8.60a	7.55b
	Roma VF	15.26a	7.40a	3.01b	0.20b	5.13a	7.54b	5.91a
SE		0.09	0.07	0.06	0.01	0.08	0.06	0.06
Storage (S)	Plastic crate	9.57a	6.01a	3.12a	0.21b	4.72a	7.16a	5.58a
	Pot in Pot	9.46ab	5.30c	2.81b	0.16c	4.40b	6.42c	5.38b
	Raffia basket	9.32b	5.78b	2.37c	0.26a	4.20c	6.75b	5.36b
SE		0.07	0.64	0.05	0.01	0.07	0.05	0.05
Treatment (T)	Ash	9.78a	5.80a	3.11a	0.19b	4.85a	6.80b	5.88a
	Rice straw	9.13c	5.85a	2.67b	0.19b	4.55b	6.63c	5.10c
	Sawdust	9.34bc	5.46b	2.66b	0.19b	3.96c	7.05a	5.17c
	Control	9.56ab	5.68a	2.62b	0.26a	4.40b	6.63c	5.61b
SE		0.09	0.07	0.06	0.01	0.08	0.06	0.06
$V \times S$		*	*	*	*	*	*	*
$V \times T$		*	*	*	*	*	*	*
$S \times T$		*	*	*	*	*	*	*
$V \times S \times T$								

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

* = Significant difference

4.8 Fungal Loads in Stored Tomato Fruits

In V1, the fungal load was 5.10cfu/g in V1PCA and showed an insignificant difference from those of V1PPA (5.17×10^3 cfu/g) and V1RBA (5.02×10^3 cfu/g). It was observed that the fungal load in V1PCR (4.27×10^3 cfu/g) was significantly different from the fungal population of V1PPR (4.27×10^3 cfu/g) and V1RBR (4.53×10^3 cfu/g) but the former and the latter were not significantly different from each other. It was also noticed in this variety that the fungal loads in sawdust-treated fruits was very low compared to other treatments. No significant difference was observed in V1PCS (3.07×10^3 cfu/g) and V1PPS (2.97×10^3 cfu/g). The highest microbial loads were observed in respective controls regardless of the storage structures (Table 24).

The highest fungal population was observed in V2PCC and was significantly different from that of V2RBC (6.08×10^3 cfu/g). Similarly, the lowest population was recorded in V2PPS (3.27×10^3 cfu/g) although it showed no significant difference from V2PCS (3.53cfu/g) and V2RBS (3.40×10^3 cfu/g). V2PCA and V2RBA had fungal populations of 5.0×10^3 cfu/g and 5.37×10^3 cfu/g respectively with no significant difference but both were significantly different from 4.27cfu/g of V2PPA. The fungal load of V2PPR (4.27×10^3 cfu/g) was more than 3.80×10^3 cfu/g of V2PCR and 3.80×10^3 cfu/g of V2RBR (Table 24).

The fungal population in V3PCA was 3.33×10^3 cfu/g, insignificantly higher than that of PPA (3.07×10^3 cfu/g) but was significantly different from V3RBA (4.06×10^3 cfu/g). V3PCR had fungal load of 2.63×10^3 cfu/g which was significantly different from 1.57×10^3 cfu/g in V3PPR but there was an insignificant difference between V3PPR and V3RBR (2.93×10^3 cfu/g). the lowest fungal population in V3 was observed in V3PPS (0.97×10^3 cfu/g), followed by the value

Table24: Fungal loads of stored tomato fruits as influenced by botanical treatments

Fungal population $\times 10^3$ (cfu/g)				
	V1	V2	V3	V4
PCA	5.10 \pm 0.26cd	5.03 \pm 0.09c	3.33 \pm 0.19c	3.06 \pm 0.26c
PCR	4.30 \pm 0.12d	3.80 \pm 0.00e	2.63 \pm 0.18d	1.82 \pm 0.42d
PCS	3.07 \pm 0.88f	3.53 \pm 0.07ef	1.07 \pm 0.09e	0.97 \pm 0.12e
PCC	7.07 \pm 0.88a	7.00 \pm 0.12a	5.07 \pm 0.45a	3.73 \pm 0.12b
PPA	5.17 \pm 0.15cd	4.27 \pm 0.07d	3.07 \pm 0.03cd	1.67 \pm 0.26e
PPR	4.27 \pm 0.45e	4.27 \pm 0.07d	1.57 \pm 0.24e	0.77 \pm 0.03d
PPS	2.97 \pm 0.12f	3.27 \pm 0.20f	0.97 \pm 0.07e	0.53 \pm 0.15e
PPC	5.47 \pm 0.33c	5.13 \pm 0.15c	4.17 \pm 0.18b	3.17 \pm 0.15bc
RBA	5.20 \pm 0.21cd	5.37 \pm 0.09c	4.00 \pm 0.06b	3.27 \pm 0.15bc
RBR	4.53 \pm 0.13de	3.80 \pm 0.26e	2.93 \pm 0.09cd	2.87 \pm 0.20c
RBS	4.10 \pm 0.34e	3.40 \pm 0.25ef	1.60 \pm 0.25e	1.63 \pm 0.17d
RBC	6.17 \pm 0.15b	6.00 \pm 0.06b	5.53 \pm 0.32a	4.87 \pm 0.24a

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF

PCA = Plastic Crate + Ash

PCR = Plastic Crate + Rice Straw

PCS = Plastic Crate + Sawdust

PCC = Plastic Crate + Control

PPA = Pot in Pot + Ash

PPR = Pot in Pot + Rice Straw

PPS = Pot in Pot + Sawdust

PPC = Pot in Pot + Control

RBA = Raffia Basket + Ash

RBR = Raffia Basket + Rice Straw

RBS = Raffia Basket + Sawdust

RBC = Raffia Basket + Control

recorded in V3PCS (1.07×10^3 cfu/g) with no significant difference. V3RBS had the fungal population of 1.60×10^3 cfu/g. The highest fungal population in V3 was 5.53cfu/g which was observed in V3RBC. The value recorded in the latter was insignificantly higher than 5.07cfu/g obtained in V3PCC (Table 24).

V4PCA had the fungal load of 3.06cfu/g which was significantly higher than that of V4PPA (1.67cfu/g) but lower than the value recorded in V4RBA (3.27cfu/g). Similarly, in V4RBR, the fungal population was 2.87cfu/g and significantly higher than both V4PCR (1.82cfu/g) and V4PPR (0.77cfu/g). Both the former and the latter had no significant difference. The lowest fungal population across all the varieties used was observed in V4PPS (0.53cfu/g). the value showed no significant difference from 0.97cfu/g of V4PCS but significantly different from 1.63cfu/g of V4RBS. However, the highest fungal load was recorded in V4RBC (4.87cfu/g). The one observed in V4PCC (3.73cfu/g) was not significantly different from 3.17cfu/g of V4PPC (Table 24).

4.8.1 Influence of Varieties, Storage and Botanicals on the Fungal Load of Stored Tomato Fruits

Hausa variety had highest fungal load and significantly different from those obtained from other varieties (Table 25). Raffia basket encouraged fungal population and the fungal load value in this storage structure was significantly higher than the other two structures (plastic crate and pot in pot refrigerator) (Table 25). The botanicals had significant effects on the fungal load of stored tomato as the control had highest value of fungal load. Significant differences were observed in all the interactions between the fixed factors (Table 25).

Table 25: Fungal load of tomato fruits as influenced by variety, storage and botanicals

Factor	Level of Factors	Fungal load $\times 10^3$ (cfu/g)
Variety (V)	Hausa	4.78a
	Yoruba	4.53b
	Tropimech	2.99c
	Roma VF	2.36d
SE		0.06
Storage (S)	Plastic crate	3.79b
	Pot in Pot	3.14c
	Raffia basket	4.08a
SE		0.05
Treatment (T)	Ash	4.04b
	Rice straw	3.08c
	Sawdust	2.27d
	Control	5.28a
SE		0.06
$V \times S$		*
$V \times T$		*
$S \times T$		*
$V \times S \times T$		*

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

*= Significant difference

4.9 Isolation and identification of Isolates

Five fungi were isolated in total and they were identified as *Aspergillus japonicus*, *Rhizopus oryzae*, *Curvularia geniculata*, *Fusarium proliferatum* and *Fusarium oxysporum*. These fungi were isolated from all the varieties used. The morphological and molecular descriptions of each isolate are given below.

4.9.1 Morphological Description of the Fungal Isolates

Isolate 1 (Aspergillus japonicus)

The colonies grown on PDA were white first, then turned black with the formation of visible and distinct conidiophores and conidia (Plate 1). The hyaline septate hyphae were conspicuous. The conidiophores were long and globose at a terminal end (Plate 2).

Isolate 2 (Rhizopus oryzae)

The colonies of the isolates grown on PDA were cottony white at first, then turned brownish-grey to blackish-grey and grew aggressively (Plate 3). Both sporangia and sporangiophores were clearly visible under the light microscope. The columella were globose. The sporangiospores were also visible but dispersed (Plate 4).

Isolate 3 (Curvularia geniculata)

The colony was black-brown velvety with black reverse (Plate 5). The conidia were bent at a sharp angle (geniculate) and assumed a boat shape. Conidia had three septations. Central cells were larger and darker than the end cells. They were straw coloured to dark brown. Conidiogenous cells were polytretic and sympodial (Plate 6).



a



b

Plate 1: The pure culture of *Aspergillus japonicus* in PDA. a) Front view b) Reverse view

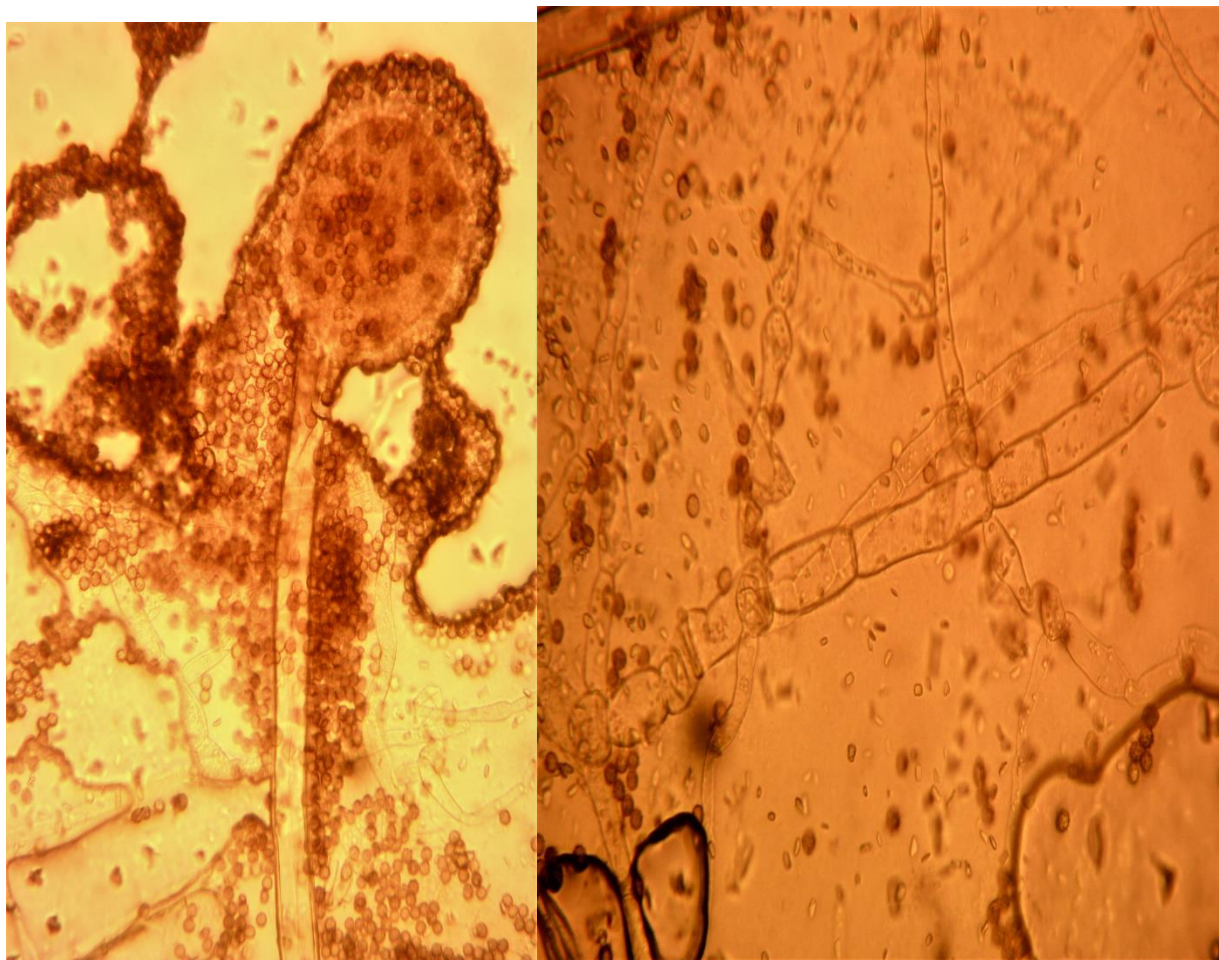
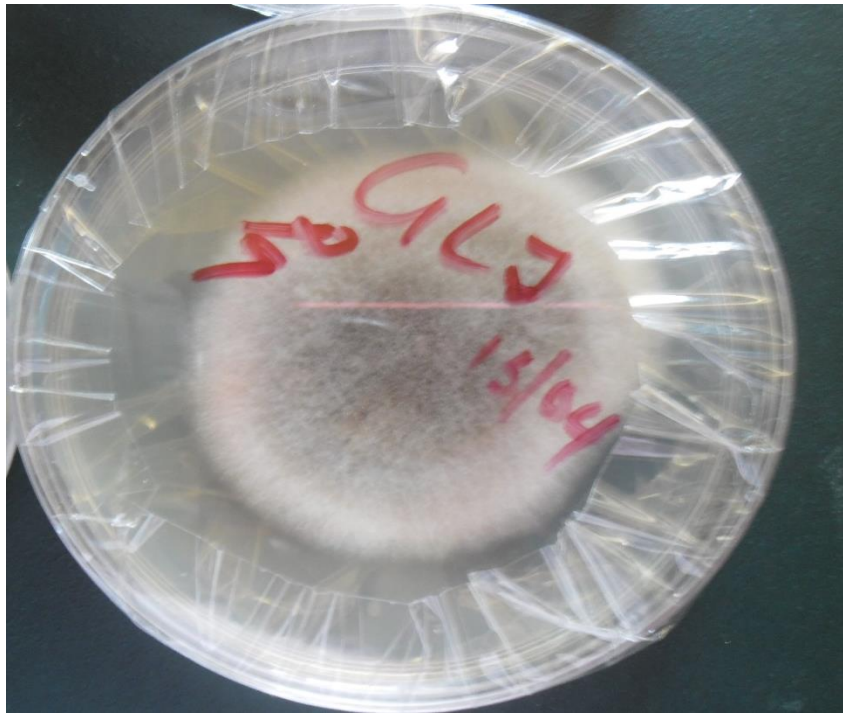


Plate 2: Microscopic view of *Aspergillus japonicus*



a



b

Plate3: Pure culture of *Rhizopus oryzae* raised in PDA. a) Front view b) Reverse view

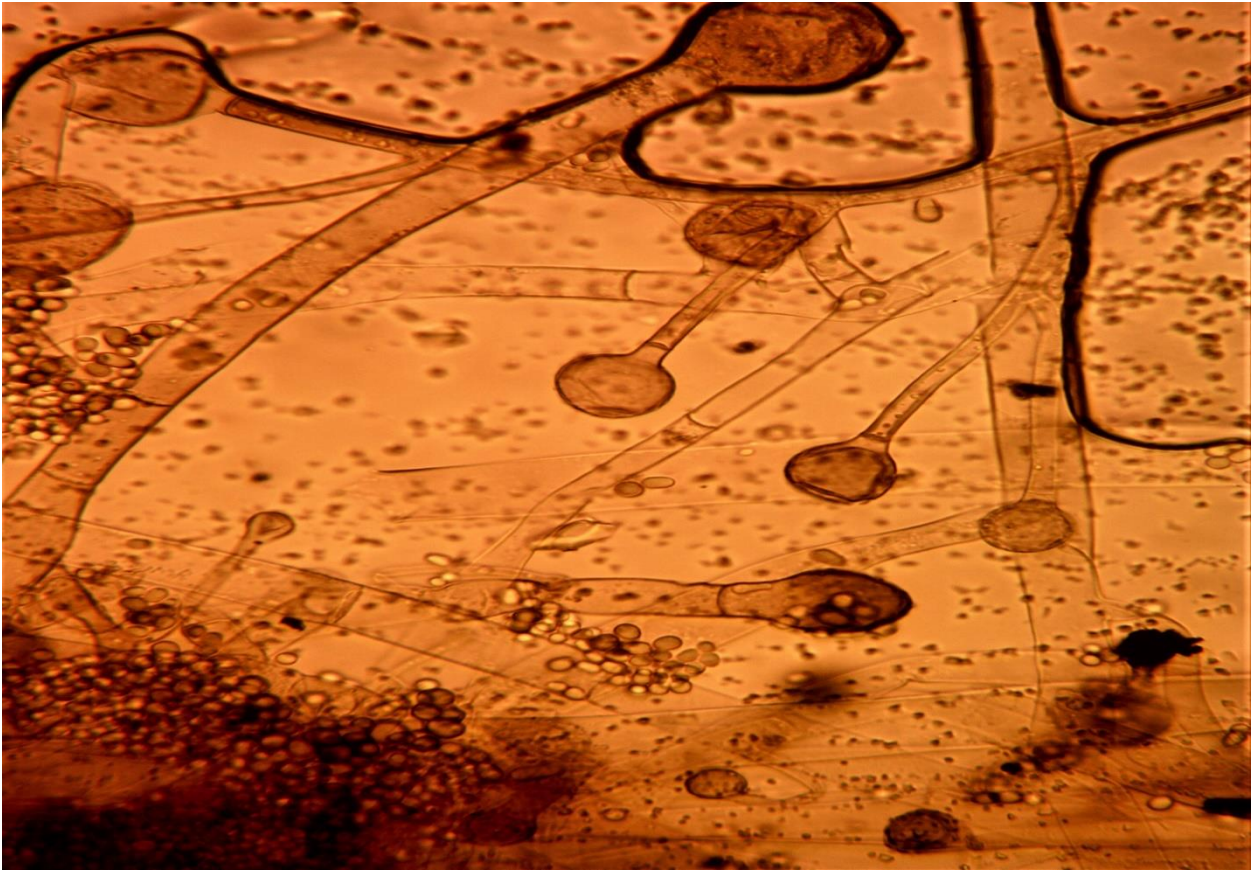
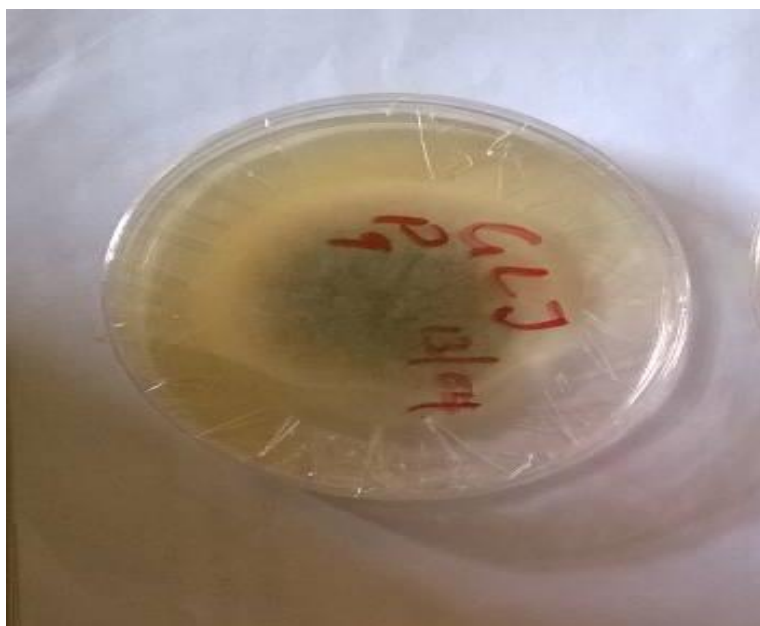


Plate4: Microscopic view of *Rhizopus oryzae*



a



b

Plate5: Pure culture of *Curvularia geniculata* in PDA. a) Front view b) Reverse view



Plate 6: Microscopic view of *Curvularia geniculata*

Isolate 4 (*Fusarium proliferatum*)

The mycelium was extensive and cotton-like in culture with tinge of pink on PDA culture medium (Plate 7). The conidiophores were short, slender and branched irregularly. Conidia (phialospores) were hyaline and variable. Macroconidia were several-celled, bent at the pointed ends and assume canoe-shaped. Microconidia were single-celled, oblong and borne singly (Plate 8).

Isolate 5 (*Fusarium oxysporum*)

Sporodochium was present bearing conidiophores on its surface. The aerial mycelium appeared white and loosely floccose (Plate 9). The conidiophores are branched. The conidia are septate and gradually tapered toward both ends (Plate 10).

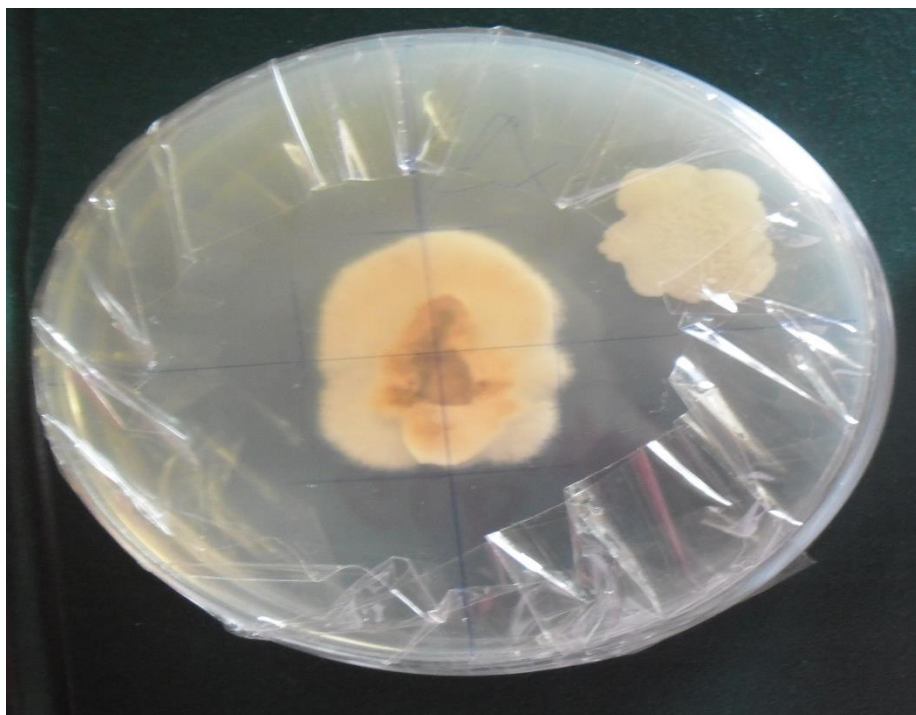
4.9.2 Molecular Identification of fungal Isolates

The consensus sequence of each of fungal isolates (Figure 29-33) was compared to those obtained from Gene Bank of NCBI. It was observed that the isolate 1 was *Aspergillus japonicus* with a homology of 100% (Figure 34). The phylogenetic tree of isolate 1 showed a complete alignment with species of *Aspergillus japonicus* and completely separated from *A. fumigatus*, *A. oryzae* and *A. flavus* (Figure 35). This confirmed that the isolate 1 was *Aspergillus japonicus*.

Basic Alignment Search Tool (BLAST) revealed that isolate 2 was *Rhizopus oryzae* with 99% identity (Figure 36). This isolate clustered with other *Rhizopus oryzae* (synonym of *R. delemar*) species generated from NCBI (Figure 37). The fungus distinguished itself from *R. stolonifer* and *R. microsporus*.



a



b

Plate 7: Pure culture of *Fusarium proliferatum* in PDA. a) Front view b) Reverse view

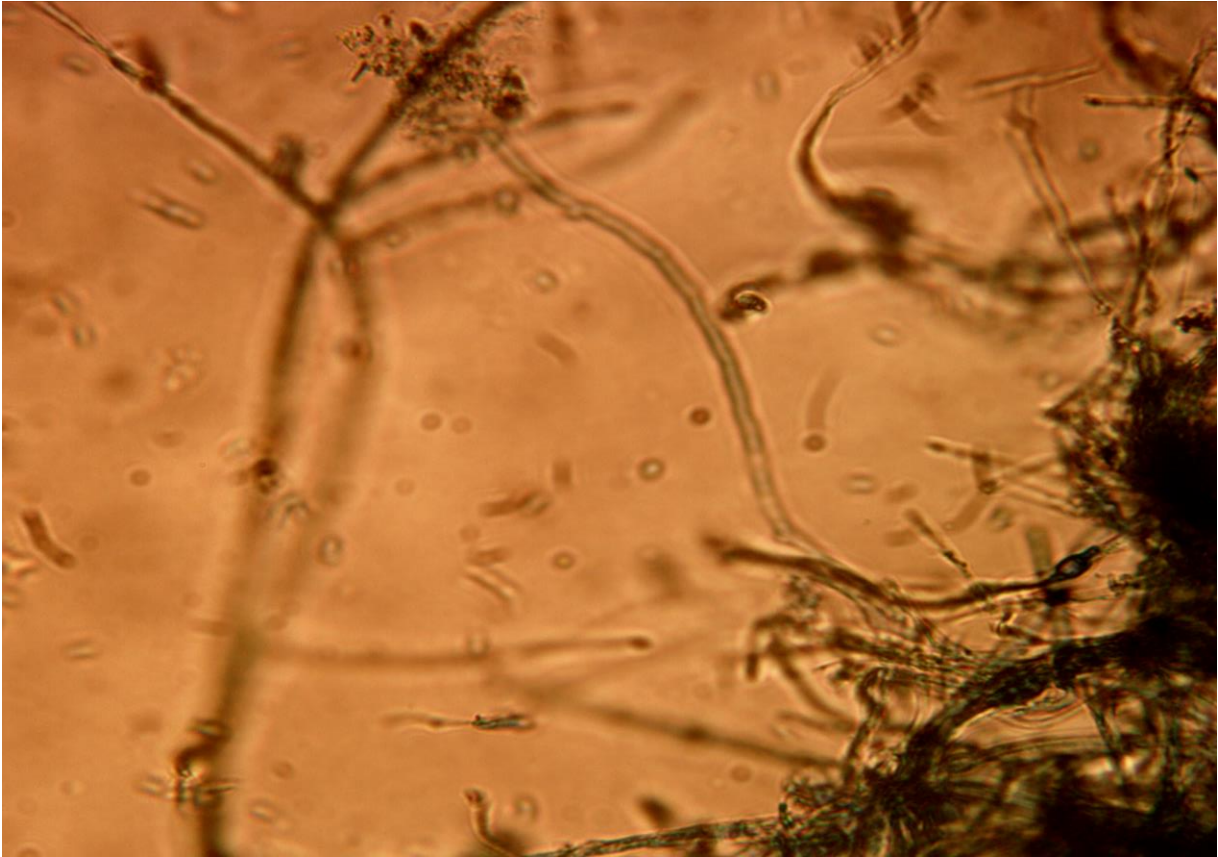
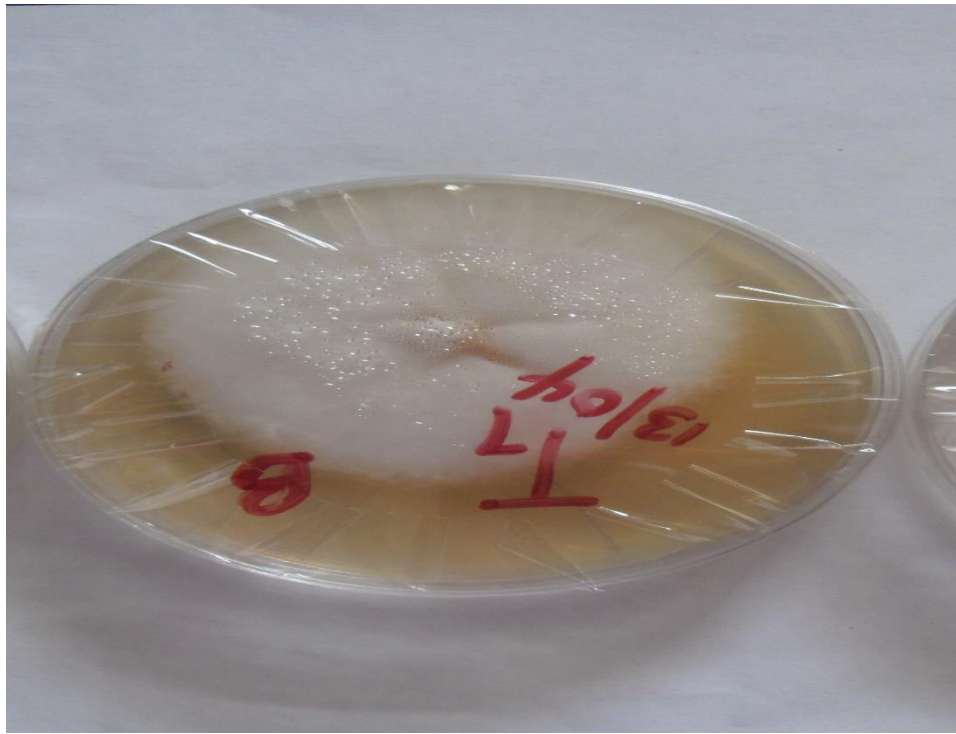
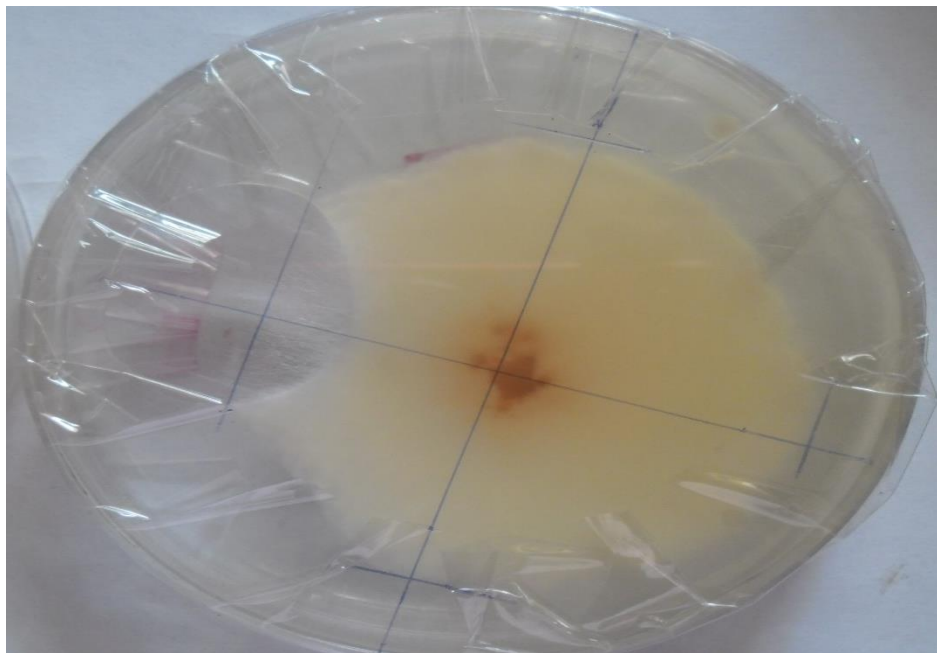


Plate 8: Microscopic view of *Fusarium proliferatum*



a



b

Plate 9: Pure culture of *Fusarium oxysporum* in PDA. a) Front view b) Reverse view

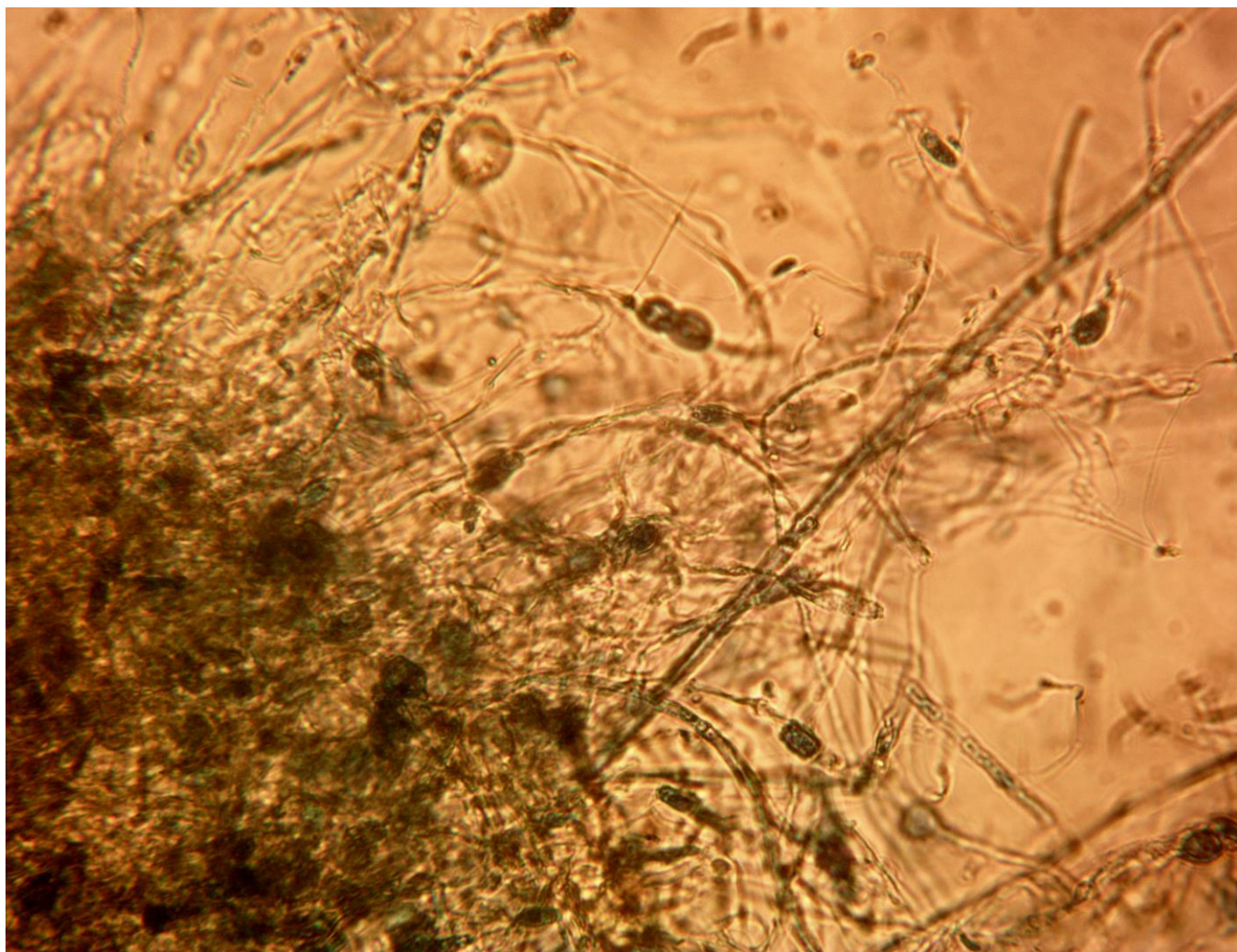


Plate 10: Microscopic view of *Fusarium oxysporum*

TAGGTGAACCTGCGGAAGGATCATTACCGAGTGCTGGGTCCTTCGGGGCCCAACCTC
CCACCCGTGCTTACCGTACCCTGTTGCTTCGGCGGGCCCGCCTTCGGGCGGCCCCGGG
GCCTGCCCCCGGGACCGCGCCCGCCGGAGACCCCAATGGAACACTGTCTGAAAGCG
TGCAGTCTGAGTTGATTGATACCAATCAGTTAAACTTTCAACAATGGATCTCTTGG
TTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCA
TGCCTGTCCGAGCGTCATTTCTCCCCTCCAGCCCCGCTGGTTGTTGGGCCGCGCCCCC
CCGGGGGCGGGCCTCGAGAGAAACGGCGGCACCGTCCGGTCCTCGAGCGTATGGGG
CTCTGTCACCCGCTCTATGGGCCCCGGCCGGGGCTTGCCTCGACCCCAATCTTCTCA
GATTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA

Figure 29: Consensus DNA sequence of *Aspergillus japonicus*

CGTAGGGACTGCGGAAGGCATTAATTATGTTAAAGCGCCTTACCTCTTAGGGTTTCC
TCTGGGGTAAGTGATTGCTTCTACACTGTGAAAATTTGGCTGAGAGACTCAGACTGG
TCATGGGTAGACCTATCTGGGGTTTGATCGATGCCACTCCTGGTTTCAGGAGCACCC
TTCATAATAAACCTAGAAATTCAGTATTATAAAGTTTAATAAAAAACAACCTTTAAC
AATGGATCTCTTGCTTCTCGCATCGATGAAGAACGTAGCAAAGTGCGATAACTAGTG
TGAATTGCATATTCAGTGAATCATCGAGTCTTTGAACGCAGCTTGCACTCTATGGTTT
TTCTATAGAGTACGCCTGCTTCAGTATCATCACAAACCCACACATAACATTTGTTTAT
GTGGTAATGGGTCGCATCGCTGTTTTATTACAGTGAGCACCTAAAATGTGTGTGATT
TTCTGTCTGGCTTGCTAGGCAGGAATATTACGCTGGTCTCAGGATCTTTTTCTTTGGT
TCGCCCAGGAAGTAAAGTACAAGAGTATAATCCAGCAACTTTCAAACCTATGATCTG
AAGTCAGGGGGTACCCGCTGAACTTAAGCATATCA

Figure 30: Consensus DNA sequence of *Rhizopus oryzae*

GTAGGGACCTGCGGAGGGATCATTACCAATAAACATATGAAGGCTGCACCGCCAAC
AGGCGGCAAGGCTGGAGTATTTTATTACCCTTGTCTTTTGCGCACTTGTTGTTTCCTG
GGCGGGTTCGCCCCGCCTCCAGGACCACATGATAAACCTTTTTTATGCAGTTGCAATC
AGCGTCAGTACAACAAATGTAAATCATTTACAACCTTTCAACAACGGATCTCTTGTT
CTGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATG
CCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTGTTGGGCGTTTTTTGTCTT
TGTTTTGTCCAAAGACTCGCCTTAAAACGATTGGCAGCCGGCCTACTGGTTTCGCA
GCGCAGCACATTTTTGCGCTTGCAATCAGCAAAAGAGGACGGCACTCCATCAAGAC
TATATCACTTTTGACCTCGGATCGGAGGGATACCCGCTGAACTTAAGCATATCT

Figure 31: Consensus DNA sequence of *Curvularia geniculata*

AACAGGTCTCCGTTGGGACAGCGGAGGGCATTACCGAGTTTCAACTCCCAAACCCCT
GTGAACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACG
GCCCCGCCAGAGGACCCCCAACTCTGTTTCTATATGTAACCTTCTGAGTAAAACCATA
AATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAAC
CCTCAAGCCCAGCTTGGTGTTGGGACTCGCGAGTCAAATCGCGTTCCCCAAATCGAT
TGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCG
TCGCGGCCACGCCGTTAAACCCCAACTTCTGAAGTGACC

Figure 32: Consensus DNA sequence of *Fusarium proliferatum*

GGATCACATCACACWCCCCTGWGCGCATACCTATMCSWWGCCTCGGAGGATCRAA
CCGCGMCCCCGKACAAGGGACGAACCGMCCGAAGACCCCTAAAMTCTTTTTTTAGTG
GAACTTTTGAGTAAAACAAACATATAAATCAGAACTTTCAACAAAGGATCTCTTGGA
ATCTGGCATCGATGAAAAACGCAAGWAATGGAACGAGTAATGYGAATTGCAGAAT
TCCGGGAATCATGSAAGCTTTGAACGCACWTTGMGCCCGCCMGTATTCTGASGKGS
ATGACTGKACSAGAGKAATTTCAACCCTCAWGCTCAGCTTGGYGTTGCKACTCGSGA
KAACCCGTGKTCCCCAAATCTATTGTTGTTTACGKSGASSTTCGRTAGCGKAGAAMT
CATACWCCTCGAYACTGRWAATCTCSACGTCCACTCCGKAAAAACCACCCTTTTAA
ATGWWGACCTCGGAAAGSGRAGAATACCCGCTGAACTTAAGCATATCATAAGCGG
AGGAA

Figure 33: Consensus DNA sequence of *Fusarium oxysporum*

Score	Expect	Identities	Gaps	Strand	
1038 bits(562)	0.0()	562/562(100%)	0/562(0%)	Plus/Plus	
Features:					
Query	1	TAGGTGAACCTGCGGAAGGATCATTACCGAGTGCTGGGTCCTTCGGGGCCCAACCTCCCA			60
Sbjct	216	TAGGTGAACCTGCGGAAGGATCATTACCGAGTGCTGGGTCCTTCGGGGCCCAACCTCCCA			275
Query	61	CCCGTGCTTACCGTACCCTGTTGCTTCGGCGGGCCCGCCTTCGGGCGGGCCGGGGCCTGC			120
Sbjct	276	CCCGTGCTTACCGTACCCTGTTGCTTCGGCGGGCCCGCCTTCGGGCGGGCCGGGGCCTGC			335
Query	121	CCCCGGGACCGCGCCCGCCGGAGACCCCAATGGAACACTGTCTGAAAGCGTGCAGTCTGA			180
Sbjct	336	CCCCGGGACCGCGCCCGCCGGAGACCCCAATGGAACACTGTCTGAAAGCGTGCAGTCTGA			395
Query	181	GTTGATTGATACCAATCAGTTAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGAT			240
Sbjct	396	GTTGATTGATACCAATCAGTTAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGAT			455
Query	241	GAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGT			300
Sbjct	456	GAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGT			515
Query	301	CTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTT			360
Sbjct	516	CTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTT			575
Query	361	CTCCCCCTCCAGCCCCGCTGGTTGTTGGGCCGCGccccccGGGGGCGGGCCTCGAGAGAA			420
Sbjct	576	CTCCCCCTCCAGCCCCGCTGGTTGTTGGGCCGCGCCCCCCCCGGGGGCGGGCCTCGAGAGAA			635
Query	421	ACGGCGGCACCGTCCGGTCTCTCGAGCGTATGGGGCTCTGTACCCGCTCTATGGGCCCGG			480
Sbjct	636	ACGGCGGCACCGTCCGGTCTCTCGAGCGTATGGGGCTCTGTACCCGCTCTATGGGCCCGG			695
Query	481	CCGGGGCTTGCCTCGACCCCCAATCTTCTCAGATTGACCTCGGATCAGGTAGGGATACCC			540
Sbjct	696	CCGGGGCTTGCCTCGACCCCCAATCTTCTCAGATTGACCTCGGATCAGGTAGGGATACCC			755
Query	541	GCTGAACTTAAGCATATCAATA	562		
Sbjct	756	GCTGAACTTAAGCATATCAATA	777		

Figure 34: BLASTN alignment between the query sequence and the subject sequence of *Aspergillus japonicus*

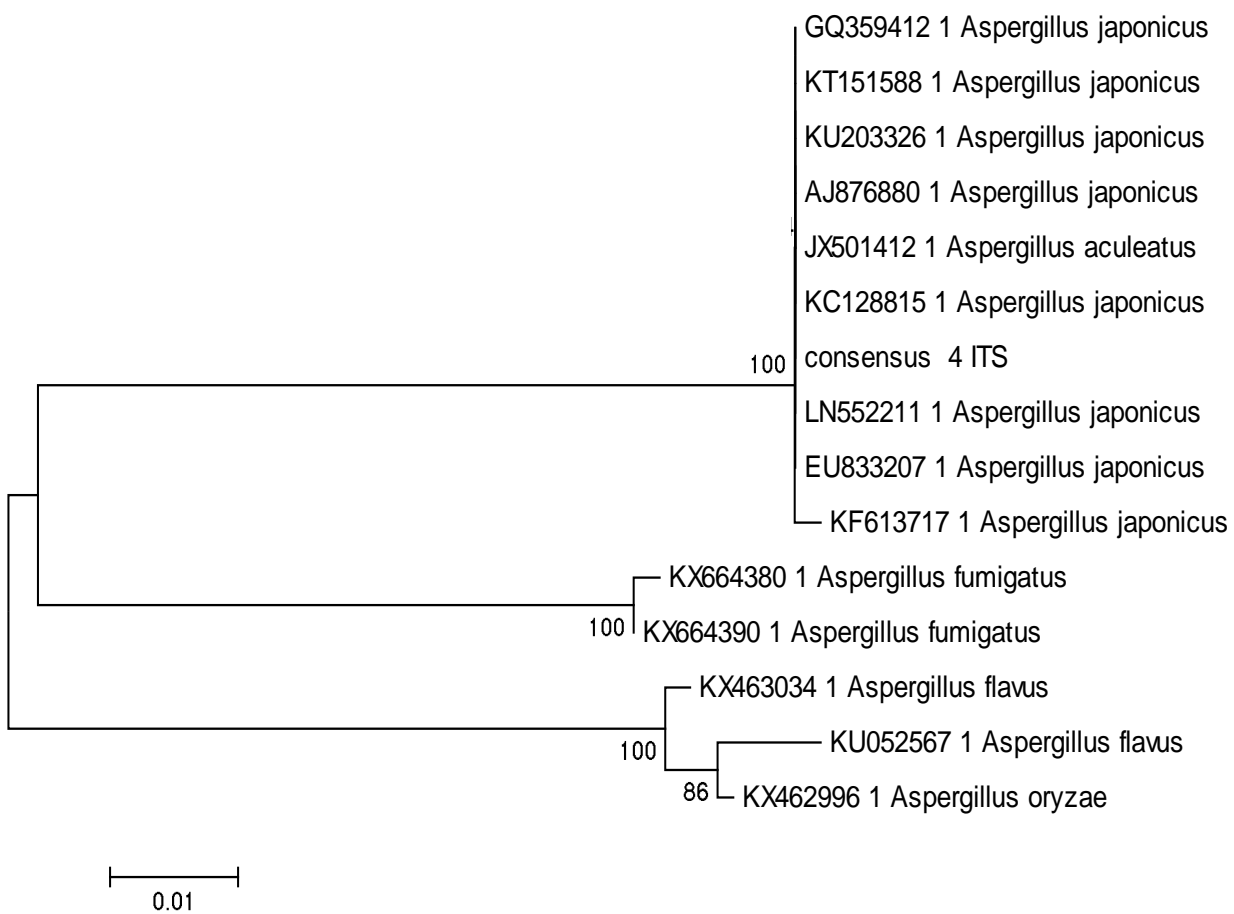


Figure 35. Molecular Phylogenetic analysis of *Aspergillus japonicus* by Maximum Likelihood method

Score	Expect	Identities	Gaps	Strand
1085 bits(587)	0.0()	607/615(99%)	8/615(1%)	Plus/Plus
Features:				
Query	1	CGTAGG-G-A-CTGCGGAAGG--CATTAATTATGTTAAAGCGCCTTACCTCTTAGGGTTT		55
Sbjct	3	CGTAGGTGAACCTGCGGAAGGATCATTAATTATGTTAAAGCGCCTTACCTCTTAGGGTTT		62
Query	56	CCTCTGGGGTAAGTGATTGCTTCTACACTGTGAAAATTTGGCTGAGAGACTCAGACTGGT		115
Sbjct	63	CCTCTGGGGTAAGTGATTGCTTCTACACTGTGAAAATTTGGCTGAGAGACTCAGACTGGT		122
Query	116	CATGGGTAGACCTATCTGGGGTTTGATCGATGCCACTCCTGGTTTCAGGAGCACCCTTCA		175
Sbjct	123	CATGGGTAGACCTATCTGGGGTTTGATCGATGCCACTCCTGGTTTCAGGAGCACCCTTCA		182
Query	176	TAATAAACCTAGAAATTCAGTATTATAAAGTTTAATAAAAAACAACCTTTAACAATGGAT		235
Sbjct	183	TAATAAACCTAGAAATTCAGTATTATAAAGTTTAATAAAAAACAACCTTTAACAATGGAT		242
Query	236	CTCTTGGTTCTCGCATCGATGAAGAACGTAGCAAAGTGCGATAACTAGTGTGAATTGCAT		295
Sbjct	243	CTCTTGGTTCTCGCATCGATGAAGAACGTAGCAAAGTGCGATAACTAGTGTGAATTGCAT		302
Query	296	ATTCAGTGAATCATCGAGTCTTTGAACGCAGCTTGCACTCTATGGTTTTTCTATAGAGTA		355
Sbjct	303	ATTCAGTGAATCATCGAGTCTTTGAACGCAGCTTGCACTCTATGGTTTTTCTATAGAGTA		362
Query	356	CGCCTGCTTCAGTATCATCACAAACCCACACATAACATTTGTTTATGTGGTAATGGGTCG		415
Sbjct	363	CGCCTGCTTCAGTATCATCACAAACCCACACATAACATTTGTTTATGTGGTAATGGGTCG		422
Query	416	CATCGCTGTTTTATTACAGTGAGCACCTAAAATGTGTGTGATTTTCTGTCTGGCTTGCTA		475
Sbjct	423	CATCGCTGTTTTATTACAGTGAGCACCTAAAATGTGTGTGATTTTCTGTCTGGCTTGCTA		482
Query	476	GGCAGGAATATTACGCTGGTCTCAGGATCTTTTCTTTGGTTCGCCCAGGAAGTAAAGTA		535
Sbjct	483	GGCAGGAATATTACGCTGGTCTCAGGATCTTTTCTTTGGTTCGCCCAGGAAGTAAAGTA		542
Query	536	CAAGAGTATAATCCAGCAACTTTCAAACATATGATCTGAAGTCAGG-GG--GTACCCGCTG		592
Sbjct	543	CAAGAGTATAATCCAGCAACTTTCAAACATATGATCTGAAGTCAGGTGGGAGTACCCGCTG		602
Query	593	AACTTAAGCATATCA 607		
Sbjct	603	AACTTAAGCATATCA 617		

Rhizopus oryzae

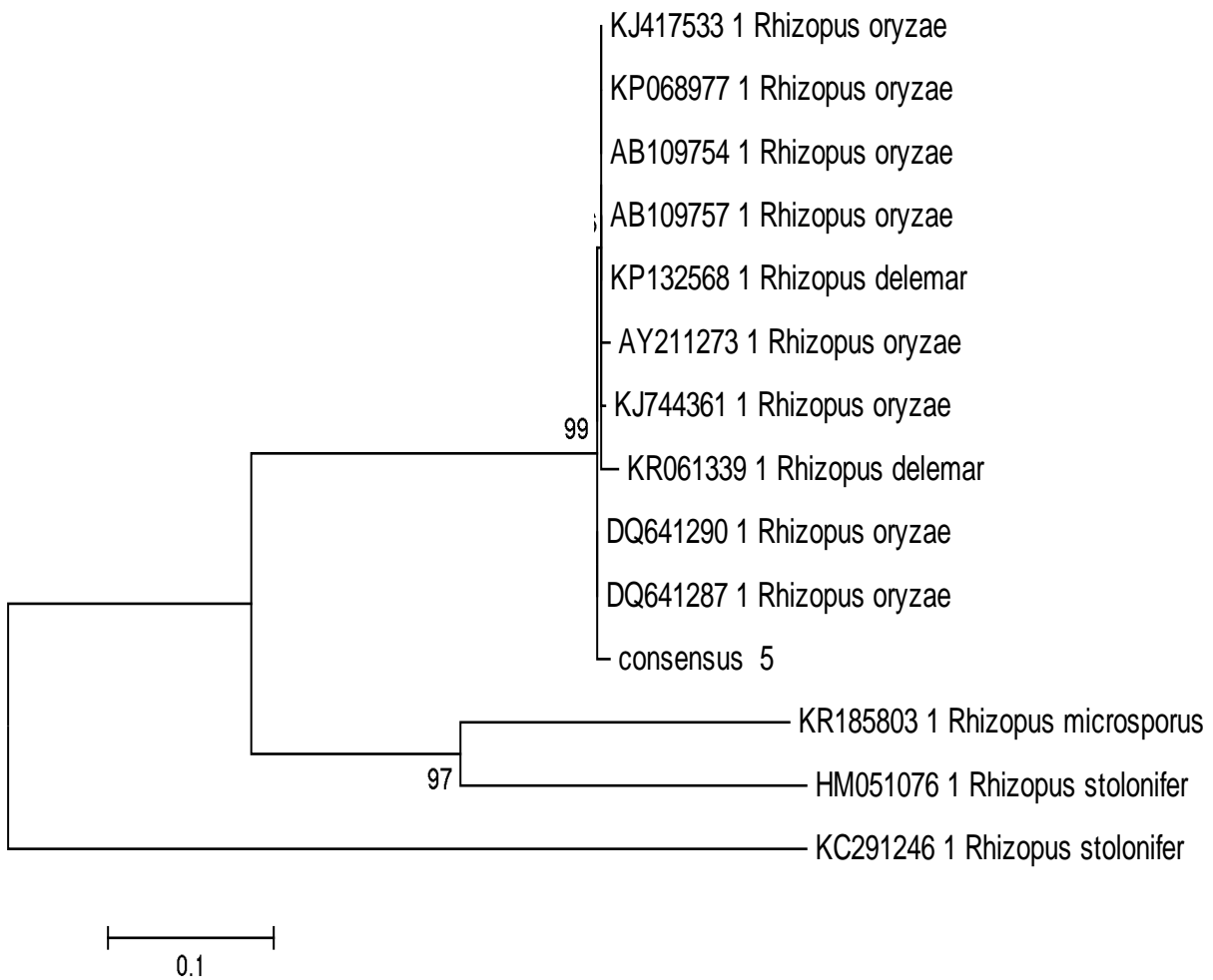


Figure 37: Molecular Phylogenetic analysis of *Rhizopus oryzae* by Maximum Likelihood method

Curvularia geniculata (isolate 3) had 98% identity with reference species from NCBI with 0% gap (Figure 38). Phylogeny tree confirmed the identity of the fungus (Figure 39). *Cochilobolus geniculatus* is the telemorph of *C. geniculata*.

Molecular analysis corroborated the morphological identity of isolate 4 to be *Fusarium proliferatum* with 98% identity and gap of 1% (Figures 40 and 41). The isolate showed a great affinity with *F. proliferatum* and clearly segregated from *F. sporotrichioides*, *F. solani* and *F. oxysporum*. The identity of isolate 5 was not confirmed by molecular characterization but showed that it belonged to *Fusarium* species with 79% homology (Figure 42). The isolate 5 was clustered with *F. chlamydosporium*, *F. oxysporum*, *F. equiseti* and *F. lateritum*, and this suggested that the isolate might be one of those aforementioned *Fusarium* species (Figure 43) but morphological identification considered the isolate to be *F. oxysporum*.

4.10 Pathogenicity Test of Fungal Isolates from Tomato Fruits

The pathogenicity test confirmed the assumptions that all the fungal isolates were pathogenic and responsible for the rot observed in all the varieties. The degree of virulence varied among the isolates. The two species of *Fusarium* displayed higher pathogenicity than other isolates. The diameter of lesion instigated by *Curvularia geniculata* was significantly lower than other isolates. However, V2 and V4 fruits were most susceptible and resistant to fungal pathogens respectively (Table 26). The symptomatic abnormalities displayed by inoculated fruits varied depending on the type of inoculum that was being inoculated. The symptom caused by each fungal isolate was summarized in Table 27.

Score	Expect	Identities	Gaps	Strand	
1018 bits(551)	0.0()	566/575(98%)	4/575(0%)	Plus/Plus	
Features:					
Query	1	GTAGGNGANCCTGCGGAGGGATCATTACNCAATAAACATNATGAAGGCTGCACCGCCAAC			60
Sbjct	3	GTAGGTGAACCTGCGGAGGGATCATTACACAATAAACAT-ATGAAGGCTGCACCGCCAAC			61
Query	61	AGGCGGCAAGGCTGGAGTATTTTATTACCCTTGTCTTTTGCGCACCTGTTGTTTCCTGGG			120
Sbjct	62	AGGCGGCAAGGCTGGAGTATTTTATTACCCTTGTCTTTTGCGCACCTGTTGTTTCCTGGG			121
Query	121	CGGGTTCGCCCCGCTCCAGGACCACATGATAAACCTTTTTTATGCAGTTGCAATCAGCGT			180
Sbjct	122	CGGGTTCGCCCCGCTCCAGGACCACATGATAAACCTTTTTTATGCAGTTGCAATCAGCGT			181
Query	181	CAGTACAACAAATGTAAATCATTTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATC			240
Sbjct	182	CAGTACAACAAATGTAAATCATTTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATC			241
Query	241	GATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCG			300
Sbjct	242	GATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCG			301
Query	301	AATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTCGAGCGTCA			360
Sbjct	302	AATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTCGAGCGTCA			361
Query	361	TTTGTACCCTCAAGCTTTGCTTGGTGTGGGCGTTTTTTGTCTTTGGTTTTGTCCAAAGA			420
Sbjct	362	TTTGTACCCTCAAGCTTTGCTTGGTGTGGGCGTTTTTTGTCTTTGGTTTTGTCCAAAGA			421
Query	421	CTCGCCTTAAACGATTGGCAGCCGGCCTACTGGTTTCGCAGCGCAGCACATTTTTCGCGC			480
Sbjct	422	CTCGCCTTAAACGATTGGCAGCCGGCCTACTGGTTTCGCAGCGCAGCACATTTTTCGCGC			481
Query	481	TTGCAATCAGCAAAAGAGGACGGCACTCCATCAAGA--CTATATCACTTTTGACCTCGGA			538
Sbjct	482	TTGCAATCAGCAAAAGAGGACGGCACTCCATCAAGACTCTATATCACTTTTGACCTCGGA			541
Query	539	TNCNGGNAGGGATACCCGCTGAACTTAAGCATATC		573	
Sbjct	542	T-CAGGTAGGGATACCCGCTGAACTTAAGCATATC		575	

Figure 38: BLASTN alignment between the query sequence and the subject sequence of *Curvularia geniculata*

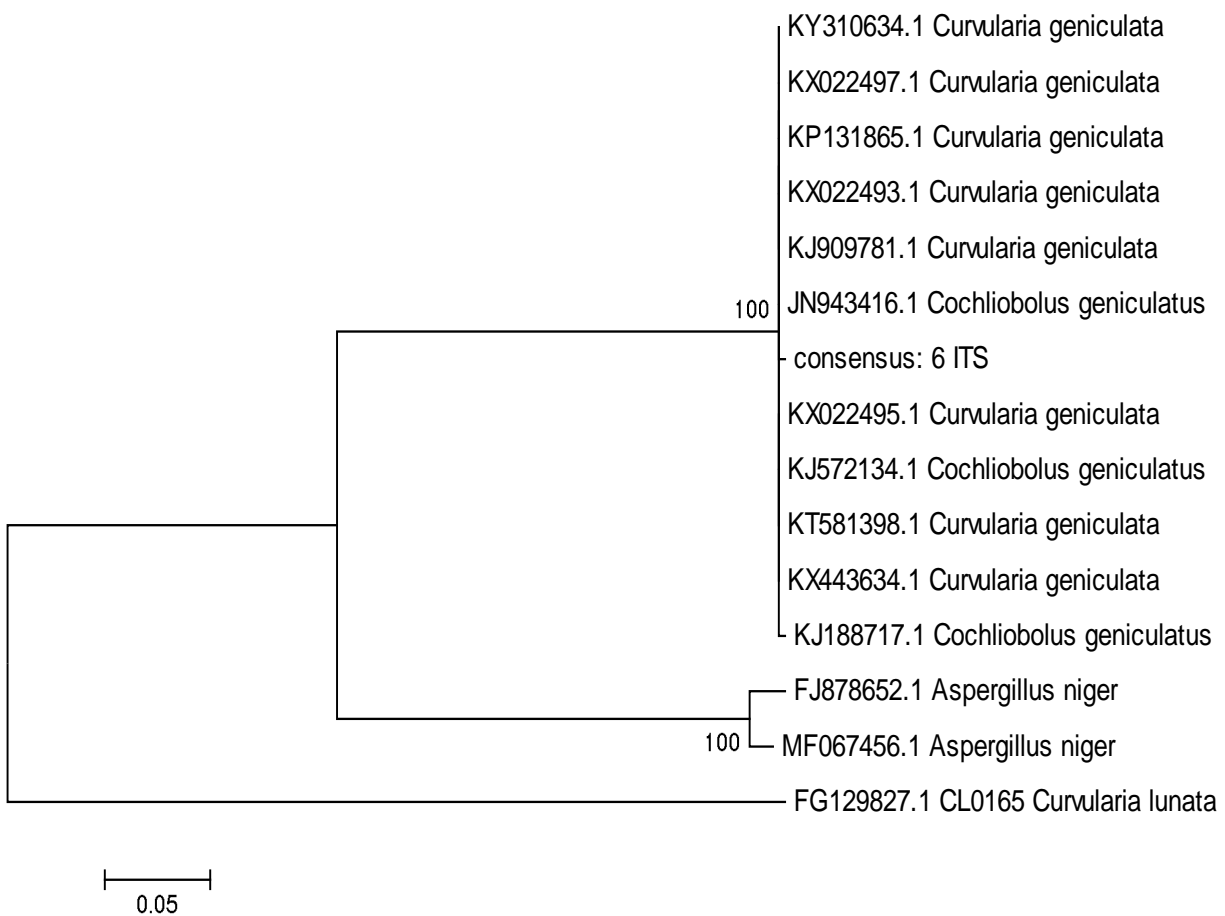


Figure 39: Molecular Phylogenetic analysis of *Curvularia geniculata* by Maximum Likelihood method

Score	Expect	Identities	Gaps	Strand	
870 bits(471)	0.0()	493/502(98%)	8/502(1%)	Plus/Plus	
Features:					
Query	1	AAC-AGGTCTCCGTTGG-G-A-CAGCGGAGGG--CATTACCGAGTTT-CAACTCCCAAAC			53
Sbjct	39	AACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACCTCCCAAAC			98
Query	54	CCCTGTGAACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACG			113
Sbjct	99	CCCTGTGAACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACG			158
Query	114	GCCCGCCAGAGGACCCCCAACTCTGTTTCTATATGTAACCTCTGAGTAAAACCATAAAT			173
Sbjct	159	GCCCGCCAGAGGACCCCCAACTCTGTTTCTATATGTAACCTCTGAGTAAAACCATAAAT			218
Query	174	AAATCAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAA			233
Sbjct	219	AAATCAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAA			278
Query	234	TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG			293
Sbjct	279	TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG			338
Query	294	CGCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCAG			353
Sbjct	339	CGCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCAG			398
Query	354	CTTGGTGTGGGACTCGCGAGTCAAATCGCGTTCCCCAAATCGATTGGCGGTACGTCGA			413
Sbjct	399	CTTGGTGTGGGACTCGCGAGTCAAATCGCGTTCCCCAAATTGATTGGCGGTACGTCGA			458
Query	414	GCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAA			473
Sbjct	459	GCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAA			518
Query	474	ACCCCAACTTCTGAA-GTGACC	494		
Sbjct	519	ACCCCAACTTCTGAATGTGACC	540		

Figure 40: BLASTN alignment between the query sequence and the subject sequence
of *Fusarium proliferatum*

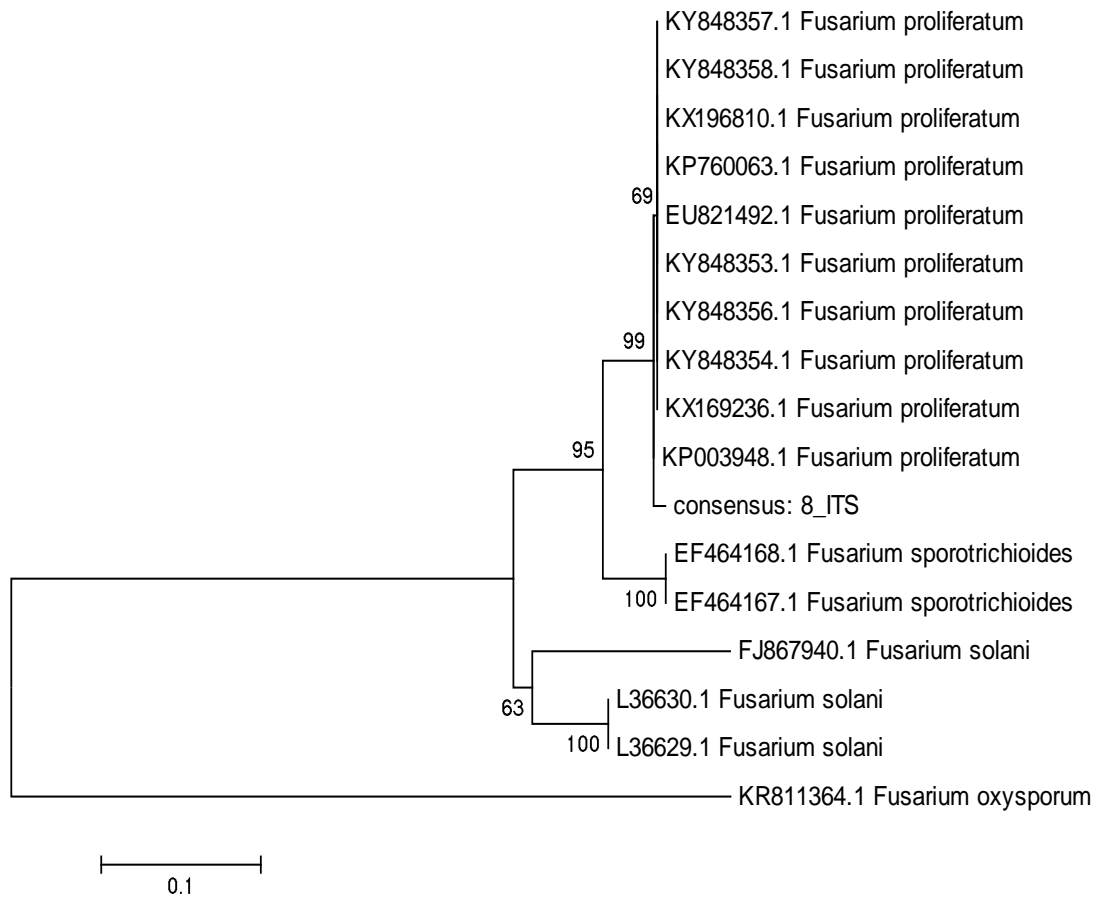


Figure 41: Molecular Phylogenetic analysis of *Fusarium proliferatum* by Maximum Likelihood method

Score		Expect	Identities	Gaps	Strand
401 bits(217)		8e-108	382/482(79%)	10/482(2%)	Plus/Plus
Query	26	CATACCTATMCSWWGCCTCGGAGGATCRAACCGCGMCCCGKACAA-GGGACGAACCGMCC			84
Sbjct	2	CATACCTATACGTTGCCTCGGCGGATCAGCCCGCGCCCCGTAAAACGGGACGGCCCGCCC			61
Query	85	GAAGACCCCTAAAMTCtttttttAGTGGAACCTTTGAGTAAAACAAACATATAAATCAGA			144
Sbjct	62	GAGGACCCCTAAACTCTGTTTTTAGTGGAACCTTCTGAGTAAAACAAACAAATAAATCAAA			121
Query	145	ACTTTCAACAAAGGATCTCTTGGAATCTGGCATCGATGAAAAACGCAAG-WAATGGAACG			203
Sbjct	122	ACTTTCAACAACGGATCTCTTGG-TTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA			180
Query	204	AGTAATGYGAATTGCAGAATTCGGGAATCATGSAAGCTTTGAACGCACWTTGMGCCCCG			263
Sbjct	181	AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC			240
Query	264	CMGTATTCTGASGKSATGACTGKACSAGAGKAATTTCAACCCTCAWGCTCAGCTTGGYG			323
Sbjct	241	CAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTG			300
Query	324	TTGCKACTCGSGAKAACCCGTGKTCCCCAAATCTATTGTTGTTTACGKSGASSTTCGRTA			383
Sbjct	301	TTGGGACTCGCGGTAACCCGCGTTCCCCAAATCGATTGGCGGTCACGTCGAGCTTCCATA			360
Query	384	GCGKAGAAMTCATACWCCTCGAYACTGRWAATC-TCSACGTCCACTCCGKAAAAACCACC			442
Sbjct	361	GCGTAGTAATCATACACCTCGTTACTGGTAATCGTC-GCGGCCACGCCG-TAAAACCCCA			418
Query	443	-CTTTTAAATGWWGACCTCGGA-AAGSGRA-GAATACCCGCTGAACTTAAGCATATCATA			499
Sbjct	419	ACTTCTGAATGTTGACCTCGGATCAG-GTAGGAATACCCGCTGAACTTAAGCATATCATA			477
Query	500	AG 501			
Sbjct	478	AG 479			

Figure 42: BLASTN alignment between the query sequence and the subject sequence
of *Fusarium oxysporum*

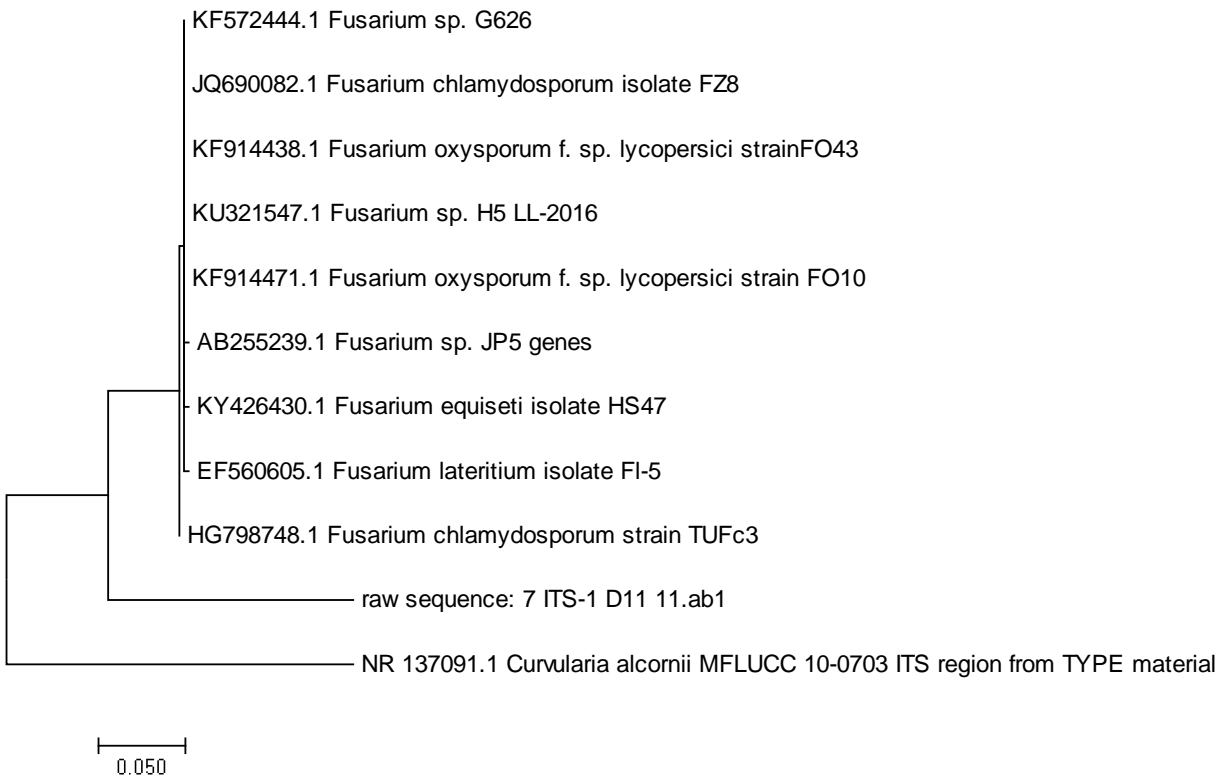


Figure 43: Molecular Phylogenetic analysis of *Fusariumoxysporum* by Maximum Likelihood method

Table 26: Pathogenicity of fungal isolates from spoilt tomatoes fruits after 7 days of inoculation

Diameter of rot (cm)				
Fungi	V1	V2	V3	V4
<i>Aspergillus japonicus</i>	3.23±0.03b	3.57±0.03c	2.27±0.12c	2.03±0.03c
<i>Rhizopus oryzae</i>	2.29±0.04c	2.32±0.00d	1.78±0.04d	2.01±0.58c
<i>Curvularia geniculata</i>	0.88±0.02d	1.16±0.02e	0.87±0.00e	0.84±0.04d
<i>Fusarium proliferatum</i>	3.22±0.00b	3.66±0.01b	3.15±0.02b	2.90±0.04b
<i>Fusarium oxysporum</i>	4.20±0.05a	3.88±0.04a	3.47±0.04a	3.10±0.00a

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF Variety

Table27: Symptomatic characterization of tomato fruits during pathogenicity

Fungal isolate	Symptom observed
<i>Aspergillus japonicus</i>	Dark and sunken spots on the fruit surface
<i>Rhizopus oryzae</i>	Soft rot with leakage of electrolyte
<i>Curvularia geniculata</i>	Black discolouration and sunken appearance
<i>Fusarium proliferatum</i>	White mycelia emerging at the point of inoculation
<i>Fusarium oxysporum</i>	Soft rot with appearance of white mycelia mat

4.11 Frequency of Occurrence of the Fungal Isolates

The results revealed that in V1, *R. oryzae* appeared more frequently than other fungal isolates with percentage frequency of 54% and next to this was *F. proliferatum* with 46%. *F. proliferatum* and *F. oxysporum* had percentage frequency of 54% and 48% respectively in V2. *A. japonicus* had highest percentage of occurrence (56%) in V3 while in V4, *R. oryzae* was more dominant (52%) than other isolates. In all varieties used in this present study, *C. geniculata* had the lowest percentage of occurrence (Figure 44).

4.12 Physiological Studies of Fungal Isolates

The colony diameter of pure of culture of *A. japonicus* was 2.50cm at Day 3 after inoculation and gradually increased to 6.60cm at Day 7 (Figure 45). The mycelia of *R. oryzae* grew aggressively and covered the space of 8.30cm of the plate at Day 7(Figure 46). The diameter of mycelia of *C. geniculata* at Day 2 was 2.47cm and increased to 4.97cm and 6.50cm at Days 6 and 7 respectively (Figure 47). The mycelial growth of the two *Fusarium* species were very slow compared to other three isolates. At Day 7, the colony diameter of *F. proliferatum* and *F. sp* were 3.75cm and 4.78cm respectively (Figures 48 and 49).

Among all the carbon sources used in the present study, *A. japonicus* grew best in fructose with mycelial dry weight of 2.80g and this was significantly higher than those recorded in other two sources including the control. Highest mycelial dry weight for *R. oryzae* (2.46g) was observed in sucrose. Both sucrose and starch enhanced weight of mycelia of *C. geniculata* as well as *F. proliferatum*, and no significant difference was observed between the two carbon sources. *Fusarium oxysporum* had highest mycelial dry weight of 1.76g in starch and lowest (0.99g) in fructose carbon media (Table 28).

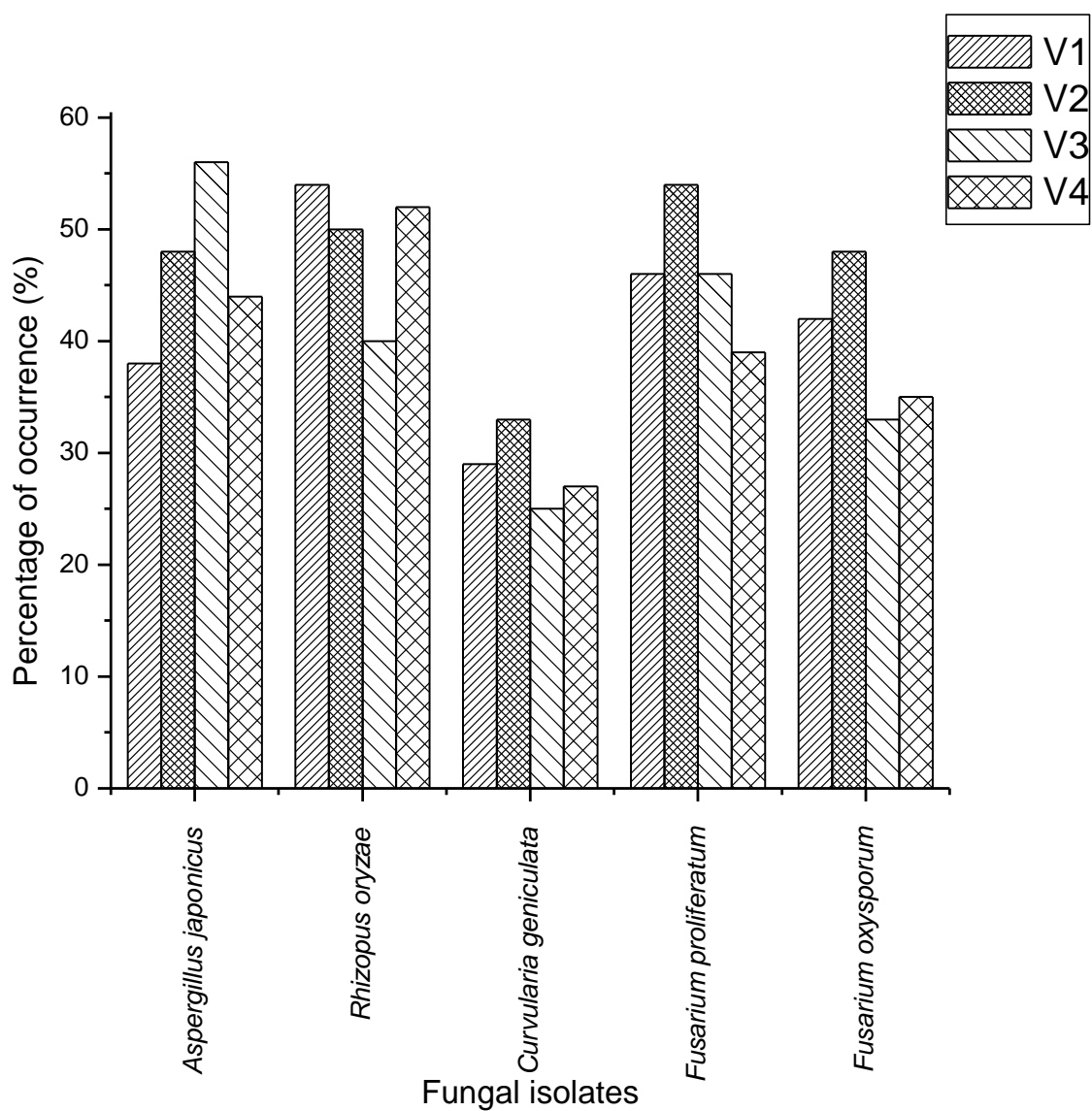


Figure 44: Frequency of occurrence of each of the fungi isolated from tomato fruits

V1 = Hausa Variety

V2 = Yoruba Variety

V3 = Tropimech Variety

V4 = Roma VF Variety

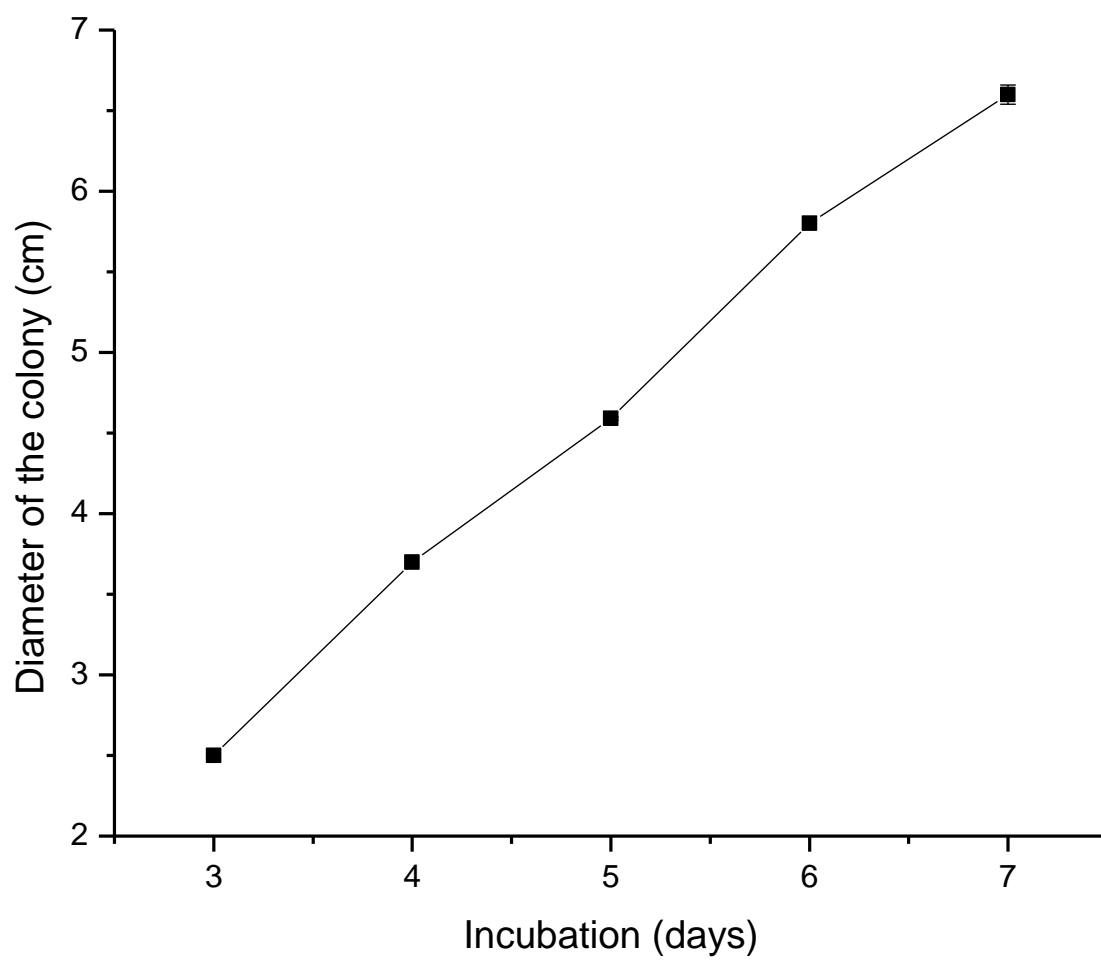


Figure 45: Growth of *Aspergillus japonicus* on PDA medium

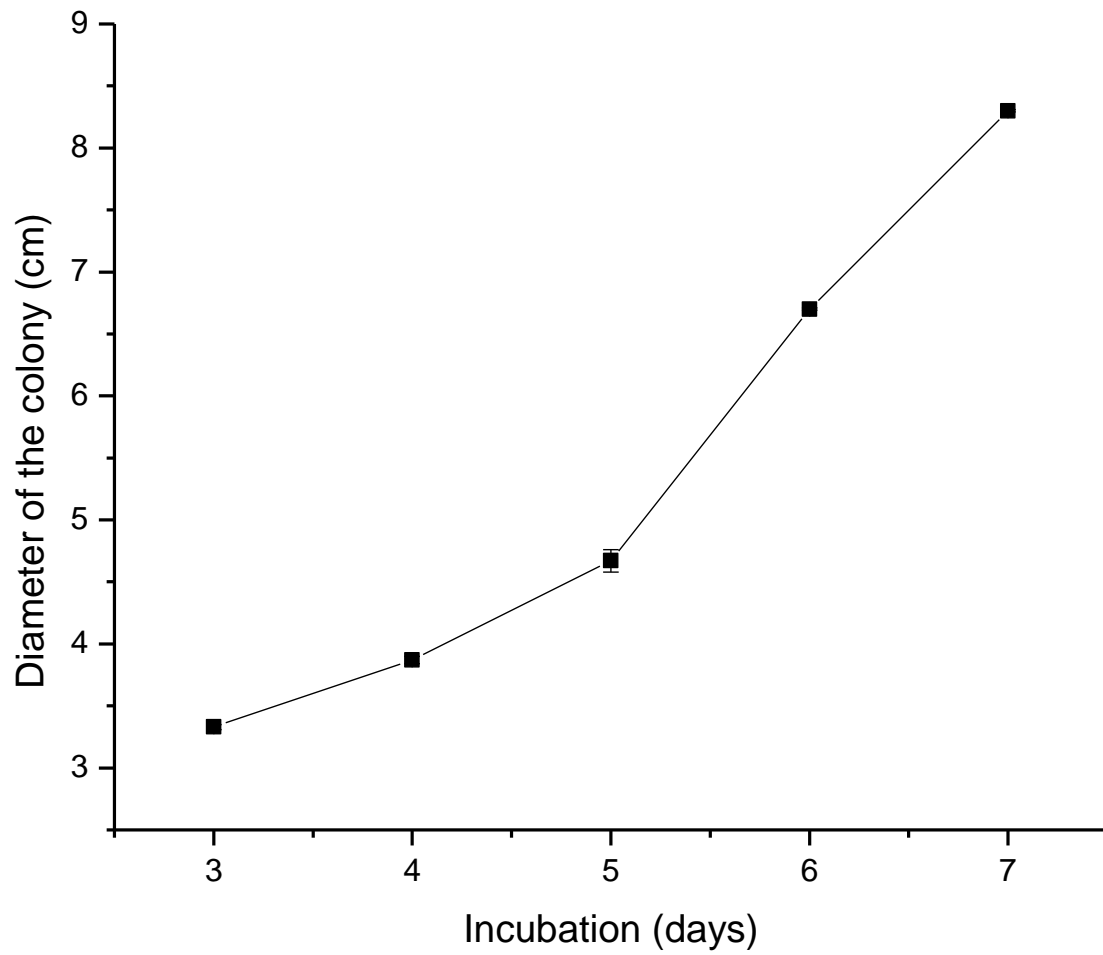


Figure 46: Growth of *Rhizopus oryzae* on PDA medium

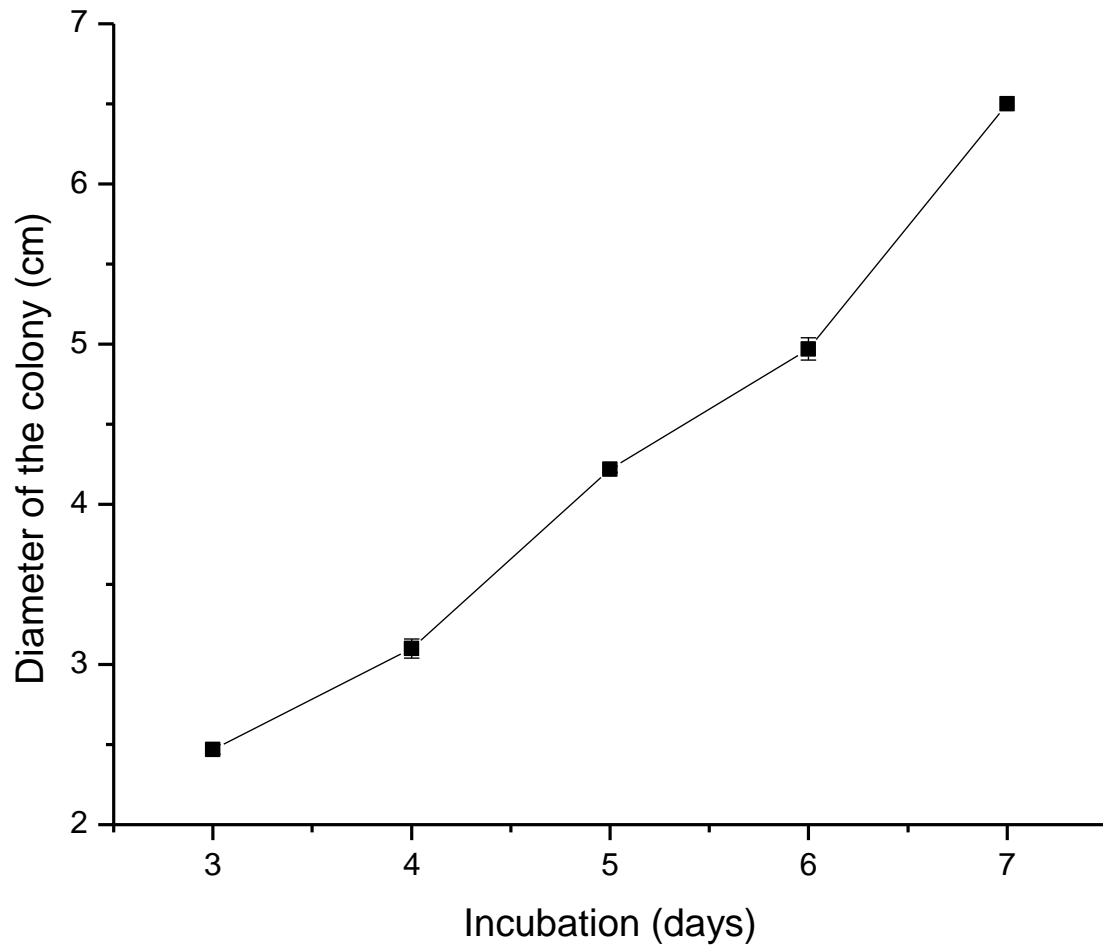


Figure 47: Growth of *Curvularia geniculata* on PDA medium

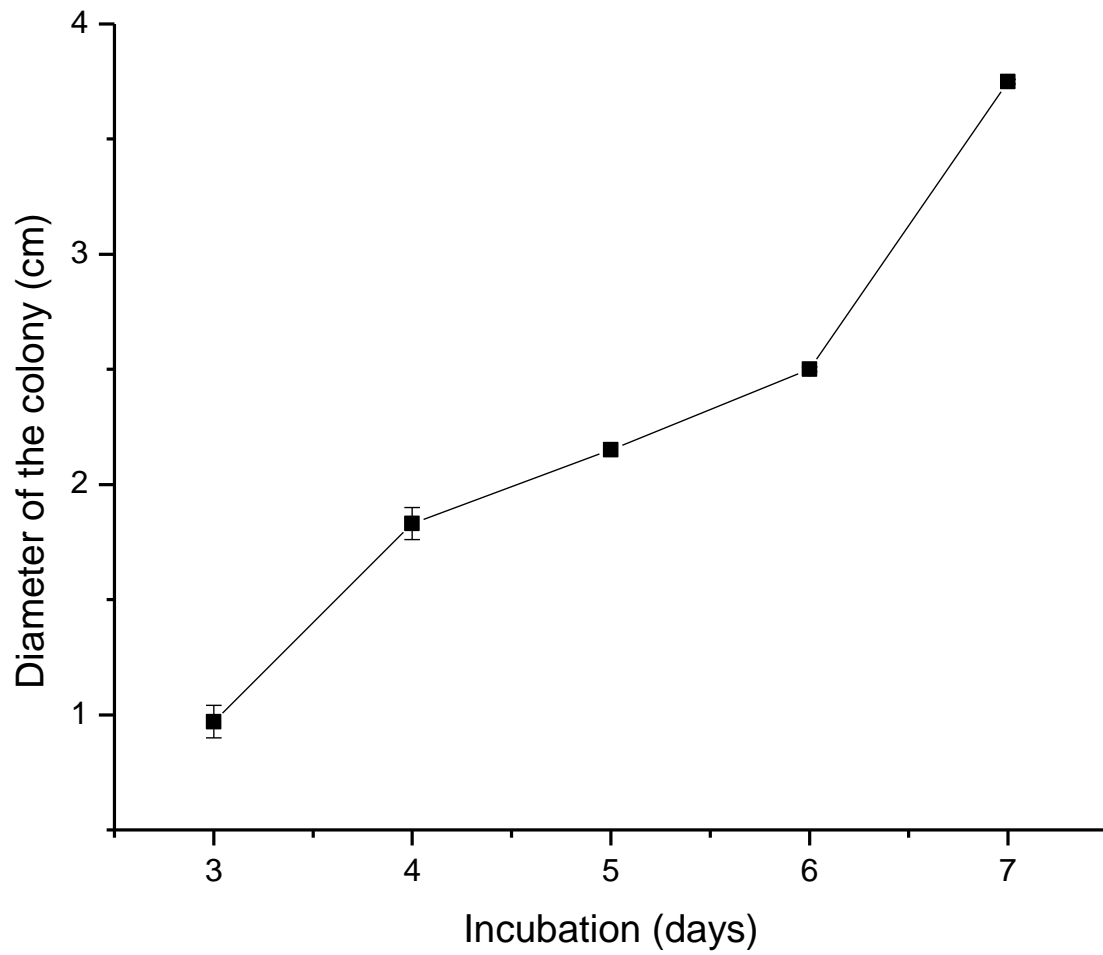


Figure 48: Growth of *Fusarium proliferatum* on PDA medium

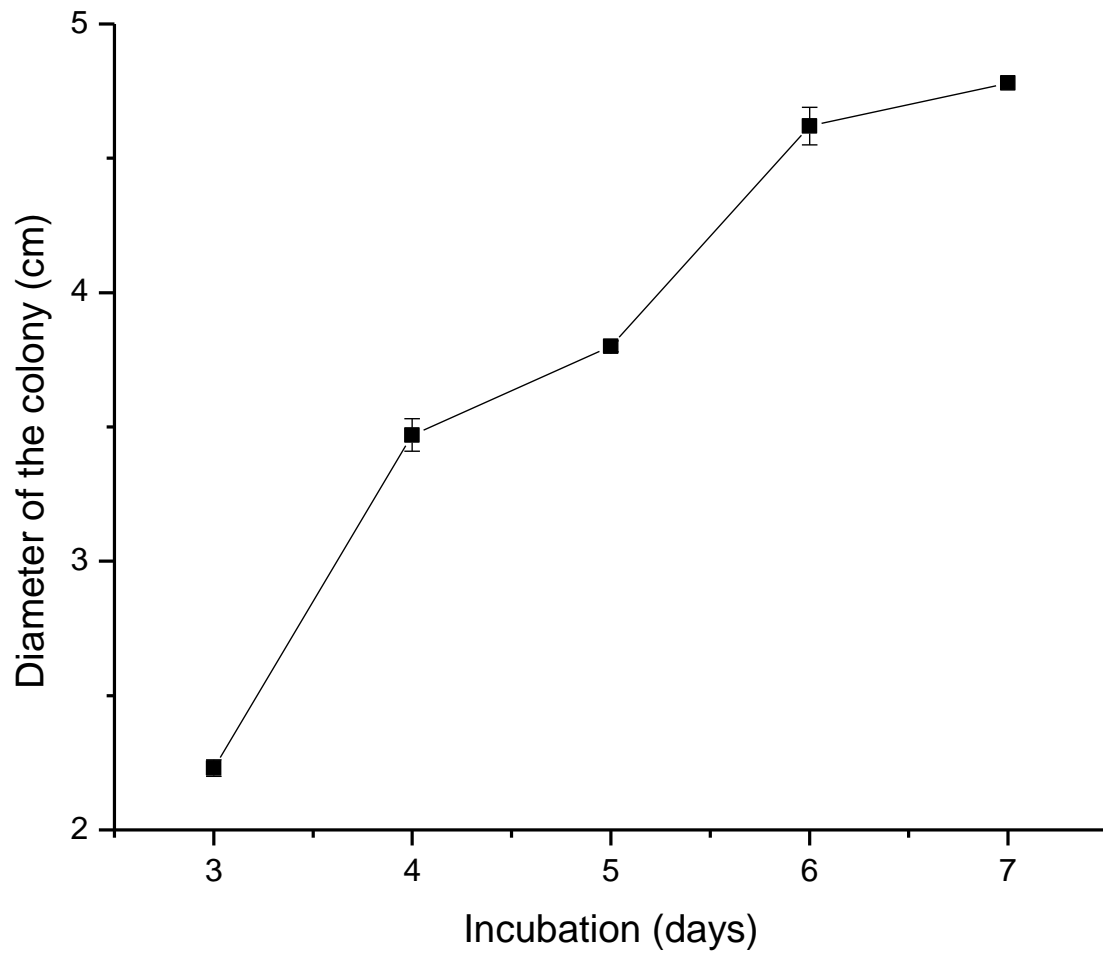


Figure 49: Growth of *Fusarium oxysporum* on PDA medium

Table 28: Mycelial dry weight of isolated fungi on different carbon sources

	<i>Aspergillus japonicus</i>	<i>Rhizopus oryzae</i>	<i>Curvularia geniculata</i>	<i>Fusarium proliferatum</i>	<i>Fusarium oxysporum</i>
Fructose	2.80±0.06a	0.85±0.33c	1.06±0.13b	0.71±0.07b	0.99±0.07c
Sucrose	0.88±0.05c	2.46±0.02a	1.71±0.09a	1.97±0.35a	1.38±0.10b
Starch	1.57±0.12b	1.97±0.04b	1.97±0.19a	2.24±0.28a	1.76±0.10a
Control	0.79±0.08c	0.61±0.02d	0.96±0.15b	0.90±0.10b	0.67±0.06d

Means followed by the same letter along the same column are not significantly different at $p \leq 0.05$

On the utilization of nitrogen, urea had 2.10g of mycelial dry weight which was significantly higher than other three sources. *R. oryzae* and *C. geniculata* utilized sodium nitrate better than other nitrogen sources and had mycelial dry weights of 2.94g and 1.78g respectively. Calcium nitrate appeared to be poor source of nitrogen that the mycelia dry weight of *F. proliferatum* but strived best in urea. Also, 1.82g of mycelial dry weight of *F. oxysporum* was recorded in ammonium chloride. No significant difference in the mycelial dry weight of *F. oxysporum* in calcium nitrate, sodium nitrate and urea media (Table 29).

Table 29: Mycelial dry weight of isolated fungi on different nitrogen sources

	<i>Aspergillus japonicus</i>	<i>Rhizopus oryzae</i>	<i>Curvularia geniculata</i>	<i>Fusarium proliferatum</i>	<i>Fusarium oxysporum</i>
Calcium Nitrate	0.76±0.050cd	1.62±0.04c	1.13±0.13ab	1.08±0.01d	0.97±0.01b
Sodium Nitrate	0.94±0.05c	2.94±0.04a	1.78±0.22a	1.51±0.03c	1.17±0.14ab
Ammonium Chloride	1.63±0.05b	1.88±0.09b	1.33±0.10ab	1.89±0.11b	1.82±0.37a
Urea	2.10±0.08a	0.86±0.04d	1.50±0.32a	2.40±0.11a	1.03±0.20b
Control	0.64±0.03d	0.42±0.03e	0.73±0.18b	0.46±0.03e	0.61±0.15b

Means followed by the same letter(s) along the same column are not significantly different at $p \leq 0.05$

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physical qualities and Shelf life of Tomato Fruits

Hardenburg *et al.* (1986) mentioned that storage under relatively low temperature is the most efficient method to maintain quality of most fruit and vegetables due to its effects on reducing respiration rate, transpiration, ethylene production, ripening, senescence, and rot development. It is generally agreed that mature green tomato can be stored for relatively longer period at a temperature of 10–15°C and 85–95% relative humidity (Castro *et al.* 2005). In this background, it is interesting to note here that the temperature of the storage room also offered similar conditions except that the relative humidity was low. Hardenburg *et al.* (1986) mentioned that storage under relatively low temperature is the most efficient method to maintain quality of most fruit and vegetables due to its effects on reducing respiration rate, transpiration, ethylene production, ripening, senescence, and rot development.

A fruit may change in texture during maturation, especially during ripening when it may become rapidly softer. there was a loss in firmness of fruits from very firm to very soft. Lana *et al.* (2005) and Gharez *et al.* (2012) indicated that the firmness of tomatoes decreased during storage, which is in agreement with the present findings. One of the physical factors that attract consumer of tomato fruits is firmness and this quality is proportional to ripeness stage. Firmness contributes immensely to vulnerability of tomatoes to physical deterioration (Raffo *et al.*, 2002). The skin toughness and internal fruit structure determined the textural quality of the fruits (Gharez, 2012).

Colour changes are one of the indications of physicochemical developmental stages in tomato fruits. During the storage period, there was a general change of tomato fruit skin colour from mature green to red ripe form were observed. Campbell *et al.* (1990) that during normal ripening of tomato fruit, tissue colour changes from green through orange to red, which coincides with ethylene biosynthesis and a climacteric rise in respiration reported similar observations. Colour changes were subjected to genetic control in view of the variation in colour development across cultivars

Tomato fruits, generally, are succulent and perishable and as a result have short shelf life. Good and protective storage methods are required to enhance their shelf life as well as their physical qualities (Saeed *et al.*, 2010). It becomes imperative to develop a simple storage method to elongate the storage period of tomato fruits. All the fruits were harvested at green mature stage following the recommendation of (Anju-Kumari *et al.*, 1993) that the longest shelf life of tomato cultivars can only be achieved when the fruits are harvested at this stage. The highest shelf life was recorded in pot in pot refrigerator. This finding was supported by the result of Idah *et al.*, (2010) who reported that evaporative cooler system is a promising storage structure that enhances the shelf life of fruits and vegetable. However, with some careful modifications in the pot-in-pot storage system, preserving fruits and vegetables in the rural areas will be more effective in Nigeria.

Chemical preservatives had previously used to preserve fruits but attention had been shifted because of their cumulative effects on the consumers. Nasrin *et al.* (2008) reported that chlorine-treated tomato fruits, stored in perforated polyethylene bag at room temperature had shelf life of 17 days which fell within the range of pot-in-pot storage system without preservative. The use of chlorine to preserve fruits had been banned in several European countries such as Germany,

Belgium, Switzerland and Netherland due to its possibility to form carcinogenic chlorinated compounds in water (Ahmed *et al.*, 2012). Tomato fruits treated with calcium carbide prolonged the storage life of tomato up to 18 days but the health implication is that it causes cancer, mouth ulcer, food poisoning and eye contact may result in permanent blindness (Asif, 2012). Botanical (bio-preservatives) are very effective in prolonging the storage period of fruits, inhibiting the growth of pathogens and increasing the physical qualities of fruits (Draughon, 2004). The use of botanicals in lieu of chemicals as preservatives of fruits is efficacious and less toxic to human (Irokanulo *et al.*, 2015). These botanical preservatives are affordable and convenient for local farmers. The botanicals used were efficacious at varying degree. According to Theu (2017), the action of sawdust to maintain the freshness and firmness of tomato for a long period is more effective than that of ash and this assertion supported the findings of this work.

5.2 Lycopene Contents of Tomato Fruits

One of the major sources of lycopene is tomato fruits (Moraru *et al.*, 2004). It is a pigment that confers red colour to the fruits. The lycopene contents of the four varieties were insignificantly different from one another but there was a report that varietal types of tomato fruits respond differently to storage conditions (Abba *et al.*, 2017). All the varieties used are rich in lycopene. The increase in lycopene content during storage as observed in this study agreed with the result of Tadesse *et al.* (2015) who reported that the green pigment in fruits called chlorophyll continues to reduce during storage time and the increment in colour intensity of the fruits is an indication of accumulation of lycopene. Lycopene comprises up to 80-90% of the pigments present (Gharezi *et al.*, 2012) and Sulaeman *et al.* (2001) reported that 3 to 5mg/100g of lycopene is present in tomato. Lycopene is an essential pigment with a great nutraceutical values. This carotenoid pigment is a natural antioxidant loaded with all kinds of health benefits (Djuric

and Powell, 2001). Attention has been shifted to extraction and derivation of natural antioxidants from fruits and vegetables (Asaduzzaman *et al.*, 2013). Eating fruits rich in lycopene has been ascribed to lower chance of having cardiovascular diseases (Willcox *et al.*, 2003; Sesso *et al.*, 2003). Lycopene helps in dealing with free radicals that may cause abnormal cell formation such as prostate cancer and reduces the risk of cardiovascular disease and age-related eye disorders (MFMER, 2017; Bhowmik *et al.*, 2012).

The lycopene content in tomato fruits stored in both plastic crate and raffia basket was significantly higher than that of fruits stored in pot in pot refrigerator. The process of chlorophyll degradation is slow at low temperature which will consequently affect lycopene synthesis (Abba *et al.*, 2017).

5.3 Nutritional Composition of Tomato Fruits

The results of proximate analysis revealed that in all the varieties, moisture content was higher than other elements analyzed irrespective of storage methods and this was in agreement with the works of Agbemaflé *et al.* (2015) and Idah *et al.* (2010). The fruits of all the varieties of tomato used are potential sources of water as it plays an indispensable role in biochemical metabolism of consumers. Water not only hydrates the body but also serve as thermoregulator and maintain the fluid balance (Popkin *et al.*, 2010). The ash content of a food substance depicts the total crude minerals. Roma VF had highest ash the value fell in range of 0.47% - 0.98% as reported by Agbemaflé *et al.* (2015). Plants accumulate these nutrient minerals by the action of the roots to absorb minerals along with water but this action decreases especially in water stressed plants (Akinci and Losel, 2012). The highest ash content in Roma VF may be as a result of its ability to absorb minerals from the soil (Agbemaflé *et al.*, 2015).

The amount crude minerals of fruits are unchanged during storage except when there are leakages from the fruits and with the fact that they are not metabolized (Hui, 2006). The variances in ash content within each variety may be as a result of storage methods coupled with the influence of botanicals. The range of protein content of all the varieties used was 0.07% – 0.92% lower than 1.0% - 1.1% as reported by USDA (2005). The differences may be as a result of varietal influence, environmental conditions and other agronomical practices during production (Agbemafle *et al*, 2015). The differences in protein content can also be attributed to botanicals which may have differential effects on the activities of cell wall enzymes such as α -galactosidase, β -galactosidase, β -mannosidase and β -glucosidase. These are also responsible for the rotting softening of the fruit (Emadeldin *et al.*, 2012).

Fruits contain low amount of protein but aged tissues such as overripe fruits usually have higher amount of non-protein nitrogen (Vincent, 2009). Tropimech had highest percentage lipid of 0.14%, lower than 0.20% estimated by Idah *et al.* (2010). Agronomical activities during production may also account for dissimilarity. Fatty acids are very essential in physiological functions of human as they participate primarily to produce hormone-like substances which control blood pressure, blood clotting, the immune response, blood lipid levels and the inflammatory response (Vincent *et al.*, 2009). All varieties used contain considerable amount of fibre in varying quantities. Onifade *et al.* (2013) revealed that the percentage crude fibre in Yoruba variety of tomato was 2.50%, comparatively higher than not only the similar variety but also other three varieties considered in this current study. The principal components of dietary fibres are lignin, cellulose, hemicelluloses, pectins, resistant starch and non-digestible oligosaccharides. The cell wall makes up to 1% to 2% of the fresh weight of fruits and cellulose constitutes about 33% of that amount (Vincent *et al.*, 2009). Brummell (2006) reported that the

quantity of cellulose fluctuates during fruit ripening. Dietary fibre is indigestible component of food that enhance peristaltic movement of bowels. It prevents constipation as well as colon cancer (Terry *et al.*, 2001). It modulates function of intestinal tract and characterized with low calories (Marlett *et al.*, 2002). Carbohydrate is an essential nutrient in the body as it is the major energy source in the body.

The amount of carbohydrate is second to moisture in all the varieties. It was observed that there is an interplay between the moisture and carbohydrate contents without influence of storage methods. This assertion was supported by Idah *et al.* (2010) that the percentages moisture and carbohydrate are increasing and decreasing respectively as the storage period increasing. Carbohydrate exists in different forms but glucose and fructose form a major portion of total sugar in fruits (Vincent *et al.*, 2009). Starch as a form of carbohydrate hydrolyzed into monosaccharides such as glucose and fructose, and disaccharides like sucrose as ripening progresses (Raffo *et al.*, 2002). Carbohydrate, a macromolecule, constitutes a major structural framework of cells and serve as storage of energy reserve.

It was observed in this study that tomato fruits contained phosphorous, potassium, magnesium, copper, iron, zinc and calcium. It was revealed that the amount of previously mentioned minerals depended on the varietal type. The four varieties are a good source of minerals and the variation may be as a result of different responses of each variety to environmental conditions of area where they were raised (Olaniyi *et al.*, 2010). All the analyzed minerals are beneficial. Potassium plays a role in protein synthesis and stability and also partake in carbohydrate synthesis.

5.4 Fungi Isolated from Spoilt Tomato Fruits during Storage

Aspergillus japonicus, *Rhizopus oryzae*, *Curvularia geniculata*, *Fusarium proliferarum* and *Fusarium* sp were isolated. Matthew (2011) reported that *Aspergillus niger*, *Rhizopus nigricans*, *Rhizopus stolonifer*, *Candida yeast*, *Penicillium* spp. and *Mucor* spp. are responsible for postharvest loss of most of the fruits during storage. The isolates in this work agreed with the results of Kalyoncu *et al* (2005) who isolated 7 fungal genera from tomato fruits including *Aspergillus*, *Rhizopus* and *Fusarium*. Similarly, Kutama *et al.* (2007) reported that *Aspergillus*, *Rhizopus* and *Alternaria* species are commonly associated with stored tomato fruits. According to Wogu and Ofuase (2014), *Aspegillius* spp, *Penicillum* spp, *Fusarium* spp and *Saccharomyces* spp. are implicated to cause spoilage in tomato fruits. Most of the associated fungi are traceable to poor handling practices during harvest, transportation and distribution, marketing practices and storage conditions (Effiuvwevwere, 2000; Akinmusire, 2011).

Generally, the fungi responsible for spoilage of fruits are toxigenic and can bring about infection or allergies (Monso, 2004; Al-Hindi *et al.*, 2011). *Aspergillus* spp and *Fusarium* spp are potential sources of harmful secondary metabolites called mycotoxins (Frisvad *et al.*, 2002; Akinmusire, 2011). *Aspergillus* spp. are known to produce these metabolites such as Ochratoxins which poses health threat to both man and livestock. Ochratoxins affects normal functions of the kidney in humans and animals (Pfohl-Leskowicz and Manderville, 2012). *Rhizopus oryzae* is naturally ubiquitous and found in association with decaying organic substances (Meussen *et al.*, 2012). It is pathogenic to plant and its pathogenicity is attributed to ability to degrading enzymes such as pectinases, cellulases and hemicellulases (Ghosh and Ray, 2011).

R. oryzae is the most common causative agent of zygomycosis, an infection among immunocompromised patients (Ibrahim *et al* 2005; Ma *et al.*, 2009). The disease is rarely cured

with antifungal therapy except with surgical removal of infected focus (Husain *et al.*, 2003). Chayakulkeeree *et al.* (2006) confirmed that *Rhizopus* are most common etiological agent of health condition known as mucormycosis. Contrarily, the role of *R. oryzae* in food industries has been identified. The fungus is used to produce traditional recipes by fermentation to stimulate sensory changes in food at different levels and new flavors appears in various degree depending on the fermented product (Cantabrana *et al.*, 2015). Besides, *R. oryzae* had been found useful in the production of an enzyme called amylase from industrial waste in acquisition of glucose (Freitas *et al.*, 2014).

A host non-specific mycotoxin known as Fusaric acid (FA) (5-butylpicolinic acid) is produced by many species of *Fusarium* both in-vitro and in-vivo conditions (Selim and El-Gammal, 2015). High concentration of Fusaric acid (> 10 M) is very toxic to host plant, animals and human beings (Eged, 2005). Besides, another most common mycotoxins produced by *Fusarium* include deoxynivalenol (DON), 3-acetyl deoxynivalenol (3-ADON), 15-acetyl deoxynivalenol (15-ADON), nivalenol (NIV) and fusarenon X (Fus-X); T-2 toxin, HT-2 toxin, neosolaniol (NEO) and diacetoxyscirpenol (DAS); zearalenone (ZEN), fumonisin B1 (FB1) and fumonisin B2 (FB2) (Bottalico and Perrone, 2002; Schollenberger *et al.*, 2006; Tian *et al.*, 2016). All these toxigenic substances are potential causative agents of many acute and chronic diseases in both plants and animals (Stoev, 2013). *Fusarium* not only causes fruit rot but also induces wilting in the field and can remain dormant in soil and debris for many years, until healthy plants are grown in the infested field (Scheuerell *et al.*, 2005; Ignjatov *et al.*, 2012).

5.5 Pathogenicity and Frequency of Occurrence

Major cause of fruit loss during storage is from the actions of pathogenic fungi (Grahovac *et al.*, 2011). All the fungal isolates (*Aspergillus japonicus*, *Rhizopus oryzae*, *Curvularia geniculata*, *Fusarium proliferarum* and *Fusarium oxysporum*) were able to cause lesion on healthy tomato fruits though having varying degrees of severity. *R. stolonifer* was initially reported to cause rot in fruits (Kwon and Jee, 2008) and *R. oryzae* induced soft rot in apple and banana fruits (Kwon *et al.*, 2011; Kwon *et al.*, 2012), and this result supported the findings in this present study. Despite the virulence of *R. oryzae*, the fungus is industrially important as it produces large amount of lactic acid and ethanol using sugar component of mycelial biomass (Abedinifar *et al.* 2009; Vially *et al.*, 2010). The pathogenicity of *A. japonicus* was also evident as the isolate caused more rot in local varieties of tomato than improved ones. *A. japonicus* belongs to black group of *Aspergillus* species (Palencia *et al.*, 2010).

Aspergillus spp. are commonly found in spoilt fruits and black *Aspergillus* species produces cell wall degrading enzymes such as xylase, cellulase, α -amylase and polygalacturonase. These aforementioned enzymes distort the integrity of the cell wall (Al-Hindi *et al.*, 2011). The essential pathogenicity factors of *A. japonicus* are attributed to Xylases and polygalacturonases (Di matteo *et al.*, 2006). Although *A. japonicus* is pathogenic, the conidia of the fungus are useful in adsorption of a mycotoxin known as zearalenone as a way of decontaminating animal fodders (Jard *et al.*, 2009).

Vishnoi *et al* (2005) reported that *C. geniculata* is also pathogenic to mammals by invading mammalian tissues such as liver and kidney. Contrarily, all species of *Curvularia* are not phytopathogenic because Motlagh (2011) discovered that *C. lunata* is potent as bioagent to

control the population of weeds of rice paddies. *Fusarium* species are destructive and most common pathogenic fungi of tomato. They infect tomato in all stages (Chehr, 2016). *Fusarium* are also responsible for storage rots of fruits and feeds (Etcheverr, 2002) and result to economic due to production of harmful metabolites called mycotoxin (Ignjatov *et al.*, 2012). Steinkellner *et al.* (2005) identified *F. oxysporum* as most virulent species of *Fusarium* in tomato field affecting both the vegetative and fruit. Pathogenic species of *Fusarium* were also known to produce pectinase, cellulase and α -amylase disintegrate the cell wall component (Di Pietro *et al.*, 2003). High level of Xylanolytic enzyme and cellulase is produced by *F. oxysporum* (Simoes *et al.*, 2009; Ramanathan *et al.*, 2010).

5.6 Physiology of fungi Isolated from Spoilt Tomato Fruits

Fungal physiology involves all metabolic activities and death of fungal cells (Thaker and Maharshi, 2012). The results of physiological studies revealed that all the three carbon sources used supported, at different rate, the mycelial growth of isolated fungi. This agreed with the findings of Sulaiman and Akaajime (2010). Carbon is said to be an essential component of fungal cell need for their growth and development (Islam, 2015). Fructose enhanced mycelial growth of *A. japonicus* and this result was corroborated by the findings of Sulaiman (2010). Bolla *et al.* (2010) stated that fructose is an effective carbon source for fungi to produce mycelial biomass and exopolysaccharides. The media containing both monosaccharides, disaccharides and starch are good sources of organic carbon that support biomass production of fungi (Guler and Ozkaya, 2008). *F. proliferatum* grew best in starch medium and Zhou and Yang (2010) had earlier reported that the mycelial growth of fungal colony of *F. acuminatum* was the best with soluble starch. Xiaol *et al.* (2016) reported that carbohydrate as a macromolecule forms a

weighty component of cytoskeleton and an essential nutritious requirement for the overall development of higher fungi.

Eaton and Ayres (2002) reported that the utilization of carbohydrate by fungi depends on the quantity of nitrogen source present. Fungal development is dependent on the level and nature of nitrogen that is used for both structural and physiological processes (Sulaiman,2005). Nitrogen is useful in protein synthesis and other important functions. All fungal isolates were able to grow in the basal media supplemented with different nitrogen sources. The culture media are usually supplemented with carbohydrate and nitrogenous substances so as to enhance the microbial growth conditions and production of enzymes by fungi (Philippoussis *et al.*, 2011) This connotes that the pathogens are adapted to utilize nitrogen in spite their difference reactions to environmental factors (Bouras *et al.*, 2016). Concentrations of nitrogen in the media alter the pH value and have impact in the growth and synthesis of metabolite (Hermann *et al.*, 2013; Pedria *et al.*, 2015).

Fungi are known to absorb inorganic form of nitrogen and this may be due to anthropogenic sources (Itoo and Reshi, 2014). Urea and ammonium chloride favoured biomass production of *A. japonicus*. *Aspergillus* spp. have potential to use wide range of nitrogen sources such as ammonium, nitrate, histidine, elastin and collagen (Krappmann and Braus, 2005). *R. oryzae* had lowest ability to utilize urea as the sole nitrogen source and this may be as a result of absence of specific transport system or lack of urease enzyme (Ewase *et al.*, 2007). Itoo and Reshi (2014) confirmed that ammonium is the best nitrogen source for biomass production of fungi. Contrarily, the mycelial dry weight of *Curvularia geniculata* in calcium nitrate and ammonium chloride was lower than other nitrogen sources and this agreed with the result of Pedri *et al.* (2015) who reported that ammonium sulfate and potassium nitrate did not support the growth of

certain fungi. Nitrogen is one of limiting factors that determines growth of microbes (Farjalla *et al.*, 2006). *Fusarium proliferatum* and *F. oxysporium* utilized all nitrogen sources to a varied extent. According to Islam (2015), sodium nitrate and ammonium nitrate media supported the growth and sporulation of *Fusarium* spp. This result conformed to the observation that nitrates favoured the mycelial growth and varied among *Fusarium* spp. (Khailare and Ahmed, 2012).

5.7 Conclusion

Tomato fruit is an important berry with minimal shelf life. The shelf life of the fruit depends on varietal type, storage conditions and presence or absence of preservatives. The storage period for tomato fruit was improved through appropriate storage method (pot in pot refrigerator). Sawdust and rice straw that seldom considered as wastes are good bio-preservations and they can be transformed into more usable forms.

Tomato fruit is nutritious containing considerable amount of dietary fibre, protein, carbohydrate and moisture. Also, potassium, phosphorous, magnesium, copper, iron, zinc and calcium are present in varying quantity. The fruit is very rich in antioxidant lycopene that have a number of health benefits. Storage conditions influence the lycopene content in tomato fruits and this carotenoid determines the colour of the fruit. The amount of lycopene increases along with ripening.

Fungal spoilage of tomato fruits is common during postharvest activities. *Aspergillus japonicus*, *Rhizopus oryzae*, *Curvularia geniculata*, *Fusarium proliferarum* and *Fusariumoxysporum* were implicated to instigate rot in the fruit during storage. These isolates caused different forms of rot and their degree of pathogenicity differs. Fungal spoilage ravages the fruits and plays a contributory role in postharvest losses. However, health implication of the fungal isolates is yet

to be studied but identification of these pathogens will definitely pave way for subsequent research.

5.8 Recommendations

1. Farmers and consumers should be mindful of storage techniques with high degree of specificity as all agricultural produce cannot be stored under the same storage conditions.
2. Farmers should create an intimacy between them and the researchers so that pertinent information will be gotten at the right time. This will definitely minimize postharvest losses and consequently enhance food security in the country.
3. Researchers should endeavour to work on pressing issues that will reduce agricultural losses and make results of their work available in an understandable form. They can make their findings available through modern platforms such as mass media, social media, agricultural extension agents, publications, exhibitions and the likes.
4. Enlightenment programmes should be put in place to educate farmers and consumers on post-harvest management.
5. Government should develop a policy against import of chemical preservatives which are known with accumulative or residual side effects and at the same time encourage the use of botanicals to elongate the storage life of agricultural produce.
6. Government should give supports to researchers in any form that will assist them in identifying research gaps and proffer solution at preharvest and postharvest stages.

5.9 Contributions to Knowledge

- I. Pot in pot refrigerator was an innovative storage structure designed to maintain the storage temperature below ambient temperature. This innovation promoted the shelf life of tomato fruits without having any significant effects on the quality of the fruits.

- II. Chemical preservatives had been previously used to elongate the shelf life of tomato fruits but researchers discouraged the use of this form of preservation due to its long time cumulative effects on the consumers. This research work confirmed the efficacy of botanical preservatives (also known as bio-preservatives) as alternative and was found successful.
- III. Botanicals, especially sawdust of *Khaya ivonresis* and *Oryza sativa* straw, have different degrees of antifungal properties with ability to minimize fungal loads in tomato fruits during storage. This potential reduces postharvest loss and enhances physical qualities of the fruits.
- IV. Pathogens were responsible for tomato fruit spoilage. This study revealed that the improved varieties (Tropimech and Roma VF varieties) were more resistant to fungal attack than local varieties.
- V. Interactions among all the factors (varieties, storage structures and botanicals) had significant impacts on the proximate and mineral compositions of all the varieties at $p \leq 0.05$

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