THE USE OF HOT WATER TREATMENT BY SMALL HOLDERS FOR THE CONTROL OF *ALTERNARIA ALTERNATA*, THE CAUSE OF BLACK MOULD DISEASE OF TOMATO

Mufutau Olatunde Animashaun

A thesis submitted for the degree of Doctor of Philosophy (PhD) in Horticulture

Department of Horticulture, Writtle College

University of Essex (April, 2015)

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Supervisor:

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Abstract

There are many vegetable fruits recognized in Nigeria, but tomato, a vegetable fruit is a major food component, an ingredient utilized by every house hold and constitutes the national food security programme. The record confirmed that Nigeria produces approximately 1.8 million metric tons of fresh fruits for domestic consumption, with national demand of about 2-3 million tons per annum with a demand gap of about 500,000 metric tons. Tomato production is an important source of income to farmers unfortunately diseases such as *Alternaria alternata* greatly increase food losses by an approximately 20-30% and methods of using synthetic chemical compounds can be costly and dangerous if applied by an unskilled operator and are often not available at the time when required. As a result this study focused on the effect of hot water dipping as a non-chemical method to control the black mould disease caused by *Alternaria alternata* on red tomatoes. Hot water dip at 50^{O}C for 5 or 10 min was carried out on *Alternaria alternata* spore suspension (*in-vitro*), the results showed a significant ($P \leq 0.05$) reduction in germination of spores after 48 h. The *in-vivo* hot water treatment was carried out in three groups, viz-a-viz; first group consists of 30 and 50^{0}C and tomato fruits were heated in hot water for 30 and 60 min respectively. The second group was 30, 40 and 50^{0}C and fruits were dipped in hot water for 20 min. In the third group the temp was at 40, 45 and 50^{0}C and fruit were dipped in hot water at these temperatures respectively for 10 min. Furthermore, the hot water temp was increased to 50 and 55^{0}C and inoculated fruits were immersed for 5 min in separate hot water bath. In this trial the result showed that dipping artificially inoculated fruit at 50 or 55^{0}C for 5 min significantly reduced ($P \leq 0.05$) decay development caused by *A. alternata*. Conidia germination was more sensitive than mycelia growth to 50^{0}C, but inhibition of both processes increased with the duration of time of treatment. The *in-vitro* hot water treatment of *Alternaria alternata* spores at 50^{0}C

for 30 min significantly reduced the spore germination and mycelia elongation of the fungal pathogen in 48 h. The *in-vitro* result obtained was attributed to the direct effect of heat on the spore germination as well as mycelia growth resulting in the reduction of the growth of the fungus on the inoculated red fruit. Splitting was observed on the pericarp (skin) at the point of inoculation of fruits before hot water treatment at 55°C for 5 min. The hot water treatment of the tomatoes had the following effects on the attributes of quality: the Brix degrees measurement showed a negligible difference in 40 °C or 50°C compared with the control for 30 min heat treatment after 24 h storage. Also there was no effect of heat on the total soluble solid likewise, the firmness measurement on flesh of tomato showed no significant difference when compared with the control. In this study the change in colour after heat treatment was not statistically significant. Similarly, in the taste test there appears no real difference recorded in the attributes of juiciness, flavour and overall acceptance except that the skin of the tomato was recorded "softer" by some of the taste panellists. This study has shown that prestorage hot water treatment may be a useful non-chemical method of controlling *A. alternata* postharvest disease pathogen without adverse consequence on the fruit quality.

Acknowledgement

To God be the Glory!

I give glory to God Almighty who in His infinite mercy made it possible for me to fulfil my ambition of obtaining a PhD degree. This achievement is the end of a story that started at the Writtle Agricultural College spanning through 40 years.

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List of Abbreviations

ACC = 1-aminocyclopropane-1-carboxylic acid

AME = Alternariol monomethyl ether

AOH = Alternariol

CABI = Commonwealth Agricultural Bureau

CI = chilling injury

CMA = Corn meal agar

DWPCA = Distilled water principal component analysis

ECS = Evaporative Coolant System

EPA = Environmental Protection Agency

Eth= Ethylene

HIS = Hue, Saturation, and Intensity

HSP = Heat Shock Protein

HWB = Hot Water Spraying and Brushing

HWRB = Hot Water Rising and Brushing

Hwt = Hot Water Treatment + Sodium Bicarbonate Solution (Sbc)

HR = Hypersensitive Reaction

IMI = International Mycology Institute

IMS = Industrial methylated spirit

JA = Jasmonic Acid

LSD = Least Significant Difference

LOX =Lipoxygenases

MT = Maritime tropical

NADPH = Nicotinamide-adenine-dinucleotide-phosphate

NAS = National Academy of Sciences

PDA = potato dextrose agar

PG = polygalacturonase

PCA = Principal Component Analysis

RH =Relative humidity

ROS = Reactive oxygen species

SA = Salicylic acid

SAM =S-adenocylmethionine

SBC = sodium bicarbonate solution

SDW = sterile water

SEM = Standard Error of Mean

SOPP = Sodium ortho-phenylphenate

TA = titratable acidity

TA = tenuazonic acid

TBZ = thiabendazole

TMI = tomato maturity index

TSS = total soluble solids

TWA = Tap Water Agar

V/v = volume/volume

WS = Wheat Straw

W/v = weight per volume

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Tomato (*Lycopersicon esculentum* Mill.) fruit is an abundant crop used by many people all over the world as fruit salad, stew and different culinary delicacies. Recent studies have also emphasised the importance of the major constituent of the fruit; lycopene which is used medicinally to prevent cardiovascular disease and prostate cancer (Ejechi *et al*., 1999). It is an important food crop that can be cultivated all year round, but if not properly handled during postharvest management this will create an opportunity for disease pathogen infection that cause fruit rot in storage.

The cultivation of fruit and vegetables in Nigeria is undertaken by small farmers who usually have a small land holding of less than two hectares. As a result the yield is low and coupled with inadequate postharvest experience, lack of storage facilities and postharvest diseases have made fresh tomato fruit unavailable abundantly all year round in the market in the country. The lack of postharvest management experience, sanitation of the environment of the farm and problem of handling and transportation may lead to pathogen infection which affects the quality of tomatoes. Large quantities of fruits and vegetables are produced and staggering yield figures are quoted as annual production. For example 6 million tonnes of tomatoes was reported as the annual yield (Idah *et al*., 2007). However, it is the amount of the produce available to the consumer that is more important.

The cost of energy and erratic power supply limits the use of low temperature storage also the chilling injury will be another contending issue. As a result farmers who have information about food processing industry harvest red ripe tomato for sale to the company. Tomatoes that are used for culinary purposes are mostly available in the processed form such as sauce, puree and peeled in cans and bottles. Thus, there is the need for an alternative method of preserving fresh tomatoes

which lay waste in the open market and on roadsides during the period of abundant yield in the country.

Fruits and vegetables contain high percentage of water when fresh. Physiological function of respiration is continuing and at this stage of the life cycle of the produce they are more susceptible to pathogen infection in transit and storage. Reports have shown that one of the contributing factors to enormous losses and high price of fresh tomato fruits in Nigeria market is the postharvest pathogen disease infection of the fruit in transit and storage (Idah *et al*., 2007). But there is no evidence to show the exact data for postharvest loss estimates of fruits and vegetables between harvest and consumption except on controlled experimental basis. However, it is reported that losses as high as 50 % are common in these produce between rural production and town consumption in the country (Idah *et al*., 2007). The resultant effect will be low production and short supply to the market creating increase in price.

Some of the diseases include; *Alternaria alternata*, *Botrytis cinerea*, *Rhizopus stolonifer* and *Fusarium* species (Ejechi *et al.,* 1999). *Alternaria alternata f. sp. lycopersici* was reported to cause stem canker disease of fresh market tomatoes. The fungus is a distinct pathogen capable of primary infection of leaves, stems, and on the fruit of susceptible cultivars. Tomato crop was reported to have been the only host of this fungus and only 25 % of 265 cultivars in San Diego County of Southern California, USA were susceptible (Grogan *et al.,* 1975). The studies also showed that when some green fruits were inoculated through artificial wounds into the carpel wall they were almost completely rotted and the surfaces became blackened by dense sporulation, which is the black mould.

The highly perishable nature of tomatoes needs careful treatment during handling after harvest because improper handling such as physical injury during postharvest management may lead to infection by pathogenic diseases. Infection of postharvest diseases which is common through physical injury, together with long distance the produce is transported to the market has limited the availability of fresh tomatoes in the market.

Postharvest technologies for long term storage and disease control are being developed and refined to expand marketing of fresh fruits. Variability in storage potential of fruit with certain quality parameters such as colour grade, maturity, and firmness might become a serious problem for the grower, distributor and retailer to achieve when developing appropriate storage techniques. Due to the restrictions in the registration of the use of inorganic chemicals on fresh produce, the cost of application and the availability of chemicals resulted to attention to be shifted by researchers and farmers to finding an alternative method in controlling disease pathogen of fruit and vegetables. As a result, research efforts are directed towards the development of the appropriate non-chemical treatments to control disease pathogen and maintain fruit quality.

Major improvements in postharvest technology for producing fresh tomatoes can come from refinement of pre-harvest management, harvesting and non-chemical postharvest treatments. Non-chemical treatments which were considered include heat, irradiation, biological control, host resistance and controlled atmosphere storage (Ferguson *et al.,* 2000; Panhwar, 2006; Droby *et al.*, 2009; Schirra *et al.,* 2000). Since consumers have not accepted gamma (ɣ) irradiation method because of the very high capital cost and centralised treatment, host resistance and heat are plausible options for postharvest treatment of fruit and vegetables to control disease pathogen.

The extent of postharvest damage due to spoilage fungi is reportedly dependent on tomato variety (Etebu *et al.,* 2013). The report showed that whilst up to 44 % of postharvest spoilage was attributed to microorganism in a given tomato variety, only 14-23 % of spoilage was attributed to the same microorganism among other varieties. Pre-harvest production practices may seriously affect postharvest return. For example losses due to soil borne fungi like *Phytophthora capsici* is common (Hausbeck and Lamour, 2004). *P. capsici* cause late blight resulting to wilting in tomato crop. *Alternaria solani* causes early blight, *Septoria lycopersici* is responsible for *Septoria* leaf sport and *Fusarium oxysporum* causes wilting in tomato (Etebu *et al.,* 2003). In other to overcome these post-harvest losses due to fungi infection, a resistant variety could be so developed that will

have a wide spectrum of resistant attributes covering these microorganisms. This approach could be recommended for the future.

Another approach to host resistance in fruit and vegetables is to involve the activation of defence mechanisms by non-pathogenic elicitors referred to as induced resistance (Wisniewski *et al.* 2001). Plants generally possess a number of morphological structures that act as a defence to protect them from infection by pathogenic organisms. Many of these resistant features are expressed during normal development, while some are activated in response to pathogen invasion or induced by elicitors. Wisniewski *et al.* (2001) defined the function of elicitors as the activation of defence mechanisms to cause an induced resistance in the crop and are non-pathogenic. These defence mechanisms can be characterised as: (i) structural barriers such as deposition of lignin and waxes on the skin surface (epidermal layer) of the fruit; (ii) attainment of constitutive inhibitors such as antimicrobial compounds; phytoalexins, and; (iii) activation of pathogenesis related proteins including chitinases, glucanases etc. Also Thakur and Sohal (2013) reported that elicitors are compounds, which activate chemical defence in plants, such as salicylic acid, methyl salicylate, benzothiadiazole, benzoic acid, chitosan, which affect production of phenolic compounds and activation of various defence related enzymes in plants.

Furthermore the activation of these defence mechanisms can be by the application of microbial antagonists, such as *Pichia guiliermondii* (yeast), which was reported to be more effective than heat treatment in reducing *Alternaria alternata* in cherry tomato (Zhao *et al*. 2010); whilst *Alternaria* growth was inhibited but not prevented when tomatoes were dipped in hot water at these following temperature and time regimes; thus, 3 min at 55 °C, 5 min at 50 °C and 10 min at 45 °C respectively (Lurie *et al*. 1998; Fallik *et al*. 1993). Another study by Liu *et al.* (2012) reported that heat treatment triggered the accumulation of reactive oxygen species (ROS), which resulted to the collapse of mitochondrial membrane activities and a decrease in intercellular adenosine triphosphate (ATP) processes in *Monilinia fructicola* disease pathogen in peach fruit. The report showed that heat induced the expression of defence-related genes including chitinases,

β-1 and 3-glucanase and phenylalanine ammonia lyase, as a result increase the activities of these enzymes in peach fruit.

There are three methods in use to apply heat to produce; hot water dips, vapour heat and dry hot air (Lurie *et al.*, 1998). Consideration of hot water was the original concept of use to control fungal pathogens on commodities, but was extended to disinfestations of insects (Lurie *et al.*, 1998). Another study showed that dry heat is effective against insect pests, but heat alone was reported not to have adequately control some of the disease pathogens in mangos (Nyanjage, 1999). Most studies have focussed on the effects of heat treatment on mature green and pink colour tomato and disease pathogens; whereas little is known about the effect of heat on red fruit. Fallik *et al.* (1993) reported that most research on the effect of heat treatment on tomatoes laid emphasis on the physiological rather than the phytopathological effects and has focused more on mature green rather than pink or red fruit. However, consumer preferences for tomatoes that are as close as possible to vine ripe are preferred in Nigeria market. Etebu *et al.* (2013) reported in their study that dipping tomato in hot water at 50 °C delayed ripening of pink/light red tomatoes also reduced chilling injury and controlled postharvest diseases of tomatoes.

There is a dearth of knowledge of this method of treatment as result the traditional method of heat treatment engaged in preserving fruit and vegetables is solar drying which is relatively low cost has compared to ware-housing cool storage system. Although there are limitations to this method; only small yield is involved and the fruit is sliced before it can be properly sun dried. As a result most farmers produce tomato for the processing industry so as to secure their investment and guarantee an appreciable income.

The quantity of fresh tomato available in the market is low compared to the yield that goes into the processing industry. As a result the study focused on the need to have an understanding of the overall impact of hot water treatment of red tomatoes. Fallik *et al.* (1993); Barkai-Golan (1989); and Tohamy *et al.* (2004) reports stated that *Alternaria alternata* pathogen inoculated into mature

green tomato caused black mould disease on the fruit. Current methods for the control of this pathogen rely on chemical compounds yet, due to resistance developed by the pathogen and the human perception of chemicals on food, the environment and health, cost and availability, natural chemical of plant and plant materials, non-chemical and environmental friendly control method were sought. Some of these chemicals reported in some studies include chlorine and essential oils for example, plant extracts; red thyme (*Thymus zygis*), clove buds (*Eugenia caryophyllata*) and cinnamon leaf (*Cinnamomum zeylannicum*) were used to control the growth of *Botrytis cinerea* on fruits (Panhwar, 2006). However, chlorination is one of the treatment options available to help manage postharvest diseases. Chlorine dioxide has been used in some sanitizing processes and its action is effective when used with proper postharvest handling practices (Sholberg and Conway, 2004). Thyme oil and essential oil from dill (*Anethum graveolens L.)* were respectively used against fungal spoilage of cherry tomatoes to control *Alternaria alternata* in-vitro and in-vivo as fumigant and contact treatments (Feng *et al*., 2011; Tian *et al.,* 2011). Fumigation with thyme oil was reported not to cause any visible disorders and off-flavour to the fruits after 3 days of incubation.

Feng and Zheng (2007) reported that cassia oil and thyme oil both exhibited antifungal activity against *A. alternata.* The treatment of tomatoes with these essential oils had no adverse effect on quality of fruit such as visible disorders and off-odour to the fruits after 5 days of storage. Another study reported that volatile compounds from plants such as essential oils can inhibit the fungal growth of pathogens while leaving few residues. Apparently, some of these `residues` are normal constituents of the human diet and are unlikely to be of any health risk (Feng *et al.,* 2008). The report of Tian *et al.* (2011) stated that the *in-vitro* and *in-vivo* study of dill (*Anethum graveolens* L.) as an essential oil against fungal spoilage of cherry tomatoes reported that the minimum inhibitory concentration of oil for the four tested fungi; *Aspergillus flavus, Aspergillus oryzae, Aspergillus niger* and *Alternaria alternata*; was 2.0 µl/ml, and the mycelia growth inhibition measured on the 9th day.

It was concluded that the promising results of both studies showed that employing *Anethum graveolens* oil has a significant potential as a fumigant during the usual storage or prolonged transport period. As a result the use of essential oils can not only improve food safety by eliminating fungal spread but also leave no detectable residues after storage. These essential oils would be economical in applications with a comparable commercial importance as a fumigant in storage containers.

For a developing country, preservation of food must be simple and inexpensive. In a study carried out by (Anonymous, 2013) it was reported that each year countries around the world produce some four billion tonnes of food but between 30 % and 50 % of this total, which amount to 1.2 to 2 billion tonnes, never get eaten by the consumers. Likewise, the Institution of Mechanical Engineers reported that as much as half of all the food produced in the world, amounting to two billion tonnes worth ends up being thrown away. The waste is caused by poor infrastructure and lack of storage facilities (Anonymous, 2013).

In another development the central bank of Nigeria made a proclamation to improve the yield of tomato by providing agriculture loan to farmers (Ibeabuchi, 2012). The money will provide infrastructure such as irrigation, seeds, packaging and storage facilities as a result the farmer will be able to plant two crops within one year. The cumulative effect of this assistance will increase yield from 2 million to 10 million tonnes per annum and also the income of the farmers. For an example the farmers will sell a raffia basket (30kg) of tomato for US$3.00 (₦450). During peak season farmers that have limited storage facility can hold on and sell for an equivalent of US$15.00 (₦2200) towards the end of the season (Ibeabuchi, 2012).

1.2 Nigeria as a country

Nigeria is a country in West Africa and is estimated to be the most populous country in Africa with an estimated population of 155,215,573 inhabitants (Taylor and Esan, 2012). Geographically Nigeria is located between longitude 4° and 14° and latitude 2° and 14 °E, making it a country

with a tropical climate type where seasons are damp and very humid. She shares land borders with the Republic of Benin in the west, Chad and Cameroon in the east, and Niger in the north (As indicated in **Figure 1**). Its coast lies on the Gulf of Guinea in the south and it borders Lake Chad to the northeast (Taylor and Esan, 2012). The climate of Nigeria is seasonally damp and very humid which is typical of a tropical country. Nigeria is affected by four climate types; these climate types are distinguishable, as one travels from the southern part of Nigeria to the northern part of the country through the middle belt region.

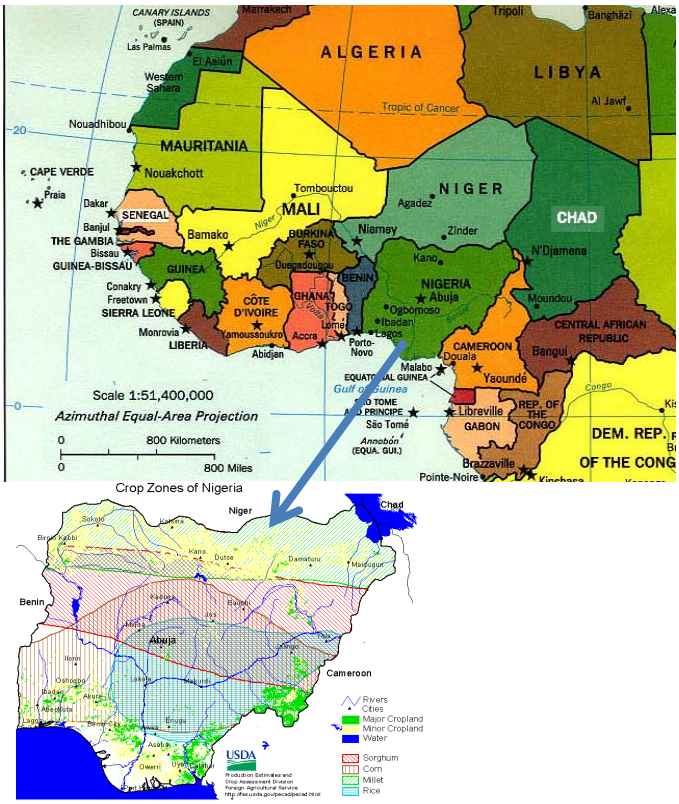

Figure 1: West African countries and the crop zones in Nigeria (USDA, 2002/03)

1.3 Climate types found in Nigeria

The Tropical rainforest climate or the Equatorial monsoon is found in the southern part of the country. This climate is influenced by the monsoons originating from the South Atlantic Ocean, which is brought into the country by the maritime tropical (MT) air mass, a warm moist sea to land seasonal wind (Briney, 2010). Also the Tropical rainforest climate in the country has a very small temperature range such that the temperature ranges are almost constant throughout the year, for an example, a

town (Warri) in the southern part records a maximum temp of 28 °C for its hottest month while its lowest temperature is 26 °C in its coldest month which is suitable for tomato production all year round.

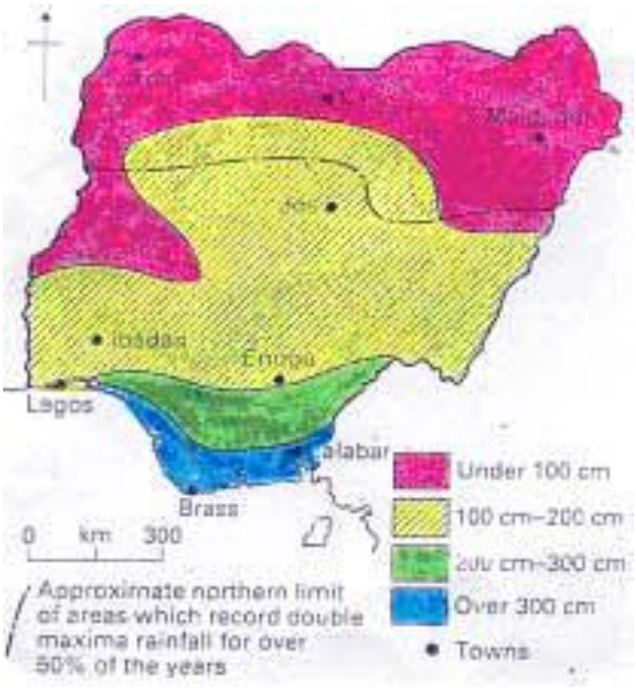

Figure 2: Rainfall pattern in Nigeria (Source: FAO, 2005)

There is heavy and abundant rainfall in the southern part of the country which results into storms and heavy flooding due to the regions proximity to the equatorial belt, as a result the annual rainfall in this region is usually above 2,000 mm (78.7 in), particularly in the south east (Briney, 2010). About 4,000 mm (157.5 in) of rainfall is recorded for a coastal town (Brass) in the Niger Delta area (**Figure 2**). The first rainy season begins in March and last to the end of July with a peak in June, this is followed by a short dry break in August known as the August break; mainly for harvesting and is a short dry season for 2-3 weeks. There is another short rainy season starting early September lasting to Mid-October and is followed by long dry season that stays till March with peak dry conditions between December and February (Briney, 2010). The Tropical Savannah climate is extensive in area and covers most of Western to central Nigeria beginning from the Tropical rainforest climate boundary in the south to the central part where it has an enormous influence on the region - **Figure 3** (Anonymous, 1997).

The tropical savannah climate exhibits a well-marked rainy season and a dry season with a single peak known as the summer maximum as a result of its distance to the equator. Average temperatures are above 18 °C throughout the year (Briney, 2010). Other climates in the country where tomatoes are successfully grown include; the Sahel Climate or Tropical dry climate, and is the predominant climate type in the northern part of the country. The annual rainfall totals are lower compared to the southern and central part of Nigeria. Alpine climate or highland climate or mountain climate are found on highland regions. Highlands with the alpine climate are over 1,520 metres (4,987 ft.) above sea level. Due to their height in the tropics, this elevation is high enough to reach the temperate climate in the tropics thereby giving the highlands, mountains and the plateau regions standing above this height a cool mountain climate which make these areas conducive to tomato production (Briney, 2010).

Consequently, the climactic difference in the country has influenced the growth of crops between zones and seasons. Tree crops such as cocoa, oil palm, avocado pear and other fruit trees are found in the southern part leading to the central region, while crops that require less amount of water for production, for example vegetables; tomato and pepper are cultivated mostly from the central to savannah zones. For example the cropping of tomatoes during the wet and dry seasons contributes immensely to the national yield average of 114 tonnes/ha but the bulk of fresh tomato production is from the dry season cropping particularly in southern states (Olaniyi *et al.*, 2010). The production of tomato (*Lycopersicon esculentum*), in dry season is mainly by irrigation in the northern part due to low rainfall. But marketing and consumption are widespread throughout the country. As a result fresh produce travelled a long distance between the farm and the market.

Tomato is transported by road and the system is associated with many problems that affect the quality of the fresh produce. The study by Idah *et al.* (2007), reported that there are no clearly defined routes for any particular produce, but the market forces dictate the handlers` choice of market. The delivery period of the produce from the farm to the market on the average normally

spends four to five days in transit, out of which two days are spent on movement for example from Kano (north) to Lagos (south) and the remaining days are spent at the source market coupled with ten days of shelf life. Aba *et al.*, (2012); Idah *et al.*, (2007) also reported that losses up to 14-20 % occurred in the consignment of fresh tomatoes transported from the production areas in northern part to an urban wholesale market in South West Nigeria, a distance of about 1000 km. These losses, it is noted occurred during transportation, storage and marketing. As a result the expected post-harvest life of tomatoes in Nigeria is between ten and fifteen days.

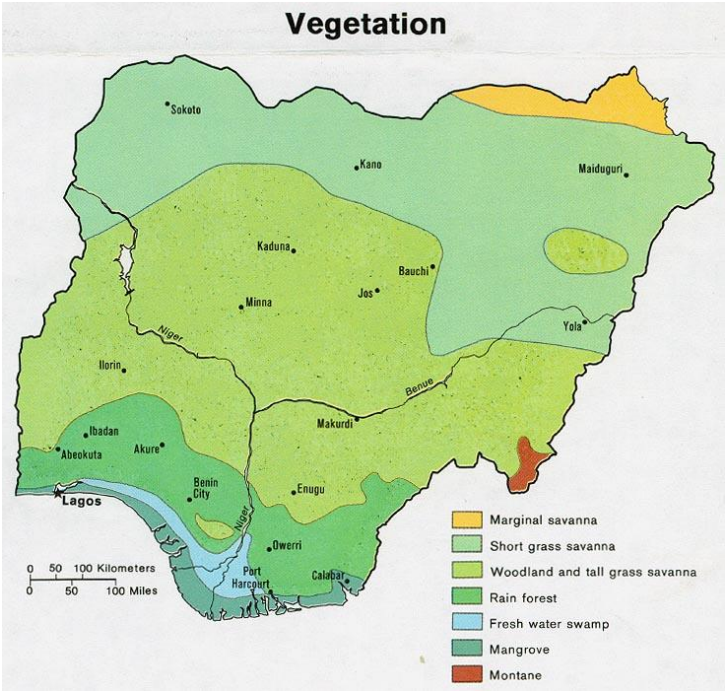

Figure 3: Nigeria vegetation

1.4 Tomato production in Nigeria

Nigeria is a leading producer of large amounts of tomatoes, pepper and other vegetables which are grown in its diverse agro-ecological zones that range from humid in the south to sub-humid in the middle belt and semi-arid/arid in the north. Fresh fruits and vegetables are inherently more liable to deterioration under tropical conditions characterised by high ambient temperatures and humidity, and high incidence of pests and diseases. Consequently, many studies including (Anonymous, 2013), have reported postharvest losses of fruits and vegetables are very high in the

country (30-50 %), and this situation was worsened by poor marketing, distribution and storage facilities.

Large scale cultivation of the crop is often practised in the northern part of the country vis-à-vis in the Savannah region. Nigeria has two main rainfall seasons; the first raining season starts in March-June the second rainfall begins in Oct-Nov. The rainfall pattern is bimodal that is having two peak period. It is at the lesser peak that tomato production gives the potential maximum yield (Oct-Nov). The climatic condition at this season is conducive to the production of tomato; since the crop is self-pollinated and requires mild rainfall for pollination. The total yield of tomato is about 1.7 million tonne per year from a total area of one million hectares used for tomato cultivation (Etebu *et al.,* 2013; Costas and Heuvelink, 2005), most of which go in to the processing industry. The quantity of produce that goes into high wastage is staggering due to the inability of farmers to provide post-harvest treatment and storage infrastructures.

Nigeria is one of the top twenty producers of tomato in the world and the second largest producer in Africa (Etebu *et al.,* 2013), having a total production of 1,701,000 tonnes per year which make the country the 13th largest producer in the world (Costas and Heuvelink, 2005; FAOSTAT, 2008). An average yield is 25 tonnes per hectare which is compared low to what is obtained in Egypt with 9.2 million tonnes per year and 50.8 tonnes per hectare a similar developing country like Nigeria. Information about the actual loss of food between harvest and consumption are not documented, also the available data for post-harvest loss estimates for fruits and vegetables are difficult to substantiate except on limited controlled experimental basis. But Idah *et al.* (2007) reported that losses as high as 50 % are common in fruits and vegetables between rural production and town consumption in the tropical regions. These losses, it is noted, occurred during transportation, storage and marketing.

Etebu *et al.* (2013) also reported that 21 % of tomato harvested in Nigeria was lost to rot in the field while an additional 20 % was due to poor storage system, transportation and marketing. In

Mauritania, Bishop and Ramma (2012) reported in their study that only 25 % of the tomato fruit fully ripened for market within a week of harvest the balance is the estimated loss through dehydration, disease and inadequate postharvest resources and management. These losses have prompted the need for simple, but effective and economical methods to control pre- and postharvest diseases and other losses in tomato.

Figure 4: Traditional packaging and transportation of tomato in jute bags.

Postharvest practices such as sanitation of the environment and packaging material should be without blemish that will encourage disease infections caused by microorganisms. The main factors affecting postharvest losses of tomatoes include; moisture loss through storage at too high temperature and low relative humidity; disease development through physical contact and storing tomato fruit at temperature conducive to disease growth. Mechanical damage can occur as a result of fruits stacked in jute bags resting on top of each other on a trailer truck – **Figure 4**. This abrasion damage could cause increased moisture loss and increase likelihood of disease entry since part of the skin layer (pericarp) has been removed. The depth of stacking in the jute bag can also cause compression damage.

Other factors that inhibit large quantity of fresh produce being provided at the markets include very limited storage facilities, poor crop cooling system, and the logistics of transportation from the farms located in the northern part of Nigeria to the south where the produce are sold at a

premium price (**Figure 4**). The picture is a typical example of how tomatoes are packed in jute bags and stacked on top of each other to the market. For example, a substantial loss (up to 20 %) typical of this transport system was reported to have occurred in fresh tomatoes being transported from the production areas in northern Nigeria (e.g. Kano, Kaduna and Sokoto States) to an urban wholesale market (Shasha) in Ibadan south-western Nigeria (Aworh, 2009). This situation can cause bruising of fruits and compression damage in the bag which can lead to an infection of disease pathogen. Consequently, many farmers have resolved to produce the crop for the processing industry where they are put in cans and bottles as tomato sauce, plum and puree.

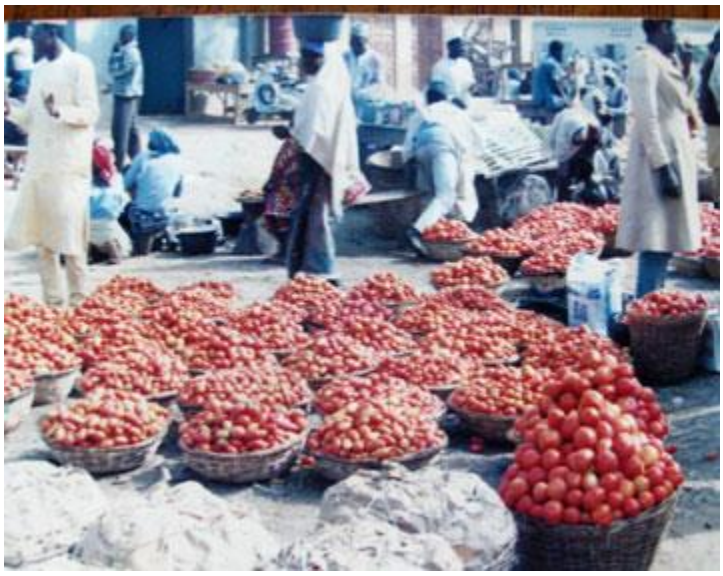

Figure 5: Tomato in basket ready for sale

Figure 5 showed red ripe tomatoes traditionally packaged in basket and covered with brown paper ready for sale in the market. This method of packaging can cause bruising of fruits and compression damage in the basket which can lead to disease infection. Diseases such as *Botrytis cinerea* (grey mould) and *Alternaria alternata* (black mould) were common causes of tomato fruit disease pathogens that cause fungal rot (Fallik *et al*., 1993; Barkai-Golan, 1989; Tohamy *et al*., 2004). The conventional method of controlling these diseases is through the application of fungicides such as: chlorothalonil, benlate, rovral, and sumisclex (Davis *et al.*, 1997); Abdell-Mallek *et al.* (1995). At 1000 ug/ml benlate, rovral and sumisclex completely prevented *Alternaria* rot (Abdell-Mallek *et al.*, 1995), while chlorothalonil, a chloronitrile fungicide serves as a broad spectrum tomato disease

management agent. For example, it is recommended in Florida for management of target spot, and also control early and late blight of tomato (Anonymous, 2014). The price of chlorothalonil is estimated as $10.32 per pound of active ingredient, and the approximate cost of a maximum application of 2.1 Ib active ingredients per acre in Florida is $21.67 (Anonymous, 2014).

To show the similarity between potato and tomato, in terms of the cost and application of fungicides to control early and late blight diseases, the mean cost per hectare of the fungicide and application to control these diseases on potatoes was $316 per hectare ($128 per acre) in the Columbia Basin of Washington, whilst the cost of the active ingredient is $7.33 per Ib. (Johnson *et al.,* 2000). In another study it was reported that the cost of the fungicide i.e. Chlorothalonil and its application, a single dose applied to plots of tomatoes would give the grower $160.47 net return per hectare (Davis *et al.,* 1997). The cost implication of these chemicals and the availability in the local market is relatively high for small scale farmers.

Studies have shown that many postharvest treatments have been developed to control the development of the fungal pathogen causing tomato fruit rot through non chemical method using these treatments individually or in combination with food preservative compounds and solutions (Ejechi *et al.,* 1999; Yan Zhao *et al.,* 2010). Lurie and Klein, 1991, reported that mature green tomato fruit kept for 3 days at 36, 38 and 40 °C respectively, before storing at 2 °C for 3 weeks did not develop chilling injury, while the unheated fruit placed in the storage at the same temp developed chilling damage. The heated tomatoes also had lower levels of ion leakage and higher phospholipid content than unheated tomatoes.

Heat treatment has also been identified by various reports to be one of the most promising non chemical control methods for post-harvest fungal diseases. The efficacy of pre-storage heat treatment either as dry or hot water dips was reported to reduce storage rots on bell peppers and tomatoes. The best results were obtained with hot air at 38 °C for 48-72 h or hot water at 50 to 53 °C for 2-3 min (Lurie, 1998; Fallik *et al.,* 2001). Fallik, (2004) reviewed the latest developments in hot water immersion treatment and hot water rinsing and brushing technologies and concluded that these

treatments kill disease pathogens that cause surface decay, while maintaining fruit quality during storage and marketing. These studies showed that heat treatment inhibited germination of fungal spores and the development of mycelia, thus effectively reducing inoculum population and lesion development on the produce (Ferguson *et al*., 2000; Lurie *et al*., 1998; Fallik *et al.*, 1993; Couey, 1989).

Studies have shown that heat treatment can be applied to fruit and vegetables as; (i) hot water dips, (ii) vapour heat and (iii) hot dry air. For an example dipping for 1-2 min in water heated to 55 °C was the optimal anti-fungal treatment reported for the control of *Alternaria, Fusarium, Rhizopus*, and *Mucor* species on melon fruit (Teitel *et al*., 1991; Fallik *et al*., 1993; 2001). Vapour heat was used on strawberries for 1hr at 44 °C, while pear fruit was exposed for 48 h at 37 °C prior to storage, inhibited the decay caused by these pathogens (Spotts and Chen, 1987). While in another study by Jacobi and Wong (1992) reported that mango (*Mangifera indica Linn.)* treated with hot water at temperature of 47 °C from 7.5-30 min shortened the fruit softening time but caused internal and external injury.

Heat treatment on the effect of hot air on tomatoes, showed that the temperature of effective control was between, 36-40^{0}C (Fallik *et al*., 1993; Lurie *et al*., 1998; Lurie, 1998; Ferguson *et al*., 2000). For an instance hot air at 38 °C, for 24 h was enough to inhibit germination of *Botrytis* and *Penicillium* spores; while after 96 h at 38 °C, *Alternaria* still showed 20 % germination (Lurie *et al*., 1998). In this instance the decay caused by *Botrytis* in tomato, *Botrytis* and *Penicillium* in apple were reduced. Another report showed that germination of *Alternaria* could only be inhibited by extended temperature above 40 °C and time period, for example 96 h at 46 °C or 72 h at 42 °C (Lurie *et al.*, 1998; Tohamy *et al.*, 2004). This shows that high temperature heating for long period is common with hot air heat treatment.

Fruits are treated in hot water for shorter times at high temperature than hot air. Lurie et al., (1998) reported that ten minutes at 45 °C decreased *Botrytis* germination to below 10 % while 5 min at 50 °C prevented it. Although, germination of *Alternaria* was more resistant to heat because some germination was reported to have occurred at all time and temperature regimes tested. For example, 3 min at 55 °C, 5 min at 50 °C and 10 min at 45 °C stopped the development after germination of *Botrytis* spores while *Alternaria* growth was inhibited but not prevented by all treatments. These reports on the effect of temperature on Alternaria disease served as a background knowledge to know the temp range to start with and also the heating time that will control the pathogen. As a result this study focused on the *in vitro*, *in vivo* and the effect of heat treatment on the quality of tomato. These temperatures 30, 40, 45, 50 and 55 °C together with 5, 10, 20, 30 and 60 min were variously combined to test the effect of heat on *Alternaria in vitro* and *in vivo* trials.

Most research reports on the effect of heat treatments on tomatoes has focused on the physiological rather than the phytopathological effects and has involved the use of mature green rather than pink or red fruit (Fallik et al., 1993). However, the consumers prefer tomatoes that are as close as possible to vine ripe as a result the farmers prefer to harvest the fruit for market at pink rather than the mature green stage. The report of reviews of Klein and Lurie (1992) stated that ripening of fruits is accompanied by a change in the ground colour, for example in apple the colour changes from green to yellow. Also prestorage heat treatment accelerated the colour change. Similarly, heated tomatoes were redder than non-heated fruit after storage at 12 °C and shelf life at 20 °C (Klein and Lurie, 1992).

Treating inoculated red pepper fruits for example in 3 min with 50 °C hot water completely inhibited decay development caused by *Botrytis* and significantly reduced decay caused by *Alternaria*; likewise a reduction of decay in area of infection was observed with *Botrytis* infected fruit dipped for 1 and 5 min hot water (Lurie *et al.,* 1998; Fallik *et al.,* 1996). Similarly, dipping *Alternaria* infected fruit at 50 °C for 5 min reduced decay compared to control fruits, although

heat injuries such as cracking on the skin surface of tomatoes were observed on fruit dipped for 5 min (Lurie *et al.*, 1998; Lichter *et al*., 2002). Hot air treatments required a long term heating at high temperatures and are unlikely to become a commercially attractive method. But hot water is least expensive, more effective and takes a shorter time to reach the inner core of the fruit compared to hot humid air. In another report the use of hot water dips or vapour heat at 39-52 °C for 2-10 min has been reported to control *in vitro* and *in vivo* spore germination and decay development of postharvest fungi on tomato and pepper (Fallik *et al.,* 1993; Lurie *et al.*, 1998).

The potential of hot water treatment to control fungal pathogen diseases is promising since the capacity of water to transfer heat is faster than air. For an instance, it was reported that when tomatoes were immersed in water at 20 °C; the interior of the fruit reached the water temp within 30 min while it took 4 h in air (Lurie *et al.,* 1998). Most of the studies on the use of hot water treatment to control the microorganisms that cause diseases on tomato reported the importance of high temperature in a short time period, failing which can easily result to damage of fruit tissue if the recommended exposure time is exceeded. Thus, 3 min at 55 °C, 5 min at 50 °C and 10 min at 45 °C were recommended in other to prevent injury to the fruit surface (Fallik *et al*., 1996; Lurie *et al.,* 1998). For example, tomatoes dipped in hot water at 45 °C above 10 min suffered injury such as shrivelling of tomato fruit pericarp also pepper fruit dipped at 55 °C above 3 min had significant water loss and softness due to heat damage causing cracks as well as pitting on the surface of the treated fruit (Fallik *et al.,* 1996; Lurie *et al*., 1998). Consequently, these has informed this study to determine the appropriate heat treatment temperature and time period that will control postharvest disease pathogens of tomato without causing injury to the fruit surface.

1.5 Hypothesis

Improvement on the quality and shelf-life of mature green tomato fruit following hot water treatment was exhaustively reported in relation to the effect on chilling injury and postharvest fungal disease control in storage. Many studies have also shown that the control of postharvest decay of fruit with hot water involved a direct inhibitory effect on the postharvest pathogens and a

resistance development in the host plant. The studies also showed no significant adverse effect of heat treatment on the fruit quality, aroma and taste.

The hypothesis of this study is that the reports of the above mentioned effect of heat treatment on mature green tomato were encouraging then this quality improvement method could be tested on red ripe tomato through experimentation and possible get the same results by using hot water as a non-chemical and sustainable disease control method of *Alternaria alternata* disease pathogen.

1.5.1 Aims and Objectives

The objectives of this study were therefore largely two fold. First to evaluate the efficacy of hot water treatments as a non-chemical control method of *Alternaria alternata* disease pathogen of tomato. Second objective also included the effect of heat treatment on the fruit quality and shelf-life. The study will conduct experiments to determine the effectiveness and confirm: (1) the temperature and time to control Alternaria alternata disease pathogen spores germination (*in-vitro*). Also to look at the effect of hot water dipping on red ripe fruit (*in-vivo*) and the required time to reduce the decay caused by the pathogen; (2) other parameters to be evaluated will include: changes in fruit quality after heat treatment for example, a*/ b* value for colour change, firmness of the fruit, total soluble solids and weight loss. In addition, untrained taste panelists will be set up to evaluate the effect of hot water treatment on the aroma, texture, juiciness, flavour and overall acceptability of the fruit compared with non-heat treated tomato.

1.5.2 Experiments Layout

- Method of counting the number of *Alternaria alternata* Conidia Spores using Haemocytometer
- The Colony Forming Unit (CFU) method to count the number of Conidia Spores of *Alternaria alternata* Spore Suspension
- The production of *Alternaria alternata* Spores on Culture Plates using Mycelia Plug
- The use of Single Spore to Test the Pathogenicity of *Alternaria alternata* Isolates on Culture Media

- The Dilution ratio method to Count the number of Conidia Spores of *Alternaria alternata* Suspension
- Experiment of Hot Water at 45 and 50 °C for 5 and 10 min Treatment of *Alternaria alternata* Spore Suspension
- Experiment of Hot water at 30, 40 and 50 °C treatment for 20, 30 and 60 min respectively of *Alternaria alternata* Spore suspension using Eppendorf vial
- The effect of hot water at 40, 45 and 50 °C treatment for 10 min on inoculated tomato fruits
- The effect of hot water at 50, 40 and 30 °C for 20 min treatment of inoculated tomato fruit
- The effect of hot water at 50 and 55 °C for 5 min on inoculated tomato fruit
- The effect of hot water treatment on the physiological processes during colour development of tomato
- The effect of heat treatment on tomato fruit firmness
- The effect of hot water treatment on total soluble solids (TSS) of tomato
- The effect of hot water treatment on weight loss of tomato fruit
- The effect of hot water treatment on the aroma, texture, juiciness, flavour and overall acceptability

The above experiments will be carried out so as to show how postharvest hot water treatment of tomato fruit can reduce pathogen infection, reduce decay development and subsequently improve the quality and storage shelf life. The effect of hot water on the skin of the fruit e.g. ethylene production, colour development and softening will be explored so as to establish a relationship between these variables and ripeness, firmness, aroma and taste which are the most important

factors for determination of tomato quality as reported by Lurie *et al.*, (1996); Thanh and Alcedo, (2006).

The shelf life is a period of time which starts from harvesting and extends to the start of rotting of fruits (Nasrin *et al.*, 2008). Rotting occurs as a result of excessive softening (Meli *et al.*, 2010) that limits the shelf life which can lead to microbial infection consequently produce poor quality fruit. Also this study will investigate the effect of heat on the fruit after 24, 48, 72 and 96 h storage. Furthermore, reports have shown that pre-storage hot water dip of inoculated mature green and pink tomato fruits inhibited decay caused by postharvest pathogens of tomatoes (Lurie *et al.*, 1998; Fallik *et al.*, 1993). Therefore, this study will conduct experiments to show the effect of heat treatment as a method of inhibiting the decay caused by *Alternaria alternata* disease pathogen on red ripe tomato based on the reports of mature green fruit.

CHAPTER 2: LITERATURE REVIEW

2.1 Tomato botany and importance

Payal (2010) stated that tomato was considered as a vegetable simply because it is a plant that is grown primarily because of the fruit which is the edible part. Botanically, tomato can also be regarded as a fruit (berry), since it fits into one of the two meanings given to a fruit which is the popular term, `Knowledge puts tomato as a fruit but wisdom puts it as a vegetable`. The common name fruit is that part of a plant which is eaten as a desert after the main meal or a snack because of the sweetness. Similar analogy is often used for apple and peach which are regarded as fruit. Botanically, a fruit is the mature ovary and also the reproductive organ from which the plant was developed.

2.1.1. Classification of the cultivated tomato

Heuvelink (2005) and Marshall (2006) classified the tomato to belong to the family *Solanaceae* (the nightshade family), genius *Lycopersicon*, sub family *Solanoideae* and tribe *Solanaceae*. The plant family Solanaceae include such important crops such as chilli and bell peppers (*Capsicum* spp), potato (*Solanum tuberosum*), aubergine (*Solanum melongena*), tomatillo (*Physalis ixocarpa*) and tobacco (*Nicotiana tabacum*).

In the 18th century, the famous Swedish botanist, Linnaeus, introduced his Binomial Nomenclature of plants, where he named tomato *Solanum lycopersicon*. Fifteen years later, another renowned botanist (Phillip Miller), changed the Linnaean name with *Lycopersicon esculentum*. Lately, the taxonomists have reintroduced *Solanum lycopersicon*, the name given by Linnaeus (Heuvelink, 2005). The taxonomic classification of the tomato has remained debatable till today. The widely accepted and used name in both text books and journal articles is *Lycopersicon esculentum Mill* (Heuvelink, 2005; Marshall, 2006)

Taxonomic classification

Common name: Tomato

Latin name: *Lycopersicon esculentum*

Family: Solanaceae

Chromosome number: Diploid; 2n = 24

Plant group: Dicotyledon

Growth habit: Perennial; grown as annual

Climate: Tropical crop; frost sensitive

Origin: Andean region (Chile, Colombia, Ecuador, Bolivia and Peru).

Source: (Heuvelink, 2005; Marshall, 2006; Purdue University, 2007)

2.1.2 Origin and evolution of the cultivated tomato

Many of the related wild species of tomato originated from the Andean region , that includes , parts of Chile, Colombia, Ecuador, Bolivia and Peru (Marshall, 2006; Heuvelink, 2005). The ancestor of the cultivated tomato is the wild species of *Lycopersicon esculentum* variety *cerasiforme* (cherry tomato) and they are indigenous of sub-tropical and tropical America. The extensive domestication of tomato started in Mexico, which is quite a distance to its ancestral home (Heuvelink, 2005; Marshall, 2006; Encyclopaedia of Food and Culture, 2010).

2.1.3 Domestication of the tomato in Europe

The Spanish were the people that introduced the tomato into Europe in the early 16th century (Heuvelink, 2005; Marshall, 2006). The report stated that the Spanish came across tomatoes after their conquest of Mexico began in 1519. The plants were disseminated first to the Caribbean, and then to Spain and Italy. The Europeans were slow in accepting tomato as a cultivated crop and as an inclusion of their culinary recipes (Marshall, 2006). Tomato was cultivated as an ornamental plant, simply because the fruits were considered poisonous, and also regarded as a close relative to the deadly nightshade (*Solanum dulcamara*) family (Heuvelink, 2005). By the mid-16th century tomato became widely cultivated and consumed in south European countries; like Italy, and

Spain, but became widespread by the end of 18th century in the north Western Europe (Heuvelink, 2005).

2.1.4 Introduction of tomato in Sub-Sahara Africa

In the Encyclopaedia of Food and Culture (2010), it was reported that by the time tomatoes were consumed in southern and north Western Europe, in mid-16th century and in late 17th century, the first known tomato recipe appeared in the cookbook – Lo scalo alla moderna and was written by Antonio Latini. During this period, tomatoes were also consumed in eastern Mediterranean and North African countries. Later tomato cookery expanded into northern and eastern European countries, and finally spread to sub-Sahara Africa, South and East Asia.

Tomato (*Solanum lycopersicum* L.), a plant species in the *Solanaceae* family, was reported to have originated in the Americas. Like its close relatives, chilli peppers and potato, tomato was probably introduced to Africa in the 16th century (Wesonga and Kahane, 2011). According to the statistics the largest area and the highest production in Africa are found in northern Africa, including Egypt, Morocco, and Algeria, and the smallest area and the lowest production in southern and central Africa respectively (Wesonga and Kahane, 2011). The total production area in Africa increased from 159,593 ha in 1961 to 660,215 ha in 2007 and production increased from 1,968,812 tons in 1961 to 14,918,554 tons in 2007 (Wesonga and Kahane, 2011). The average yields range from 6 t/ha in central Africa to 34 t/ha in southern Africa. South Africa production contributed to this higher productivity.

2.2 TOMATO PRODUCTION AND QUALITY

Morphologically, tomato fruit is a berry and the seeds are embedded in a jellylike proteinous matrix with the ovary wall developing in to the flesh of the fruit. The total world production stands at 152.9 million ton with a value of $ 74.1 billion (Rakha *et al.*, 2011).

2.2.1 Tomato market and types

Growth habits: There are three different types of cultivars:

(i) Determinate: In this cultivar the primary shoot produces 0 to 2 leaves followed by the flower cluster but no further vegetative shoots (Payal, 2010). This process makes the main stem much shorter. The side shoots which are many arise from the main stem and all terminate with a flower cluster. Determinate or the Bush tomato, as otherwise called, is mainly used in the food processing industry and is the most important commercial outdoor type (Taylor, 1986). It is common with this cultivar to have one time flowering period followed by fruit set.

(ii) Semi Determinate: Shoots of this type produce many flower clusters at the side of the main stem just similar with what happens with indeterminate varieties (Marshall, 2006). Eventually, the shoot terminates in a flower cluster, thereby making the plant to behave as determinate.

(iii) Indeterminate: Payal (2010) showed that the main stems of indeterminate varieties of tomato can grow indefinitely and can reach a height of up to 3-6 meters. Furthermore, the characteristic of this cultivar was stated that the shoot continues to grow upward and flower clusters develop to the side of a main stem. Also the growth of the primary shoots usually end up with the formation of the first flower and the upward growth will continue to produce a side shoot that will give rise to three more leaves before terminating in a flower cluster. This process of producing new growth and side shoot from the last leaf initiated before the flower cluster continues indefinitely (Payal, 2010). The indeterminate or the vine variety of tomato is used for the production in the glass/green houses. The varieties produce flower inflorescence and set fruit throughout the life of the plant (Marshall, 2006).

Another classification method of the varieties of tomato was reported by Costa and Havelink (2005) which was based on the difference between the characteristic of fresh and processed tomato. Varieties of processing and fresh-market tomatoes have different growth habits. The major characteristics of processing tomatoes are; determinate growth, dwarf habit, uniform fruit set and ripening, tough skin and a high soluble solids content (Costa and Heuvelink, 2005). The plants are grown mostly in the field.

The fresh markets varieties are grown largely in the greenhouses, indeterminate type and require trailing on galvanised wire or polystyrene string (Payal, 2010) – **Figure 6**. Much larger number of varieties and cultivars are available for fresh market production: (Costa and Heuvelink, 2005; Marshall, 2006; Payal, 2010).

Figure 6: Breaker stage of round tomatoes in trailing and galvanised wire

Classic round tomatoes: The most popular varieties have a round shape and contain two to three locules. The average weight of a fruit is between 70-100 g, and the diameter is 4.7-6.7 cm.

Cherry and cocktail tomatoes: The fruit is smaller than the classic tomato and having a weight ranging between 10-20 g and diameter 1.6-2.5 cm. The fruit of cherry tomato is common with the red colour, but there are some other colours like; golden, orange and yellow. They are usually left on vine to ripe.

Plum and baby plum tomatoes: The fruits are small and oval in shape; whereas the flesh is firm and less juicy in the centre (**Figure 7**).

Figure 7: Plum tomatoes in basket ready for market

Beefsteak tomatoes: They are larger than the traditional round tomato; the weight is between 180-250 g. It contains five or more locules (**Figure 8**).

Figure 8: Beef-steak tomatoes display in the market

Vine or truss tomatoes: They can be either classic round or cherry type. The most important market quality required is the aroma which is produced by the fruiting trusses (**Figure 9**).

Figure 9: Vine tomatoes attached to the stalks

Tomato is one of the most important vegetable crops grown all over Nigeria. It is the world`s largest vegetable crop after potato and sweet potato but it tops the list of canned vegetables. The largest producer of tomato is China, followed by USA as indicated in **Table 1**.

Nigeria is listed the 13th world`s largest producer of the crop but came second after Egypt among African countries (GEOHIVE, 2013). Some of the varieties commonly grown include; Tropical, Roma VF, UC82B, Ibadan local and Ogbomosho local (Olaniyi *et al.,* 2009). In Nigeria the crop is regarded as the most important vegetable after onions and pepper. Tomato fruit contains good source of vitamins such as vitamins A, C, and E and minerals that essential to the body as protection against diseases. The total worldwide production of both fresh and processed tomatoes, based on 2003 estimates was 110 million tonnes (**Table 1**), produced from an area of about 4.2 million hectares (Costa and Heuvelink, 2005).

Table 1: The top twenty tomato producing countries of the world

Regions of Tomato production (tonnes)	
People's Republic of China	33,911,702
United States	13,718,171
Turkey	10,985,355
India	10,303,000
Egypt	9,204,097
Italy	5,976,912
Iran	4,826,396
Spain	3,922,500
Brazil	3,867,655
Mexico	2,936,773
Russia	1,938,710
Uzbekistan	1,930,000
Nigeria	1,701,000
Ukraine	1,492,100
Greece	1,338,600
Morocco	1,312,310
Chile	1,270,000
Tunisia	1,170,000
Syria	1,163,300
Portugal	1,147,600

(Source: GEOHIVE, 2013)

2.2.2 Structure, composition and physiology of tomato

The genus *Lycopersicon* is believed to consist of the cultivated species, *Lycopersicon esculentum* and seven other wild species (Taylor, 1986). Earlier taxonomy of the genus was not relied upon because species and races have increased from the origin of diversity, South America (Taylor, 1986; Purdue University, 2007).

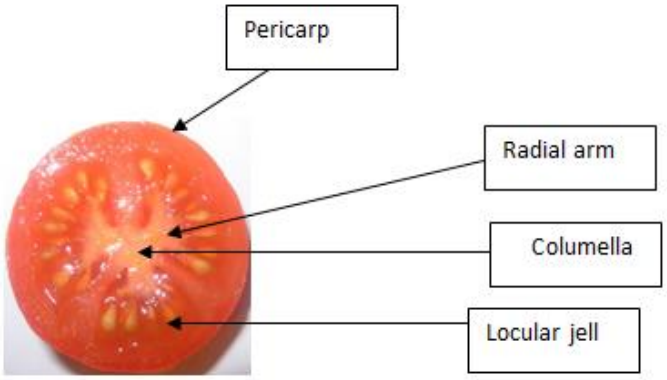

Figure 10: Anatomy of tomato fruit with multi-locular structure showing transverse section (Walls, 1989).

The anatomy of a tomato fruit consist of pericarp, radial arm, columella, and locular jell as illustrated in **Figure 10**. The pericarp originated from the ovary wall and consists of an exocarp or skin, a parenchymatous mesocarp with vascular bundles and a single-celled layer of endocarp lining the locules (Ho and Hewitt, 1986). The radial arm (septa) of the pericarp separates adjacent locules and the inner wall (columella), while the columella is located in the centre of the fruit. The septa and columella similarly have parenchyma cells. Locular jell is parenchymatous and is found around seeds (Ho and Hewitt, 1986).

2.2.3 Physiology

Tomato flowers are self-pollinated. During flower opening ‘Anthesis’, the stigma is about two days ready to receive the pollen and can remain so for up to four days or more (Ho and Hewitt, 1986). Once the flower is pollinated, the pollen tubes start to grow down the style to reach the

micropyle of the ovule within 12h at 25 °C. Most of the ovules will be fertilized within 30 h at 20 °C (Ho and Hewitt, 1986). In another development, Ahrens and Huber (2006) correlated the process of tomato fruit development to ripening and the characteristic changes that take place during this process are genotype dependent. By using the intrinsic variation in firmness, polygalacturonase activity and other ripening parameters, including rate (days from mature-green to full red) and intensity (rate of ethylene production at climacteric peak) of ripening, a quantitative relationship was developed between these quality variables.

Texture, respiration and ethylene production were measured and used as benchmark to monitor the immature-green through the red ripe stages of fruit development. The results showed that in all fruit the polygalacturonase activity was highly correlated with pericarp softening, but only moderately correlated to the whole fruit. Polygalacturonase was also reported to have been involved in the cell wall autolytic activity in changing the fruit colour from pink to red. Fruits having firmer genotypes also exhibited low rate of respiration and ethylene production during ripening (Ahrens and Huber, 2006).

2.2.4 Biochemistry

Several metabolic changes are initiated after the harvest of fruits. In fruits an increase in the biosynthesis of the gaseous hormone ethylene serves as the beginning of physiological changes for the initiation of the ripening process (Paliyath and Murr, 2008). All plants produce a low level of ethylene, during ripening process some fruits evolve large amounts of this gas, sometimes defined as an autocatalytic increase in ethylene production. This process correlates with an increase in respiration referred to as the respiratory climacteric (Paliyath and Murr, 2008). Fruits are generally classified into climacteric and non-climacteric based on the ethylene produced and their responses to external ethylene added. The climacteric fruits characteristically demonstrate an increase in ethylene production and respiration as noticed by the evolution of carbon dioxide. In climacteric fruits such as apple, tomato and avocado, ethylene evolution can reach 30-500 ppm/(kg/h),whereas in non-climacteric fruits such as orange, strawberry, and pineapple, ethylene

levels range from 0.1 to 0.5 ppm/(kg/h) during ripening (Paliyath and Murr, 2008). Since ethylene has been linked to fruit ripening the biosynthesis of the gas can be regulated technologically in storage so as to improve the shelf life and quality in fruits. Controlled atmosphere storage with low oxygen reduces ethylene production, while scrubbing is another biotechnological practice in storage facilities for the same purpose.

Many studies including Lurie (2008) stated that in climacteric fruits such as tomato and apple, which rely on ethylene for their coordinated ripening processes, heat treatment of fruits with high temperature inhibits ethylene evolution and subsequently inhibit many ripening processes, including fruit softening, colour changes, and aroma development.

2.2.5 Nutritional value

Fruits and vegetables are very essential in our diet and important to our day to day life. They are rich sources of vitamins, minerals, fibres, and antioxidants which are essential to a wealthy living. Consumers' perception of good quality fruits is based on their colour, texture, taste and nutritive values. A series of physiological, biochemical, and organoleptic changes occur during ripening process that changes the status of an in edible fruit to an optimal quality material. Examples of the products of the processes which take place during ripening are: biosynthesis of anthocyanins, such as lycopene and carotenoids; degradation of chlorophyll, acceleration of activity of cell wall degrading enzymes, and production of aroma/volatile compounds (Sharma *et al.*,2008).

Tomatoes contain no starch, but rich in sugars (fructose, glucose, and sucrose). They also contain some amount of food fibre, including cellulose and lignin in the seeds and the skin (Payal, 2010). Tomato is one of the most commonly consumed fresh vegetable and the most frequently consumed canned and processed fruit in human diet. Epidemiological study by Canene-Adams *et al*. (2005) concluded that there is concerted evidence that an increase in tomato consumption may lead to a reduced risk for both cardiovascular disease and prostate cancer. Tomato products are said to be good sources of potassium, folate, and vitamins A, C, and E (**Table 2**). In addition,

tomatoes are valuable in phytochemicals, such as carotenoids and polyphenols. For example, lycopene which forms the red pigments in the fruit, *B*-carotene, and the precursors of vitamin A compounds are contained in high quantity in fresh tomatoes and products (Canene-Adams *et al.*, 2005; Payal, 2010). Flavonol is another typical example of polyphenols contained in the fruit and in a high quantity as well. If all these phytochemicals are properly annexed and vigorously pursued they will contribute in no small way to reduce the risk of human ailments such as cardiovascular disease and prostate cancer.

Table 2: Nutrient composition of tomatoes and related tomato products

	Tomato products (*per 100g*)[2]				
Nutrient	Raw tomatoes	Catsup	Tomato juice	Tomato sauce	Tomato soup
Potassium *mg*	237	382	229	331	181
α-tocopherol *mg*	0.54	1.46	0.32	2.08	0.50
Vitamin A, *IU*	833	933	450	348	193
Vitamin C, *mg*	12.7	15.1	18.3	7.0	27.3
Total folate, µg	15	15	20	9	7

Source: (Canene-Adams *et al.*, 2005)

2.3 HARVEST MATURITY, RIPENESS INDICES AND MEASUREMENTS

The first step in the postharvest life of fruit is the moment of harvest. For most fresh fruit, harvest is done manually, so the picker will determine whether the fruit has reached the correct maturity stage for harvest. Tomatoes are perishable commodity and their maturity has a bearing on their storage life and quality and may affect the way fruits are handled, transported, and marketed. Harvest maturity is regarded as that stage at which the fruit has reached a sufficient stage of development that after harvesting and post-harvest handling, including ripening, its quality will be at least the minimum acceptable standard to the ultimate consumer. Harvest maturity varies with markets e.g. in Nigeria for processing market tomatoes are harvested at red ripe vine stage also fresh fruit is harvested at pink stage and become red while being transported from the farm to the market; which can be a long distance. In comparison to the United Kingdom market consumers prefer fresh tomato with good quality; as a result the fruit is harvested at mature green stage and become red in storage at temp of 10 to 29 °C (Anonymous, 2015). Only cherry tomatoes are harvested red ripe on the vine.

2.3.1. Physical indices

A wide range of physical features, such as size, shape, and surface characteristics are used to assess the maturity of fruits and vegetables (Reid, 2002). The changes in these features are the parameters often used as maturity indices. Choi *et al.* (1995) used the United States Department of Agriculture colour code classification as a physical index for measuring maturity of tomato fruit. Photo images of the standard classification: green, breakers, turning, pink, light red, red and blue were captured on camera, and converted to Hue, Saturation, and Intensity (HIS) measurements. The classification was based on the average percentage of the surface area which falls below hue angles. A tomato maturity index (TMI) was developed from this method to indicate the degree of maturity within each stage and to provide a continuous index throughout the complete maturity range. The results obtained from the study correlated to 77 % of hand grading. Developing a linear model from the colour image analysis will save time, labour and energy involved in

determining maturity of fruit using surface characteristics. **Figure 11** indicates the different colour stages of tomato and the colour chart:

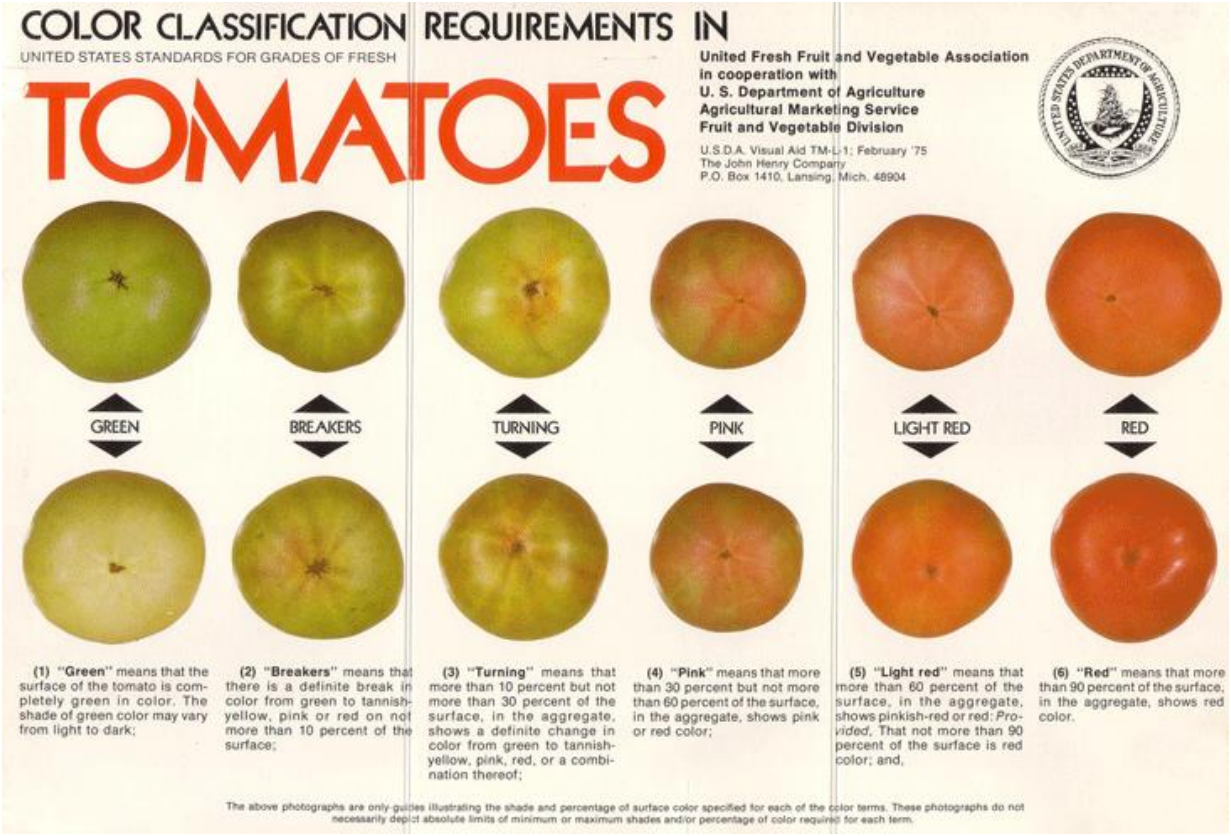

Figure 11: All about ripening of Tomato - Colour chart (Source: Ripening-fruit, 2013).

In another development a laboratory study carried out by Lien *et al*. (2009) indicated that a non-destructive method for assessing the maturity of tomatoes is feasible using the mechanical properties of the fruit, such as the falling impact test. The method was used for firmness measurement and classified tomato into; unripe, half ripe, and ripe classes. The system also achieved an accuracy classification of 75 % and above which is considered good enough for practical application. Hence, this classification of tomato technique could be developed into a computer programme whereby tomato maturity could be sorted on line.

2.3.2 Chemical indices

The definition of maturity of a commodity, such as a fruit, as the stage of development giving minimum acceptable quality to the consumer implies a measurable point in the fruit's

development, and it also involves the need for techniques to measure maturity. Therefore, maturity index for a commodity is a measurement or measurements that can be used to determine whether a particular commodity is ready for harvest. Saltveit (2005) classified tomato ripeness on the external colour of the fruit. The traditional six ripeness stages for fresh market tomatoes are based on the external colour change from green to red. When the fruits reach about 80 % of their final size they continue to develop and ripe normally after harvest, they are considered to be mature-green. Under proper temperature and humidity conditions, tomato fruits develop through the six defined stages to the red-ripe colour stage (**Table 3**).

Table 3: Ripeness classifications of fresh-market tomatoes based on changes in external and internal colour and tissue softening.

Stage	Description
0. Immature	The fruit is not sufficiently developed to ripen to an acceptable level of horticultural quality. Many immature fruit will eventually ripen, but to an inferior quality.
1. MG* -	The fruit will ripen to an acceptable level of horticultural quality. The entire surface of the fruit is either green or white, no red colour visible. Stages within the MG classification include:
MG1 -	firm locular tissue, knife cuts seeds
MG2 -	softened locular tissue, seeds not cut with knife
MG3 -	some gel in the locule, no red colour
MG4 -	locular tissue predominantly gel, some red colour in columella
2. Breaker	There is a definite break in colour from green to tannish yellow, pink or red on the blossom end of the fruit
3. Turning	More than 10 % but less than 30 % of the surface of the fruit shows a definite colour change to tannish-yellow, pink, red, or a combination of colours
4. Pink	More than 30 % but less than 60 % of the surface of the fruit shows pink or red colour
5.Light- red	More than 60 % but less than 90 % of the surface of the fruit shows red colour
6. Red-ripe	More than 90 % of the surface of the fruit shows red colour

*MG means mature green (Source: Saltveit, 2005)

Colour change that accompanies maturation in fruits, for example, tomato is widely used as a maturity index. Human eye is not capable of giving a good evaluation of a single colour as is extremely sensitive to colour (Reid, 2002). Accurate devices with the state of the art electronics

and optics, including Minolta colour meter are now the equipment used for objective measurements for fruits.

2.3.3 Horticultural maturity and harvesting

Previous findings defined maturation of fruits and vegetables as a stage of development leading to attainment of physiological maturity or horticultural maturity (Sudheer and Indira, 2007; Mir, 2008; Dhatt and Mahajan, 2007). However, horticultural maturity is the stage a plant or plant parts reached in its development that is considered to be ready for utilization by consumer for a particular purpose such as a food. Vegetables are recommended to be harvested when they attain certain size characteristic of the crop, while delayed harvesting beyond this stage will result to a bigger size which is not acceptable to the consumer.

Most fruits are harvested at proper maturity stage which is often quite earlier than ripening stage (Sudheer and Indira, 2007; Mir, 2008). Tomato fruits for example, are harvested at the mature green stage and are allowed to ripe in store. Mir (2008) reported that the optimum maturity of a product at harvest should be the objectives of the grower or producer since this process is the determinant factor to the quality of the product. Commodity can be horticultural mature at any stage of development or physiologically mature depending on the species. Furthermore it was stated that all plant or plant parts are harvested when they are horticultural mature but may be physiologically immature or mature. Some examples of crops which mature horticultural but remain immature physiologically are: sweet corn, pears, snap beans, summer squash and cucumber. Tomato, pepper, eggplant, melons and winter squash are classified as crops which have both of their developmental processes in mature stage (Mir, 2008).

The question now is what are the implications of harvesting these crops too early or too late? Harvesting fruit too early or green the tomatoes will either never ripen or take so long to ripen so they are poor quality. Late harvesting will result to too much water in the fruit and also over ripened. Thus the main role of the postharvest treatment is to devise methods by which

deterioration of produce is restricted as much as possible during the period between harvest and consumption. For example a situation whereby farms are located near towns and cities, harvesting the crop late will reduce postharvest treatment and the produce quickly disposed before serious wastage can occur. In countries which encompass a wide range of climatic regions, fresh fruit and vegetables are frequently grown at locations remote from the major centres of population. Thousands of tonnes of produce are now transported daily over long distances both within countries and internationally. As a result produce can be harvested early and put in storage to undergo its postharvest treatment.

2.3.4 Economic effects of disease pathogen on tomato

It is important to recognise the enormous crop losses that occur between harvesting and final consumption of tomatoes. The losses incurred happened through postharvest handling and led to diseases in storage. Waller (2002) divided postharvest diseases into those in which infection occurs in the field and those that happen during or after harvest. The postharvest loss far exceeded the damage caused by field diseases because of large investments in the overall processes the product undergoes from harvest until it reaches the customer; such processes include harvesting, sorting, packaging and shipping (Barkai-Golan, 2001). The attack of micro-organisms during handling of produce is a serious cause of postharvest loss in fruits and vegetables such as tomatoes. The physical damage that occurs exposes the fruit and vegetables to infection by pathogens and subsequently affects the physiological processes (Waller, 2002; Booth and Burden, 1983). These pathogens caused quantitative losses of fresh produce and also reduce the quality.

For example, *Fusarium*, *Botrytis*, *Alternaria* and *Rhizopus* species are capable of infecting tomato from wound caused by physical damage during handling or through the stomata or lenticels (Waller, 2002). The infection can occur at pre or post-harvest period at the sites of mechanical injury or through the stomata during harvesting and handling operations. At this point of entry the pathogen has the capacity to attack undamaged epidermal tissues thereby causing a substantial disease spread in storage (Barkai-Golan, 2001). Consequently, the physiological processes that

occur after harvest are impaired and may lead to decay of fruit. For instance, Schirra *et al*., (2000) reported that the increased susceptibility of tomatoes to decay during ripening was linked with the decrease and disappearance of mRNA encoding an anionic peroxidase. The reports also provided a corollary that if mature green tomatoes are heat treated the degradation rate of mRNA will be delayed and subsequently maintain antifungal resistance in the fruit tissue to decay (Schirra *et al*., 2000).

Although, another study reported that there was no significant effect of handling treatment for example, mechanical impact known to cause bruises and create entry points for spoilage microorganisms on ethylene production which stimulates ripening process, but the effect of storage temperature was significant at 20 °C than at 12 °C (Mutari and Debbi, 2011).

2.3.5 Other physiological defects

A number of defects affect the quality of fresh market tomatoes. These disorders result from a combination of environmental, production and handling procedures or are generic in origin. An example of such defects is electrolyte leakage of the cell wall which impedes the electrical transfer ions across the cell membrane.

2.3.5.1 Electrolyte leakage

An electrolyte is a liquid that reacts chemically when electricity is passed through it. It may be an aqueous solution of an acid, base, or salt. Tucker and Grierson (1987) stated that the biochemical reactions in fruit pericarp cells are associated with ripening result in the production of various organic salts and acids as well as other products. Chilling sensitivity of tomato fruit is related to ripening and senescence (Autio and Bramlage, 1986). It was reported that post chilling ion leakage, respiration, and ethylene (C_2H_4) biosynthesis elucidated the degree of chilling injury to fruit of tomato. Another report by Lacan and Baccou (1996) stated that the amount of electrolyte leakage is directly proportional to increased cell membrane permeability caused by fruit ripeness or damage induced by stressful conditions.

Sharom *et al*. (1994) also reported that chilling injury results in damage to cellular membranes and ensure leakage. This is evidenced in the formation of water-soaked patches in chill-injured tomato fruit (Sharom *et al.,* 1994), and an enhanced electrolyte leakage from chill-injured tissue. Electrolyte leakage is measured as an increase in conductivity and is thought to reflect leakage across the plasma lemma. In another development the electrolyte leakage was reduced in heat treated fruit and the fruit turned red faster and showed less CO_2 than non-treated fruit (Saltveit, 2005). The trial concluded that red tomatoes both unheated and heated showed the lowest visible chilling injury, CO_2 and ethylene production, but the highest electrolyte leakage. This phenomenon is common with tomato production in developed agricultural countries and occurs during the postharvest management of the produce.

2.3.5.2 Electrical properties

When electricity flows through a solution of electrolyte, chemicals split up in a process known as electrolysis. Current flows through electrolyte due to electrical potential difference (V) (Zhang *et al.,* 1990). Anions are oxidised at the anodes, whereas cations are reduced at the cathodes. Electrolyte could be molten salts or aqueous solution of salts, acids, or bases. Electrolytic conductivity of a solution depends on the concentration of the ions in a solution. Water, ammonia, bases and most organic acids are weak electrolytes having only a fraction of molecules split into ions in a solution. Such solution thus formed will contain some ions that are in equilibrium with non-ionised molecules. Incomplete ionisation results to low conductivity. Generally, the number of ions in a solution of an electrolyte depends on the total number of molecules available and the degree of ionisation.

Fruit ripening is a complex series of changes involving cell wall degradation, alteration of membrane condition and function, changes in metabolic activities and formation of various organic compounds associated with climacteric respiration and ripening. These changes result to fruit softening and development of juicy texture (Harker and Dunlop, 1994). The juice contains bioelectric properties because of organic acids, salts and water present in it. Therefore fruit can

behave as an electrical apparatus having anode and cathode terminals with resistances. Previous report by Furmanski and Buescher (1979) used electrical impedance measurements as maturity index during fruit development and as a method for following physiological changes in storage and fruit ripening.

2.4 POSTHARVEST PHYSIOLOGY IN TOMATO

The type of respiration of a fruit determines its postharvest physiology. Generally fruits can be divided according to whether they are climacteric or non-climacteric; whereas climacteric fruits have a corresponding rise in respiration rate. Harvested tomatoes have a climacteric rise in respiration (i.e. carbon dioxide production and oxygen consumption) and ethylene production that coincides with the beginning and progression of ripening (Saltveit, 2005). During this process other physiological changes take place in the fruit.

Respiration is the oxidation of carbohydrates (i.e. primary sugars) and organic acids to carbon dioxide and water, followed by the release of energy and production of intermediate carbon compounds. Apparently, the climacteric rise in respiration is necessary during ripening to produce energy for the tissue and the intermediate compounds required for the synthetic reactions involved in ripening. Although tomato is considered as a climacteric produce but there is as much variability in the rate of respiration among cultivars, growing locations and individual fruits as is within a fruit as it progresses through the climacteric during ripening (Saltveit, 2005). Grierson and Kader (1986) reiterated the afore mentioned statement in their studies showing that the rate of carbon dioxide produced by a tomato fruit at a turning stage of ripeness is twice what it was for the same fruit at the mature-green stage of ripeness.

2.4.1 Fruit ripening

Fruit ripening is the final stage of maturation when the fruit develops the characteristic colour, flavour and aroma which are the parameters that determine quality. Tomatoes belong to the class of fruits and vegetables that demonstrate a climacteric respiratory behaviour during ripening. With

the onset of a climacteric rise in respiration, ethylene is produced; level of carbon dioxide is increased and coincided with the first appearance of red colour (Saltveit, 2005). Whether ripening occurred naturally or induced by ethylene, it is accompanied by many biochemical changes in organic acids, proteins, amino acids, and lipids which influence flavour quality of the product (Kader, 2002). Tomato fruits which are meant for distant markets or export are often harvested mature green. When this happens it is important to pick the fruits which are advanced in colour stage first such as `turning′ and separate them into groups based on the time the produce will arrive in the market.

2.4.1.1 Ethylene production and respiration

Ethylene was discovered along with the evolution of land plants which dated more than 450 million years ago from a lineage of freshwater algae (Ju *et al.,* 2015). Another study described ethylene as a substance which is physiologically active in minute quantities, but its activity cannot be traced to known mechanisms of substrate degradation (Anonymous, 2015). As a result of the pronounced effects that ethylene apparently has upon the physiology of both individual plant organs and entire plants, its metabolic origin could not be determined. Therefore, it was agreed that only living or respiring tissue produces this active emanation. Likewise, Kader (2002) defined ethylene (C_2H_4), as a natural occurring plant product and a simple organic compound that affects the physiological processes in plants. It is produced by the tissues in higher plants during metabolism and micro-organisms.

Amongst the organic compounds present in plants, ethylene is regarded as a hormone that regulates growth, development, and senescence. 1-aminocyclopropane-1-carboxylic acid (ACC) is regarded as the precursor of ethylene and this product in turn was produced from S-adenocylmethionine (SAM) by the enzyme ACC synthase (Saltveit, 2005; Kader, 2002). Through this process the biosynthesis of ethylene can be controlled (Kader, 2002), by preventing the oxidative processes of ACC oxidase to produce ethylene.

It was noted that the activities of ACC synthase and ACC oxidase are gene linked and can be affected by the environment including temperature and concentrations of oxygen and carbon dioxide. As maturity process progresses the ethylene production rates increase and also increase during physical injuries, disease attack, increased temperature (30 °C), and water stress. In contrast low temperature reduced the production of ethylene by reducing the level of oxygen(less than 8 %), and raise the carbon dioxide levels (more than 2 %) in storage (Kader, 2002; Saltveit, 2003).

2.4.1.2 Colour change

For the consumer, the changes in physical appearance are the characteristics that attract buying. However, consumer satisfaction is also linked to pleasant texture, taste and freshness, which are all considered as part of the ripening phase. Lurie (1998) stated that ripening of most climacteric fruit is characterised by softening of flesh, an increase in the sugar: acid ratio, enhanced colour development, and increases in respiratory activity and ethylene production. By exposing fruit to high temperatures some of these processes can be terminated while others can be enhanced. This situation resulted in heated fruit being more advanced in some ripening characteristics than non-heated fruit. As a result the produce quality is retained longer during shelf life at 20 °C (Lurie, 1998).

Among these attributes, fruit colour is probably the most important that determines overall quality. Quite a number of changes happen when tomatoes progress from the mature-green to red-ripe stage. During this complex ripening process in tomato, the breakdown of chlorophyll, synthesis of lycopene and the activity of polygalacturonase increase. Accumulation of lycopene is the most obvious external change and is the characteristic responsible for the colour (pigment) of the fruit (Saltveit, 2005). The inhibition of ripening by heat may be caused by reduced production of ripening hormone ethylene, although this process will commence when the fruit is returned to room temperature (Lurie, 1998).

For an example, hot air treatment of 35-38 °C was reported to inhibit ethylene synthesis in few hours in both apples and tomatoes. Increase in these temperatures can cause endogenous ACC to accumulate in apple and tomato tissue at the same time with the decrease in ethylene. Likewise, a rapid loss of ACC oxidase activity was reported to occur in many fruit exposed for a few hours to hot water immersion at 42-46 °C. It is due primarily to decrease in ACC oxidase mRNA and termination of enzyme synthesis, the hormone responsible for ripening. During the heating period, not only is endogenous ethylene production inhibited, but fruits will not respond to exogenous ethylene (Lurie, 1998).

Colour changes in tomato fruit development differ between varieties, but for red cultivars they are normally considered to be in six different colour stages: Mature green, breaker, turning, pink, light-red, and red (**Table 3**). These colour changes take place on cellular and molecular levels and are involved in the conversion of chloroplasts to chromoplasts, which contain red or yellow carotenoids (Heaton and Marangoni, 1996). These colour stages are important in tomato enterprise for both pre-and postharvest fruit management. For instance, tomatoes that are produced for processing remain on the vine until they are red ripe while fruit that are meant for fresh market are harvested at mature green – breaker. This fruit will ripe either in transit or storage and determine the cool chain practices in postharvest handling of the crop and the subsequent shelf life. Also, colour plays a vital role at harvest because by picking the fruit too early will reduce some quality and taste characteristics later on, in fact harvesting tomato at a stage when still firm is best for shipment or transporting to long distance. If the fruit is allowed to stay longer on the plant it will develop aroma and taste factors. The adverse effect of allowing the fruit to ripen on the plant is that the fruit will be too soft and thereby lose its market quality.

Saltveit (2005) stated that the synthesis of lycopene and β-carotene is almost the same for both harvested mature green fruit and fruit left to ripen on the plant. Also by exposing mature green fruits to ethylene stimulate normal ripening, synthesize and accumulate lycopene and β-carotene greater than fruit left to ripe without ethylene stimulation. This confirms the method adopted by

producers and handlers whereby ethylene is introduced into storage of mature green tomatoes to accelerate ripening and quicken the change to red colour. During these stages of development and ripening, major structural changes occur in the cell walls which initiated and precipitated the action of ethylene and polygalacturonase (PG), leading to loss of galacturonic acid residues, and degrading of polyuronide from the cell wall (Stommel and Gross, 2001; Alexander and Grierson, 2002).

In another study by Farneti *et al.* (2012), it was reported that temperatures below 12 °C resulted in lycopene loss in red-ripe tomatoes and substantial colour loss as well. Prior hot water treatment did not prevent lycopene loss. Furthermore the storage of red ripe tomatoes at chilling temperatures reduces the nutritional and health promoting substance lycopene and also affects fruit colour. Red colour development was inhibited by heat treated fruit, likewise softening (Mitcham and McDonald, 1992). These reports also confirm earlier studies that heat treatment inhibited ripening-associated cell wall modification and the rate of loss of cell wall galactose and arabinose was reduced in heat-stressed tomatoes.

2.4.1.3 Cell wall metabolism

During ripening it is understood that the activities taking place in the cell wall are crucial to softening and textural changes. As ripening advances the cell wall becomes increasingly hydrated as the pectin in middle lamella becomes modified and partially hydrolysed (Alexander and Grierson, 2002). It is the pectin gel that holds the cells of the lamella together, once this change the cells are separated from one another, which in turn affects the final texture of the ripe fruit. The tomato fruit polygalacturonase has been implicated as the major cell wall polyuronide degrading enzyme (Brummell and Harpster, 2001), and considered to be ethylene dependent ripening elements. Some studies have shown that low PG fruits are more resistant to splitting, mechanical damage and pathogen infection (Alexander and Grierson, 2002).

In another study by Mitcham and McDonald (1992), it was reported that tomato fruit harvested at mature green stage and kept for 4 days at 21 °C or 40 °C and later stored at 21 °C; showed that red

colour development was inhibited in heated fruit as well as softening. Furthermore, fourteen days later the heat stressed fruit were twice as firm as the control. This demonstrated that heat treatment inhibited ripening associated cell wall modification so that 14 days after treatment, the heated fruit contained only one-third the amount of soluble ployuronides that was present in the control.

Mature red cherry tomato fruit (*Lycopersicon esculentum* var. *cerasiforme*) were treated with hot water treatment (HWT) at 45 °C, and/or with 2 % (w/v) sodium bicarbonate solution (SBC), alone or in combination (HWT + SBC) for 10min then stored at 20 °C for 6 days (Shao *et al*., 2011). The report showed that hot water treatment alone caused cracking on the fruit surface, while no cracking was observed on sodium bicarbonate solution treated or on combined treatment fruits. It was also reported that after storage the fruit from combined treatment had higher skin firmness and titratable acidity and with a lower infection of grey mould rot caused by *Botrytis cinerea* compared with untreated control.

2.4.1.4 Volatile compounds

The concentration of organic acids and sugars is very important to the taste of ripening fruits. The characteristic flavour of fruits is as a result of the aroma volatile compounds produced within the fruit during ripening and cutting (Alexander and Grierson, 2002). It was determined in the study by gas chromatography that volatile profile of fruit includes many alcohols, aldehydes and esters. Brauss *et al.* (1998) stated that the difference in flavour between tomato varieties is due to variation in aroma volatile production. Hobson and Grierson (1993) also detected that tomato fruit contains over four hundred volatile compounds. Among these lot, only a group of seven including some amino acids (3-methylbutanal and 3-methylbutanol) and unsaturated fatty acids (hexanal and hexenol) are amongst the most important contributors to fruit aroma. In tomato fruit these aromatic volatile compounds are produced through different chemical pathways. For example, some of these hydrocarbon compounds are produced by the deamination and decarboxylation of amino acids, whereas others are formed by the lipid oxidation of unsaturated fatty acids on slicing of fruit (Hobson and Grierson, 1993; Griffiths *et al.*, 1999a). Lipoxygenases (LOX) are the

enzymes identified in this process. Through analysis ethylene is said to have played a role as the regulator of the different genes on the enzymes acting on unsaturated fatty acids in tomato during fruit ripening (Griffiths *et al.*, 1999a). Therefore, in order to maintain a good flavour, harvesting is best carried out when the fruits are at the physiological maturity (mature green) stage and ripening begins with initiation of ethylene.

Boukobza and Taylor (2002) carried out their trials to show the effect of postharvest treatment on flavour volatiles of tomato; using storage conditions to mimic typical storage/transport scenarios. The results indicated that the storage under low oxygen conditions, followed by a recovery period in air (4-6 h) had less effect on volatile compounds. Whereas low temperature storage which is the usual practice for storing fresh fruit tomatoes, caused a significant decrease in volatile concentrations, the effect was not even reversed after long recovery period (72 h). A situation should arise in which these two conditions are put together so as to exploit the favourable advantages of each to increase the storage life of tomato. For example, ripening of most climacteric fruit is characterised by enhanced colour development, increase in respiratory activity and ethylene production (Klein and Lurie, 1992). Carbon dioxide production rises as oxygen decreases as climacteric fruit ripen. The presence of ethylene, either exogenous or endogenous usually promotes respiration, therefore a simultaneous increase in CO_2 production with the consequent ethylene reduction occurring during exposure of tomato to high temperatures could be utilised to promote increase in shelf life.

2.4.2 Physiological disorders

Physiological disorders are abnormalities in fruit colour or appearance that caused by environmental factors. Sometimes these abnormalities are often confused with damage caused by pathogens or insects. Physiological disorders are different from a single nutrient deficiencies, physical, chemical or herbicide injury (Peet, 2009). The causes of these anomalies may be as a result of genetic susceptibility, environmental factors, watering practices, nutrition and storage temperature.

2.4.2.1 Storage temperature

Tomato is regarded as one of the most important vegetables grown for edible fruits consumption in every home in Nigeria. Its production is carried out during the hot rainy season (Babatola *et al.*, 2008). Lack of postharvest storage expertise of tomato constitute a major cause of seasonal fluctuation in availability, deterioration in quality and other economic disadvantages as a result of inefficient means of storage thereby the fruit goes for canning (Babatola *et al.*, 2008). Environmental factors such as soil type, temperature, frost and rainy weather at harvest can also have an adverse effect on storage life and quality of tomatoes (Ndukwu, 2011; Iwuagwu *et al.*, 2013).

An aspect to consider when handling fruits and vegetables is the temperature and relative humidity of the storage environment. For example, freshly harvested produce will need method that will increase the relative humidity of the storage environment so as to slow the rate of water loss and other metabolic activities (Ndukwu, 2011). Although, refrigeration is very popular but it was observed that many fruits and vegetables, such as tomato, banana, plantain etc., cannot be stored in the domestic refrigeration for a long period because of susceptibility to chilling injury (Ndukwu, 2011). The irregular power supply and low income of farmers make refrigeration method not feasible. As a result an appropriate technology needs to be developed. But in another study by Babatola *et al.*, (2008) it was reported that deep freezer storage condition at temperature of 0 °C and 95 % Relative Humidity (RH) was the best among other conditions such as ambient storage environment 32 °C; RH 85 %; room refrigerator 12 °C; RH 85 %; and storage incubator 8 °C; 80 % RH in terms of good quality tomatoes.

Therefore, a proposition can be made for an appropriate technology that is adequate, affordable and easily adoptable by farmers, for example the Evaporative Coolant System (ECS) which can be useful for postharvest treatment of tomato (Iwuagwu *et al.*, 2013; Ndukwu, 2011). The performance of cooler was evaluated for temperature; evaporative effectiveness and cooling

capacity for freshly harvested tomatoes. The result showed that the evaporative cooler reduced the ambient temperature to up about 10 % (32-40 °C to 24-29 °C) and increase the relative humidity of incoming air from 40 % to 92 %. Consequently, the cooler was able to preserve freshly harvested tomato for 19 days before deterioration in colour, weight of fruit, and infection of fungal pathogens (Ndukwu, 2011).

Tomatoes for fresh consumption are commonly harvested at the mature green or early red-ripe stages and transported to retailers under controlled conditions, such as temperature, atmosphere and relative humidity (Boukobza and Taylor, 2002). The report of studies showed the best method of transporting fresh tomato is to have the fruits in storage under low oxygen conditions followed by recovery period in (4-6 h), and at ambient temperature (21-22 °C). Reports have shown that during this period, ethylene will evolve through the autocatalytic reaction (Alexander and Grierson, 2002) then trigger the respiratory climacteric and ripening process will begin once the fruits reach their destination. The resultant effect is that storage of fresh tomatoes under reduced oxygen level has less effect on volatile compounds. Low temperature storage caused a significant decrease in volatile compound concentrations even after a long recovery period.

Results of the studies conducted by Maul *et al.* (2002) indicated that in ripe tomatoes stored at 20 °C showed a significant increase in aroma, sweetness and flavour in fruits, as opposed to other temperature regimes; 5 °C, 10 °C, and 12.5 °C.

2.4.2.2 Chilling injury

Exposure of susceptible plant tissue to temperatures below 10-12 °C induces a physiological disorder called chilling injury (Saltveit, 2005). The extent of this disorder will depend on the environment to which the tissue was exposed previously, tissue type; such as unripe or ripe fruit, temperature, length of exposure and post-chilling conditions. Ding *et al.* (2002) stated that tropical and subtropical fruit and vegetable crops are susceptible to chilling injury when stored at low temperatures after harvest. For an example chilled tomato fruit lost the ability to develop full

colour, developed sunken areas on the fruit causing blemishes, and more importantly showed increased susceptibility to Alternaria rot and decay (Ding *et al.,* 2002; Safdar *et al.*, 2008; Saltveit *et al.,* 2005).

Farneti *et al.* (2012) reported that low temperature induced lycopene degradation in red ripe tomato which is a major component of vitamin C. Furthermore temperatures below 12 °C resulted in lycopene loss in red-ripe tomatoes and a substantial colour loss. Pre-storage hot water treatment did not prevent the loss of this essential nutrient contained in red tomatoes. The conclusion showed that storage of red ripe tomatoes at chilling temperatures reduces the nutritional and health promoting value and affects fruit quality.

Chilling injury is a physiological disorder caused by the exposure of fruits to low temperatures above freezing point, resulting to reduction in fruit quality. Studies have shown how high temperature treatments and other stress factors have controlled this anomaly and improve the quality of fruit and extend shelf life (Lurie, 1998; Polenta *et al.,* 2006; Ding *et al.,* 2002; Whitaker, 1994; McDonald *et al.,* 1999). These studies indicated that heat treatment (38-45 °C) administered for 5-60 min prior to chilling reduces the incidence and severity of chilling injury in tomato fruit (Farneti *et al.,* 2012). Also partial ripening of tomato has been shown to reduce chilling sensitivity (Whitaker, 1994).

In another study carried out on the effect of heat treatment uniformity on tomato ripening and chilling injury, a significant difference was found between the heated and unheated tomato halves in terms of colour and chilling injury (Lu *et al.,* 2010). The objective of this investigation was to demonstrate the effect in tomatoes of heat treatment uniformity on quality attributes and chilling injury (CI) by subjecting only one half of each tomato to a specific treatment and evaluating the effect of the treatment on the two halves separately. It was believed that submitting the two halves of the same tomato fruit to two different conditions would ensure that such treatment would be a fair representative of tomatoes submitted to non-uniform treatment (Lu *et al.,* 2010).

Saltveit (2002) reported that a phase transition or lateral phase separation in portion of cell membranes was significant in chilling injury. In addition one of the outcomes of a temperature-induced phase transition in cellular membranes would be an alteration in their biophysical properties thereby resulting to change in their function. This procedure resulted to increased membrane permeability and increased rates of ion leakage associated with chilling of sensitive tissue. Tomato fruit exposed to non-freezing temperatures below 10 °C causes an increase in the subsequent rate of ion leakage from the pericarp (Saltveit, 2000, 2002; Marangoni *et al.*, 1996). Another study reported that chilling sensitive tomato fruit is related to ripening and senescence (Autio and Bramlage, 1986). Ion leakage was used to measure the degree of chilling injury to fruit of tomato. The process first declined as the tomatoes began to ripen and then increased during the late stages of ripening. But in the non-ripening genotype, chilling sensitivity did not show the early decline but showed the increase during senescence. It is evident that ion leakage is as a consequence of chilling injury.

Ding *et al.* (2002) also reported that with low concentrations of some inorganic compounds such as methyl jasmonate or methyl salicylate tomato fruit substantially developed resistance to chilling temperature and decrease the incidence of decay during low- temperature storage. In another study, a correlation was drawn between heat shock protein (HSP) and thermal tolerance in many organisms, but recently found that a heat stress can condition plants to low temperature and subsequently develop a resistance to chilling injury in tomatoes (Lurie, 1998; Saltveit, 2005).

2.4.2.3 Effect of mechanical injury on tomato

Tomatoes are stage harvested in Nigeria and UK for different purposes. The fruits meant for the processing are harvested red-ripe and immediately taken to the processing plant. Whereas fruits proposed for the fresh market can be harvested at mature-green through to red-ripe stages. It is a common practise to harvest greenhouse tomatoes riper than field grown fruit and is therefore more prone to mechanical injuries. Riper fruits are softer and have a shorter shelf-life than fruits

harvested at mature green stage (Saltveit, 2005), and their thin skins and locular walls are valued by consumers, but these characteristics make them prone to mechanical injury.

Horticultural products such as tomatoes are exposed to several external forces during their postharvest life. When these forces exceed a threshold for tissue failure they will cause mechanical damage to the fruit. Tomato bruising is one of the different types of mechanical damage that exit. Studies have shown that bruises are not always immediately visible but they become noticeable during subsequent handling and shelf-life (Van linden *et al*., 2006). Reports of mechanical impact on tomato bruise development stated that the pericarp tissue over the locules was much more sensitive to bruise development than radial wall tissue (Van linden *et al.,* 2006; Milczarek *et al*., 2009). In addition tomato susceptibility to bruising increased substantially with ripening and loading conditions (Van linden *et al.,* 2006).

Arazuri *et al.* (2007) reported that the influence of mechanical harvest on the physical properties of tomato such as firmness and skin resistance are the most relevant quality characteristics in the canning industry. As a result most of mechanical actions affecting tomatoes are produced during harvest and transport causing low quality fruit. A simulated transport study carried out in the laboratory conditions to assess the performances of the traditional raffia basket, the only packaging container for tomato fruit in Nigeria, compared with plastic container which is currently not in use. The result showed that 40 %, 37.50 % and 45 % of the samples of tomato fruits from the top, middle and bottom of the basket respectively were severely bruised after four hours of excitation while the samples contained in the plastic container had the corresponding values of 44.18 %, 30.23 %, and 18.60 % respectively (Aba *et al.,* 2012).

Similarly, another study evaluated the impact of handling at three different harvesting periods: 15 days, 30 days, and 45 days after the beginning of harvest (Ferreira *et al.,* 2005). Fruits were classified according to ripening stage and diameter and were also evaluated for mechanical damage and external defects caused by harvesting procedures after storage. The results indicated

that the highest % fruit damage occurred during the 30 days period than the other two periods. Also fruits harvested during this period and stored for 21 days showed higher losses due to mechanical injury.

Van linden *et al.* (2006) reported that tomato susceptibility to bruise damage is dependent on the location of impact and cultivar and was related to the applied energy input. The Impact assessment also showed one-third loss in firmness in tomato fruit from the bottom of the trailer and a substantial loss of skin resistance to crack in transit (Arazuri *et al.,* 2007; Van linden *et al.,* 2006; Milczarek *et al.,* 2009; Van linden *et al.,* 2006). In another study tomato fruits subjected to injuries as a result of high impact energy showed a significant increase in respiration in storage (de Paiva *et al.,* 2012); and consequently increase CO_2 and ethylene (C_2H_2) production in Roma tomato at mature-green, breaker, or pink ripeness stages (Lee, 2005).

It is a customary practice in most industrialized countries to harvest processing tomatoes by machine. The harvest operations begin when at least 90% of the fruit are ripe (Sargent and Moretti, 2002). During the operation the fruits are dumped into field bins, as a result bruises are sustained at impact. The mechanical injuries inflicted on the fruit as well as the bruises encountered are not severe enough to be rejected for the processing. But in comparison the fresh market produce has a long period between harvest and consumption, therefore allows sufficient time for water loss, ion leakage, pathogenic infections, and the stimulation of CO_2 and C_2H_2 production (Kader, 2002). Consequently, the injuries sustained during picking and packing in to basket thereby renders the fruits unacceptable to the fresh market.

In another development Lee and Kader (2000) stated that bruising and other mechanical injuries, such as trimming, lower the retention of vitamin C in horticultural crops. Many studies have shown that vitamin C is one of the constituents of tomato fruit and an essential nutrient for that matter, has many biological activities in the human body (Opena et al., 1989; Ejechi *et al.*, 1999;

Ilic and Fallik, 2007; Ndukwu, 2011; Iwuagwu *et al.*, 2013;. Therefore, efforts should be made to minimise the injury inflicted on the fruit skin during mechanical harvesting.

2.5 POST-HARVEST PATHOLOGY

Barkai-Golan (2001) defined postharvest pathology as a process that is required to protect and sustain fruit and vegetables against microorganism attack that causes decay during storage. The development of a decay which happened as a result of pathogen infection during storage is the main cause of deterioration of the fresh produce and can become the limiting factor in prolonging the shelf-life of fruits and vegetables. After harvest ripe fruits and vegetables can be attacked by various microorganisms which were unable to attack them during their growing period on the field. These are mainly weak pathogens such as fungi and bacteria commonly found on harvested and stored fruits and vegetables.

The disease resistance contained in the plant organ meant for storage became weakened as a result of its separation from the mother plant. In addition, picked fruits and vegetables are rich in moisture and nutrients which form substrate that favours the development of pathogens. During ripening fruit and vegetables are more susceptible to injury and, therefore, more prone to the attack of these microorganisms that require an opening or damaged tissue to facilitate their entry in to the produce (Barkai-Golan, 2001).

Losses caused by postharvest diseases of fruits and vegetables may be specifically classified into parasitic, nonparasitic, or physical (Sholberg and Conway, 2004). The parasitic causes are more common and are of microbiological in origin and can begin as latent infections before harvest or occur at or after harvest during storage (Cappellini and Ceponis, 1984). The losses are generally more than what people realize because prices of these produce continue to rise as they are taken from the field to the consumer. The postharvest losses are estimated to range from 10 to 30 % in the Europe and other developed world even with modern storage facilities and techniques (Waller, 2002). The estimates can be more in developing countries which lark sophisticated postharvest

storage facilities and often faced with incessant electricity power fluctuations. Conservative estimate losses of 40-50 % of fruits and vegetables may not be uncommon in the tropics (Tian, 2007).

Several diseases (**Table 4**) cause postharvest decay of tomato fruits, however, the main diseases are *Alternaria* rot, anthracnose, bacterial soft rot, *Cladosporium* rot, grey mould, *Rhizopus* rot, and watery rot (Waller, 2002).

Table 4: Diseases of Tomato

Commodity	***Disease/disorder***	***Causal organism or condition***
Tomato	*Alternaria* rot	*Alternaria spp.*
	Anthracnose	*Colletotrichum spp.*
	Bacteria canker	*Clavibacter michiganensis subsp. Michiganensis*
	Bacteria soft rot	*Erwinia carotovora subsp. carotovora*
	Blight	*Phytophthora infestans*
	Cladosporium rot	*Cladosporium herbarum*
	Early blight	*Alternaria solani*
	Fruit rot	*Didymella lycopersici*
	Grey mould	*Botryotinia fuckeliana*
	Phoma rot	*Phoma destructive*
	Rhizopus rot	*Rhizopus stolonifer*
	Sclerotium rot	*Corticium rolfsii*
	Soil rot	*Thanatephorus cucumeris*
	Watery rot	*Geotrichum candidum*

(Source: Waller, 2002)

2.5.1 Some postharvest disease pathogens of tomato

Studies have shown that postharvest diseases of fruits and vegetables are caused by microorganisms such as fungi and bacteria (Barkai-Golan, 2001; Snowdon, 1991). Sholberg and Conway (2004) reported that bacterial pathogens commonly affect vegetables because of less acid contents than fruits. They are capable of rapid multiplication under favourable conditions such as, pH, temperature, and nutrition. But ripening fruits are prone to attack by fungi both at pre-harvest or postharvest stages.

The term "vegetable" generalises the use of a range of plant parts, and the common definition is that of a culinary function being used as part of main dishes rather than a dessert food (Snowdon, 1991). In tomato, fruit is the plant part that is consumed, therefore by definition; tomato can be classified as a vegetable in botanical sense instead of a fruit. Other crops that are in the same category are; peppers, squashes, and cucumbers. Tomatoes are susceptible to many fruit decays, both bacterial and fungal. For example; bacterial decays include soft rots (*Bacillus spp*., *Erwinia carotovora spp., Pseudomonas spp.*, and *Xanthomonas campestris*); and fungal decays comprise *Alternaria* rot (black rot) (*Alternaria alternata*); Fusarium rot (*Fusarium spp.);* Grey mould rot (*Botrytis cinerea*); Mucor rot (*Mucor mucedo*); Rhizopus rot (*Rhizopus stolonifer)*; Sour rot (*Geotrichum candidum*), to mention just a few that are common in tropical Africa (Sargent and Moretti, 2002; Sholberg and Conway, 2004; Waller, 2002).

Generally, *B. cinerea* is the major cause of loss in temperate countries, whereas in hotter climatic areas the common causes of decay are *A. alternata, R. stolonifer, G. candidum* and *E. carotovora.* All of these pathogens have high optimal growth temperatures (Barkai-Golan, 2001). These disease pathogens often develop via wounds, bruised tissue and sometimes during fruit ripening and softening. A common incidence in tropical vegetable production, particularly during postharvest management, is the penetration of these pathogens through cross- contamination from diseased fruits, dirty harvest containers and poor sanitation during handling and packing.

2.5.2 Effects of fungal pathogens on the postharvest quality of tomato (*Lycopersicon esculentum Mill.*) in Nigeria

Tomato (*Lycopersicon esculentum Mill.)* is a globally grown vegetable fruit rich in vitamins and minerals. It is used for culinary purposes and in the production of fruit drinks (Etebu *et al.,* 2013). Reports have shown that tomato (*Lycopersicon esculentum Mill.*) genotype is one of the most popular and widely grown crop plants in the world, likewise the most important vegetable worldwide in terms of the amount of vitamins and minerals it contributes to food (Osemwegie *et al.,* 2010). Global production of fresh tomato fruit is about 89.8 million metric tonnes cultivated from 3,170,000 ha (Asgedom *et al.,* 2011). Nigeria is second largest producer of tomato in Africa after Egypt (Costa and Heuvelink, 2005). The total annual cultivation of the crop is about one million hectares (Etebu *et al.,* 2013). Tomato fruits contribute about 18 % of the average daily consumption of vegetables as paste or puree which are used for cooking and as fruit drinks.

2.5.3a Disease pathogens control methods

Fruit vegetables of the Solanaceae family include tomatoes, peppers and eggplants (Barkai-Golan, 2001). These three vegetable crops have common pathogens that affect their fruits; such as *Botrytis cinerea, Alternaria alternata, Erwinia carotovora, Rhizopus stolonifer, Mucor spp., Geotrichum candidum, Fusarium spp., Phytophthora spp.* and *Rhizoctonia solani*. Among these lot, *Botrytis cinerea, Alternaria alternata* and *Erwinia carotovora* are the common most important pathogens that affect the fruits.

Very small proportion of fungicides are presently used as postharvest treatments for control of a number of decay causing microorganisms compared to pre-harvest pest control products (Sholberg and Conway, 2004). Most of the products for postharvest treatments of fruits are no longer permitted for use because of the public concerns with chemical residues in food and possible toxic effects. Furthermore, some of these products have lost their relevance due to persistence use of the fungicides and consequent development of resistance by the target pathogen (Sholberg and Conway, 2004).

Utkhede *et al*. (2001) demonstrated the biological and chemical control methods by applying Root Shield (*Trichoderma harzianum*) and yeast strain (S33) of *Rhodosporidium diobovatum* on tomato plant leaves to control tomato stem canker caused by Botrytis cinerea. Apparently, it is the synergy of these two microorganisms that really reduced the growth of the pathogen. It was suggested that *R. diobovatum* was antagonistic to *B. cinerea* on potato dextrose agar, therefore, it may be inferred that antibiosis may be one of the mechanism responsible for the control of this disease. The treatments with Root Shield, Soil Gard, and *R. diobovatum* produced significantly more total fruits than the inoculated control. Two strains of *Bacillus subtilis* (BACT-O and BACT-10), did not reduce the lesion length caused by *B. cinerea* on tomato plant, but they significantly increased the fruit yield and number of tomato plants (Utkhede *et al.,* 2001).

Feng and Zheng (2007) demonstrated in their studies the inhibitory effects of five essential oils (thyme, sage, nutmeg, eucaptus and cassia), against *Alternaria alternata* at different concentrations (100-500ppm) *in vitro*. The studies showed that both cassia oil and thyme oil have antifungal activity against *Alternaria alternata.* Spore germination and germ tube elongation of the pathogen were completely inhibited on potato dextrose agar with 500 ppm cassia oil. Natural oil contained in plant for example the thyme gave a lower degree of inhibition 62 % at 500ppm. Ejechi *et al.* (1999) study showed that phenolic and essential oil extracts of pepper fruit (*Dennetia tripetala*) inhibited the growth of these pathogenic fungi common in tomato. Some of the fungi include *Saccharomyces cerevisiae, Candida tropicalis, Cryptococcus sp., Geotrichum sp., Rhizopus stolonifer, Aspergillus niger* and *Fusarium sp.;* they are rot fungi of tomato. The combination of phenolic and essential oil extracts below 2.5 and 1.5 mg/ml of tomato/glucose medium respectively, significantly retarded the growth of these disease pathogens. Thus, the pepper fruit extracts could be used to control food spoilage as a potential source of food preservative.

2.5.3b Host resistance of disease pathogens

The cuticle and the epidermal layer stand as barriers to pathogen penetration in to the plant or its organs. Treatments that cause damage to the cuticle accelerate infection by various pathogens (Barkai-Golan, 2001). Therefore, the cuticle may function not only as a physical barrier but also as chemical barrier, since it may contain antagonistic substances to the pathogen (Egusa *et al.*, 2009). For example, it was reported that Salicylic acid (SA) and Jasmonic acid (JA) are produced by tomato to combat the infection of host-specific AAL-toxin of *Alternaria alternata* fungal pathogen.

The development of pathogen within the host is related to the activity of cell wall degrading enzymes which are responsible for the degradation of organic molecules which then become the source of attack by the pathogen (Barkai-Golan, 2001). As a result the liberated nutrients led to the stimulation of pathogen growth and accelerated disease development. Reports showed that sugars present in the produce, for example glucose, which serve as available nutrients for the pathogen and stimulate its growth is responsible for the inhibition of pectolytic and cellulolytic enzyme production and activity. Other studies showed that another class of inhibitors of cell wall degrading enzymes includes the polygalacturonase (PG) inhibitory proteins present in plant tissue (Barkai-Golan, 2001).

Furthermore, a PG inhibiting proteins from a plant tissue may act on peptic enzymes produced by different fungal species. Reports have shown that pepper fruits cell wall proteins of the host plant inhibited pectolytic enzyme production by *Glomerella cinigulata,* but the pectolytic activity of Botrytis cinerea was much less affected by PG (Barkai-Golan, 2001). Another study by Chung (2012) reported that plants produce toxic reactive oxygen species (ROS) as a defence mechanism against pathogens. This is in response to the microorganism invasion, the plant cells produce excessive amounts of H_2O_2 by a specific plasma membrane NADPH oxidase, called the hypersensitive reaction (HR), which resulted to cell death and consequently to cellular defence against pathogen attack (Niks and Rubiales, 2007).

2.5.4 Mechanism of resistance

Mitcham and McDonald (1992) reported that the cell wall uronide content increased in heat treated tomato fruit while it remained unchanged in the control at 21 °C. As a result heat treatment inhibited ripening associated cell wall modification consequently decrease the infection of the fruit by disease pathogen. Vicente *et al*. (2005) reported that strawberries (75 % red colour) heat treated fruit remained firmer because the activity of cell wall degrading enzymes is prevented. This process would have been responsible for cell wall tissue degradation and the release of nutrients available to the pathogen that cause infection of fruit. The role played by glucose which serves as available nutrients for the pathogen to promote its growth has been reported. Therefore, if the activity of the cell wall degrading enzyme is prevented, glucose production is also unavailable for the fungal pathogen, as a result increases the fruit resistance to disease.

One of the most important characters to consider in plant breeding programmes is the resistance to pathogens that cause diseases (Niks and Rubiales, 2002). It is also recognised that growing resistant cultivars is the appropriate and cost effective approach to crop improvement. But there is a drawback in having durable resistance; the production of host-selective toxins by necrotrophic fungi for example *Alternaria alternata* (Chung, 2012), and host specificity and durability of the resistance incorporated in the cultivars (Niks and Rubiales, 2007) will prevent this proposition to be feasible in the long run. Also, there is a variation in varietal resistance between the indigenous and exotic cultivars imported in to the country. Invariably, it is the imported varieties that the farmers prefer to grow, but the plants cannot withstand the harsh environment.

In other to overcome this problem, Niks and Rubiales (2007) reported that using upright plant habit decreased fungal spore infection on cereals in the field. The reduction was possible because of the crop structure, spacing and the micro-climate as a result the plant arrangement will have better aeration in the environment thereby reduces the chances of infection by disease pathogen. This method is feasible with tomato grown on trellis or metal wire in the field.

2.5.5 *Alternaria alternata* (Black Mould) disease of tomato

Alternaria alternata (Fr.) Keissler is regarded as one of the major storage decay agent of tomatoes, giving the fungus about 80 % infections same as the entire range of fungi that are isolated from the surface of harvested tomatoes (Barkai-Golan, 2001). Grogan *et al.* (1975) described *Alternaria alternata* as a stem canker disease of fresh market tomatoes (*Lycopersicon esculentum* Mill.) causing serious disease infection such as leaf spot, rots and blights on many plant parts. At various times, these microorganisms have been unavoidably identified with many names including: *Alternaria fasciculata*, *Alternaria rugosa* (McAlpine), *Alternaria tenuis* (Nees), *Macrosporium fasciculatum* and *Torula alternata* (Fr.). Mahovic *et al.* (2004) identified Black mould rot disease of tomato as the fungus that appears on the shoulders by the stem scar or on the blossom end of fruit that have been injured by chilling, calcium deficiency, sun exposure and some climactic factors.

Several different pathogens were reported to have caused black mould rots in tomato including *Alternaria sp.* and *Stemphyllium sp*. Invariably in Grogan *et al.* (1975) study it was stated that stem canker is caused by a virulent pathotype of *Alternaria alternata* (Fr.) Keissler which is synonymous to *A. tenuis Auct.* Furthermore prolonged storage that subjects tomatoes to chilling temperatures was reported by Segal and Hayslip (1966) to have increased tomatoes susceptibility to *Alternaria tenuis* infection. The optimal growth temperature of the fungus is 28 °C, but it may continue to grow in relatively high temperatures in storage. The fungus survives on plant debris and its conidia are present in the atmosphere which makes the control of infection by conidiophores difficult.

2.5.6 Vegetative structure of *Alternaria alternata*

The vegetative structure of *Alternaria alternata* is the conidia with multiple cells. This develops into conidiophores that germinate on plant tissue or organ and subsequently elongate to form the mycelia. The fungal spores are found in the air and in soil, also survive on plant debris (Snowdon, 1991). The survival of the fungus in the field soil is based on the widespread distribution of the

inoculum in air (Grogan *et al.*, 1975) and likewise the soil. Spore germination is a preliminary stage to fungal penetration into the host. The environmental condition including temperature, water or moisture and sometimes, available nutrients transferred from the host into the water do encourage spore germination. In addition Barkai-Golan (2001) stated that atmospheric gases such as oxygen (O_2) and carbon dioxide (CO_2) levels in the store where fruits or vegetables are located can also affect germination. For example it was stated that an atmosphere that has 32 % or more of carbon dioxide (CO_2) can only inhibit the germination of *Alternaria alternata* spores.

2.5.7 Conidia and spore formation

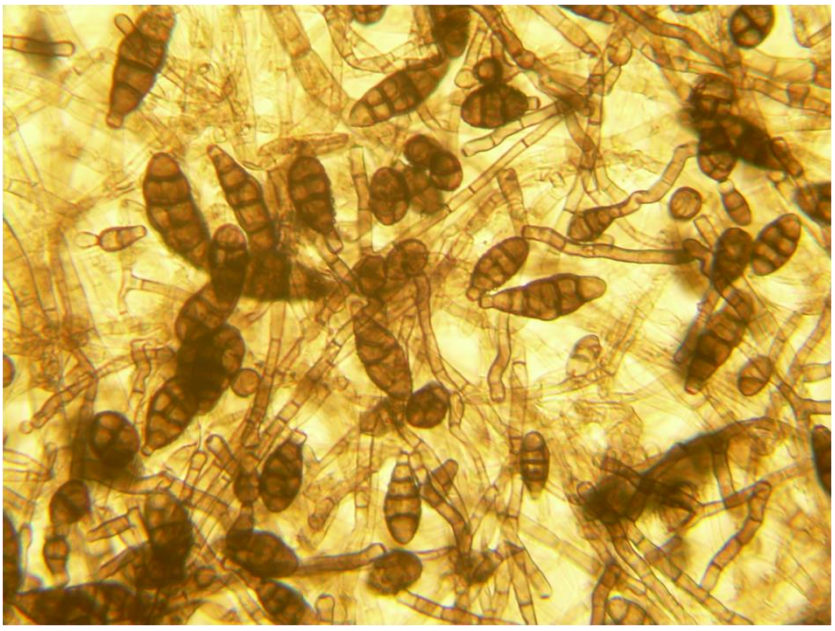

Figure 12: *Alternaria alternata* conidia spores (Barnett and Hunter, 1972)

Alternaria alternata (spores shown in **Figure 12**) was reported to have been causal agent of leaf spot and other diseases such as black mould and stem canker on over 380 host plants (Barnett and Hunter, 1998). It is regarded as an imperfect pathogen having many hosts and causing disease infections such as leaf spots, rots and blights on many plant parts. Conidia the vegetative structure is pale brown to light brown in colour with short conical beak at the tip or sometimes beakless. The conidia are produced in an often branched long chain more than five conidia. Studies have

shown that the pathogen penetrated the host tissue through stomata, lenticels and micro cracks in the epidermis (Swart *et al.*, 1995).

In another studies by Wolf *et al.* (2010) it was reported that by elevating the atmospheric carbon dioxide concentrations increases *Alternaria alternata* sporulation on timothy grass (*Phleum pratense*). Also the leaf carbon-to-nitrogen ratio was greater and leaf biomass than at the lower $C0_2$ concentrations.

2.5.8 Factors that affect the development of the disease

Alternaria alternata is a weak fungal pathogen it requires an opening or injured tissue for penetration and development (Snowdon, 2001). The fungus survives on water that forms on the surface of ripening fruit from rain or dew, the spores germinate in response to soluble nutrients on the fruit surface (Barkai-Golan, 2001). Swart *et al.* (1995) reported that under conditions of high humidity, the fungus grew out of the stomata, lenticels and micro cracks of table grapes and formed an extensive superficial growth within 7 days. The fungus was able to grow into adjacent epidermal cells that surround wounds. This shows the extensive surface growth pattern of the pathogen on tissues and organs of fruit after cold storage.

2.5.9 Host factors that affect the development of *Alternaria* Rot

The point of entry of the fungus can be via growth cracks, opening on fruit skin, mechanical damage or bruises, but infection can also be through the calyx scar or stem scar of the fruit (Barki-Golan, 2001). The fungus may appear on the margin of the stem scar but it remains inactive unless the fruits are subjected to weakening conditions such as chilling injury, sunscald or over ripening (Barkai-Golan, 2001; Snowdon, 2001). Also the production of non-specific toxic metabolites in the infected fruits may prevent the development of *A. alternata* decay. Barkai-Golan (2001) reported that the main toxin in infected tomato fruits was tenuazonic acid, while others such as alternariol, alternariol monomethyl ether and alternuen were found in small quantities.

Swart *et al.* (1995) found that the pathogen penetrated the host tissue through stomata, lenticels and micro cracks in the epidermis. In another studies by Jia *et al.* (2012), it was reported that phytohormone molecules such as ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) have key roles in the disease response to nectrophic fungal pathogens. Both the ET and jasmonic acid (JA) pathways are necessary for susceptibility, while SA response promotes resistance to *Alternaria alternata* f. *sp. lycopersici* infection. Kepczynska (1994) also suggested that endogenous ethylene synthesis and action are essential for the development *of A. alternata*.

2.5.10 Pathogen factors that affect the development of *Alternaria* Rot

The development of *A. alternata* in tomatoes is reported to have been associated with the production of host-selective toxins by the fungus, this is essential for pathogenesis to take place as a result of cellular stresses (Chung, 2012; Barkai-Golan, 2001). In citrus for an example, the fungal infection causes induction of lipid peroxidation on the leaves as well as other organs resulting to rapid accumulation of hydrogen peroxide (H_2O_2), and possibly cell death (Prasad and Upadhyay, 2010; Chung, 2012). The genus Alternaria includes both plant pathogenic and saprophytic species which may affect crops in the field or cause postharvest decay of plant products (Pose *et al.,* 2010). In another study by Wu *et al.* (1997) it was reported that H_2O_2 acted as disease resistance radical in potato as well as a broad range of plant pathogens.

Many studies have reported that *Alternaria alternata* has been the most frequent fungal species invading tomatoes (Barkai-Golan, 2001; Pose, *et al.*, 2010; Graf *et al.*, 2012). The production of mycotoxin by *Alternaria alternata* was adduced to be one of the pathogen factors that assisted in causing the black mould disease in tomato (Graf *et al.*, 2012; Pose *et al.*, 2010). Alternariol (AOH), Alternariol monomethyl ether (AME), and tenuazonic acid (TA) are some of the examples of *Alternaria* mycotoxins that can be found in tomato.

2.5.11 Environmental factors that affect the development of *Alternaria* Rot

The fungus is very common in the air, soil and plant debris. It infects plants that are disposed to the favourable condition for sporulation and areas that maintain inadequate sanitation during

postharvest management (Snowdon, 2001). Environmental conditions during growth, for example, unfavourable high or low temperature, wind, rain or dew, can all affect the crop in terms of yield but also the quality of the produce when put in store (Barkai-Golan, 2001). For example, when free water forms on the surface of ripening tomato fruit from rain, dew or overhead irrigation, spores *of A. alternata* germinate in response to water soluble nutrients on the fruit surface (Egusa *et al.*, 2009; Jia *et al.*, 2013). High temperature was found to increase *Botrytis cinerea* infection of tomatoes through the flower as a result of increase in flower development and senescence (Barkai-Golan, 2001).

Consequently, all these necrotrophic pathogens enter through growth cracks, insect bites or mechanical bruises but infection is often initiated at the calyx scar or stem end of the fruit (Barkai-Golan, 2001). Chilling injury, sunscald or over-ripening which subject tomato fruit skin to weakening conditions can also be points of entry of the pathogens. Environmental conditions may also affect the pathogens directly. Many pathogens remain in the soil or survive on plant debris in the field, but their dispersal to the next hosts will depend on wind, rain and other dispersal agent**.**

2.6 CONTROL METHODS OF *ALTERNARIA ALTERNATA* DISEASE

Barkai-Golan (2001), reiterated that the condition of the environment during growing of fruit and vegetable crops is of a great importance in order to prevent infection of disease pathogens. Examples of such condition are: high or low temperature, wind, rain or hail. High temperature increases the susceptibility of *Alternaria alternata* infection of tomato resulting to an increase in the rate of flower production consequently increases senescence (Barkai-Golan, 2001). Therefore, growing tomato with suitable temperature conditions, such as 20-25 °C will decrease the amount of flower produced thereby control *Alternaria* infection. Wider plant spacing within the row against narrower spacing was reported by Legard *et al.* (2000) to have reduced the incidence of decay rot on strawberries. Cultural practices, such as pruning of trees and destruction of crop debris, can affect the survival of pathogenic fungi.

2.6.1 Cultural control method

Cultural practices improvement can also reduce the level of inoculum through sanitation or modifying the crop canopy to produce a microclimate leading to conditions less favourable for pathogen infection (Legard *et al.*, 2000). Likewise increased plant density was reported to reduce the efficacy of fungicide applications by reducing plant coverage. Mahovic *et al.* (2004) reported that fruits and vegetables vary in their internal ability to resistance to decay. For example, crops that have active wound healing system are pear fruit (Spotts et al., 1998), jade leaves, tomato fruit and bean pods (Dean and Kolattukudy, 1976) are more resistant than crops that don't have these attributes. The mechanism of wound healing is through the protective suberin layer e.g. in potato tubers and tomatoes forming a natural abscission zone at the stem scar; use these processes as defence mechanism against infection from decay pathogens.

The potential for development of fruit decay is reduced if the produce is harvested dry and free of disease pathogen infection at the time of harvest. Although, harvested fruit may have some level of disease pathogens infection which are not visible at the time of harvest, but with good sanitation practices the development of this microorganism into fruit decay will be reduced during postharvest handling. The process should develop sanitation that will provide clean environment in the pack house and packing materials.

2.6.2 Chemical control method

At present the export trade in fruits and vegetables rely on chemical compounds to protect the produce against entry of microorganisms that cause diseases in storage during transit. For example, fungicides and bactericides are used for the purpose of killing or controlling the development of pathogens (Barkai-Golan, 2001). The chemical has to make contact with the pathogen before it can be more effective. Sometimes the chemical substance and concentration of efficacy is pathogen dependent or the chemical compound will act in synergy with another compound before it becomes effective. Chemical treatments can be applied with these three methods: (i) pre-harvest application to prevent infection in the field; (ii) to carry out sanitation

procedure to reduce the level of inoculum in the environment of the injured fruits or vegetables present; (iii) postharvest application to prevent infection through wounds and to wipe out or weaken already existed infections, in order to prevent their development and spread in storage (Barkai-Golan, 2001).

The current study is to elucidate the importance of postharvest fungicides against fungal pathogens of fruit and vegetables but laying emphasis on *Alternaria alternata* (Black mould) disease of tomato. It is important that the open wounds created during harvesting, handling and packaging, which are the major sites of invasion by postharvest wound pathogens, should be protected by chemical compounds so as to considerably decrease decay in storage.

During the last five decades, thirty or more organic compounds have been identified to control decay caused by postharvest pathogens, such chemical fungicides include biphenyl (diphenyl), sodium ortho-phenylphenate, dicloran and sec- butylamine (Barkai-Golan, 2001). Their selection was based on these three criteria: (i) sensitivity of the pathogen to the chemical substance; (i) ability of the chemical to reach infection site through penetration of surface barriers; (iii) tolerance of the host to both the injury and any toxic effect of the pathogen, as well as the adverse effect of the quality of the chemical compound (Eckert and Ogawa, 1985).

Biphenyl was extensively used on citrus fruit to inhibit sporulation of *Penicillium* species on decay fruits meant for export, but was reported not to be active against *Alternaria*, *Rhizopus*, *Mucor, Phytophthora* and *Geotrichum* species (Barkai-Golan, 2001). In addition, thiabendazole (TBZ) which is a benzimidazole compound and a systemic fungicide is not effective on these microorganisms (Barkai-Golan, 2001). In another study by Eckert and Ogawa (1998), it was reported that due to the limitations experienced in the use of TBZ against some important postharvest pathogens, such as *Alternaria, Rhizopus, Phytophthora* and *Mucor* therefore benzimidazole compounds may be used in addition with other chemical to control decay in storage. In another report Barkai-Golan (2001), stated that Iprodione and vinclozolin, which are

dicarboximide fungicides were used as alternative to TBZ on products such as cucumbers, tomatoes, strawberries, eggplants and grapes. Iprodione itself is effective against *Alternaria*, and considerably reduces the infection of this pathogen on mangoes (Prusky *et al.*, 1983).

In another study by Kumar *et al.* (2013), it was reported that out of twelve fungicides tested, only five proved to be effective fungicides as they completely inhibited the growth of *Alternaria alternata* on chilli pepper. The chemical compounds include: Bavistin, Indofil M-45, Chlorothalonil, Vitavax and Thiram. Another chemical compound introduced in to the market for postharvest treatment, called Imazalil a systemic fungicide, inhibited the biosynthesis of ergosterol, an essential component of fungal membrane was effectively used on Alternaria (Barkai-Golan, 2001; Brown, 1984). An effective and considerable control of both stem-end rot and anthracnose was achieved when imazalil was applied with hot water at 53 °C (Spalding and Reeder, 1986[b]). The chemical compound inhibits spore germination and mycelial growth of *Alternaria* and suppresses decay development caused by the pathogen in the following crops: apples, pears, persimmons, tomatoes and bell peppers (Spalding, 1980; Prusky and Ben-Arie, 1981).

The study carried out by Prusky *et al.* (2006), reported that application of a combination of hot water spraying and brushing (HWB) for 15-20s, followed by spraying with 50mM HCl effectively controlled *Alternaria* rot in stored mango fruit. Hot water brushing (HWB) treatments with increased concentration of prochloraz were more effective than with the acid alone. Also hot water dip treatment of persimmon fruit followed by 50mM HCl reduced *Alternaria* rot. Sodium ortho-phenylphenate (SOPP) was reported to be an example of broad spectrum fungicide against fungal pathogens and anti-bacterial activities. For example solutions of SOPP with a pH of 11.5 is effective and save on many fresh fruits and vegetables which include citrus, apples, pears, peaches, tomatoes, peppers, cucumbers, carrots and sweet potatoes (Barkai-Golan, 2001).

The inhibitory effects of essential oils and plant extracts as antifungal chemical compounds on fruits and vegetables deserve adequate mentioning in this study. Barkai-Golan (2001) reported

that the major plants having the highest antifungal activity were pepper (*Capsicum sp*.) and garlic (*Allium sp.*). Gel derived from Aloe Vera plants was found to contain antifungal compounds that act against postharvest pathogens such as *Alternaria alternata*, *Botrytis cinerea, Penicillium expansum* and *Penicillium digitatum* (Barkai-Golan, 2001). The report further stated that its natural gel suppressed germination of spores as well as mycelial growth of fungi, with *P. digitatum* and *Alternaria alternata* the most sensitive species.

The cassia oil and thyme oil were reported to have exhibited an antifungal control on Alternaria alternata disease pathogen which infected cherry tomato (Feng and Zheng, 2007). For example the cassia oil inhibited completely the growth of *A. alternata* at 300-500ppm, while thyme oil inhibition was 60 % at 500 ppm (Feng *et al.*, 2011; Feng and Zheng, 2007). Similarly, Tian *et al.* (2011) determined the antifungal activity *in vitro* of the essential oil extracted from the seeds of dill (*Anethum graveolens L*) subsequently use the oil to control *Alternaria alternata* contained in the poisoned food. The effect of the dill seed oil on the inhibition of decay development on cherry tomatoes was tested in vivo by exposing inoculated and control fruit to the oil vapour at 120 µl/ml and 100 µl/ml concentrations respectively. The oil was found to inhibit the mycelial growth after nine days.

2.6.3 Biological control method

The concern of official and the public is becoming greater now about the presence of chemical fungicide residues on food, the development of pathogens resistance and most importantly, the withdrawal of a number of fungicides used to control postharvest diseases of fruits and vegetables are some of the reasons for the increased interest in the potential of using biological control as an alternative non-chemical means of control of fungal decay. For instance Klein and Lurie (1992) reported that some chemicals including a fumigant ethylene dibromide have lost their Environmental Protection Agency (EPA) USA registration, while others may follow soon. In another study conducted by National Academy of Sciences (NAS), an agency of EPA, concluded

that fungicides constitute 60 % of the cancer risk among all the pesticides used on food (Wilson *et al.,* 1991).

The cost of developing, testing, and registering a new fungicide is increasingly prohibitive; hence there is a need for research into alternative methods of control. As a result biological control method could be considered as one of the alternatives to non-chemical means of decay suppression.

The biological control is defined as the use of naturally found microorganisms which antagonizes postharvest pathogens of some fruits and vegetables (Barkai-Golan, 2001; Wilson *et al.,* 1991). Wilson *et al.* (1991) and Wisniewski *et al*. (2001) defined the biological control of plant disease as `the decrease of inoculum or the disease-producing activity of a pathogen accomplished through one or more organisms, including the host plant but excluding man`. Droby *et al.,* (2009) reported that antagonism between microorganisms involves fungi (e.g. yeast) and bacteria (Wilson *et al.,* 1991; McSpadden, 2002), which naturally present in the soil and the surfaces of plants. To identify a suitable antagonist as promising agents for disease control, a screening system should be developed such that it simulates natural inoculation and the inoculum should be applied to the infection sites and at the appropriate time (Barkai-Golan, 2001).

Studies have shown that the use of antagonistic microorganisms has been explored to control various plant pathogens, but the research has been successful largely in the laboratory than the field (Wilson *et al.,* 1991). Furthermore, the antifungal activities were concentrated on these various stages: (i) the antagonist inhibits the pathogen directly by secretion of antibiotics, for example, *Pseudomonas cepacia* controlled *Botrytis* and *Penicillium* rots of pome fruit by producing an antibiotic, pyrrolonitrin; (ii) the antagonist competes with the pathogen for nutrients or space; an example yeast antagonist *Trichoderma* species (US-7) act against the citrus pathogen, *Penicillium digitatum*; (iii) the antagonist induces resistance in the host; for example, by indirectly changing the chemical and osmotic environment at the wound site to favour the antagonist over

the pathogen; (iv) the antagonist directly interacts with the pathogen, an example of such process is the isolation of the antagonistic fungi, *Trichoderma* species, to reduce the *Botrytis* responsible for pre- and post-harvest rotting of strawberries (Wilson *et al.,* 1991). All these processes focussed on the promotion and management of natural epiphytic antagonists inhabiting fruit and vegetable surfaces.

In another report by Barkai-Golan (2001), which stated that fungicides may change the micro flora on the plant organs thereby affecting the microorganisms other than the pathogens against which control were intended, thereby changes can occur that will affect the pathogen resistance of the host. This situation can be modified and suit the purpose of promoting beneficial antagonistic microflora against the pathogen (Shafique and Shafique, 2012; Barkai-Golan, 2001). Plant secondary metabolites such as natural plants derivative compounds can contribute a lot in controlling disease pathogens (Wilson *et al.,* 1991; Shafique and Shafique, 2012). For example, *Alternaria alternata* species was subjected to biological control by *Tagetes erectus* L. root extract which caused the reduction of the fungus biomass as much as between 81-92 % (Shafique and Shafique, 2012); flavour compounds such as acetic acid, jasmonates, glucosinolates, propolis, chitosan, essential oils and plant extracts also reduce fungal rotting of fruit and vegetables thereby prolonging shelf life (Tripathi and Dubey, 2004).

Application of *Trichoderma harzianum* spore suspension to tomato fruits decreased disease infection significantly at higher concentrations of 10^8 cells per mil and at high filtrate culture the pathogen spore germination on the surface of tomato fruits was reduced leading to decrease of rot symptoms (El-Katatny and Emam, 2012); grape, pear, kiwi and strawberry (Ahmad-Odeh, 2006).

The combination of biocontrol agent and other methods such as heat treatment was reported to be one of the most effective techniques at controlling postharvest fungal spoilage in tomato (Zhao *et al.,* 2010). Other areas open to control postharvest diseases of fruits and vegetables reported by Wilson *et al.,* (1991), included the use of plant-derived fungicides from secondary plant metabolites, and the manipulation of resistance responses in harvested products. In the study of

Zhao *et al.* (2010), it was reported that either heat treatment or *Pichia guilliermondii* reduce decay caused by *Botrytis cinerea*, *Alternaria alternata* and *Rhizopus stolonifer* on tomato. But the combination of heat treatment followed by the application of *P. guilliermondii* had the best efficacy in protecting tomatoes from fungal rot caused by these pathogens.

Attention has been focused on naturally occurring yeast species which do not produce antibiotic substances in order to act as antagonists. They colonize the surface of the wound quickly and for long periods. They produce extracellular polysaccharide compounds, which enhance their survival and make use of host nutrients to reproduce rapidly and are affected minimally by pesticides (Barkai-Golan, 2001). In another study by Wang *et al.* (2008), where it was reported that the yeast *Rhodosporidium paludigenum* Fell and Tallman isolated from fruit wounds and marine resources e.g. south of East China Sea, showed that washed cell suspension of *R. paludigenum* gave a better control of *A. alternata* than any other treatment. Furthermore, its efficacy was largely dependent on the concentration of the antagonist inoculum. For example, the concentration of the washed yeast cell suspension of 1x 10^9 cells per ml reduced the percentage rate of black rot of cherry tomato fruit to 37 %, which was remarkably lower than water (control).

2.6.4 Non-chemical control method

In the past years there has been a steady increase in the interest of finding alternatives to the use of synthetic fungicides for postharvest disease control. This has led to considerable research in physical treatments that could serve as alternatives to fungicides or the use of microbial antagonists as protective agents in as much the same way as chemical compounds for disease control (Lurie, 1998). Examples of such treatments include: heat treatments, salt solution, induced resistance, microbial antagonists, yeast, bacteria, chitosan, ionizing radiation and ultraviolet illumination (Wisniewski *et al.*, 2001; Barkai-Golan, 2001).

Heat treatments can be applied to the commodity by hot water dips and sprays, hot water vapour or dry air, infrared or microwave radiation (Fallik *et al.*, 1993; Fallik *et al.*, 2001). *In vitro* studies

have shown that spore inactivation increases with both temperature and duration of treatment; as a result conidia of *Alternaria alternata* may be inactivated equally by treatment for 2 min at 48 °C or 4 min at 46 °C. The conidia of the pathogen may be inactivated by dipping tomatoes for 1-2 min in water heated to 55 °C which was the optimal antifungal treatment for control of *Alternaria alternata* (Fallik *et al.,* 1993; Lurie *et al.,* 1998). In another study it was reported that germinated spores as well as elongated germ-tubes or young hyphae were more sensitive to heat than non-germinated spores; as a result *Alternaria* rot was effectively controlled in tomatoes heat treated 8 h after inoculation than in those treated immediately after inoculation (Barkai-Golan, 2001).

2.7 HEAT TREATMENT OF FRUIT

Many studies including Ben-Yehoshua and Porat (2005) reported that postharvest heat treatments of fruit started at the beginning of the first decade of the 20th century where they were used on a commercial scale to control fungal diseases and pest infestation of horticultural crops. However, by the discovery of Thiabendazole, a synthetic, systemic, and selective fungicide, heat treatment was abandoned because the chemical cost less, effective and easy to apply. Consumers are wary of food products that contain chemical residues as a result the use of agrochemicals in fruits and vegetables is declining in a slow progression.

Another reason for the decline is that many fungi are developing resistant strains because of the improper and prolonged use of these chemicals; as a result their efficacy has diminished (Adaskaveg *et al.,* 2002). The prohibitive costs of registering new synthetic chemicals and the difficulties in maintaining the registration of the approved ones have resulted for search for a reliable, cost effective, and environmentally friendly system. Invariably, postharvest heat treatment was favoured by satisfying all these requirements.

2.7.1 Development of early heat treatments

Postharvest heat treatment of fresh fruits and vegetables was the process that was involved in preserving horticultural food products after the First World War (Ben-Yehoshua and Porat, 2005). The system started with hot water, 44-48 °C, to clean the fruit in a tank as well as a partial disease

control of green and blue moulds (*Penicillium digitatum* and *Penicillium italicum*). Many studies have shown that postharvest decay is one of the major factors limiting the storage life of fresh commodities (Kader, 2002), with losses ranging between 5-25 % in developed countries and 20 to 50 % in developing countries. Therefore, it will be of priority to develop reliable methods to minimise losses caused by postharvest pathogens.

It is well understood through various studies that the most important environmental factor that affects decay development is temperature. For example, storage of horticultural commodities at low temperature that does not cause chilling or freezing injuries will slow pathogen growth and reduce decay (Schirra *et al.,* 2000a). In the same manner applying high temperature to fruit for a short time before storage at low temperature have a double effects on the product i.e. disinfestation and reduction in decay incidence. Furthermore, the studies described the mode of action of heat treatment on the causal agents; they exert their effects either by slowing pathogen growth or killing its germinated spores. Heat treatment can also enhance host pathogen defence responses (Lurie, 1998), thus renders the commodity to have more resistance. High temperature heat treatment may also partially melt the epicuticule surface of fruits or vegetables, thus resulting to sealing of micro-cracks and wounds which could serve as entry points to pathogens (Schirra *et al.,* 1999b).

2.7.2 Types of heat treatment

Time immemorial fresh fruits and vegetables have been part of the human diet although fruits and vegetables have always provided variety in the diet through their differences in colour, shape, taste, aroma, and texture, but the importance of their postharvest treatments have been emphasised by various studies to improve their shelf life and sustainability. Postharvest decay is the major factor limiting the extension of storage life of many fresh harvested commodities. All fresh fruits and vegetables for domestic or export markets should be free of dirt, dust, pathogens and chemical residues before they are packed. The susceptibility of freshly harvested produce to postharvest

diseases increases during packaging, transporting and prolonged storage resulting to physiological changes that enable pathogens to develop in the fruits.

The use of synthetic chemicals on harvested fresh produce is becoming more difficult to justify mainly because consumers are sceptical on the residual effect of these compound on human health. Therefore, the interest in non-chemical methods for postharvest decay control of horticultural crops has been increasing (Schirra *et al.*, 2000; Ferguson *et al.*, 2000; Fallik, 2011). The progress made in agronomic, processing, preservation, packaging, shipping, and marketing technologies globally have enabled the fresh fruit and vegetable industry to supply consumers with a wide range of high quality produce all year round. However, the economic losses caused by postharvest pathogens can be high and the avoidable losses between the farm and the consumer could be minimised. These losses together with the costs of harvesting and handling have increased the cost of the produce several times as they are moved from the farm to the consumer.

The diseases caused by postharvest pathogens have always relied on chemical control for ensuring the quality of harvested produce; various methods to control postharvest decay are being developed. Such methods include heat treatments which have been effective in controlling pathogens that are the main causes of pre-storage and postharvest decay development (Lurie, 2008; Barkai-Golan, 2001; Fallik, 2011). Studies have shown that postharvest heat treatments to control decay development during storage are applied for a relatively short time; minutes, because the decay causing agents are found on the outer skin layer of the fruit or in the first few cell layers under the skin (Barkai-Golan, 2001). There are different forms of heat applied to control the decay causing agent of freshly harvested produce; these include hot water dipping, vapour heat, dry hot air and very short hot water rinsing and brushing (Lurie, 2008; Fallik, 2004).

2.7.3 Hot water dipping

The exposure of various fruits to high temperatures (50-60 °C) either as a wet or dry treatment has been demonstrated to have a significant impact on controlling both postharvest diseases and fruit

quality. Many fruits and vegetables tolerate exposure to water temperatures of 50-60 °C for up to 10 min, but they can be exposed to shorter time and still control many postharvest plant pathogens (Lurie, 1998). In comparison, hot water dips for fruit require 90 min exposure to 46 °C. Fallik (1993) reported that dipping melon fruits in hot water at 55 °C for 1-2 min resulted in optimal antifungal treatment for control of *Alternaria, Fusarium, Rhizopus* and *Mucor* species. Also inoculated mature green and pink tomato fruits were held for 3 days at 38 °C completely inhibited decay caused by *Botrytis cinerea*.

The effectiveness of a pre-storage hot water dip of bell peppers and tomatoes was investigated to reduce rots; the results showed that hot water dip at 50-53 °C for 2-3 min significantly inhibited the disease pathogens (Lurie *et al.,* 1998). Another study reported that a minute dipping of rock melon to 60 °C hot water was the optimum time and temperature combination for the control of decay development caused by *Alternaria sp., Fusarium sp.,* and *Colletotrichum sp*. after storage for 3 weeks at 5 °C. Likewise immersing bell peppers (*Capsicum annum*) in hot water at 45 °C and 53 °C for 15 min and 4 min, respectively; and storing bell peppers at 8 °C inhibited fungal disease pathogen infections (Fallik, 2011).

2.7.4 Vapour heat

Vapour heat at 44 °C for 1h was reported to have been used against *Botrytis cinerea* in strawberries, while exposing pear fruits to 37 °C for 2 days pre-storage inhibited decay due to *Mucor piriformis E. Fisch* (Fallik, 1993). Water is regarded as the most effective heat transfer medium, as a result for air treatments the moisture content greatly influences heat transfer and heated moist air is generally more effective at killing pathogens than dry air at the same temperature (Mitcham and Cantwell, 2002). In an another study, the effect of vapour heat at 46 °C on the export quality parameters of fresh tomato was investigated using two maturity stages i.e. breaker and green stage; and secondly two fruit sizes; between 280-450 g and between 200-279.9 g (Hurtado *et al.,* 2009). The quality variables measured are external appearance, internal appearance, and flavour; while the quantitative ones are weight loss, firmness, soluble solids, pH,

and citric acid. The report concluded that treatment of the tomato fruits with vapour heat caused a non-acceptance of the produce for export purposes. The main parameters of rejection were the qualitative variables and the external appearance.

2.7.5 Dry hot air

Pre-storage hot air treatment of apples at 38 °C in combination with calcium chloride has also been shown to significantly reduce storage decay caused by *Penicillium expansum* and *Botrytis cinerea*; it was also used in combination of a biocontrol agent, *Pseudomonas syringea* to control these rots (Wisniewski *et al.*, 2001). Other studies on *in vitro* heat treatment 38 °C of fungal pathogen (Lurie *et al.*, 1998; Zhao *et al.*, 2010), for example, *Botrytis* and *Alternaria* species on tomato spoilage, it was reported that dry hot air markedly inhibited the mycelia growth of these fungi. After 24 h hot air treatment on *Botrytis* the inhibitory rate was 89 % but further treatment for 48 h or 72 h completely inhibited the mycelia growth. In the case of *Alternaria* species twenty four heat treatment inhibited 18 % of the mycelia elongation, while 65 % inhibition was obtained after 72 h treatment.

2.7.6 Hot water rinsing and brushing (HWRB)

This a technology based on a brief hot water rinsing and brushing for cleaning and disinfestations of fresh produce at the same time. The system was introduced commercially to be used on fruits and vegetables and worked by rinsing and brushing at temperatures of 48 °C to 62 °C for 15 to 25 s, depending on the commodity (Fallik, 2011). Also by treating pink tomatoes with hot water rinse and brushing at 52 °C for 15 s or dipping the fruit in water at 52 °C for 1 min significantly reduced decay development caused by *Botrytis cinerea* after 3 weeks in storage at 2 °C or 12 °C followed by an additional 5 days at 20 °C. Fallik (2004) reported that rinsing and brushing bell peppers immediately after harvest at 55 °C for 12 s and 52 °C for 15 s on red tomatoes reduced decay incidence significantly and also maintain fruit quality as compared with untreated fruit. Furthermore, the HWRB 52 °C treatment enhanced resistance against artificially inoculated Botrytis cinerea when tomatoes were inoculated 24 h after treatment. Ripening inhibition was also

reported to have occurred indirectly in HWRB - treated fruit by inhibited colour development in melons and tomatoes. A short hot water brushing treatment at 62 °C for 20 s was reported to have controlled green mould (*Penicillium digitatum*) decay in citrus fruit, improved the colour retention in litchi fruits and also improved the quality and shelf life of pomegranate (Wisniewski *et al.*, 2001). As a result of the various studies this simple technology has demonstrated that a combined treatment of a hot water rinse and brushing improved the overall quality of fresh harvested produce and reduced postharvest decay development while maintaining fruit quality.

2.8 Pathogen control through heat treatment

Pre-storage heat treatment has been demonstrated as a promising method of postharvest control of fungal rot. It is also a safe and environmentally friendly procedure with increasing acceptability in commercial operations. It is used successfully, to control the incidence of postharvest disease in many commodities (Fallik, 2004). Studies have shown that heat treatments have a direct effect on pathogens by slowing down germ tube elongation or inactivating or outright killing germinating spores, thus reducing the effective inoculum size and minimising rots. Water is the preferred medium of application as it is a more efficient medium of heat transfer than air (Lurie *et al.*, 1998). Also pre-storage hot water treatments, methods of hot water immersion and treatment duration have been reviewed by Fallik (2004).

Heat treatment can control decay development through physiological responses of the fruit tissue. These responses include inducing antifungal-like substances that inhibit fungal development in the fruit tissue, such as PR proteins- chitinase and β-1, 3 glucanase and stabilise membranes (Schirra *et al.*, 2000). This type of response of the fruit tissue to pathogen infection will reduce postharvest treatments with agrochemicals as a result research efforts can be focused on the enhancement of host resistance to pathogens through physical, chemical or biological methods (Wilson *et al.*, 1994; Ben-Yehoshua *et al.*, 2000).

Many studies have shown that pre-storage hot water heat treatment appears to be one of the most promising methods in postharvest control of rot decay (Couey 1989; Ben-Yehoshua *et al.*, 2000; Fallik *et al.*, 1995; Wijeratnam *et al.*, 2005). These studies showed that generally effective heat

treatments are applied for short periods of time for example three to five minutes because the target micro-organisms are found on the surface or within the first few outer cell layers of the fruit or vegetable. The reports of Mitcham and Cantwell (2002) stated that for a given species of fungi, spore inactivation increases with both temperature and duration of treatment. For example, spores of *Botrytis cinerea* may be inactivated equally by treatment for 2 min at 48 °C or for 4 min at 46 °C on grape tomato. Germinated fungal spores are much more sensitive to heat than non-germinated spores. As a result the length of time between inoculation and heat treatment can be a factor which determines the effectiveness of a heat treatment.

The essence of heat on the decay organism is to eradicate or simply to control the rot. Therefore, some factors can be responsible for effective heat treatments on the pathogen these include; disease organism, temperature of heat treatment, length of heat period, age of spores, moisture content of spores, and germination of spores (Mitcham and Cantwell, 2002). In carrying out some experiments on the effect of heat on the fungal spores, for example in the study carried out by Zhao *et al.* (2010) the report stated that the *in vitro* heat treatment markedly inhibited the mycelia growth of *Botrytis cinerea*. After 24 h treatment, the inhibitory rate was 89%, further heat treatment for 48 or 72 h completely inhibited the mycelia elongation. *Alternaria alternata* growth was inhibited by 18 % in 24 h of heat treatment but increased to 65 % after 72 h.

Species of fungi have their effective control on spore germination increasing with both temperature and duration of treatment. For example, hot water dips on *Botrytis cinerea* on green bell peppers were effective at water temperatures of 55 °C and 58 °C for 5 min and 60 °C for 2 min gave complete control of *Botrytis* rot without injury to the fruit. Dipping inoculated mature green and pink tomato fruits for 3 days at 38 °C completely inhibited decay caused by *Botrytis cinerea* one of the main postharvest pathogens of tomato (Fallik, 1993). Immersing the fruit in hot water at 55 °C for 1-2 min was the optimal antifungal treatment for control of *Alternaria* species. Lurie *et al.* (1998) reported that hot water at 50 to 53 °C for 2 to 3 min gave the best result to control *Alternaria* pathogen infection of bell peppers and tomatoes.

It was reported that heat treatment by immersion has a beneficial effect in preventing rot development in many temperate, sub-tropical and tropical fruit and vegetables. This method has a number of advantages which include relative ease of use, short treatment time, ease of monitoring of fruit and water temperatures, and the killing of skin-borne decay pathogens. Another important economic advantage of hot water immersion technology is that the cost of a typical commercial system is about 10 % of that of a commercial vapour heat treatment system (Jordan, 1993; Lurie, 1998; Fallik, 2003) which makes the system affordable to farmers in developing countries. However, the physiological responses of cultivars of different fruit species to heat treatments can vary by season and growing location. The differences in climate, soil type, season production practices, and fruit maturity at harvest might be responsible for this variation.

2.8.1 Heat damage

Many studies and reviews have shown that heat treatments affect the products in both positive and negative ways in addition to disease control (Lurie, 1998; McDonald *et al.*, 1999).Although my investigation has focussed on the positive response of commodities to heat treatment, at the same time underestimate the negative response. Lurie (1998), review stated that there is always a danger of tissue damage if fruits and vegetables are heat treated which may lead to increased decay development. Likewise, Fallik *et al.* (1996) reported that scanning electron microscopy analysis of red pepper fruit dipped in hot water at 55 °C for 3 min, showed both external and internal heat damage.

Some studies have reported the adverse effects of high temperature on tomato besides the advantage of disease pathogen control. For instance during the growing of the tomato crop (Thanh and Acedo Jr., 2006) reported that high temperature resulted to a restriction on lycopene (red colour) in the fruit. As a result the fruits developed light red or yellowish red colour since B-carotene formation is produced instead. Some damages that can be sustained during heat treatments include: increased rate of water loss, cracks (Fallik *et al.*, 1996), discolouration on the surface or internal portion of the commodity, increased susceptibility to decay if surface is

injured, loss of acidity, reduced shelf-life, and inhibition of ripening, uneven ripening (Mitcham and Cantwell, 2002).

Polenta *et al.* (2006) have also shown that heat treatments applied to mature green tomatoes resulted to an increase in anaerobic processes and the long-term heat stress inhibited colour development irrespective of storage temperature. Air heat treatment reduced titratable acidity by increasing malic acid metabolism. Acetaldehyde concentration was also increased when the fruits were immersed in hot water for 60 min which produced a low-aerobic environment (Polenta *et al.*, 2006).

2.8.2 Application of heat treatment of tomatoes

This section focuses on the effects of heat application on tomato and its postharvest quality. Such effects will include postharvest heat treatment on aspects of ripening, development of thermo tolerance and other physiological changes that occur as a result. For example: ethylene production, respiration softening, colour change and taste components such as soluble solids, acidity and volatile compounds. Studies have shown that heat treatment substitutes a non-damaging physical treatment for chemical application to postharvest tomato diseases. Many studies have also dealt with specialized aspects of heat treatments; but this section will discuss two of the three methods in use to heat tomatoes; hot water and hot air.

Lurie (1998) reported that many fruits and vegetables can tolerate exposure to water temperatures of 50-60 °C for up to 10 min, but shorter exposure at these temperatures are also effective to control many postharvest plant pathogens. McDonald *et al.* (1999) reported that hot water treatment at 42^0C reduced decay by 60 % in tomato, whereas heat treatment had no effect on ripening of the fruit. It also follows that at red ripe stage heat treatment had no effect on firmness, while fruits treated at 39 and 42 °C were preferred in terms of taste and texture. Lu *et al.* (2010), found no significant difference in the taste, the total soluble solids (TSS)/titratable acidity (TA), or sugar/acid ratio. Also partially ripened green tomatoes treated with hot water (37-50 °C)

effectively reduce chilling injury when stored at 2 °C (Whitaker, 1993; Fallik *et al.*, 1993). Dipping tomatoes for 1-2 min in water heated to 55 °C was effective to control *Alternaria* species (Fallik *et al.*, 1993).

Hot air treatment (35-40 °C) was reported to inhibit ethylene synthesis within hours in tomatoes (Lurie, 1998). Furthermore, continuous storage of tomatoes in hot air of 30-40 °C affect the fruit firmness i.e. it becomes softened more slowly compared to when held at 20 °C. Flavour characteristics of the fruit such as titratable acidity (TA) declined in hot air heated tomatoes as well. Also ripe tomatoes heated in hot air and stored at 13 °C before ripening had highest volatile compounds compared to mature green fruits (Lurie, 1998). Chlorophyll content in tomato pericarp was also reported to have decreased during a hot air treatment of 35-40 °C consequently inhibits lycopene synthesis.

CHAPTER 3: PRODUCTION OF MORE *ALTERNARIA ALTERNATA* SPORE CULTURE ON AGAR MEDIA

3.0 Introduction

The use of spores as inocula in phytopathological experiments has been reported (Morris and Nicholls, 1978). Although, it might be desirable to determine the concentration of spores in an aliquot suspension studies, as this might have an effect on the results. But in other studies it might not be relevant to carry out such procedure. There are methods of counting the number of spores in an aliquot suspension of fungal pathogens. For example, the use of haemocytometer (HC) slide, microscope, and colony forming unit (CFU).

A haemocytometer can be defined as a specialised microscope slide that permits you to easily count the number of cells in the microscope's field of view to determine the number of cells per millimetre in sample. Haemocytometer is characterised by etched glass counting chamber for estimating the number of cells in a suspension. The chamber holds a specific volume of liquid and has grid lines etched into the glass so that cells can easily be counted using a microscope.

At the centre of the chamber the etched lines form a 25 square (5 × 5 square) grid. There are actually 2 chambers on each haemocytometer. In addition, there are 16 smaller squares etched into each of the 25 squares which represent the visual aid for counting cells. This is the area of the chamber that you want to use for counting. When a coverslip is placed on the haemocytometer, the dimensions of all 25 squares are:

0.1 cm × 0.1 cm × 0.01 cm or 1/10,000th of a millimetre

Colony forming unit (CFU) is used to measure the number of bacteria and fungi spores. This process has its common use in medical mycology and plant pathology to determine the spore concentration of fungi in indoor and outdoor environment using artificial culture media. For example, the study by Takahashi (1997) showed that the concentration of fungi 'indoor' and flora 'outdoor' can be analysed using Reuter centrifugal air sampler and dichloran 18 % glycerol agar (DG18), and compared with the levels assessed with potato dextrose agar (PDA). Conidia and spores are the inoculum of infection of

pathogenic fungi, but their numbers may differ with different microorganisms and method of multiplication on agar media. Therefore, the approximate number of spores used for the control of disease pathogen will be known to support other research work.

3.1 GENERAL MATERIALS AND METHODS

3.1.1 Biological and agar media materials

Two strains of Alternaria alternata isolate were used for the experiments. They were purchased from the Commonwealth Agricultural Bureau International (CABI) and were accompanied with the following information:

IMI number: 89342 Pathogen no. (PON) 74351 – First isolate was purchased from CABI

A strain from rotted red tomato from the Writtle College, Research Glasshouse was used as the second isolate.

Growth medium: Distilled water principal component agar also tap water agar + wheat straw (TWA + WS) 23 °C.

Alternaria alternate f. sp. *Lycopersici* (Tomato pathogen)

The isolate with the pathogen no. 74351 from CABI was used as the first isolate, while the second isolate of *Alternaria* was a strain of rotten red tomato cultivar 'Delycassi' grown in the Research Glasshouse of the Writtle College, Chelmsford. Two to three millimetre (2-3mm) diameter of the infected fruit skin was cut and layered flat on PDA in a 9 cm diameter petri-dish plate for re-culturing.

Agar media

The agar media used for the experiments were potato dextrose agar (PDA) and corn meal agar (CMA). The materials were obtained from the science laboratory of the Writtle College, Chelmsford. The materials were purchased from Oxoid Ltd., Basingstoke Hampshire England.

Components of Agar media:

- PDA: Typical formula (g/l) potato extracts 4.0; dextrose 20.0; agar 15.0. Four gram (4.0g) of potato extract is equivalent to 200g infusion from potatoes – Oxoid Ltd., Basingstoke Hampshire England.
- CMA: Typical formula (g/l); corn meal extract (from 50g whole maize) 2.0; agar 15.0; pH 6.0 ± 0.2 – Oxoid Ltd., Basingstoke, Hampshire England.

3.1.2 Other materials used for culturing

Lamina flow hood was used to re-culture the isolates under aseptic environment in the micro-biology laboratory. In addition, 70 % alcohol (methylated spirit) was used to clean the cabinet. Twenty millilitre (20 ml) PDA agar plate was inoculated with 2-3 mm mycelia plug to develop fresh culture of the isolate from CABI and the strain from the rotten tomato cultivar 'Delycassi'. A 9cm diameter PDA plates were used to grow more spore culture of the isolates.

Two sets of experiments were designed for each isolate; one set of ten plates was grown under white diurnal fluorescent light (12 h) 813 lux, RH 20-30 % and 26 ± 1 °C temp; while the second set was grown in the dark (Gallenkamp incubator) for eight days under 17-20 °C temperature. After 8 days, the plates were given additional light period for two or seven days according to the aims and objectives of this study. After two weeks the culture colony developed from the inoculated plates formed the stock culture of *Alternaria* used in this study.

3.1.3 ASSESSMENT METHODOLOGY

3.1.3.1 Haemocytometer

To determine the spore concentration in the suspension the cover slip was placed over the HC cell counting chamber and by using a Pasteur pipette a drop of the spore suspension was placed at the edge of the `V′ shaped of the chamber. The suspension was allowed to be drawn in to the chamber by capillary action. The HC was placed on the microscope stage and the number of spores in 1mm square area was counted and finally adjusted to 0.8×10^5 spores per ml.

Formula:

$$C \times 1/10^{-4} \times 1/Y = \text{spores count / ml (N)}$$

Where:

C = average of spores counts / square (16 small squares/large squares)

10^{-4} = overall volume of all 25 squares of the Neubauer counting chamber (ml)

$$V = 1\ mm \times 1\ mm \times 0.1\ mm = 0.1 mm^3$$

$$1\ mm^3 = 0.001\ ml$$

$$V = 0.1\ mm^3 = 0.0001\ ml\ (10^{-4}\ ml)$$

N = spores count per ml

Y = utilized dilution (e.g.: 1/10, 1/100)

Method adopted with modifications from Leuca *et al*., (2008)

3.1.3.2 Colony forming unit (cfu)

One millilitre (1ml) of spore suspension of each of the dilution ratio was spread with sterilised glass rod on PDA plates and grown under 12 h diurnal fluorescent light (813 lux), 20-30 % RH and 26 °C temp. The number of colonies which developed on each plate was counted after the observation of visible growth in 24, 48 and 72 h respectively. The mean data of the replicates was determined by haemocytometer counting. This method is adopted with modifications from Morris and Nicholls (1978).

3.2 METHOD OF COUNTING THE NUMBER OF *ALTERNARIA ALTERNATA* CONIDIA SPORES BY USING HAEMOCYTOMETER

3.2.1 Materials and Methods

Alternaria alternata was obtained from CABI. More culture plates were developed by inoculating 9 cm diameter Petri dish with about 2-3 mm mycelia plug and allowed to grow for 2-3 weeks before being used for this trial. Spore suspensions were prepared by cutting 2 mm deep of mycelia and agar from the edge of the two week old culture plate with a sterilised fine blade. This was transferred into 10 ml sterile water containing 0.5 ml/l (v/v) Tween-20. The suspension was filtered through two layers of cheese cloth to remove adhering fungal mycelia. The number of spores in the resulting suspension was estimated with a haemocytometer (**Figure 13**).

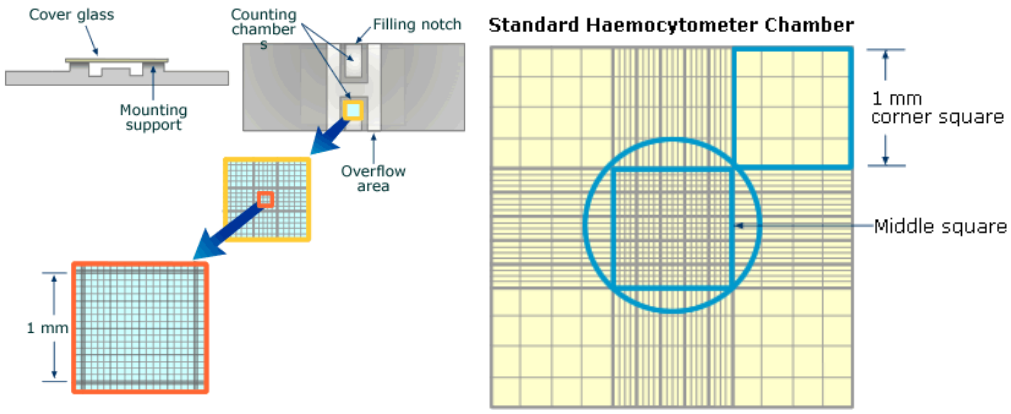

Figure 13: Haemocytometer

3.3.1 Haemocytometer:

To determine the spore concentration in the suspension the coverslip was placed over the haemocytometer counting chamber and by using a Pasteur pipette. A drop of the spore suspension was placed at the edge of the `V′ shape of the chamber. The suspension was allowed to be drawn in to the chamber by capillary action. The haemocytometer was placed on the microscope stage and the number of spores in 1 mm square area was counted.

Calculation formula:

C = n/v

Where:

C = spore concentration in cells/ml

N = average number of spores/mm² area

V = volume counted = 10^{-4}

Thus: C = n/10^{-4}

3.3.1.1 Results

The results obtained from the Haemocytometer count of conidia spores of *A. alternata* are shown in **Table 5**.

Table 5: Haemocytometer count of conidia spores of *A. alternata*

Block A	16 conidiophores	3 conidiophores	5 conidiophores
Block B	12 conidiophores	6 conidiophores	6 conidiophores

Block mean = 48 ÷ 6 = 8

C = 8 x 1/10^{-4} = 8/0.0001 = 80000

= 0.8 x 10^{5} spores/ml

Method adapted with modifications from Wang *et al.* (2010).

Method adapted with modifications from (Anonymous-Hyclone)

3.3.1.2 Remark

In conclusion, haemocytometer counting can be defined as more reliable method of inoculum preparation for fungal susceptibility testing.

3.3.2 THE COLONY FORMING UNIT (CFU) METHOD TO COUNT THE NUMBER OF CONIDIA SPORES OF ALTERNARIA ALTERNATA SPORE SUSPENSION

3.3.2.1 Materials and Methods

Alternaria spore suspensions were prepared by flooding a 2-3 week old culture on PDA Petri dish. The plate was soaked for one hour before being used for this trial. The spores were brushed off the plates with a transfer needle in to sterilized water supplemented with 0.5 ml/l Tween-20. One millilitre (1 ml) of this suspension was taken into a 10 ml measuring cylinder containing 9 ml of sterile water to make 1:10 dilution. One millilitre (1 ml) from this suspension was taken and added to 9 ml sterile water for a 1:100 dilution ratio. One millilitre (1 ml) from each of this dilution ratio was spread with sterilised glass rod on PDA plates and grown under fluorescent light (813 lux), 12 h diurnal, RH 20-30 % and 26 °C temperature. After 24, 48 and 72 h incubation at 26 °C, the colonies were counted and results were expressed as the mean number of colony forming units (CFU) ml^{-1}.

3.3.2.2 Results

3.3.2.2.1 Colony forming unit (cfu)

The result of the cfu method of sporulation is shown in **Table 6.**

Table 6: Colony forming unit method of sporulation

Ratio	24hr	48hr	72hr
1: 10	8, 9, 8	8,12, 10	29, 27, 27
1:100	3,1,3	4, 1, 5	8, 7, 9

Conclusion: It is evident that Alternaria forms 800 colony units/ml/cm^3 in 72hr at 1:100 dilutions.

72 hr: 8, 7, 9 = 24/3 = 8 × 100

= 800 cfu/ml/cm^3

3.4 Discussion

Most importantly, difficulties were encountered in sporulation of *Alternaria alternata* on artificial media. This situation led to the use of different methods of sporulation of fungal pathogen with artificial media and materials reported by some studies. For example, potato dextrose agar (PDA), corn meal agar (CMA) and tap water in 9 cm diameter petri dishes, 15 ml glass bottles, and 1.5 ml Eppendorf vials were the materials used for this study. A lot of time was expended during this period which led to the purchase of fresh *A. alternata* strain culture from CABI. The purpose for this process is to determine the concentration of the inoculum that will effectively control the fungal pathogen using haemocytometer under light microscope.

In a previous study, it was reported that *A. alternata* is an incalcitrant fungal pathogen that only forms conidiophores easily on natural substrate (Misaghi *et al.*, 1978). Also the conidia are larger, have longer beaks and uniform size than those produced *in vitro* on common agar media. Studies have shown that the morphology of the conidia produced *in vitro* was influenced by the environment such as temperature, relative humidity (RH), and the constituent of the culture media (Misaghi *et al.*, 1978).

Sporulation did occur on corn meal agar (CMA) cultures after 3 days at 20 °C when incubated at lower temperature (9 °C), but the beak are lower in size and shorter. These results indicated that *A. alternata* conidia can be produced *in vitro* on CMA medium by exposing the culture to low temperatures during the early stages of conidia formation. Subsequently, spore suspension was prepared and the concentration of inoculum was estimated with a haemocytometer under microscope.

3.5 Conclusion

Based on the results and assessment of this trial it is obvious that the different methods that were reported in many studies of spore counting in aqueous suspension ended up adjusting the concentration to that of haemocytometer count. Aberkane *et al.* (2002) reported that there is a

correlation between species distribution of fungi and percentages of agreement between colony counts and haemocytometer counts whereby, the percentage of agreement refers to the number of isolates whose cfu/ml obtained by colony counting agreed with the haemocytometer count. Hence, the final inoculum size was adjusted to a range of $1.0 \times 10^6 – 5.0 \times 10^6$ spores/ml by microscopic enumeration with a cell-counting haemocytometer. Therefore, the result of this trial is in agreement with the haemocytometer outcome of 0.8×10^5 spores/ml.

CHAPTER 4: IN-VITRO AND IN-VIVO HOT WATER TREATMENTS TO CONTROL THE DISEASE PATHOGEN OF TOMATO WITH ASSOCIATED METHOD DEVELOPMENT

4.1 Introduction

4.1.1 The production of *Alternaria alternata* spore cultures on plates using mycelia plug

Many reports have shown that most pathological studies require culturing a pathogen either to provide sufficient infective propagules for inoculation or to study its taxonomy and morphology (Dhingra and Sinclair, 1985). Plant pathologists and breeders use conidia of the fungus as inoculum to screen plants for resistance to the fungus (Shahin and Shepard, 1978). However, studies have shown that some pathogens produce relatively fewer spores on artificial media than on natural substrate due to immaturity of the spores and probably the method applied for sporulation during *in vitro* studies (Shahin and Shepard, 1978).The spores are the inoculum with which the pathogen infects the plant. As a result, the amount and maturity of spores are important for an effective pathogen infection of the plant. The quality and quantity of spores are important because the nutrient content of the propagules is related to its ineffectiveness also the quantity of spores is important as it is through this material that the concentration is adjusted in many *in vitro* studies (Dhingra and Sinclair, 1985).

Sporulation of *Alternaria* species has been enhanced by different methods; and these are peculiar to individual pathogen and specific host plant. For example, sporulation of *Alternaria solani* was enhanced by mycelia wounding, medium dehydration, and use of chemical additives (Shahin and Shepard, 1978). Temperature was reported to be an important factor in the development of disease of sunflower caused by *Alternaria helianthi* (Abbas *et al*., 1995). For instance, the conidia of *Alternaria helianthi* were produced; also the percentage germination and number of germ tubes increased at 18 compared to 26 °C (Abbas *et al.*, 1995).

The use of mycelia to produce the conidiophores has been reported. In one study the sporulation of *A. alternata* was achieved by using mycelia plug (Maiti *et al*., 2007). In this study, the fungus that caused the foliar infections of *Stevia rebaudiana* was isolated on potato dextrose agar media (PDA) and produced abundant branched septate, brownish mycelia. The conidiophores were brown, variable

in length with conidia which were in short chains. Based on the morphological characters, the fungus was identified as *Alternaria alternata* and the identification was confirmed by pathogenicity tests (Maiti *et al*., 2007).

Misaghi *et al.* (1978) reported that the conidia of *Alternaria alternata* (Fr.) Kiesler f. sp. *lycopersici* (the causal organism of tomato stem canker) collected from tomato plants in the field were significantly bigger, uniform in size and have longer beaks than those obtained through artificial culture media. Furthermore, the conidia produced *in vitro* is influenced by the environment such as temperature, relative humidity (RH), and composition of culture media, while light was not required for sporulation, and neither the size, shape, nor the number of conidia formed are significantly affected by light (Misaghi *et al.,* 1978).

Micro-organisms require some environmental conditions to reproduce asexually or sexually, such condition includes light, temperature and culture media, under which they grow to form propagules or inoculum and develop vegetative growth (Dhingra and Sinclair, 1985). The range of these conditions allowing for sporulation and mycelia elongation is divided into minimum, maximum and the best condition for different fungi. For example, *Alternaria* species thrive on water agar or any other low level nutrient culture media and are commonly found in soil or on decaying plant tissue (Thomma, 2003). The production of enough inoculum for research studies is very important likewise the concentration of the inoculum contained in the volume used is essential in the study.

The influence of environment and culture media on morphology, taxonomy and sporulation of many fungi has been reported. For example, *Monilinia laxa* (Tamm and Fluckiger, 1993); *Alternaria alternata* (Misaghi *et al.,* 1978; Grogan *et al.,* 1975); *Alternaria spp*. (Thomma, 2003), but the importance of these factors has not been emphasised on *A. alternata* conidia initiation as well as the spore count on culture media. Post-harvest pathology requires the knowledge of the fungus, method of asexual or sexual production and the causal organism of the disease for trials. In view of the reliance on spores for infection, this study was done to show the influence of temperature, light and culture media on conidiophores, spore production and infection of tomato by *A. alternata.*

4.1.2 The influence of light, temperature and culture media on sporulation of *Alternaria alternata*

The Tomato fungal pathogen *Alternaria alternata* f. sp. *lycopersici* has different isolates such that each isolate infects different part of tomato plant. Some of these isolates are pathogenic; for example the strains that infect tomato plant causing stem canker; while others are saprophytic and are found on rotting tomatoes (Misaghi *et al.*, 1978).The influence of environment on conidia sporulation and infectivity of *Alternaria* species have been reported in some studies. The number of spores produced was not affected by light but temperature has a profound effect on the initiation and size of conidia formed (Misaghi *et al.*, 1978; Abbas *et al.*, 1995). In the study, it was reported that more conidia were produced at 18-26 °C than higher temperatures.

The effect of culture media on the morphology of conidia of *Alternaria* and other fungal species has been reported by Misaghi *et al*., (1978) and Grogan *et al.*, (1975). For example, differences were noticed in the size of conidia and length of beaks of isolates of *A. alternata* resulting to variability in their morphology. However, the same morphological measurements were obtained between spores from stem cankers and conidiophores from *in-vitro* cultures on agar media (Grogan *et al.*, 1975).

Alternaria alternata f. sp. *lycopersici* have two isolates (Misaghi *et al.*, 1978). Some are saprophytes that are found in soil and on decaying plant tissues (Thomma, 2003); while some species are pathogenic that cause a range of diseases on a large variety of crops as host plants such as apple, citrus and tomato. To identify the strains in *Alternaria* species; Grogan *et al.* (1975) described the disease symptom of this pathogen as dark-brown to black cankers with concentric zonation occur on stems near the soil or above ground. These cankers got in to the plant through wounds as a result of pruning leaf petioles. Grogan *et al.* (1975) described the pathogen on culture of potato dextrose agar (PDA) under fluorescent lights as fluffy and off-white, but become darkish neutral grey with an off-white border within 48 hours. As the mycelia elongate, the colony covers the entire plate, a lot of spores are produced and the colony becomes almost black. Colonies developed on corn meal agar (CMA) was reported to be dark-brown, few and scattered, but with raised concentric rings and abundant sporulation.

All isolates of *Alternaria alternata* grew on corn meal agar (CMA) at temperatures in the 6-33 °C range, but beyond this range none grew in that medium (Misaghi *et al.,* 1978). Furthermore, colonies grown at 27 °C had the largest diameters while the mycelia elongation was observed on CMA cultures after 3 days of incubation at 25 °C (Misaghi *et al.,* 1978). The study showed that by exposing the inoculated culture media to temperatures below this threshold within 12 hours during the early stages of conidia development increases the size and consequently the number of spores. Therefore, the report suggested that temperature has an influence on the production and number of *Alternaria alternata* conidiophores and these can be correlated with the increase in the number of conidia produced.

Dhingra and Sinclair (1985) reported that most pathological studies that involved culturing a fungus on artificial media is either to show the morphological characteristics of the fungal pathogen or to increase the quantity of an infective inoculum. For example, the effect of culture media on sporulation of *A. alternata* isolates grown on pieces of stem *in vitro*, showed larger conidia than when grown on water agar, CMA or PDA (Grogan *et al.,* 1975; Misaghi *et al.,* 1978). Though, the beaks of the conidia formed *in vitro* on pieces of stem were slightly longer than those grown on corn meal agar. The result showed that *A.alternata* has pathotypes that grow on natural substrate rather than on artificial culture media. The length: width ratio of conidia from cultures grown on CMA or PDA was two times more than the stem pieces grown culture (Misaghi *et al.,* 1978).

Single spore can be used to identify fungi morphology and their characteristic features. The fungal cultures obtained from single spore isolation produce more and pure culture with typical characteristics of the fungi (Choi *et al*., 1999). The implication is that the identity of the fungi is specific to the species and its isolates so that it could be compared with the culture obtained from the single spore used to carry out the pathogenicity test of the pathogen. Also the identification of fungi through single spore method can provide extra characters for identification and the connections between species. In most cases identification of the phylum of some genetic species of fungi, such as comparison of both morphological and molecular characters require single spore (Choi *et al.,* 1999). Masunaka *et al*. (2004) reported that the pathogenicity of each isolate of *A. alternata* was tested using

single spore of conidia suspension ($1x10^5$ conidia/ml) formed in to spray to inoculate potato dextrose broth (PDB). Also, single spore of conidia suspension ($1x10^5$ conidia/ml) of each isolate of *A. alternata* tested was used to determine the pathogenicity on detached leaves of citrus cv. Iyokan tangor and rough lemon (Masunaka *et al.,* 2004).

The study by Goh (1999), reported that investigations on the genetics of *Neurospora* species and yeasts necessitated making numerous single spore isolations of all spores in a single ascus. This to show that there is a connection between an ascomycete and a conidium when cultures are derived from both single ascospore and single conidiophore. In another study it was reported that the comparison of genetic analysis of both morphological and molecular characters of *Fusarium* and *Colletotrichum* involving the use of single pore culture produced profuse spores that have characteristics of these fungi (Goh and Hanlin, 1997).

The report of another study showed that *Alternaria alternata* has many pathotypes (pathogenic variants) and are known to produce Host-Selective Toxins (HST). Despite the morphological similarity of these pathotypes, it is possible to identify each one of them based on host range and this host specificity is due to the production of a particular HST (Masunaka *et al*., 2004). Therefore, the quantity of spores produced by single spore culture can be used to test the fitness of fit of isolates of *A. alternata*. This may provide more detail information of a fungus which will enable other researchers to repeat experiments and may lead to a new discovery.

In microbiological studies spores are used as inocula in plant pathological experiments of some fungal pathogen studies (Morris and Nicholls, 1978). The knowledge of fungal pathogens is important so as to determine the approximate spore concentration that causes the effective control of the pathogen on the host plant. The information obtained may be useful for future development of a management strategy for control of diseases. However, Haemocytometer has been used to estimate the concentration of many fungal suspensions in pathological studies and was found to be slow and difficult particularly when it involves large samples and fungi with tiny spores such as *Alternaria*

alternata (Morris and Nicholls, 1978). Therefore, an alternative method e.g. the dilution ratio of spore suspension and the number of spores contained therein may be used to estimate the concentration of the spore suspension.

4.1.3. *In- vitro* treatments: the evaluation of different methods of hot water treatment of *Alternaria* spore suspension

The susceptibility of freshly harvested produce to postharvest diseases increases during prolonged storage as a result of physiological changes that enable pathogens to develop in the fruits (Schirra *et al*., 2000; Fallik *et al.,* 2001). These fungi cause different mould or canker on the plant tissue and organ leading to rot in storage. An example is *Alternaria alternata* which causes black mould disease on tomato fruit and canker on the stem (Akhtar *et al.,* 2004). This fungus uses spores as the inoculum of infection through wound or opening on the skin of fruit. The *in-vitro* trial by Fallik *et al.* (1996) reported that the exposure time (ET_{50}) that will kill 50% of spore in order to prevent germination and growth of *Alternaria alternata* on tomato was 8.8, 4.2 and 1.4 min at 45, 50 and 55 °C respectively, whereas in another study 3 min at 55 °C, 5 min at 50 °C and 10 min at 45^0C inhibited *Alternaria* spore germination (Lurie *et al.,* 1998). In another study it was reported that *in-vitro* spore suspension of *Botrytis cinerea* exposed to hot water treatment at 50 °C for 7 min prevented the germination of spores, whereas that of *Alternaria alternata* failed to germinate when treated in hot water at 55 °C for 7 min (Tohamy *et al*., 2004). The optimum treatment time and temperature to control the growth of *Alternaria alternata* spores with hot water *in vitro* will be determined and the result will be used to design the *in vivo* trials.

4.1.4. *In-vivo* treatments

Many fruits and vegetables can tolerate exposure to water temperatures of 50 - 60 °C for up to 10 min, but shorter exposure at these temperatures also control many postharvest plant pathogens (Lurie, 1998). At 46 °C hot water dips of fruit will require 90 min before it can be regarded as being effective. In contrast, hot air treatment temperatures range from 40 to 70 °C for 1 to 24 h (Mitcham and Cantwell, 2002). Air treatments were influenced by the water content because of heat transfer. Also heated moist air is generally more effective at killing pathogens than dry air at the same

temperature (Mitcham and Cantwell, 2000). However, Fallik *et al.* (1993) reported that most of the research on the effect of heat treatment at temperature below 40 °C e.g. 36 - 40 °C has been with hot air.

The experiments focused on the physiological process rather than the phytopathological effects and have involved mature green rather than pink or red fruit (Fallik *et al.*, 1993). Another study reported that dipping of fruits and vegetables in hot water at 55 °C for 1 - 2 min was the optimal antifungal treatment for control of *Alternaria*, *Fusarium*, *Rhizopus*, and *Mucor* species on melon fruits (Fallik *et al*., 1993). 2004). In addition, dipping cantaloupe fruits inoculated with *Fusarium simetectum*, *Cladosporium herbarum* and *A. alternata* in hot water at 50 °C for about 3, 5 and 10 min inhibited the decay caused by these fungi (Tohamy *et al.*, 2004).

The positive effects of hot water treatment of mature green tomato fruit against *Alternaria alternata* have been documented (Couey, 1989; McDonald *et al.*, 1999; Lurie *et al*., 1998). Studies have shown that hot water dipping of tomato fruit inhibited or reduced pathogen diseases (Schirra *et al.*, 2000; Mitcham and Cantwell, 2002), improved fruit resistance to chilling injury (Ferguson *et al.*, 2000; Lu *et al.*, 2010) and subsequently maintain fruit quality in storage (Shao *et al.*, 2011).

Most reports of tomato heat treatment to control fungal pathogens started the trial with mature green fruit but information is scarce regarding the use of red-ripe tomato for the trial. The aim of this experiment is to show the effect of heat treatment at 30, 40, 45, 50 and 55 °C for 5, 10 and 20 min respectively on red ripe tomato and also the control of decay caused by *Alternaria alternata*. The disease emergence of inoculated and heat treated fruits will be measured after 48, 72 and 96 h. The effect of heat treatment on the mycelia elongation will be measured after 96 h incubation period.

4.2 Materials and Methods

Two isolates of *Alternaria alternata* f. sp. *lycopersici* were used for this study. *Alternaria alternata* with isolate number 74351 was obtained from the Commonwealth Agricultural Bureaux International (CABI), Bakeham lane, Egham Surrey TW20 9TY England and served as the old stock culture. Two millimetre (2mm) sections of *A. alternata* infected ripe tomato cultivar `Delycassi`, grown in the

research glasshouse, Writtle College, Chelmsford Essex, England was cultured to serve as the second isolate. Mycelia plugs obtained from the culture colony were grown on agar plates and served as the fresh stock culture. The isolate with the pathogen number (PON) 74351 was maintained as stock culture for about six months on corn meal agar (CMA) glass bottle slants and stored at 6 °C before being used for this trial. The second isolate was a strain of the naturally infected tomatoes in the research glasshouse.

To determine the effect of various factors that induce sporulation of *A. alternata* conidia, 2-3 mm diameter plug was taken from the edge of the pathogen 74351culture and transferred to 9 cm diameter petri dishes containing 20 ml of corn meal agar (CMA) or potato dextrose agar (PDA), and taped with Parafilm. A medium size rotted red tomato obtained from the glasshouse served as the source of the second isolate. The rotted tomato was washed with tap water; surface disinfected for 1 min in 1.5 % sodium hypochlorite solution and rinsed twice with sterile distilled water (SDW). For this trial two to three (2-3) mm sections were taken from the infected surface of the fruit and were centrally placed in 90-mm diameter petri dish plates containing 20 ml of potato dextrose agar (PDA) or corn meal agar (CMA) (Oxoid Ltd., Basingstoke, Hants, England) respectively.

Effect of light—Five inoculated culture plates each were grown on CMA or PDA and exposed to fluorescent light 813 lux (12 h of light daily), temp 26 °C and RH 80-85 % for 10 or 15 days. Another set of cultures were incubated in the dark at temp of 17-20^{0}C, RH 20-30 % for 8 days after which they were kept under 12 h of fluorescent light daily for additional 2 or 7 days. Conidia germination and mycelia elongation measurements were made on 4, 8, 10 and 15 days respectively. A piece of paper strip calibrated from 0-90 mm was taped under the plates to measure the radial growth of culture in the plates.

Effect of temperature— Each of the five inoculated plates with either CMA or PDA were incubated at 26 °C under fluorescent for 10 or 15 days; while another set of cultures were incubated at temp of 17-20 °C for 8 days in the dark and later kept for additional 2 or 7 days under light. Measurement of culture growth was done after 2 or 7 days under fluorescent light.

Effect of culture media— CMA and PDA were the two culture media used to show the effect of substrate on sporulation of the conidia of *A. alternata*.

This trial was divided in to two groups for the combination of light, temperature and culture media studies. One group is made of four sets: viz-a-viz; two sets in a group has five inoculated plates each that were put under fluorescent light 23 lux (12 h of fluorescent light daily), temp 26 °C and RH 80-85 % for 10 or 15 days; another two sets were incubated in the dark at temp of 17-20 °C, RH 20-30 % for 8 days then incubated for 2 or 7 additional days at 26 °C under fluorescent light. In the first group, all the inoculated plates contained corn meal agar (CMA) as the culture medium. The second group consisted of the same materials as the former group but the only difference was that the petri dishes contained potato dextrose agar (PDA) as the culture medium. Conidia germination and measurements of mycelia elongation were made at the 4^{th}, 8^{th}, 10^{th} and 15^{th} day respectively. In addition a piece of paper strip measuring 90 mm length was placed under the petri dish plate to measure the germination of spore and mycelia elongation. This method enabled the radial growth of the culture colony on the plate to be measured without opening the petri dish. These trials were carried out to show the effect of light, temperature and culture media on conidia development, sporulation, spore count and pathogenicity between two strains of *Alternaria alternata* cultures developed at two different times.

4.2.1. The production of spore suspension from PDA culture plates

To produce more culture plates on potato dextrose agar (PDA) 3 mm diameter plugs were taken from the edge of fresh cultures that were developed from CABI isolate and the strain on the rotted tomato cultivar `Delycassi`. They were inoculated on 20 ml potato dextrose agar contained in 9 cm diameter plates. Ten inoculated plates were incubated for ten days under the temp of 26 °C, cool-white fluorescent light 813 lux and ambient RH 80-85 %. One millimetre (1mm) sample of mycelia plug containing conidia spores was taken from each of the fresh culture and was examined with sterile water on a slide under the microscope ($_{X}$400) for the presence of conidiophores of *Alternaria alternata*. After identification the spores were subsequently harvested by flooding the surface of culture plates with sterile water. The conidia suspension was prepared in 0.05 % (v/v) Tween-20. The concentration of conidia spores was estimated by using a haemocytometer after filtration of the

suspension through two layers of sterile muslin white cloth. The adjusted concentration was $1.0x10^5$ spores per cm^3. The experiment was repeated thrice with four replications and the method was adopted with modifications from Zhao *et al.*, (2010).

4.2.2. The production of spore suspension from CMA cultures plates

For the production of culture plates on corn meal agar (CMA) 3 mm diameter plug was taken from *Alternaria* culture stock from CABI isolate and the strain of rotted tomato. They were inoculated on 20 ml corn meal agar (CMA) contained in 9 cm diameter petri dish. Ten culture plates were grown on the culture medium and exposed to fluorescent light (813 lux), temp 26 °C and RH 80-85 % for ten days. After seven days five plates were transferred to a Gallenkamp incubator at temp of 17-20 °C in the dark for eight days to enhance sporulation (Misaghi *et al*., 1978).

To determine the number of conidia spores produced, four disks 1 cm diameter were cut with a cork borer at about 1-2 cm from the centre of the fresh culture plates. Conidia were washed and brushed off the disks with a transfer needle in to 5 mL of water and the number of conidia spores in the resulting suspension was estimated with a haemocytometer and adjusted to 1.0×10^5 spores/mL with sterile water. The trial was carried out thrice with four replications. The method was adopted with modifications from Misaghi *et al.*, (1978).

4.2.3. Improvised paper ruler

Figure 14 represents the improvised ruler that was used for measuring the radial growth of the hyphae in the petri-dish. The 90 mm paper ruler was attached to the base of the petri-dish.

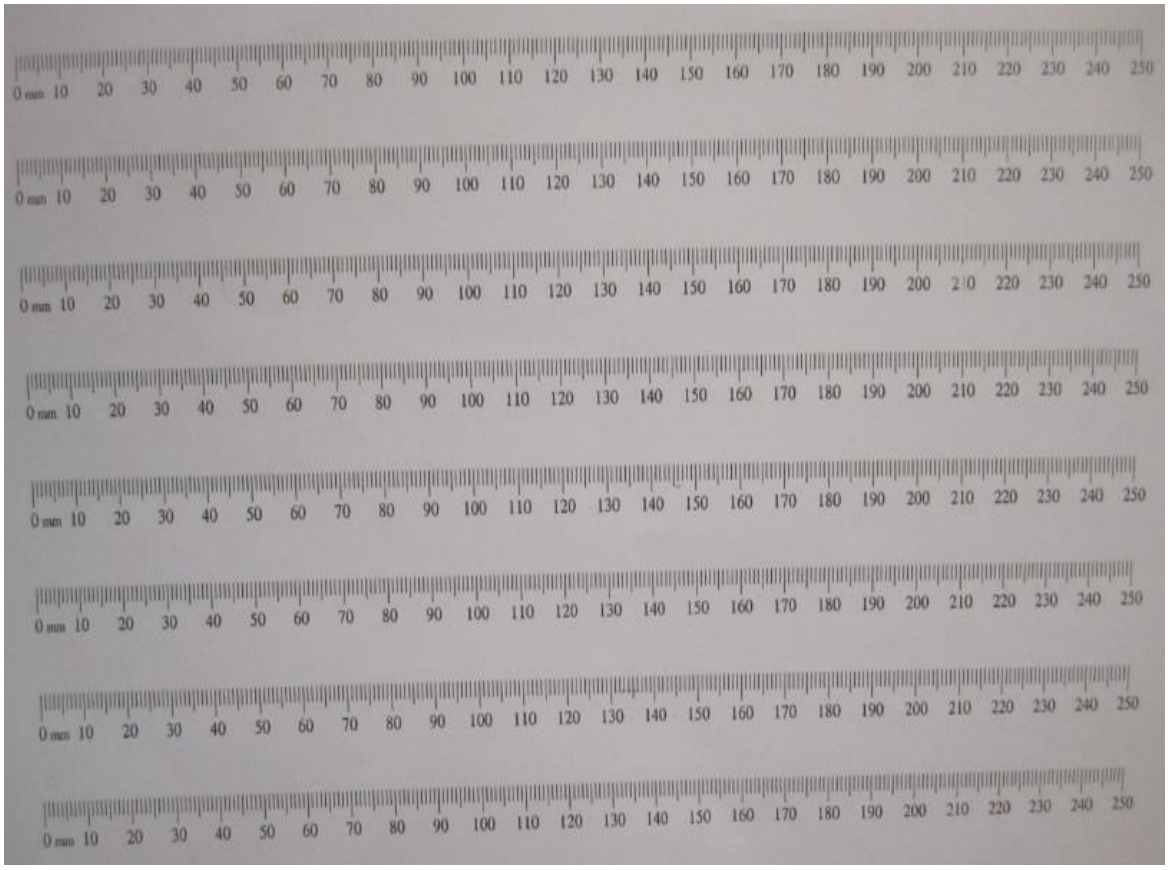

Figure 14: Improvised ruler for measuring the radial growth of the hyphae on the petri-dish

Another trial was done to test the pathogenicity of *A. alternata* spores produced from agar media such as CMA or PDA using two isolates of *A. alternata* disease pathogen. The two isolates of the fungus were grown using CMA or PDA media under different temperature, light and darkness. Single spore of these isolates were used to inoculate the petri dishes. The inoculated 9 cm diameter plates were grown under 12 h diurnal fluorescent light at 26 °C for 10 or 15 days; or incubated in the dark (Gallenkamp) at temp of 17-20 °C RH 20-30 % for 8 days after which the plates were put under light for additional 2 or 7 days. The numbers of spores on 9 cm diameter dish plates were counted using the colony counter (Scientifica and Cook electronics Ltd [BPN 4652] Bolo Bridge Road, Acton, London, W3 8AU).

To determine the number of conidia spores produced four disks 1cm diameter were cut with a cork borer at 1-2 cm from the centre of the fresh culture plate. The conidia were washed and brushed off the disks with a transfer needle into 5 ml of water and the number of conidia spore in the resulting

suspension was estimated with a haemocytometer and adjusted to 1.0 x 10^5 spores per ml with sterile water. The experiment was carried out thrice with four replications. The method was adopted with modifications from Misaghi *et al.*, (1978).

A dissection microscope with illumination from both below and above was kept in a lamina flow cabinet. A compound microscope was kept nearby to check conidia and to observe germinating spores. Alcohol (70 %) was used to clean the working surface while an alcohol lamp was also used to sterilize a fine forceps, extra fine scapel blade and a rigid picking needle. Two isolates of *Alternaria alternata* were used for this trial: one isolate from CABI with pathogen number 74351; the other was a strain of *A. alternata* obtained from an infected tomato cultivar `Delycassi′ grown in the research green house of Writtle College. Petri dishes containing potato-dextrose agar (PDA) or corn meal agar (CMA) were seeded with single conidiophore derived from isolate 74351 and from the strain of tomato cultivar `Delycassi`, using the following treatment: (i) CMA culture plate developed for 10 days under (diurnal 12 h light) and 26 °C temp or (ii) 8 days incubation in the dark at about 17-20 °C, RH 20-30 % plus 2 days light culture; (iii) CMA plate grown for 15 days under (diurnal 12 h light) and 26 °C temp or (iv) 8 days incubation in the dark at 17-20 °C, RH 20-30% plus 7 days light culture. The single spores were grown on 9 cm diameter corn meal agar petri plates.

A single spore was identified on the agar surface under light microscope and were removed by using a fine needle to put on corn meal agar plates and incubated under fluorescent light (813 lux), temp 26 °C and relative humidity 80-85 % for eight days. Germination of spores and mycelia elongation was evaluated for each treatment at 4^{th}, 8th, 10^{th}, 15^{th} day respectively. A strip of paper 100 mm length was divided in to 90 mm ruler and was taped under petri dish plates to measure mycelia elongation on the plate. The experiment was carried out thrice with four replicates in each trial.

4.2.4. Pathogenicity Tests

The Koch postulates method of testing pathogenicity on fresh media was carried out to confirm the morphology and characters of *A. alternata* (Agrios, 2005). Also pathogenicity test was carried out on fresh tomatoes using techniques of Tian and Bertolini (1995). Fresh tomato fruits were washed in

sterile distilled water and surface sterilized with 70 % sodium hypochloride solution. Two wounds (2 mm deep) per fruit were made with a sterile fine scapel blade on the equatorial surface opposite each other on the disinfected tomatoes. Two millimetre diameter plugs of mycelia from the two isolates cut from the edge of an actively growing colony on agar were introduced into the open cut of the tomato. These were kept for 8 days at 20°C temperature. The disease symptoms were established and the inoculum from the infected fruits were taken and cultured. Pure cultures were identified according to Misaghi *et al*., (1978). The symptoms were identical of naturally infected tomatoes. Morphological characteristics of conidia and mycelia of the fungus that were re-isolated from inoculated fruits confirmed Koch`s postulates (Agrios, 2005).

An identification test was carried out on the pure culture isolates obtained from the diseased tomato fruits. Each isolate was subjected to colony and microscopic examinations during which their structural features were observed. Identification of the fungus was based on the growth patterns, colour of mycelia and microscopic observation of vegetative and reproductive structures according to Misaghi *et al*., (1978).

The dilution ratio method was used to estimate the concentration of spores in *A. alternata* suspension. Single spore culture of tomato isolates pathogen number 74351 or the strain from tomato cultivar `Delycassi′ grown on PDA or CMA were used to prepare the suspension. The cultures were grown at 26 °C under (12 h diurnal light) or at 17- 20 °C in the dark for 8 days followed by an additional 2 or 7 days fluorescent (813 lux) light. Spore suspensions were made from 10 and 15 days single spore culture and the spores developed on CMA or PDA media using the following treatments: 10 days (12 h diurnal fluorescent light); 8 days dark plus 2 days light in addition; 15 days diurnal light; and 8 days dark plus 7 days additional light respectively.

The surface of culture plate was soaked with sterile water for an hour and the conidia were removed by flooding the plates with sterile distilled water. Tween 20 (0.03 % w/v) was added to the conidia suspension to prevent the conidia from sticking to either the agar surface or the petri dish walls during

collection. The resultant spore suspensions were filtered through two layers of sterile cheesecloth to remove the hyphae.

One millilitre (1 ml) of each suspension was put in to 9 ml sterile water in a measuring cylinder to produce 1:10 dilution ratio. Similarly, 1 ml of this solution was added to 99 ml sterile water using a pipette of 1000 ul (1 ml) calibration to produce 1:100 dilution ratio. 1 ml of the aqueous suspension of spores from each isolate of *A. alternata* was grown on 90 mm diameter plastic petri dishes containing about 20 ml of PDA or CMA. The suspension was spread evenly over the agar surface using a sterile bent glass rod and the plates were incubated under 12 h diurnal white fluorescent white for eight days. The spores were counted with a Colony Counter model BPN 4652 (Scientifica & Cook electronics Ltd, 78 Bolo Bridge Road, Acton, London, W3 8AU, UK). Four replicates were made for each treatment and the experiment was repeated three times.

4.2.5. *In-vitro* hot water treatments for disease control

Fifteen day old single spore cultures of *Alternaria alternata* (Fr.) Keissler grown at 26 °C were used for preparing spore suspensions in sterile water supplemented with 0.03 % Tween-20. For testing the effect of hot water treatment on germination and mycelia growth, 10 ml of suspension containing 10^5 spores ml^{-1} were added to 16 mm diameter test tubes and heated for 45 or 50 °C for 5 or 10 min, while non-heated spore suspensions were used as control. Four glass test tubes were used for each treatment. Sample aliquots (100 µl) of the suspensions were streaked aseptically on the surface of 9 cm diameter CMA plates. The spore germination and mycelia growth were measured at 48, 72 and 96 h respectively. A piece of paper cut to 10 cm length was used as a ruler and divided to 100 mm measurement. This ruler was taped under the plates to measure the growth of the spore at the respective days described above.

The spore suspension was prepared by flooding the surface of 30 day old culture plate of *Alternaria alternata*. Ten millilitres (10 ml) of sterilized water was used for flooding the surface of the culture plate and scrapped with a sterile cooled needle. The resulting spore suspension was filtered through sterile cheese cloth into 250 ml flask. The filtrate was diluted with water and spore concentration was

adjusted with haemocytometer to $1x10^5$ spores per ml. Aliquots (1 ml) of spore suspension was pipetted into 1.5 ml Eppendorf vial and subjected to hot water treatment. Ten vials were put in hot water baths (Grant JB series) held at temp of 30, 40 and 50 °C each for 20, 30 and 60 min respectively. After the heating period the vials were immediately transferred to a cool water bath at room temp for 2 min to stop the heating process.

After hot water treatment 0.2 ml of treated spore suspension was spread on six PDA plates and all the plates were incubated at room temp 26 °C under white fluorescent light. Also to serve as the control 0.2 ml aliquot of untreated spore suspension was put on ten plates and incubated under the same conditions with the treatment. The spore germination was considered to have occurred when the mycelia had exceeded one half the lengths of the conidia. The mycelia elongation was measured after 24 h. The effect of hot water treatment on the spore was determined as the mean percent germination of spores compared to the germination of the control plate. The method was adopted with modifications from: (Gramaje *et al.*, 2010).

4.2.6. *In-vivo* hot water treatments for disease control

Tomato fruit of uniform size at breaker-turning red colour stage were purchased from a farm shop (var. unknown) and used for this experiment. Ten fruits were used for each temperature treatment. They were dipped in 70 % ethanol (IMS) for one minute and transferred to distil water for the same period. The fruits were air dried and a small cut of 2-3 mm diameter was made on the fruit and inoculated with mycelia plus spores plug (3 mm diameter) from a 10 day old culture of *Alternaria i*nto the equatorial area of the skin surface (pericarp). The inoculated fruits were dipped in an insulated hot water bath (JB Grant) at 40 °C, 45 °C and 50 °C for 10 min respectively. Another ten inoculated fruits were immersed in sterilized distil water at room temperature and served as control. After the heating treatment the fruits were put in a plastic cup covered with a lid and left at room temperature for incubation for 48, 72 and 96 h. The experiment was repeated three times.

Tomatoes (var. *Vanessa*) were obtained from a commercial fruit and vegetable shop. Ten fruits were used for each temperature degree (50, 40 and 30 °C) and put in the hot water for 20 minute. Before

putting the tomatoes in the hot water each fruit was dipped in 70 % industrial methylated spirit (IMS; ethanol with 4 % methanol) for one minute and then transferred into sterile distil water (SDW) for another 1 min. All the fruits were inoculated with 100 µl of 10^5/ml conidial suspension of *Alternaria* into 3 mm deep cut on the surface. After allowing the spore suspension to dry four fruits were dipped in insulated hot water bath (JB Grant) at 50, 40 and 30 °C for 20 min. separate water bath was used for each treatment. Similarly, ten inoculated fruits were immersed in sterile water (SDW) at room temperature and served as control. Also, ten inoculated fruits were also used as a check but were not dipped in water. The purpose is to show that the effect of hot water on *Alternaria alternata* is real not that the spores were washed away by water in the bath.

Tomatoes were obtained from a commercial fruit and vegetable farm shop var. `Cossack′. Ten fruits per replicate were disinfected with 70 % Industrial methylated spirit (IMS) containing ethanol with less than 4 % methanol for 1 min before inoculation to conduct the experiment. Ten fruits were used for hot water treatment and likewise for the control as check. All the fruits were inoculated with 100 μl^5 ml^{-1} conidial suspension of *Alternaria alternata* into 3 mm cut in to the surface of the fruit. The spore suspension was allowed to air dry and ten fruit were dipped in insulated hot water bath (JB Grant) at 50 °C for 5min and another set of ten fruit were immersed in sterilized water for the same time as the control. At the end of the heating period of 5 min each fruit was put in a plastic and cover with a lid before transferred to a room temperature (20 °C) for incubation for 48 h, 72 h and 96 h.

A big plastic tank containing 0.12 m³ volume of hot water from the tap was used to demonstrate the practical application of hot water treatment in rural farm. Fifty seven red tomato fruits were put in a plastic tray basket and weighed before immersed in hot water bath contained in the tank. The weight of the fruit was measured on a weighing scale in the laboratory and also the temperature of inside (core) of the fruit was determined using a metal rod thermometer probe (Food Check- E.T.I. Ltd Worthing, Sussex). The room temperature was 29 °C. Hot water was put in the tank and stirred to the required temperature of 55 °C. The unit maintained the water temperature in the treatment tank consistently at or slightly above the set point temperature. Red tomatoes of equal size and colour were immersed in the hot water for 5 min and final temperature of hot water was taken.

4.3. Results

The mycelia plug of *Alternaria alternata* isolate pathogen no. 74351 and the strain from tomato cultivar `Delycassi` which were obtained from ten day old culture (10 day) and grown on corn meal agar (CMA), under white fluorescent light (813 lux), temperature 26 °C and RH 80-85 %; showed a significant difference ($P < 0.05$) for the main effects of isolate and media in four day period. Similarly, the isolates and media were significantly different in four days when the culture was developed on potato dextrose agar (PDA). The fourth (4^{th}) day result showed no significant difference of interaction between the isolate and media (**Figure 15**). Furthermore, the result also showed that there was a significant difference between the two isolates and media for instance on CMA, but no significant difference on the isolates and media-PDA in 8^{th}, 10^{th}, and 15^{th} day period respectively (**Figure 15**). The graph also showed a clear difference between the media for PON-74351. Also from observation the PDA gave more rapid growth than CMA.

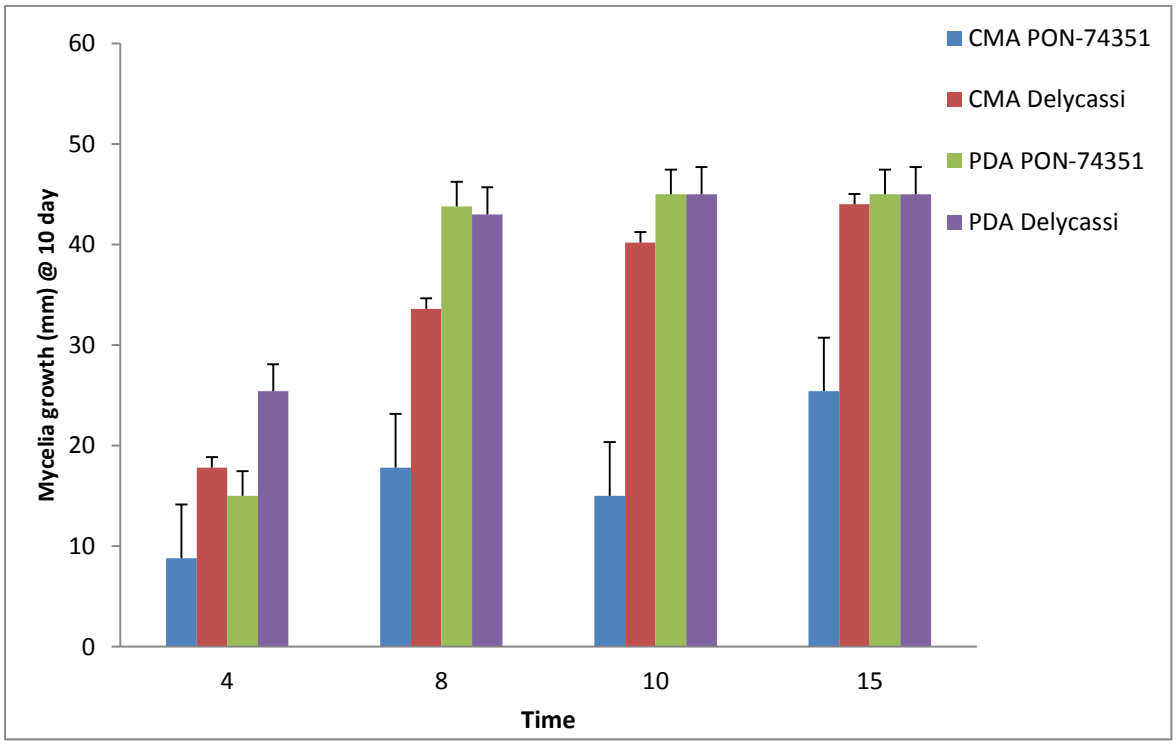

Figure 15: The mean radial growth of a mycelia plug from a 10 day old culture of *Alternaria alternata* at 26 °C at the 4^{th}, 8^{th}, 10^{th}, and 15^{th} day after inoculation on different media isolates. Each data point is the mean of 30 plates. The error bars indicate LSD of each data point.

The result of the mycelia plug obtained from the leading edge of a fifteen day (15 day) old culture plate of isolate no. 74351 and the strain of tomato cultivar `Delycassi′ culture grown on (CMA) gave a significant difference ($P < 0.05$) between the isolates and the media in 4th, 8th, 10th, and 15th day respectively. For the culture grown on PDA a significant difference was shown between the isolates and media for the 4th day but no significant difference on the 8th, 10th and 15th day respectively (**Figure 16**).

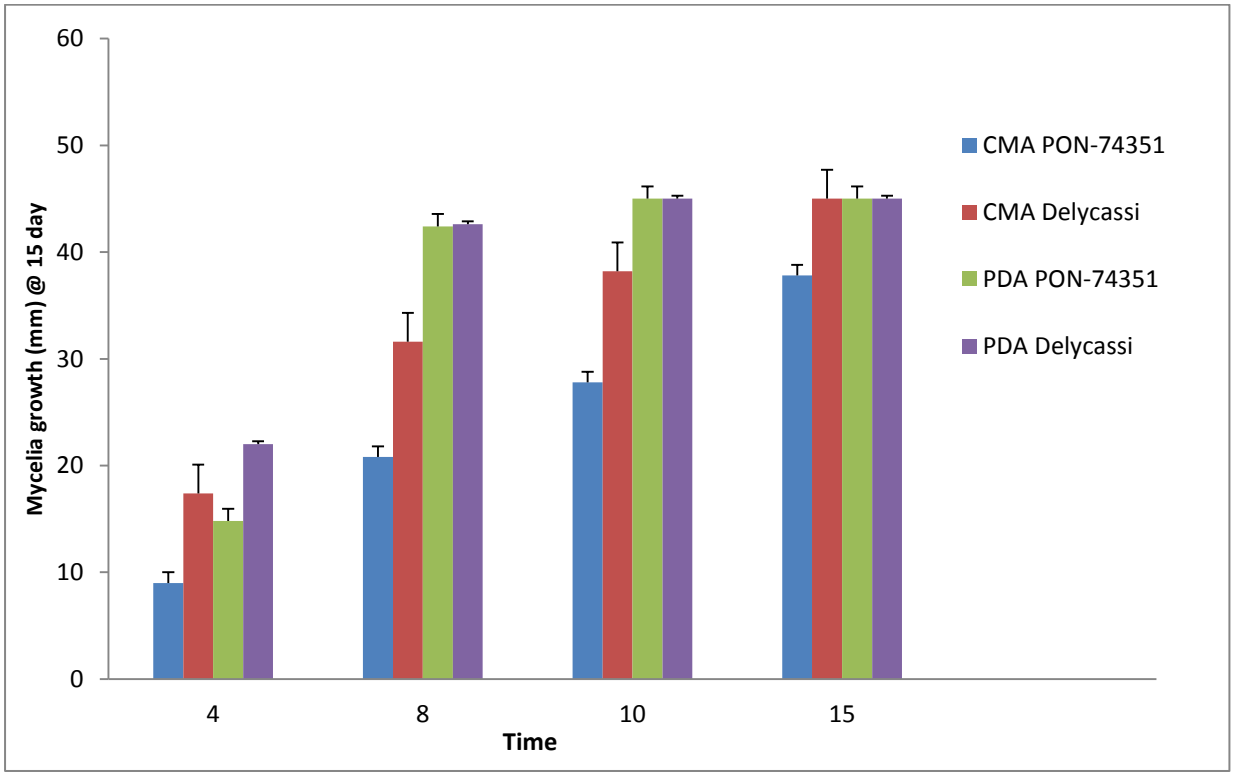

Figure 16: The mean radial growth of a mycelia plug from a 15 day old culture of *Alternaria alternata* at 26 °C at the 4th, 8th, 10th, and 15th day after inoculation on different media isolates. Each data point is the mean of 30 plates. The error bars indicate LSD of each data point.

Another experiment was carried out with these two isolates and media but under cool temperature (17-20 °C) in the dark for eight days followed by additional two or seven days under white fluorescent light as the same process described above. Two to three millimetre (2-3 mm) mycelia plug was cut from a 10 day old culture plate to carry out this trial. The result showed a highly significant ($P < 0.05$) F-value for the main effects of isolate and media in the 4th day for the two culture media. Also there was a significant difference between these variables on CMA on the 10th

and 15th day. There was no significant difference between the isolates and media in 8 days on CMA and PDA culture likewise on the 10th and 15th day period **(Figure 16**).

The analysis of variance of the tenth day old culture showed a significant difference between the two isolates and media on the 4th day. On the 8th day there was no significant difference between the isolates and media on PDA plates. Likewise, on the 10th there was no significant difference between these variables. On CMA plates there was no significant difference between the isolates and media on the 8th day but a significant difference was shown on the 10th. The 15th day showed a significant difference between these factors on corn meal agar (CMA), while there was no significant difference on potato dextrose agar (PDA) (**Figure 17**).

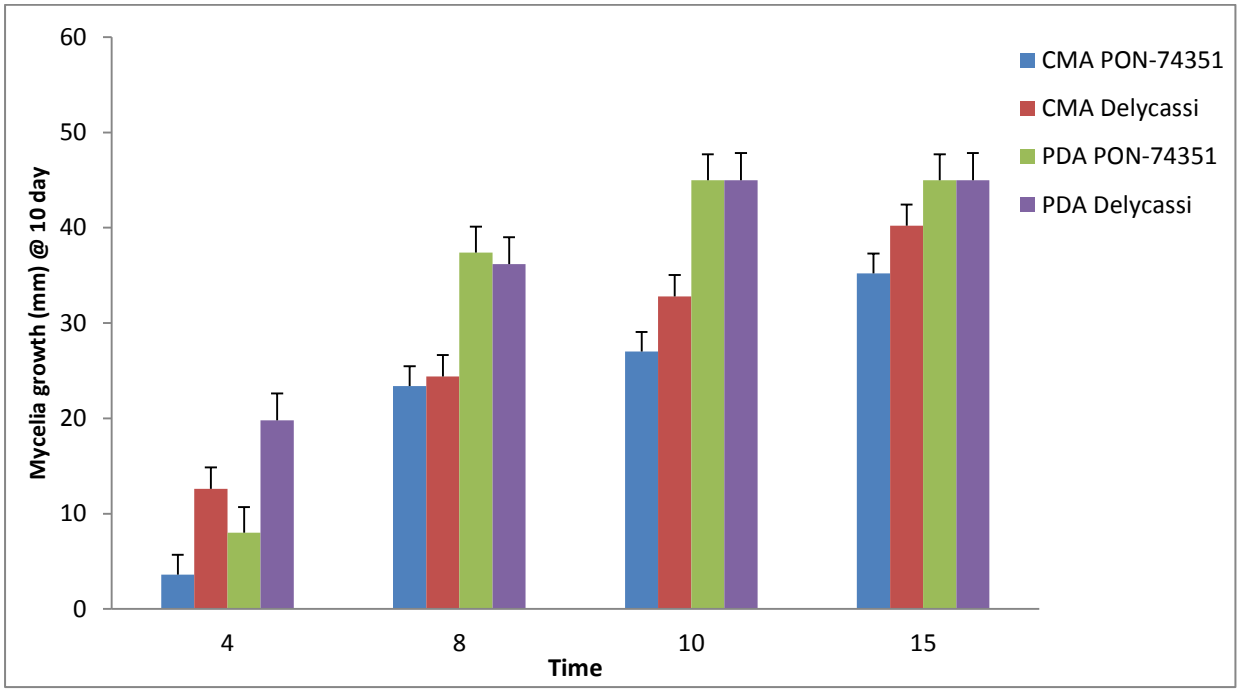

Figure 17: The mean radial growth of a mycelia plug from a 10 day old culture of *Alternaria alternata* at 17 °C at the 4th, 8th, 10th, and 15th day after inoculation on different media isolates. Each data point is the mean of 30 plates. The error bars indicate LSD of each data point.

Another trial was performed using the same set of materials and variable factors but with mycelia plug obtained from 15 day old stock culture of *A. alternata*. In the fourth day the analysis of variance of the data showed a significant difference between the isolates and the media. On PDA there was a

highly significant difference ($P < 0.001$) between isolates; for example isolate with the no. 74351 and the strain from tomato cultivar `Delycassi′ (**Figure 18**). Also the result showed a significant difference in the interaction between the isolates and culture media. The eighth day result showed no significant difference between the isolates and media on CMA but a significant difference on PDA. The tenth day result showed no significant difference between the isolates and media (**Figure 18**). On the fifteenth day a significant difference was shown between the isolates and media on CMA but the difference was not significant on PDA. The interaction between these main effects was also significant in the analysis of variance (ANOVA) of the data on the 10th and 15th day (**Figure 18**).The overall results of this trial indicated that the radial growth of the mycelia on PDA plates were better than CMA. Further elongation of the mycelia was restricted to the available space in the petri dish after the tenth day (**Figure 18**).

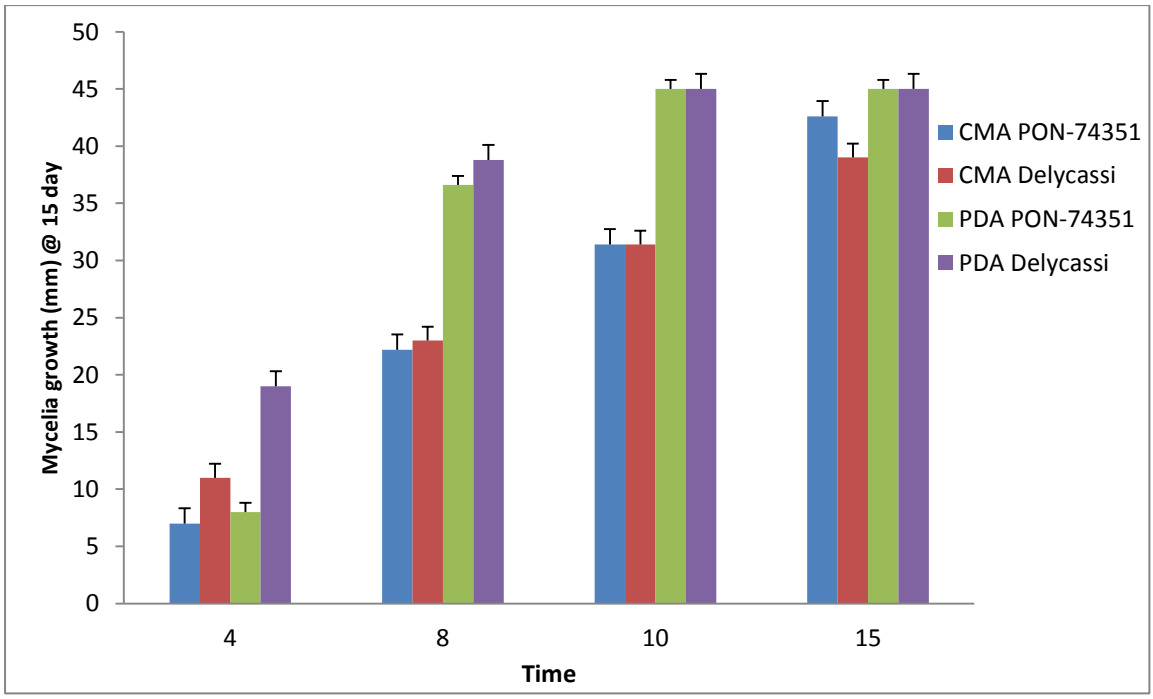

Figure 18: The mean radial growth of a mycelia plug from a 15 day old culture of *Alternaria alternata* at 17 °C at the 4th, 8th, 10th, and 15th day after inoculation on different media isolates. Each data point is the mean of 30 plates. The error bars indicate LSD of each data point.

The single conidiophore germination and mycelia growth of the isolates tested were not significantly different at 26 °C (10 day old culture) in the 4th and 8th day period of investigation. However, the

analysis of variance for these data showed a highly significant ($P < 0.001$) F-value for the main effect of culture media at the same period of time. For the interactions of isolates × media there was a significant difference in the 4th day, also after the 8th day the result showed a highly significant difference between these main factors. Furthermore, in the 10th and 15th day period of this study the results showed a highly significant difference between the isolates ($P < 0.001$), media as well as the interactions (**Table 7).**

The single spore growth of 15 day old culture at 26°C showed that the germination and mycelia growth result was significantly different between the isolates and media on the 4th, 8th, 10th and 15th day respectively, but on the 8th day the difference between the isolates was not significant (**Table 8**). The difference was highly significant ($P < 0.001$) between the media in the 8th, 10th and 15th day. Based on the analysis of variance of the data obtained it was shown that there was a significant difference in the interactions between the isolates and media in all the investigated days except on the 8th day (**Table 8**).

Table 7: Mycelia growth of germinated single spore of 10 day-old culture at 26 °C

	Treatments				**10 DC**					
					S.e.ds			**significance**		
Day	CMA		PDA							
	PON-74351	Delycassi	PON-74351	Delycassi	I	M	I×M	I	M	I×M
4	14.3	15.5	21.5	18.3	0.67	0.67	0.95	0.161	0.001	0.006
8	25.8	31.0	43.0	37.5	0.57	0.57	0.81	0.831	0.001	0.001
10	29.0	38.0	45.0	44.5	0.90	0.90	1.28	< 0.001	0.001	0.001
15	36.3	45.0	45.0	45.0	0.24	0.24	0.34	< 0.001	0.001	0.001

Table 8: Mycelia growth of germinated single spore of 15 day-old culture at 26 °C

	Treatments				**15 DC**					
					S.e.ds			**significance**		
Day	CMA		PDA							
	PON-74351	Delycassi	PON-74351	Delycassi	I	M	I×M	I	M	I×M
4	16.0	14.8	21.0	14.8	0.86	0.86	1.22	< . 001	0.013	0.013
8	32.5	31.5	43.0	37.5	1.90	1.90	2.69	0.113	0.001	0.259
10	34.3	39.0	45.0	44.5	0.89	0.89	1.25	0.034	0.001	0.012
15	39.3	43.8	45.0	45.0	0.67	0.67	0.95	0.006	0.001	0.006

The germination of spore and mycelia elongation of the single spore derived from the two isolates were also studied under cool temp of 17-20 °C and cultures were developed from the10th and 15th day old plates. The result of the radial growth of the mycelia of the single spore of 10 day old culture showed a significant difference ($P < 0.05$) between the isolates and media in the 4th, 8th and 10th day, except that there was no significant difference between the isolates on the 8th day. The result also showed that the interactions between the isolates x media was significantly different after the 4th, 8th and 10th day (**Table 9**). After the fifteenth (15th) day of the experiment, the mycelia have grown to the full diameter of 9 cm petri dish plates. As a result the analysis of variance (ANOVA) was recorded as missing data (**Table 9**).

The single spore obtained from 15th day old culture grown at 17-20 °C for eight days in the dark plus an additional seven days under fluorescent light, the mycelia length measurements showed a highly significant difference ($P < 0.001$) F-value between the main effects i.e. isolates and media and the interactions in the 4th, 8th, 10th and 15th day respectively. But the 4th day result showed no significant difference between the culture media as well as the interactions (**Table 10**). The results of this trial to produce more spores on agar media using these methods have shown that potato dextrose agar (PDA) produced more and sufficient quantities than CMA and were used for further research.

Table 9: Mycelia growth of germinated single spore of 10 day-old culture at 17 - 20°C

	Treatments				**10 DC**					
					S.e.ds			**significance**		
Day	CMA		PDA							
	PON-74351	Delycassi	PON-74351	Delycassi	I	M	I×M	I	M	I×M
4	13.5	13.3	20.8	17.0	0.59	0.59	0.83	0.005	.001	.011
8	26.0	29.0	42.8	36.3	0.95	0.95	1.35	0.091	.001	.001
10	31.0	37.0	45.0	44.3	0.63	0.63	0.88	<.001	.001	.001
15	35.0	45.0	45.0	45.0	--	--	--	--	--	--

Table 10: Mycelia growth of germinated single spore of 15 day-old culture at 17 - 20°C

	Treatments				**15 DC**					
					S.e.ds			**significance**		
Day	CMA		PDA							
	PON-74351	Delycassi	PON-74351	Delycassi	I	M	I×M	I	M	I×M
4	22.5	14.3	22.5	14.8	0.53	0.53	0.75	<.001	NS	NS
8	44.3	30.0	44.3	36.5	0.65	0.65	0.92	<.001	.001	.001
10	45.0	36.0	45.0	45.0	0.29	0.29	0.41	<.001	.001	.001
15	45.0	40.8	45.0	45.0	0.24	0.24	0.34	<.001	.001	.001

The results of the pathogenicity test of the spores obtained from the culture incubated at varying conditions are shown in **Figures 19 – 22.** An identification test was carried out on the pure culture of isolates obtained from the diseased tomato fruits. Each isolate was subjected to colony and microscopic examinations during which their structural features were observed. Identification of the fungus was based on the growth patterns, colour of mycelia and microscopic examinations of vegetative and reproductive structures according to Misaghi *et al.* (1978).

For example, when the culture was incubated in the same media at different culture ages for instance 10 days versus 15 days, no significant difference was observed in the growth of mycelia and morphology of *Alternaria alternata* culture on agar plates (**Figure 19-20**). Likewise, when same media was used and the samples incubated for different duration of time, no significant difference was observed in the growth of the mycelia and the morphology of *Alternaria alternata culture* on agar plates (**Figures 19** and **20**). The culture on PDA under fluorescent lights are at first fluffy and off-white, but become dusky neutral grey with an off-white border within 48 h. After, the colony extends over the entire plate while sporulation is abundant and the colony becomes flat and nearly black. Colonies on CMA (corn meal agar) are dark brown sparse and are lying flat but with slightly raised concentric bands with intense sporulation (**Figure 19-20**).

Pathogenicity test of *Alternaria alternata* was carried out on tomato fruits (**Figures 21** and **22**). The results showed that when the fungal pathogen was incubated either in CMA (**figure 21**) or PDA (**figure 22**) no significant difference was observed in the infectivity of the fruit irrespective of the isolate of the pathogen. The symptoms were typical of naturally infected tomatoes. Morphological characteristics of conidia and mycelia of the fungus that were re-isolated from inoculated fruits confirmed Koch′s postulates (Agrios, 2005).

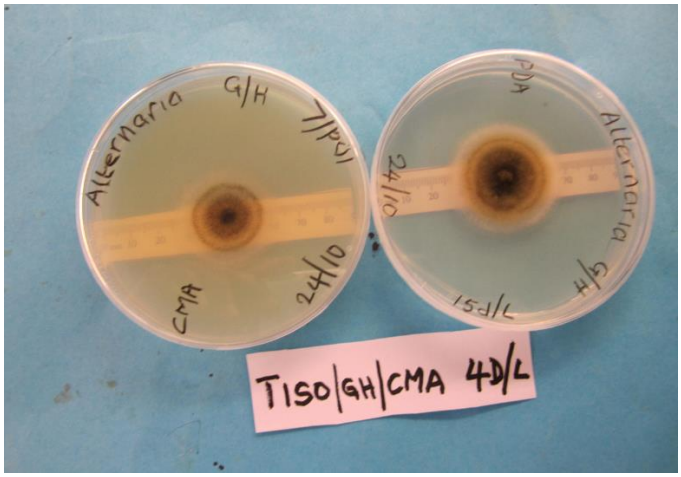

Figure 19: Pathogenicity test of *A. alternata* spore on CMA plates

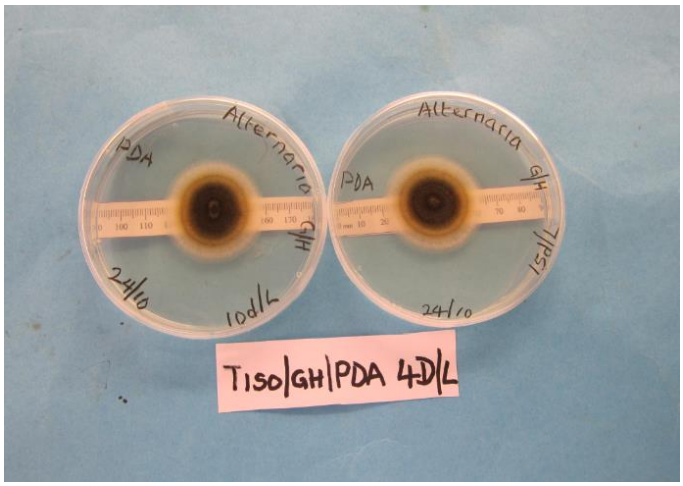

Figure 20: Pathogenicity test of *A. alternata* spore on PDA plates

Figure 21: PON-74351 'CABI' isolate

Figure 22: Strain of isolate on tomato cultivar (Delycassi) on PDA

The result of the spore count experiment using dilution ratio showed that the mean spore count was a function of dilution, media and isolates. For example, the suspension prepared from 10 day old culture, when diluted to 1:10 ratio and grown under dark at 17 °C the result showed the mean spore count of the isolates was equal under CMA. In the case of PDA the spore concentration decreased from about 700 to 500 in the culture colony (**Figure 23**). The dilution ratio 1:100 produced more spores of pathogen no. 74351 than the strain of tomato cultivar `Delycassi` under CMA and PDA (**Figure 23**).

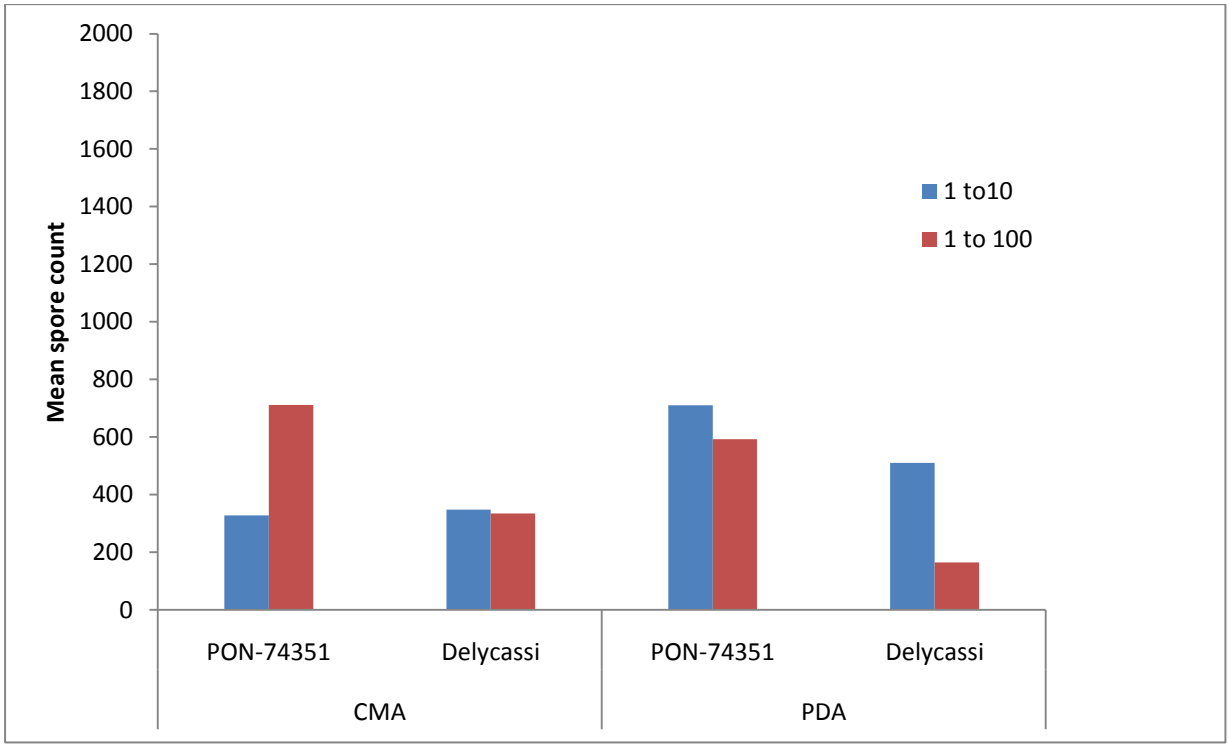

Figure 23: The mean spore count of 10 day old culture at 26°C incubation for 10 days under fluorescent light and at 17-20°C for 8 days dark plus 2 days light on CMA or PDA agar. Total number of spores produced at 26°C on CMA = 598, PDA = 2926 (1: 10 dilution); CMA = 1188, PDA = 760 (1:100 dilutions). At 17-20°C number of spores produced on CMA = 1390, PDA = 2040 (1:10 dilution); 8 days dark plus 2 days light: CMA = 1320, PDA = 660 (1:100 dilutions).

For instance, on CMA agar the mean spore count of isolate PON-74351was about 700 compared with Delycassi which was about 300 spores **(Figure 23).** There was a difference in the mean spore count between PON-74351 and Delycassi isolates under CMA or PDA agar with the exception of 1:10 dilution of spores on CMA agar medium.

When the spore concentration was adjusted to about 1:10 dilution ratio in the suspension prepared from 15 day old culture at 17 °C temp, the number of spores in the suspension of isolate PON-74351 increased from about 400 in CMA to 700 in PDA culture media. While the mean spore count of the 1:100 dilution of this isolate decreased from about 750 in CMA to about 700 as well in PDA. The suspension prepared from the strain of tomato cultivar Delycassi, in the 1:10 dilution the spore count increased from about 1100 in CMA to 1700 in PDA. Similarly, in 1:100 ratios the strain of tomato cultivar 'Delycassi' increased in CMA from about 100 to 400 in PDA (**Figure 24**). Overall more spores are produced on PDA than CMA but the two media produced sufficient quantities for further research.

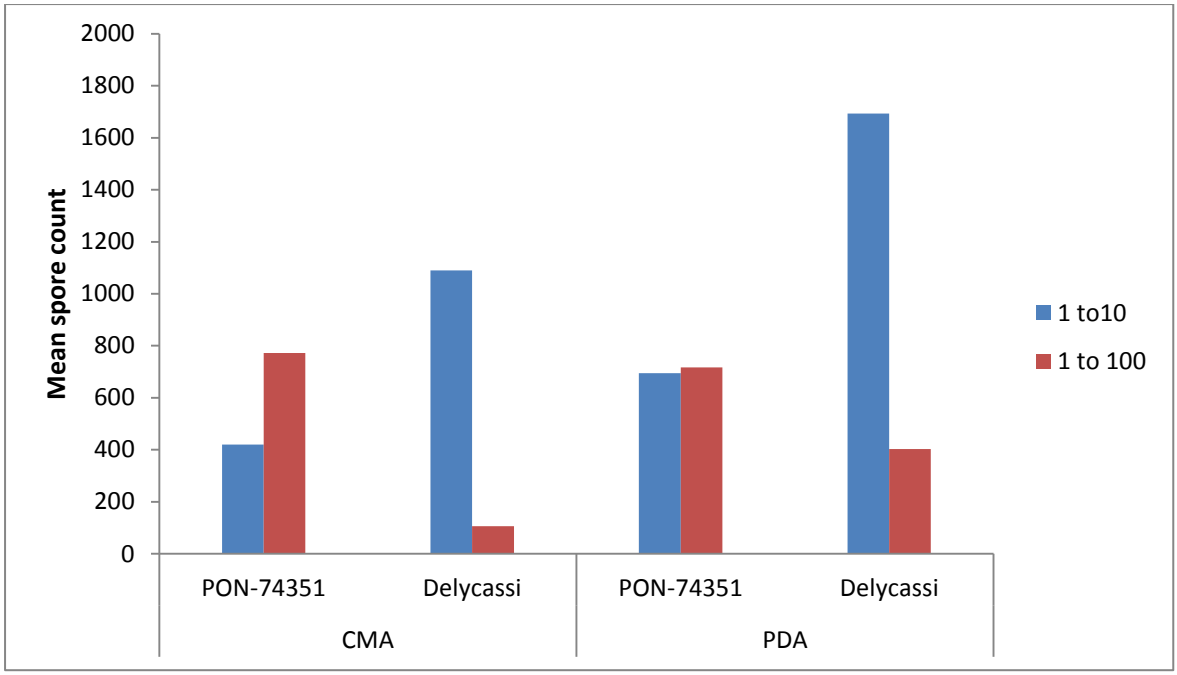

Figure 24: The mean spore count of 15 day old culture at 26°C incubation for 15 days under fluorescent light and at 17- 20°C for 8 days dark plus 7 days light on CMA or PDA agar. Total number of spores produced at 26°C on CMA = 1180, PDA = 6360 (1:10); CMA = 104; PDA = 3820 (1:100). At 17-20°C the number of spores produced on CMA = 4360, PDA = 6772 (1: 10); CMA = 424, PDA = 1612 (1:100 dilution).

There was an increase in the mean spore count of the dilution ratios of the two isolates on the agar media except PON-74351 on PDA where they have equal number of spores (**Figure 24**). The approximate number of spores in the culture colony of the suspension prepared from 10 day old culture grown at 26^0C, showed a linear increase from CMA to PDA for the two isolates in 1:10 and 1:100 dilution ratio with the exception of the isolate strain of tomato cultivar `Delycassi′ in 1:10 and 1:100 dilution on CMA and PDA. For example, the spore count of the suspension of `Delycassi` in 1:10 dilution grown on CMA is about 60 % less than the PDA, while in 1:100 ratio is about 50 % (**Figure 25**).

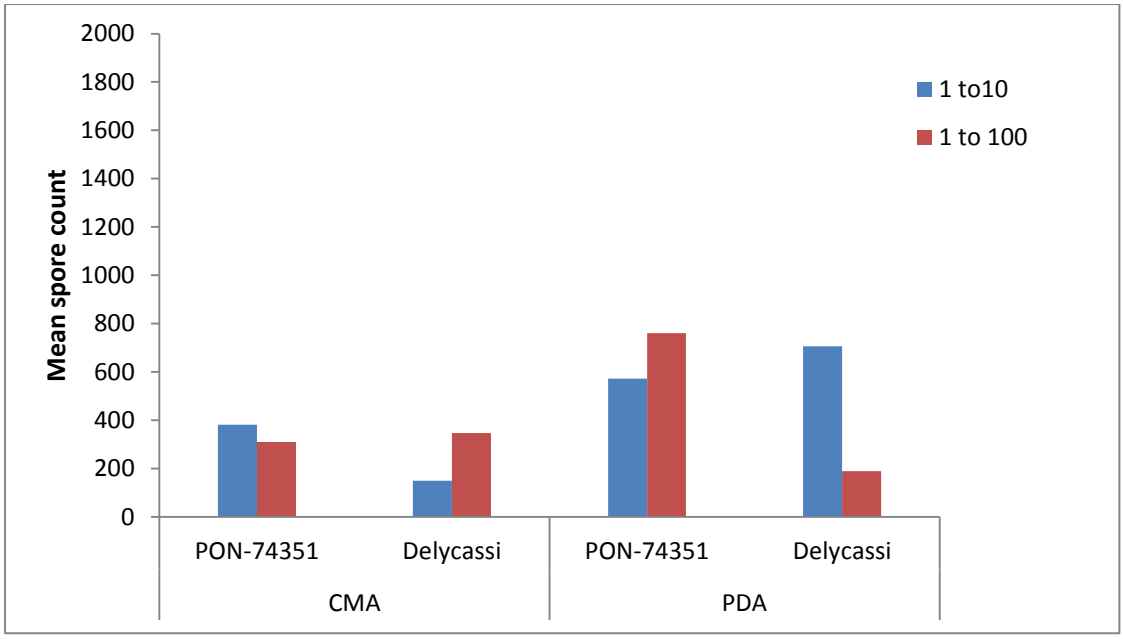

Figure 25: The mean spore count of 15 day old culture at 26 °C incubated for 10 days under fluorescent light and at 17-20°C for 8 days dark plus 2 days light on CMA or PDA agar. Total number of spores produced at 26°C on CMA = 1528, PDA = 2288 (1:10 dilution); CMA = 1240, PDA = 3040 (1:100 dilution). At 17-20°C number of spores produced on CMA = 1310, PDA = 2440 (1:10); 8 days dark plus 2 days light: CMA = 2844, PDA = 2370 (1:100 dilution).

The result of 15 day old culture suspension grown under fluorescent light and temp 26 °C showed the mean spore count of the dilution ratio 1: 10 and 1: 100 increased from about 400 per culture plate on CMA to 800 on PDA for isolate no. 74351(**Figure 26**). While the number of spore contained in the suspension from the strain of tomato cultivar `Delycassi′ increased sharply from about 300 on CMA to 1800 on PDA in 1:10, while in ratio 1: 100 it was about 50 to 900 spore count (**figure 26**). Generally, PDA agar produced more spores of the isolates than CMA of the two dilution ratios.

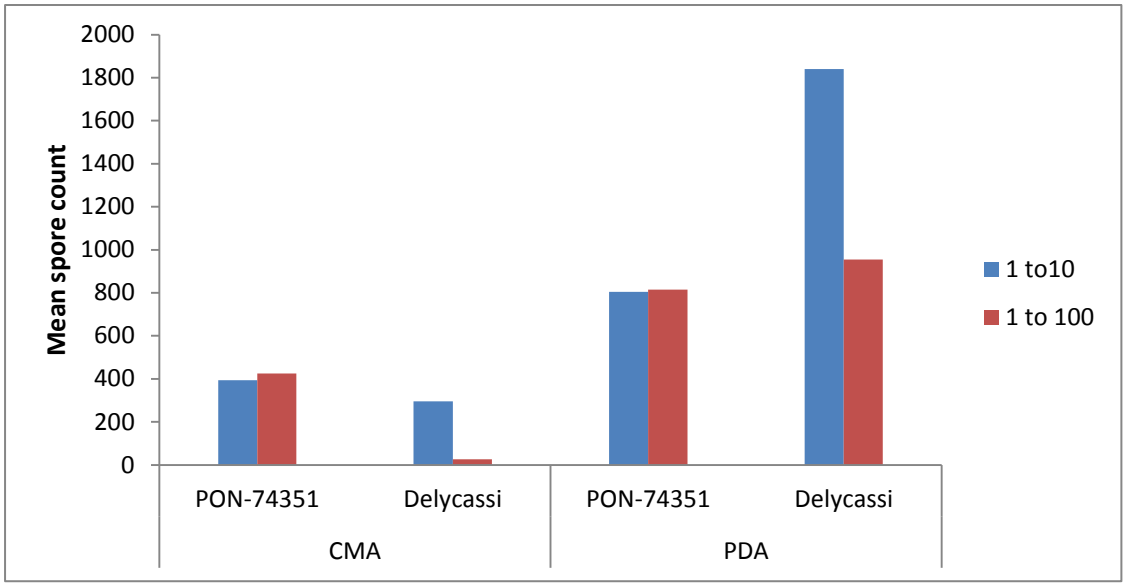

Figure 26: The mean spore count of 15 day old culture at 26 °C incubation for 15 days under fluorescent light and at 17-20°C for 8 days dark plus 7 days light on CMA or PDA agar. Total number of spores produced 26°C on CMA = 1576, PDA = 3220 (1:10); CMA = 1700, PDA = 3260 (1:100). At 17-20°C the number of spores produced on CMA = 1680, PDA = 2780 (1:10); CMA = 3088, PDA = 2868 (1:100 dilution).

The result showed that the isolates produced abundant spores for further experiments on PDA than CMA agar media.

4.3.1. *In vitro* hot water treatments for disease control

Spore germination and mycelia growth of *A. alternata* were inhibited by heat treatment at 45 and 50^{o}C for 5 and 10 min (**Figure 27**). For example, after incubation on CMA at 26 °C, the heat-treatment at 50 °C for 5 or 10 min of *A. alternata* spores exhibited a significant difference ($P < 0.05$)

in germination after 48 h compared with the heat treated spore at 45 °C (**Figure 27**). Likewise, a similar pattern was observed for hot water at 50 °C for 5 or 10 min treated spore for 72 h and 96 h. The inhibitory effect of hot water at 50 °C for 5 min sufficiently reduces the germination to the barest minimum (**Figure 27**).. At 45 °C for 5 and 10 min there was a significant difference in spore germination between the treatments compared with the control after 48 h (**Figure 27**). For instance, heat treatment of spores at 45°C for 5 and 10 min reduced the germination to about 45% and 50%.

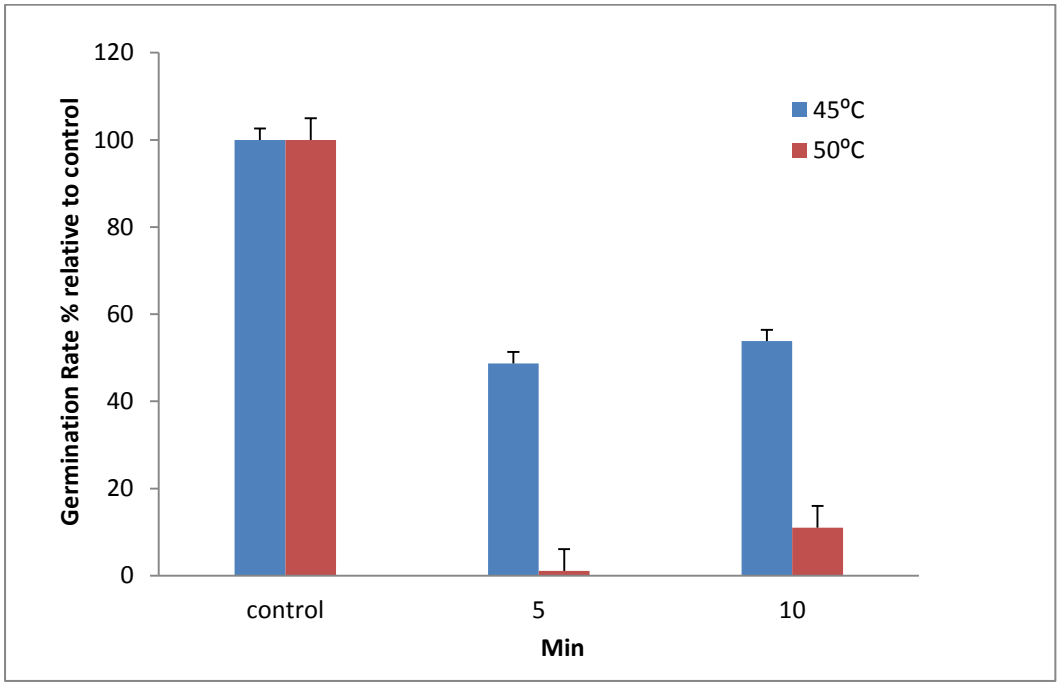

Figure 27: Hot water treatment of *Alternaria alternata* (*In vitro*) at 45 and 50 °C showing mean percentage spore germination rate after 48 h incubation. Error bars indicate standard error of mean of each data point.

The above figure illustrates the inhibitory effect of hot water treatment on spore germination of *A. alternata.* After 48 h of incubation of culture plates at 26 °C, the heat-treated *Alternaria alternata* spores at 50 °C for 5and10 min were significantly different ($P < 0.05$) in germination percentage compared to the non-heat treated control. Whereas, the inhibitory effect of heat treatment at 50 °C diminished when the treatment time was increased from 5 to 10 min. For example, the germination rate was 1 % in 5 min, while in 10 min it increased to 10 % of the control (**Figure 27**). After 48 h incubation of the culture plate at 26 °C there was a significant difference in spore germination between the heat

treated spores at 45 °C for 5, 10 min and the control. For example, in 5 min the germination percent was 45 % while in 10 min increased to 52 % of the control (**Figure 27**).

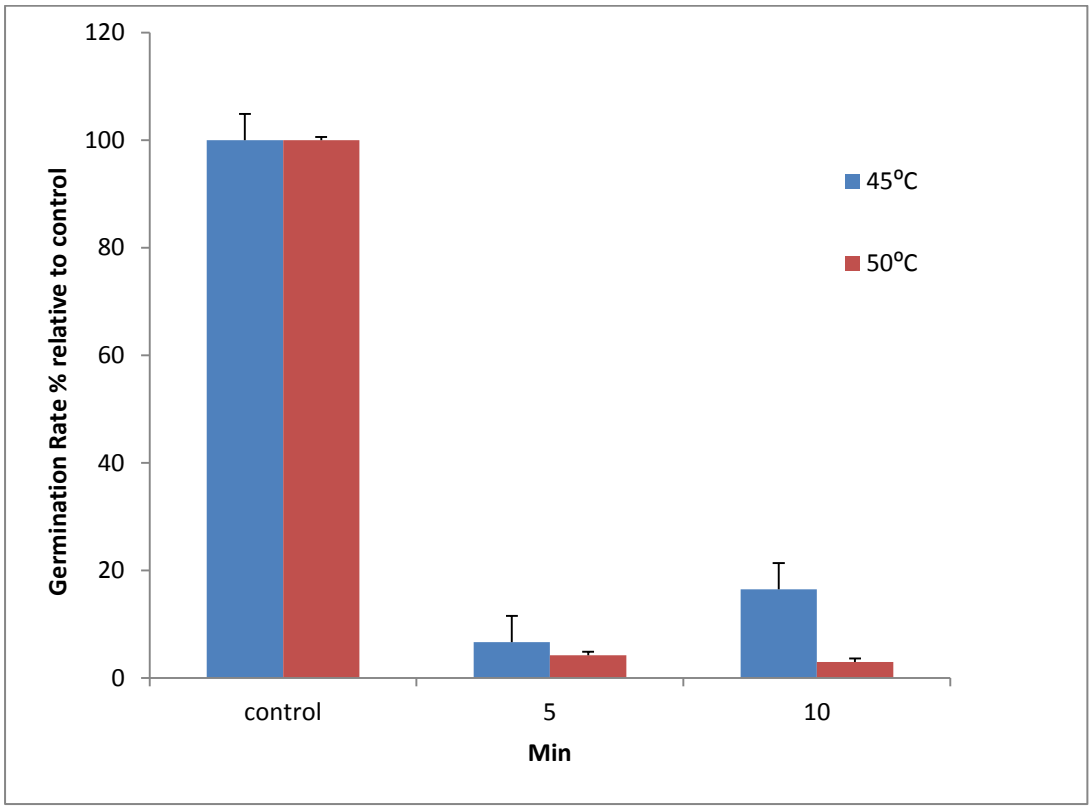

Figure 28: Hot water treatment of *Alternaria alternata* (*In vitro*) at 45 and 50 °C showing mean percentage spore germination rate after 72 h incubation. Error bars indicate standard error of mean of each data point.

When the culture colony was incubated for 72 h at 26 °C, there was no significant difference in the level of spore germination in heat treated spores at 50 °C for 5 or 10 min, but there was a significant difference in percentage germination compared with the control (**Figure 28**). But when the temp of hot water was reduced to 45 °C, the result showed a significant difference in percentage germination of 5 and 10 min treatments (**Figure 28**). For example, heat treatment in 5 min showed about 6 % germination; while in 10 min the percentage rose to 16 %. This is similar to the result obtained for hot water treatment at 50 °C in 5 and 10 min during incubation for 48 h (**Figure 28**).

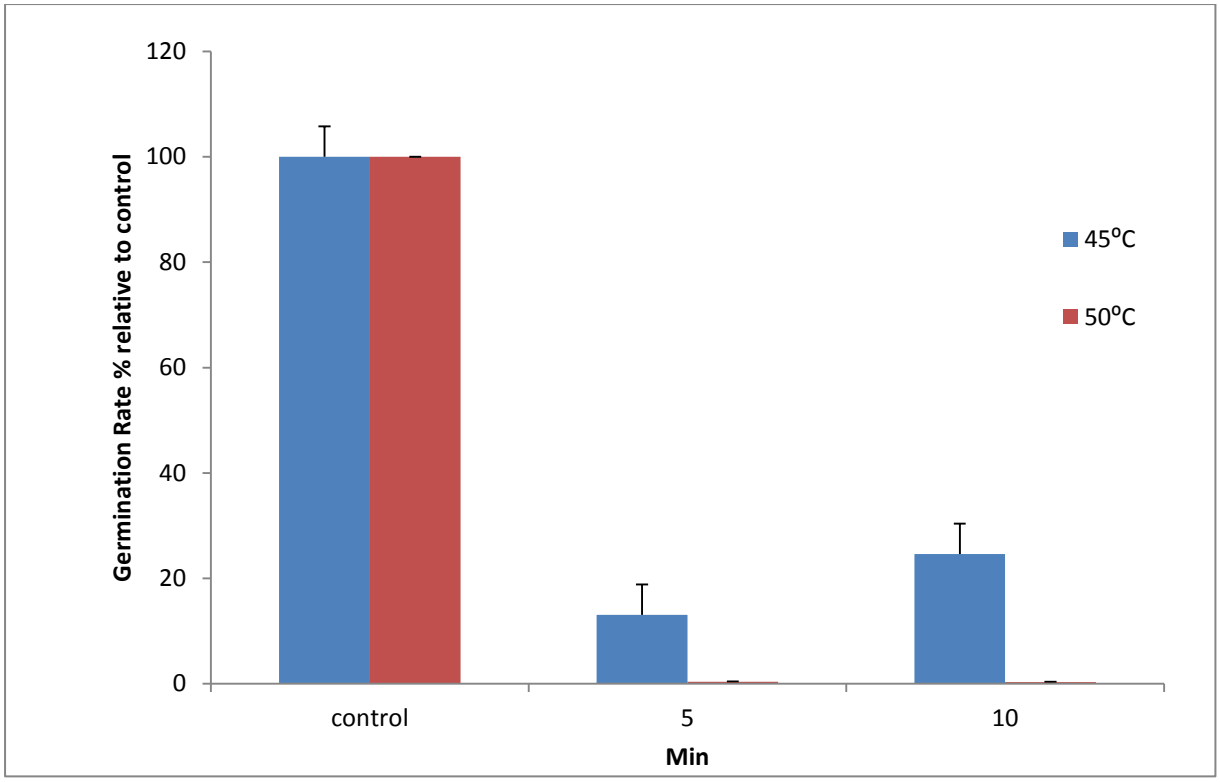

Figure 29: Hot water treatment of *Alternaria alternata* (*In vitro*) at 45 and 50 °C showing mean percentage spore germination rate after 96 h incubation. Bars indicate standard error of mean of each data point.

After 96 h, all the treated spores on agar plate failed to germinate in either 5 or 10 min hot water treatment at 50 °C. At 45 °C hot water treatment the result showed a significant difference ($P < 0.05$) of germination percentage in 5 and 10 min as well as the control **(Figure 29)**.

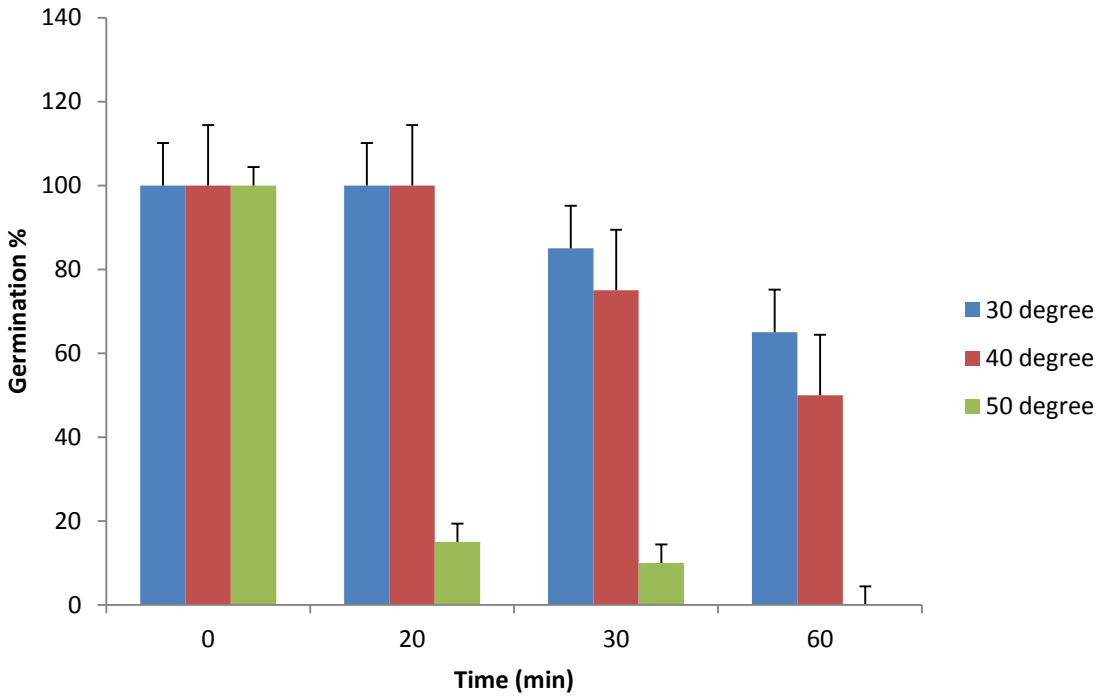

Figure 30: Hot water treatment of *Alternaria alternata* (*in vitro*) at 30, 40 and 50 °C showing percentage spore germination. Error bars indicate standard error of difference of the mean of each replication (n = 30).

The result above showed that increase in both the temperature of hot water and time of exposure to heat is inversely related to the germination of spores on all the tested temperatures. For example, the germination of spores was totally prevented at 50^{o}C heat treatment for 60 min, while for 30 min and 20 min the percentage germination was 10 and 15 % of the control (**Figure 30**). The percentage germination of hot water treatment of spore suspension at 50^{o}C for 20, 30 and 60 min were significantly different compared with 40 and 30^{o}C for 20, 30, 60 min and the control respectively (**Figure 30**). Also there was no significant difference between heat treated spores at 40 and 30^{o}C for 20 and 30 min but for 30 min exposure there was a significant difference between 40 and between 30^{o}C compared with the control. Furthermore, 60 min of heat treatment at 40 and 30^{o}C were significantly different and with the control (**Figure 30)**.

4.3.2. *In vivo* hot water treatments for disease control

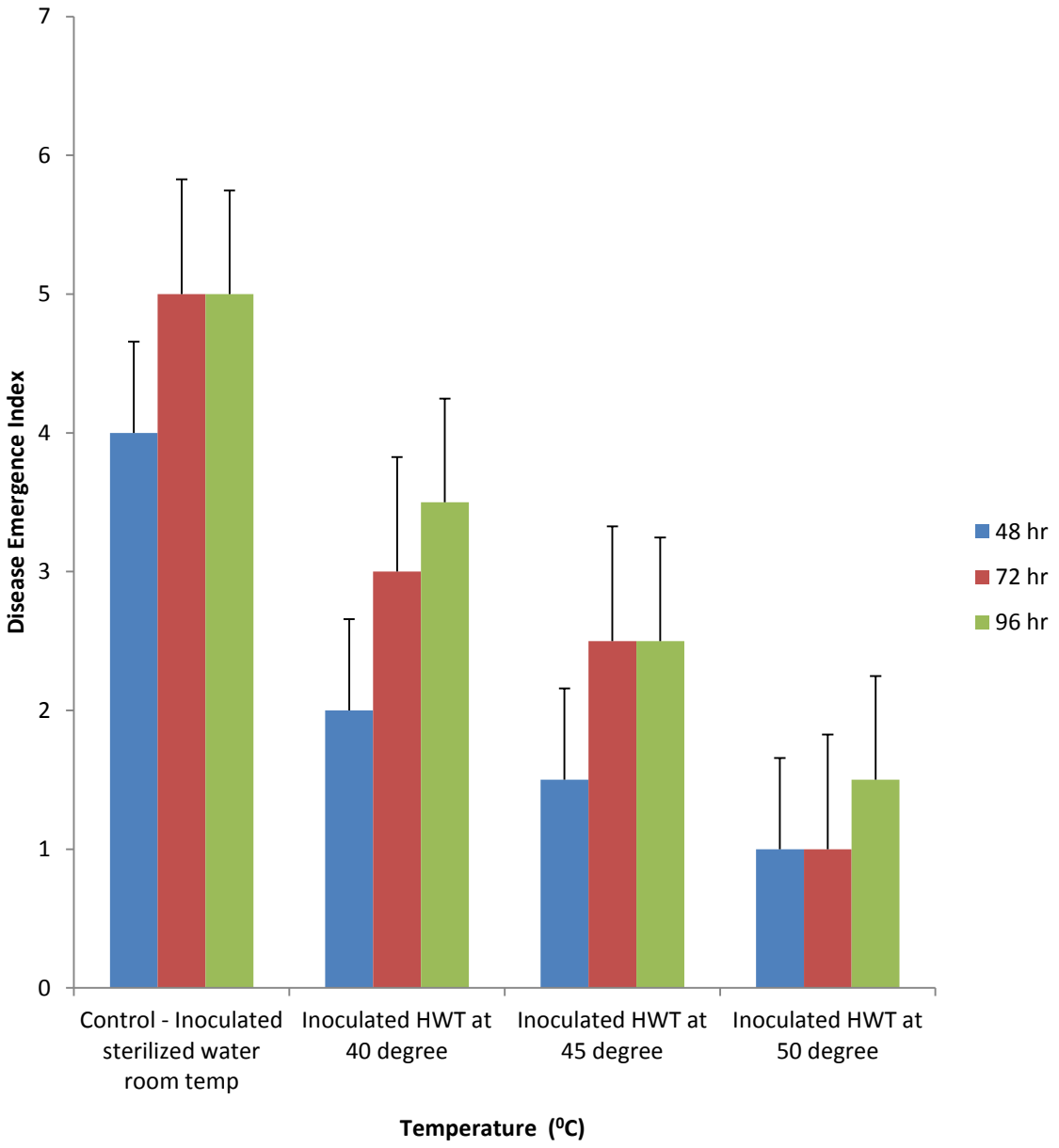

Figure 31: Disease emergence of red tomato fruit inoculated with *Alternaria alternata* and then heated at 40 °C, 45 °C and 50 °C for 10 min. Error bars indicate standard error of difference of the mean of 30 tomatoes.

Grade scale 0 = no rot; 1 = decay up to 0.5cm in diameter without sporulation; 2 = decay between 0.5 and 1.0 cm in diameter with sporulation beginning; 3 = decay between 1.0 and 2.5 cm in diameter with sporulation; 4 = decay between 2.5 and 4.0 in diameter with sporulation and

mycelium; 5 = completely rotten tomato covered with mycelium (Method adapted with modification from Lurie *et al.*, 1998).

The result of hot water treatment at 40, 45 and 50 °C for 10 min of tomato fruit showed a significant difference ($P < 0.05$) compared to the control after 48, 72 and 96 h, respectively (**Figure 31**). However, the results showed an inverse relationship between temperature and incubation time.

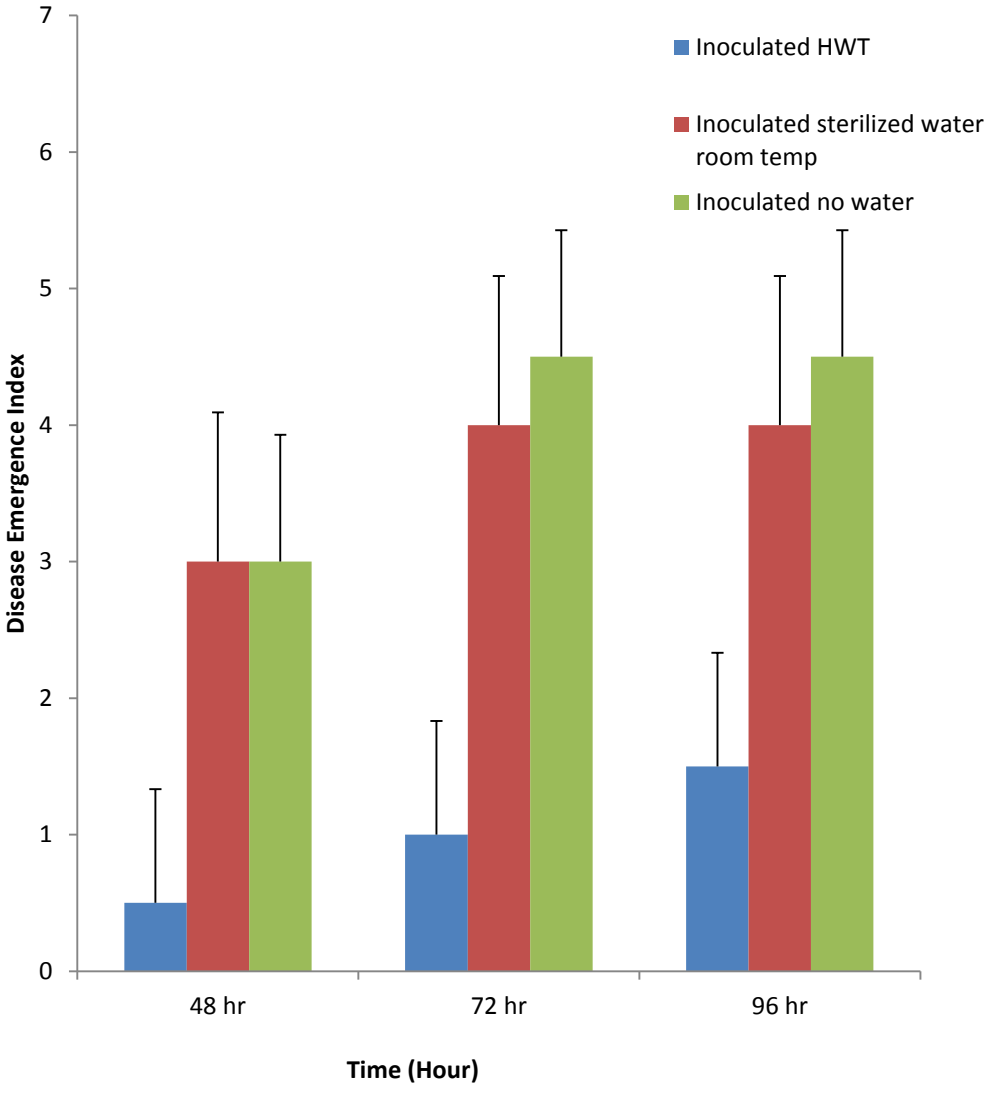

Figure 32: Disease emergence of red tomato fruit inoculated with *Alternaria alternata* and then heated at 50 °C for 20 min. Fruit was examined after 48, 72 and 96 h at room temperature. Bars indicate standard error of difference of the mean of 30 tomatoes.

Grade scale 0 = no rot; 1 = decay up to 0.5cm in diameter without sporulation; 2 = decay between 0.5 and 1.0 cm in diameter with sporulation beginning; 3 = decay between 1.0 and 2.5 cm in diameter with sporulation; 4 = decay between 2.5 and 4.0 in diameter with sporulation and mycelium; 5 = completely rotten tomato covered with mycelium (Method adopted with modification from Lurie *et al.*, 1998). HWT - Hot water treatments.

Figure 32 shows disease emergence of red tomato inoculated with *Alternaria alternata* spores heated at 50 °C for 20 min and examined after 48, 72 and 96 h at room temperature. At 48 h, 72 and 96 h respectively, the disease emergence is significantly different compared with tomato inoculated and put in sterilized water at room temperature and inoculated fruit with no water. There was no significant difference between the control treatments i.e. inoculated tomato dipped in sterilized water and inoculated but no water at room temperature.

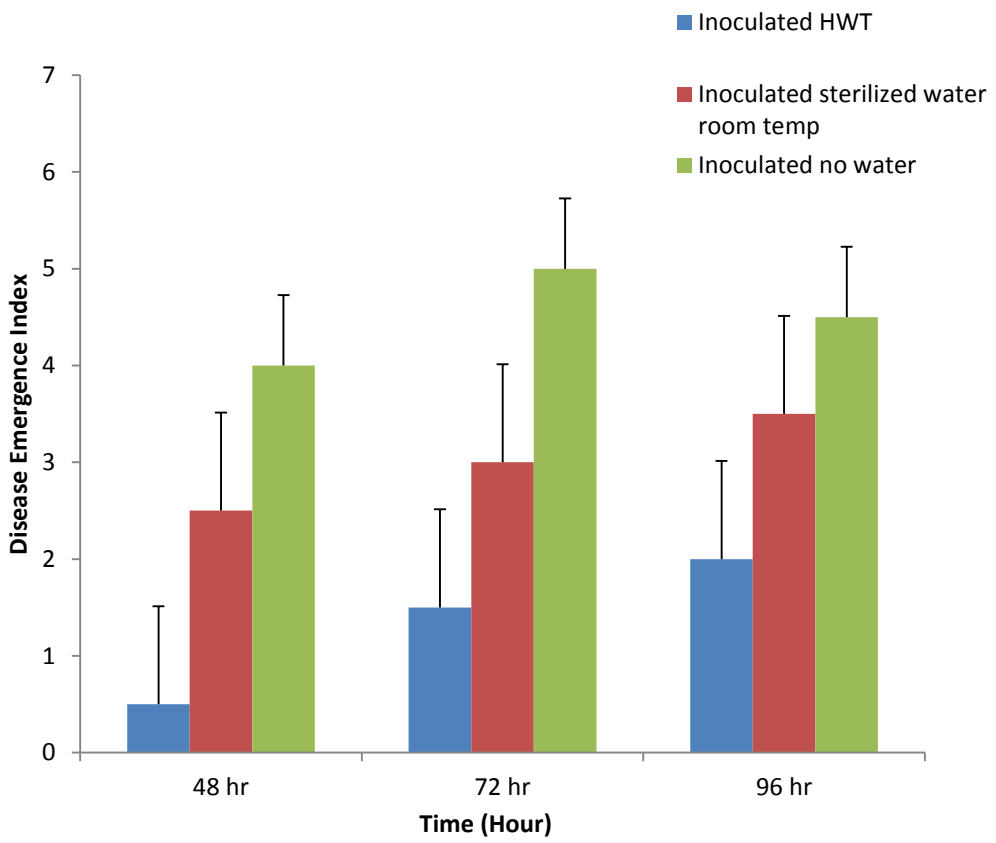

Figure 33: Disease emergence of red tomato fruit inoculated with *Alternaria alternata* and then heated at 40 °C for 20 min. Fruit was examined after 48, 72 and 96 h at room temperature. The error bars indicate standard error of difference of the mean of 30 tomatoes.

Grade scale 0 = no rot; 1 = decay up to 0.5cm in diameter without sporulation; 2 = decay between 0.5 and 1.0 cm in diameter with sporulation beginning; 3 = decay between 1.0 and 2.5 cm in diameter with sporulation; 4 = decay between 2.5 and 4.0 in diameter with sporulation and mycelium; 5 = completely rotten tomato covered with mycelium (Method adapted with modification from Lurie *et al.*, 1998).

Figure 33 shows disease emergence of red tomato inoculated with *Alternaria alternata* spores heated at 40 °C for 20 min and examined after 48, 72 and 96 h at room temperature. The result showed that at 48 h, 72 and 96 h respectively, the disease emergence was significantly different compared with the fruit inoculated and put in sterilized water at room temperature, and inoculated fruit with no water.

Also after 48, 72 and 96 h respectively, the result showed a significant difference between the two control treatments (**Figure 33**).

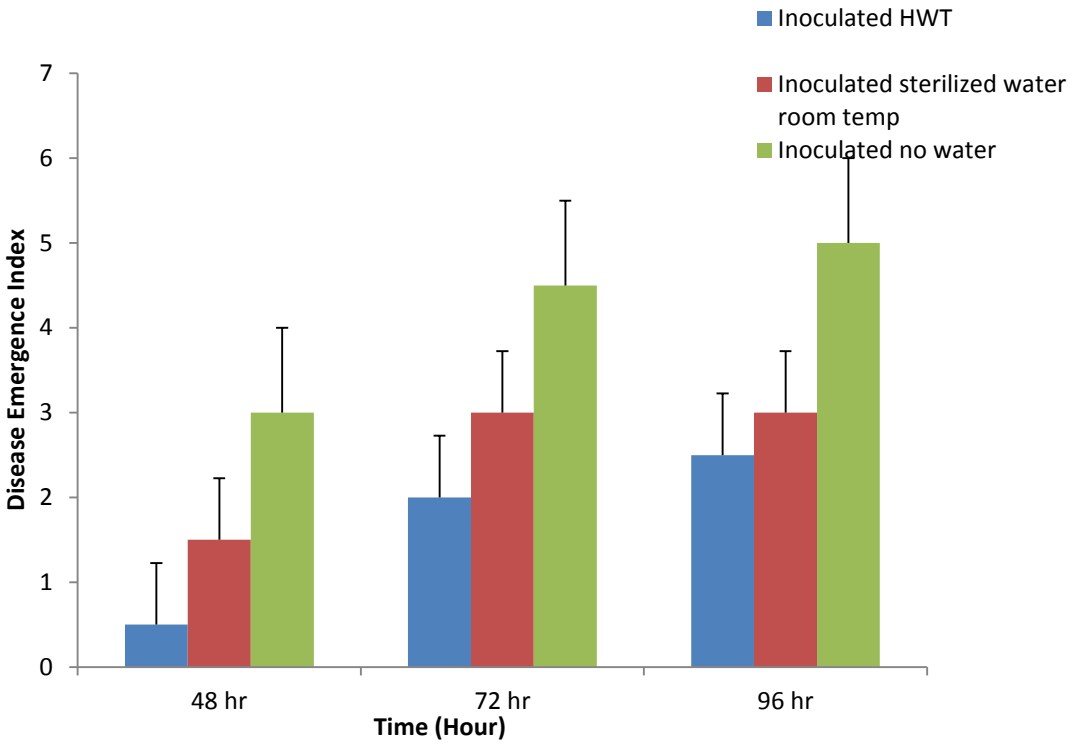

Figure 34: Disease emergence of red tomato fruit inoculated with *Alternaria alternata* and then heated at 30 °C for 20 min. Bars indicate standard error of difference of the mean of 30 tomatoes. Fruit was examined after 48, 72 and 96 h at room temperature.

Grade scale 0 = no rot; 1 = decay up to 0.5cm in diameter without sporulation; 2 = decay between 0.5 and 1.0 cm in diameter with sporulation beginning; 3 = decay between 1.0 and 2.5 cm in diameter with sporulation; 4 = decay between 2.5 and 4.0 in diameter with sporulation and mycelium; 5 = completely rotten tomato covered with mycelium (Method adapted with modification from Lurie *et al.*, 1998).

In the case of **Figure 34**, the disease emergence of inoculated hot water treated fruits at 30C for 20 min showed a significant difference between the treatment and the control after 48, 72 and 96 h respectively, with the exception of the treatment compared with the inoculated sterilized water at

room temperature For example after 48 h, the disease emergence was reduced to about 60 % compared with inoculated fruit dipped in sterilized water.

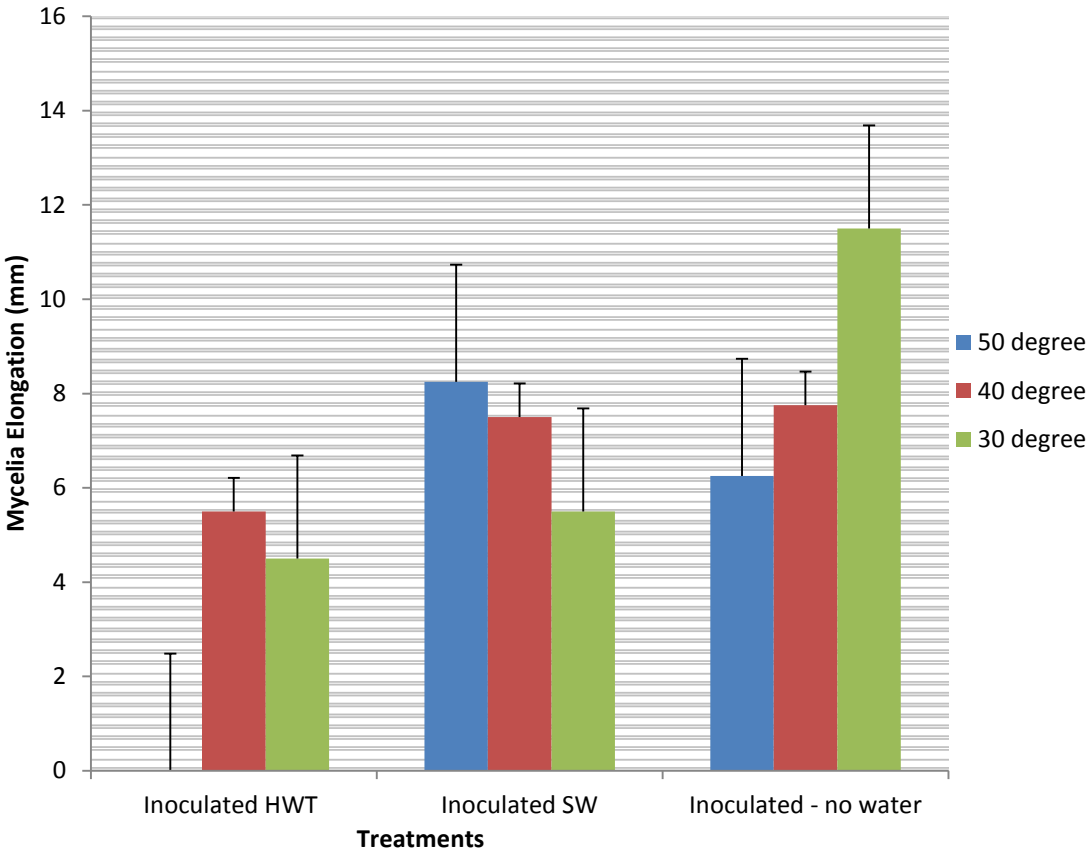

Figure 35: The effect of an increasing temperature of hot water dip at 30, 40 and 50 °C for 20 min on mycelia elongation of *Alternaria alternata* on red tomato. Bars indicate standard error of difference of the mean of 30 tomatoes. Mycelia elongation was evaluated after 96 h. Mycelia elongation is measured as per mm of affected area. HWT – Hot water treatment; SW – sterilized water.

In **Figure 35**, the effect of increasing temperature of hot water dipping was investigated. In this trial, the temperatures tested were 30, 40 and 50 °C for 20 min. At 50 °C the mycelia elongation was completely reduced and was significantly different compared with 40 and 30 °C. Furthermore, the result showed no significant difference in mycelia elongation between the inoculated and heat treated tomato at 40 and 30°C (**Figure 35**).

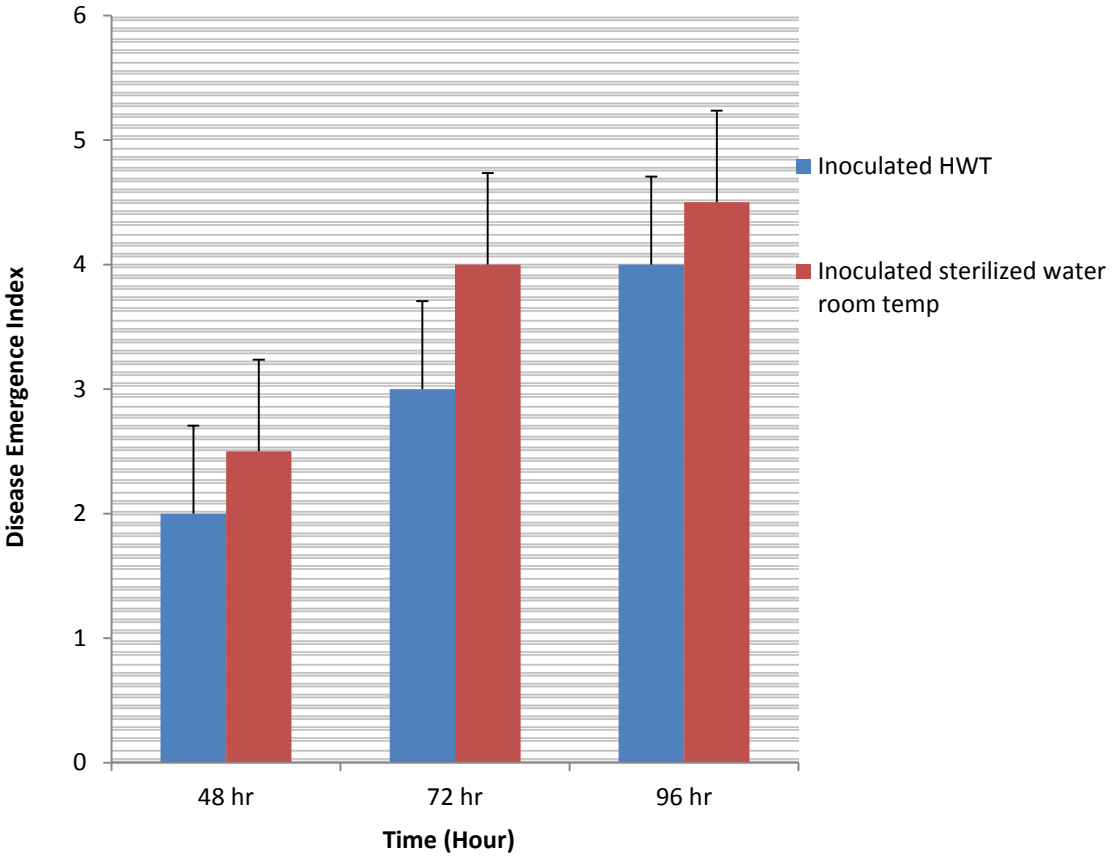

Figure 36: Disease emergence of red tomato fruit inoculated with *Alternaria alternata* and then heated at 50 °C for 5 min. Bars indicate standard error of difference of the mean of 30 tomatoes.

Grade scale 0 = no rot; 1 = decay up to 0.5cm in diameter without sporulation; 2 = decay between 0.5 and 1.0 cm in diameter with sporulation beginning; 3 = decay between 1.0 and 2.5 cm in diameter with sporulation; 4 = decay between 2.5 and 4.0 in diameter with sporulation and mycelium; 5 = completely rotten tomato covered with mycelium (Method adapted with modification from Lurie *et al.*, 1998).

The disease emergence of red tomato fruit inoculated with *Alternaria alternata* and then heated at 50 °C for 5 min examined after 48, 72, and 96 h incubation is shown in Figure 36. The disease emergence has a linear relationship with time of incubation i.e. after 48 h to 96 h. For example, the disease emergence index increased from 2 in 48 to 3 in 72 and to 4 in 96 h. Furthermore, the result also showed a significant difference in disease emergence index ($P \leq 0.05$) between the heat treated

tomatoes compared with inoculated fruit dipped in sterile water at room temperature after 72 h incubation.

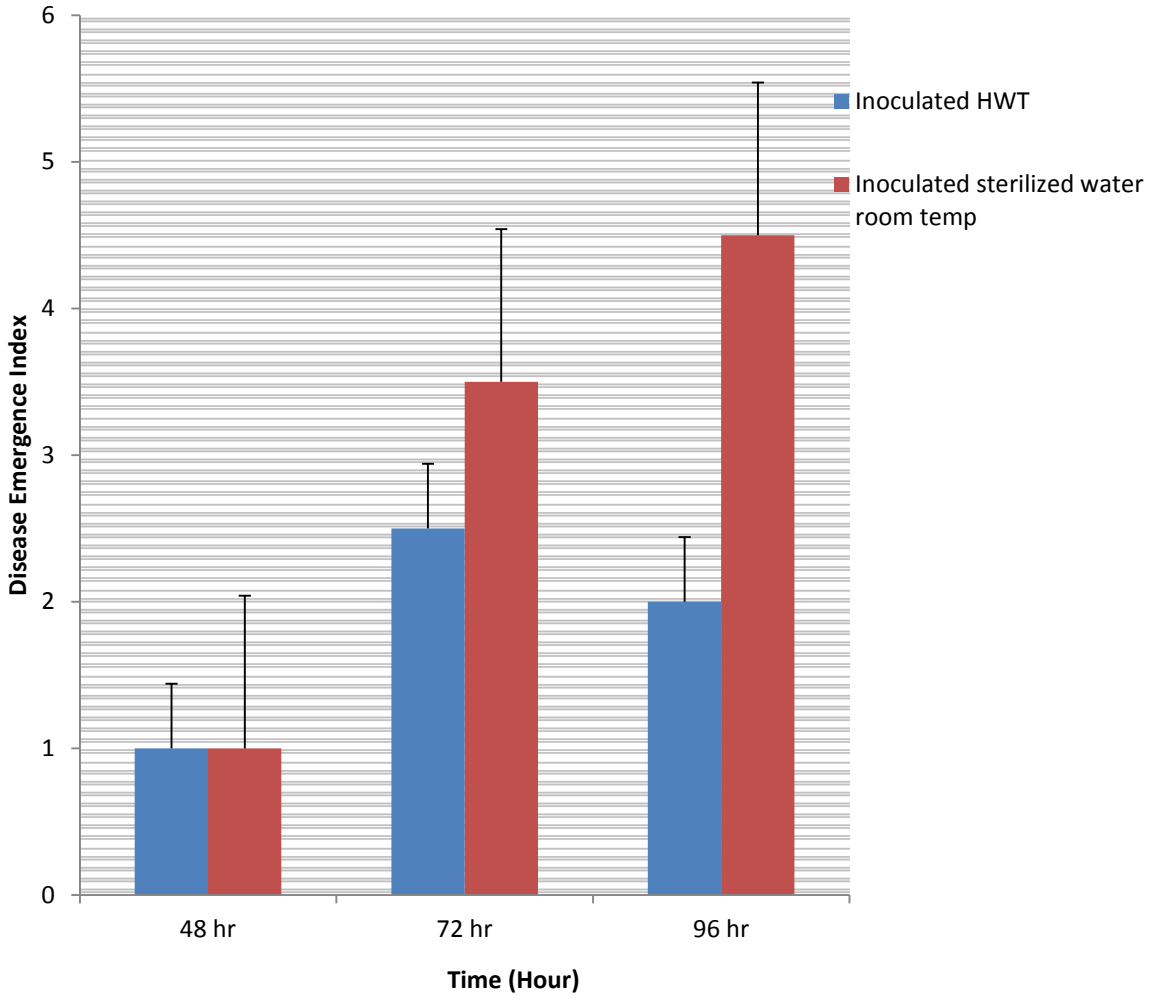

Figure 37: Disease emergence of red tomato fruit inoculated with *Alternaria alternata* and then heated at 55 °C for 5 min. Fruit was examined after 48, 72 and 96 h at room temperature. Bars indicate standard error of difference of the mean of 30 fruits. Grade scale 0 = no rot, 5 = rotten tomato covered with mycelium (Method adopted with modification from Lurie *et al.*, 1998).

Figure 37 shows the disease emergence of red tomato fruit inoculated with *Alternaria alternata* spores and then heated at 55 °C for 5 min examined after 48, 72, and 96 h incubation. After 72 and 96 h of incubation the inoculated and heat treated fruits showed a significant difference compared with inoculated sterilized water at room temperature which served as the control treatment. However, no significant difference was shown in disease emergence between the inoculated and treated fruit and

the control after 48 h. About 30 % of the fruits were observed to split following hot water treatment at 55 °C for 5 min (**Figure 38**).

Figure 38: Hot water treatment at 55 °C for 5 min of inoculated tomato fruit in a basket showing the splitting of tomato fruits (about 30 % split).

4.4. 1. Discussion

Maximum radial length was recorded for the two isolates on PDA after 8 days either with the 10 or 15 days culture colony growth at 17 or 26 °C temp. Radial growth ranged from 8.8 mm to 45 mm of *A. alternata* isolates on tested media at 25 ± 1 °C (**Figure 15** and **16**). However, the growth of the isolates was not significantly different on PDA at 8^{th}, 10^{th} and 15^{th} day respectively, except in the 4^{th} day. In general, the radial growth of the isolates was better on PDA than CMA in all days of the measurement except the 4^{th} day. In addition, it was observed that the mycelia elongated faster at the initial 4-5 days of the experiment and continue to grow fast until the 8^{th} day where the growth started to level off. The result also showed that isolate PON-74351development was better than the strain of rotted tomato either on the agar media, dark and light or at the temperature tested. This is an indication that PDA gives more rapid growth than CMA either at cool or high temperatures.

In the comparison of figure 15 and 16, figure 17 and 18 the result showed the effect of temperature on the elongation of mycelia of *A. alternata* on culture plates. For example, the isolates elongated faster at 26°C temp irrespective of the age of culture or agar media. Therefore, this result agreed with the report of Tian and Bertolini (1995), which stated that temperature is generally considered to be one of the most important environmental factors that affect the germination of spores also the elongation of mycelia of the pathogens *in vitro*. Also high temperature favours the initiation and development of infectious plant diseases *in vivo*.

The conidiophores produced in *A. alternata* are big enough to be identified on agar media compared to a single spore. In the study of Grogan *et al.* (1975) a comparison of spores and conidiophores from field and greenhouse stem cankers and *in vitro* culture on CMA and propylene-oxide-sterilised tomato stems showed that its measurement agree quite well with the published description of *A. alternata* (*Fr.*) Keissler. Although, conidiophores are produced on the mycelia hyphae as the growth continue on the culture media. In comparison the single spore growth of 10 day and 15 day old culture at 26^{o}C and at 17-20^{o}C of the isolates were similar on the agar media. This result agreed with study of Grogan *et al.*, (1975).

The influence of the composition of the media on the morphology of conidia of *A. alternata* and other *Alternaria species* has been reported by Misaghi *et al.* (1998). There was also a similarity between the stem canker organisms in morphology with *A. alternata* from rotted ripe tomato fruits. In this trial, the pathogenicity test of isolates *in vitro* on the agar media showed no difference in growth of mycelia – as indicated in Figure 19 and 20. Therefore, this result agreed with Grogan *et al.*, (1975) and Misaghi *et al.*, (1998) studies.

Grogan *et al.* (1975) reported that when fruits were inoculated through superficial wounds into the carpel wall, after 8 days and the rotted surfaces become blackened by dense sporulation – as indicated in Figure 19 and 20. However, isolation from diseased fruit yielded cultures of *Alternaria alternata* similar in all respect to the isolates used for inoculation. Despite the fact that figure 21 and 22 have different pathotypes, there is no difference in infectivity of tomato.

The study by Morris and Nicholls (1978) stated that spore suspensions are used widely as inocula in phytopathological experiments. The use of haemocytometer to count spores is slow and difficult to view under microscope especially if the size of fungal spores is small and the suspension contains large number. There are variations in the count made with the haemocytometer especially if the concentration is high, when many spores lay on boundary lines between square of the specialised microscope.

However, under fluorescent light at 26 °C the concentration of spores in the suspension is relatively equal in 1:100 dilutions. The findings agreed with the study of Tian and Bertolini (1995) which stated that sporulation was considered influenced by temperature in pathogenic fungi. Similarly, Wilcox (1989) experiment with variable inoculum levels that disease incidence was a function of inoculum concentration in addition to temperature. For example, the brown rot blossom blight of sour cherry inoculum concentration was increased from 50 to 500 and 5,000 conidia/ml at 16 °C temp; the disease incidence was 11, 47 and 81 %, respectively. However, when the temperature was increased and concentration remained the same the blight incidence rose to 26, 59 and 95 %, respectively (Wilcox, 1989). Therefore, this result showed that the dilution rate and mean spore count depend on the concentration of the suspension for infectivity irrespective of the agar media. This experiment agreed with Wilcox (1989) report, hence, at higher concentration (1:10) more spores were produced from the isolates for further research study.

4.4.2. *In vitro*

Heat treatments have a direct effect on spore and mycelia elongation or outright killing germinating spores, thus reducing the effective inoculum size and minimising rots (Schirra *et al.*, 2000; Fallik, 2004). In the present study, it was found that heat treatment directly inhibited spore germination and by implication the mycelia elongation particularly at 50 °C. The results confirmed previous findings about the effect of heat treatment on *A. alternata* and *Botrytis cinerea* (two pathogenic fungi of tomato) reported by Lurie *et al.*, 1998 and Fallik *et al.*, 1996. The studies stated that when fungi are exposed to severe abiotic stresses including heat stress, the thermal shock happened within few minutes such that germination of spores might be inhibited. However, giving the spores more time of

exposure to heat treatment may wear-out the thermal effect or the spores might have developed a resistance to heat treatment.

In this experiment the spore of *Alternaria alternata* proved to be erratic to heat treatment at high temperature and lower exposure time because the germination rate was expected to decrease on agar plates from 5 min to 10 min heat treated spores at 50 °C in 48 h incubation period (**Figure 29**). These indicated that the heat treatment only delayed the germination process for a short time of exposure and possibly resumed the process when the spores are exposed to an increase in treatment time and may have developed a resistance to heat treatment. This result agreed with the study of Lurie *et al.*, 1998 and Fallik *et al.,* 1996. Another consideration for this situation that occurred when the culture plates of 5 min treatment had a lower germination compared to 10 min after 48 h incubation at 26°C might be due to the exposure of spores to high temp and the spore suspension might have contained some germinated spores. Barkai-Golan (1989) studies have reported that germinated spores are more sensitive to heat than non-germinated spores. Consequently, the situation at 5 min exposure might be as a result of heat on the germinated spores. Therefore, the result of this trial agreed with the study of Barkai-Golan (1989).

Lurie *et al.* (1998) reported that hot water treatment for 10 min at 45^0C decreased *Botrytis* germination to below 10 % while 5 min at 50 °C prevented it. Germination of *Alternaria alternata* was more resistant to heat and some germination occurred during all the time and temperature regimes tested. For example, *Alternaria* growth was inhibited not prevented by all treatments e.g. 40 % at 45 °C in 10 min, 38 % at 50 °C in 5 min and 15 % at 55 °C in 5 min. Also the use of hot water dips or vapour heat at 39-52 °C for 2-10 min was reported to control the *in vitro* and *in vivo* spore germination and decay development of postharvest fungi in tomato (Fallik *et al*., 1993). Tohamy *et al*. (2004) also examined in vitro effect of hot water treatment on fungi. A linear growth of *Botrytis* and *Alternaria* was obtained from standardised number of spore treatment at 45 and 50 °C for different periods and was inversely related to the temperature treatments and time of dipping. Therefore, all these reports of studies agreed with the results of this trial.

The result of *in vitro* trial indicated that increase in both the temperature of hot water and time of spore exposure to heat is inversely related to the germination of spores (Figure 30). Lurie *et al.*, (1998), study has shown that germination of fungal pathogens such as *Alternaria alternata* and *Botrytis cinerea* could be inhibited or prevented by extended periods of temperature above 40 °C; for example 10 min at 45 °C decreased *Botrytis cinerea* to below 10 % while 5 min at 50 °C was enough to prevent the germination of spores and elongation of mycelia. But in *Alternaria* some germination occurred with *Alternaria* heated spores. This report agreed with this study because germination of *Alternaria* spores occurred at all time and temperature regimes except at 50 °C for 60 min. The effect of heat on the pathogen depends on the state of development of the inoculum, for example germinated spore and elongated mycelia are more sensitive to heat than non-germinated spore (Lurie *et al.*, 1998).

In another study by Barkai-Golan (1989); Barkai-Golan (2001) the reports showed that in a given species of fungi, spore inactivation increases with both temperature and duration of treatment, for example the conidiophores of *Alternaria alternata* was inactivated by heat treatment at 48 °C for 2 min or at 46 °C for 4 min (Barkai-Golan, 2001). In this study the spore germination was reduced at 50 °C with the exposure time starting from 5 min which finally resulted to total inhibition in 60 min. Also spore sensitivity depends on their physiological state such that germinated fungal spores are more sensitive to heat than non-germinated spores (Barkai-Golan, 1973). Therefore, the result of this trial might be translated to mean that the germinated spores are inhibited at 50°C as a result the percentage germination of spores was less compared to other temperature tested. Therefore, this report agreed with the study of Barkai-Golan, (1973).

4.4.3. *In vivo*

The result showed that the disease emergence and mycelia elongation which was measured by an empirical scale for disease emergence were inversely related respectively to the temperature increase at 40, 45 and 50 °C for 10 min and the length of incubation period (Figure 31). Temperature and incubation time are the factors found to be significant compared with control (Figure 31). Lurie (1998) reported that many fruits and vegetables tolerate exposure to hot water temperatures of 50 - 60 °C for up to 10 min, so also Tohamy *et al*., (2004) reported that dipping cantaloupe fruits inoculated

with *Alternaria alternata* in hot water at 50^{o}C for 10 min inhibited the decay by this fungus. This experiment also showed that the development of *A. alternata* decay of tomato was reduced to a minimal level as was observed at 50°C for 10 min after 48 h incubation period. Therefore, this result agreed with Lurie (1998) and Tohamy *et al*., (2004) studies.

The following hot water treatment of inoculated tomato fruits at 50, 40 and 30^{o}C for 20 min the disease emergence was inhibited when compared with control after 48, 72 and 96 h respectively (Figures 32 - 35). The use of water dips at 38 to 60^{o}C for 2 to 60 min has been reported to control *in vivo* and *in vitro* spore germination and decay development of postharvest fungi in melons (Klein and Lurie 1992). The result of this trial agreed with this report. The mycelia elongation measurements have shown that treatment at 40 and 30 °C reduced decay by 50 and 40 %, while at 50 °C the mycelia elongation was prevented completely (Figure 35). Barkai-Golan (1973) study showed that fungal disease of fruits was controlled by hot water at 38-50 °C for 5-60 min and as a result achieve a significant decay reduction ranging from 40-60 %. The report of Biggs *et al*., (1988) stated that heat treatment at 30^{o}C for 20 min reduced germination of fungal spore on agar media as a result reduced the mycelia elongation. Therefore, this result is in agreement with Barkai-Golan (1973) and Biggs *et al*., (1988) studies. Lurie (1998) also reported that many fruits and vegetables can tolerate exposure to heat treatment at 50-60 °C for about 90 min also this result was in agreement with this study.

In another experiment the result showed that dipping inoculated red tomato fruits in hot water at 50 (Figure 36) and 55 °C (Figure 37) reduced fruit decay caused by *Alternaria alternata* compared with control after 72 h incubation. The severity of infection of the disease pathogen was gradually reduced as water temperature rose from 50 to 55 °C (Figures 36 and 37). At 55 °C the hot water treatment reduced the decay incidence compared with control after 72 h incubation period (Figure 37). Thus, it follows from reports of studies that 3 min at 55 °C, 5 min at 50 °C and 10 min at 45 °C reduced the decay caused by *Alternaria alternata* on tomatoes (Lurie *et al.*, 1998; Lurie, 1998). Therefore, the result agreed with Lurie (1998) and Lurie *et al*., (1998) studies.

Furthermore, report of Mitcham and Cantwell (2002) stated that dipping bell pepper fruit in hot water at 55^{o}C for 5 min gave complete control of *Botrytis* rot without injury to the fruit. Therefore, the result of this trial agreed with this report. The report of Klein and Lurie (1992) also stated that the *in vivo* and *in vitro* spore germination and decay development were controlled at 38-60^{o}C for 2 to 60 min is in agreement with the result of this experiment. After 5 min of hot water treatment at 55 °C crack was observed on the skin of tomato fruit from the point of inoculation (Figure 38). Approximately 30 % of mature red fruit cracked after heat treatment. Shao *et al.*, (2011) reported that turgor pressure within the fruit is the major force causing cracking, and it increases with the temperature of water.

Ripe fruits have the potential for an increase in cracking because of lower skin firmness (Lichter *et al.*, 2002). Consequently, the heat treatment caused a rapid increase in internal temperature thereby increased the turgor pressure, as a result extended the skin of the fruit resulting to fruit cracking. Furthermore, the study showed that immersion of tomato fruit in calcium chloride reduced cracking compared with sodium carbonate, sodium bicarbonate and the control (Lichter *et al*., 2002). The result of cracking can be for further research of this study.

4.5. Conclusion

Conidia are formed on the hyphae and produced spores that germinated and form the mycelia. This study has shown that light has no effect on this process but the temp and culture media are important for the development of spores using mycelia plug on agar plates. This is in agreement with the report of Misaghi *et al*. (1978) that light was not required for sporulation, size, shape, and mycelia growth. Furthermore, it stated that *A. alternata* form spores within 6-33^{o}C temp by implication the temperature used in this study is within this range. Therefore, a conclusion can be drawn that in other to produce more *A. alternata* spores on plates, the temp could be as high as 26^{o}C without adverse effect on the pathogen. Potato dextrose agar (PDA) produced more spores than corn meal agar (CMA) and preferred in microbiology studies on petri dish plates.

The Koch′s postulates test showed no morphological differences between the isolates. When the isolates were inoculated on red tomato, after 8 days the result showed the strain of tomato cultivar

`Delycassi′ caused disease infection on the fruit. Likewise, isolate no 74351 did develop disease infection on the fruit. Therefore, it could be concluded that there was no significant difference between the two isolates when Koch′s postulate test was done. Furthermore, there was no significant difference in age of culture of the isolates as regard to temperature as shown in the analysis of variance tables. For example, in comparing the result of the single spore growth of 10 and 15 day old culture at 26°C, the tested temperature assisted the elongation of mycelia almost on equal rate. Likewise, similar situation applied to 10 and 15 day old culture at 17-20°C. .

The 1:10 dilution ratio produced more spores at 17 °C cool temp on CMA and PDA agar media compared with dilution 1:100. Usually it is desirable to determine the concentration of spores as it may affect the experimental result. In addition, the 1:10 dilution ratio of Delycassi isolates produced more spores compared to CABI isolates under PDA agar medium.

The result of this study showed that the reduction of fungal growth was due to the direct effect of heat on the spore as well as mycelia as a result of hot water treatment at the tested temperature. Consequently, the germination of spores and mycelia elongation was reduced. The results obtained for *in vitro* experiment could be applied to explain the direct effect of heat treatment on tomato during *in vivo* trial. As a result of the heat treatment that reduced fungal viability, the effective inoculum concentration that causes decay development is also reduced, consequently control the rot development. This result is in agreement with the work of Schirra *et al.*, (2000). This study showed that hot water treatment at 50 °C of *Alternaria alternata* for 5 min is enough to cause a greater reduction of conidia germination in 48 h. The result of this study has shown that increase in temperature above 40 °C could inhibit the germination of *Alternaria alternata* as a result reduces rot decay. However, fungi-static condition in *Alternaria alternata* occurs because the growth of *Alternaria alternata* was inhibited and not prevented. Based on the outcome of this trial, 30°C had no potential to reduce spore germination of *Alternaria alternata* because the result is similar to the control. Hot water treatment at 40 or 50°C has the potential to reduce the germination rate of *Alternaria* spores compared to the control and lower temperature.

A conclusion can be drawn from this result that hot water treatment at 40 °C in 10 min is sufficient to reduce disease emergence of *Alternaria alternata* on red tomato during 48 h incubation period. In this study, less than or equal to 48 h incubation time after hot water treatment of inoculated tomato fruits has the greater potential to reduce fungal disease emergence compared to higher incubation period. The common outcome in this investigation is that in all the treatments the disease emergence on the fruit was significantly reduced (0.5 index) in 48 h. Mycelia elongation was completely and significantly reduced during 50 °C hot water treatment of tomato fruit compared to the control and lower temperature; which means that 50 °C could be an important temperature threshold for managing *Alternaria* fungal disease of tomato.

Postharvest heat treatments to control decay are often applied for a relatively short time e.g. seconds to minutes because the target pathogens are found on the surface or in the first few layers under the skin of the fruit or vegetable. In this study the *in vitro* heat treatment of *Alternaria alternata* spores and *in vivo* for artificially inoculated tomato fruit indicated that hot water dipping at 50 °C or 55 °C for 5 min was sufficient to inhibit growth of the fungus and decrease the severity of infection. Though, the use of hot water at 39-52 °C for 10 -2 min has been reported to control the *in vitro* as well as the *in-vivo* spore germination of postharvest fungi and decay development in tomato fruit (Tohamy *et al.*, 2004). The outcome of this trial showed that at 50 °C hot water treatment of inoculated tomato fruit for 5 min there was a decrease in disease emergence after 48, 72 and 96 h incubation time. But the heated fruits at 55 °C showed a decrease of disease emergence index after 48 h than 50^{o}C compared to the control (Figure 37).

However, all the methods currently being employed to reduce decay by heat treatment are temporary measures because the effect is reversible. The pathogen is markedly inhibited by thermal inhibition, and possibly by enhanced resistance of the fruit against the pathogen. This enhanced resistance is could be related to the welding of the epicuticular surface, filling cracks of the cuticle and preventing the use of these occluded cracks as invasion sites for various pathogens.

CHAPTER 5: THE EFFECT OF HOT WATER TREATMENT ON THE COLOUR, FIRMNESS, TOTAL SOLUBLE SOLID AND WEIGHT LOSS OF TOMATO FRUIT

5.1 Introduction

Tomato is an important source of vitamins A and C and contains antioxidants such as lycopene (Okolie and Sanni 2012). In tomato colour serves as a measure of total quality. Consumers notice colour first and their observation often supplements preconceived idea about other quality attributes such as aroma and flavour. Colour in tomato is due to carotenoids, a class of isoprenoid compounds varying from yellow to red colour (Okolie and Sanni, 2012). The major quality attribute of ripe tomato is its red colour, which is due to the lycopene content of the fruit. Other important physicochemical parameters, which determine the quality of tomato, include; firmness, °Brix, fruit weight, flavour (Jackman *et al.,* 1990; Okolie and Sanni, 2012); aroma, texture, juiciness and overall intensity (Maul *et al.,* 2000). A fresh tomato is described by appearance, colour, texture and flavour. The best quality is attained through vine ripening; but ripe tomatoes are perishable and are susceptible to damage during handling which lead to loss of quality and waste.

Lurie (1998) reported that heat treatment of fruits increased the rate of degreening in apples; as well as the chlorophyll content in the fruit skin of apple peel, plantain peel and tomato pericarp decreased during a heat treatment at 35-40 °C. Cucumber developed yellow colour when dipped in hot water at 45 °C for 30-60 min. Klein and Lurie (1992) stated in their study that heated tomatoes became redder than unheated fruit at 45 °C. The skin colour started changing from green to yellow and later to red after storage. Colour development was attenuated during heating both mature green and pink fruit (Fallik et al., 1993). However, 10 days after harvest all fruit have attained a uniform red colour irrespective of heat treatment or stage of maturity. These studies have demonstrated the significance of colour as a determinant of good quality tomato.

Many studies showed that ripening of most climacteric fruit such as tomato is characterised by softening of flesh (pericarp), increase in the sugar: acid ratio, enhancement of colour development,

and increase in respiratory activity together with ethylene production (Lurie, 1998). When fruit are exposed to high temperatures some of these processes are retarded while others are speeded up. Consequently, heated fruit are more advanced in some ripening characteristics than non-heated fruit as a result maintaining quality and may lead to a longer shelf life. In one of the review it was stated that inhibition of ripening by heat may be related to the inhibition of ethylene by the enzyme ethylene synthesis (Lurie, 1998); for instance Biggs *et al.* (1988) and Lurie (1998) reported that hot air treatment at 35-40 °C for 20 min inhibited ethylene synthesis in both apples and tomatoes as a result the fruit become softened slowly when held in storage at 20°C temperature.

Temperature increase from 35-38 °C hot air caused endogenous 1-aminocyclopropane-1-carboxylic acid (ACC) to accumulate in apple and tomato tissue with decrease in ethylene. Immersion of fruit in hot water at 42-46 °C for few hours also resulted to loss of ACC oxidase activity as well as decrease in ACC oxidase m-ribonucleic acid (mRNA) and stoppage of enzyme synthesis (Lurie, 1998). Furthermore, ACC synthase is less sensitive to heat than ACC oxidase therefore implicating the importance of ACC oxidase.

The inhibition of ethylene is a reversible process. For an example when the fruit is removed from heat and held at 20 °C, the result showed a reduction in ethylene synthesis compared with unheated fruit; however, production eventually recovered and even exceeded the control levels (Klein and Lurie, 1992). The implication of these biosynthetic processes is that during the heating period, not only did endogenous ethylene synthesis is inhibited, but fruit may not respond to external application of ethylene. As a result it was suggested that either the ethylene receptors were lost or inactivated or it was due to the inability to continue with the subsequent series of processes that took place during fruit ripening (Lurie, 1998). No information is available on the availability of ethylene receptors in tomato due to heat treatment, but there are reports on the expression of tomato ripening genes inhibiting ethylene synthesis by high temperature (Lurie, 1998); (Barka-Golan and Kopeliovitch, 1989). Studies have shown that when apples, avocados, and tomatoes are heat treated a simultaneous increase in CO_2 production and decrease in ethylene occurred (Klein and Lurie, 1992). However, when the fruits are

removed from heating, CO_2 production fell below that of controls while ethylene increase exceeded that of the control unheated fruit.

Biggs *et al.*, (1988) reported that heat treatment at 35 or 40°C inhibited ethylene synthesis in apples and tomatoes as a result the fruit become ripened slowly when held for 7 days. The rate of ripening increased when heated fruit were returned to ambient temperature, but it was still less than that of non-heated fruit. During heat treatment there was a decrease in ethylene production compared with non-heated fruit but later the enzymatic hormone increased more than the control. As a result of this increase in ethylene production, the fruit did not become softened because of heat treatment. The `physiological injury` softening, is linked with the production of endogenous ethylene in fruit and so the injury could be overcome by heat treatment. As a result the practice of removing ethylene gas from the store of fruits and vegetables, which is considered beneficial to a long shelf life of the produce, might become unnecessary with heated fruit.

The L*a*b* are the colour space coordinates presently used for measuring object colour and commonly used in the food industry (Anonymous, 2002). In the colour space diagram, L* indicates lightness and a* and b* are the chromaticity coordinates. The a^* and b^* indicate colour directions: $+a^*$ is the red direction, $-a^*$ is the green direction, +b* is the yellow direction, and –b* is the blue direction (Anonymous, 2002). The a^* value is a good parameter for red colour development and degree of ripening in tomato while the b^* parameter shows yellow discolouration (Batu, 2004). Most studies use the USDA colour classification and the Minolta a^* / b^* values of the tomatoes correspond to the six USDA colour stages which are used for estimation of the colour values. The colour changes of tomatoes are normally recorded as a^*/ b^* values (McDonald *et al.*, 1999; Batu, 2004). Fruit colour is one of the quality factors of fresh tomatoes for consumer preference.

Lurie (1998) reported that ripening of most climacteric fruit is characterised by intensified colour development, increase in respiratory activity and ethylene production, softening of flesh and increase in acid: sugar ratio. Therefore, exposing fruit to high temperatures weakens some of these processes and possibly enhance others (Lurie, 1998). As a result this situation may lead to some heated fruit

being more advanced in some ripening characteristics than non-heated fruit and still maintain their quality. For example, McDonald *et al.* (1999) reported that fruit colour was affected by heat treatment with increasing a^*/b^* values associated with increasing treatment temperature; such that non-chilled tomato had higher a^*/b^* values than chilled fruit. Furthermore, chlorophyll levels were reported to be highest in fruit from the 48 °C hot water treatment and in non-chilled compared with chilled fruit. Lycopene was reported not to be affected by the heat treatment (McDonald *et al.,* 1999). Another study by McDonald *et al.,* (1996) reported that there was no significant heat treatment effects on colour (L^* and a^*/b^* values), ripeness, lycopene, chlorophyll, percent soluble solids, and fruit firmness in red-ripe fruit. Based on these reports it was shown that heat treatment inhibited the ripening of tomatoes.

The two quality attributes that are most important to buyers and consumers are texture and skin colour for fresh tomatoes (Batu, 2004); and texture is influenced by flesh firmness and strength of the skin. For example changes in firmness were highly correlated with surface appearance characteristics of tomatoes and was related to colour, shape and perceived feel for firmness at the time of purchase. Therefore, the degree of fruit firmness can be used to determine the quality and may be the final index by which the consumers decide to purchase tomatoes.

McDonald et al., (1999) reported that heat treatment has no effect on fruit firmness. For instance red tomatoes remained firmer as a result of heat treatment and storage at chilling temperature; showing that heat treatment had no effect on firmness but on chilling injury. Another study described the effect of continuous storage of fruit at elevated temperatures on fruit firmness; for instance plums, pears and tomatoes softened more slowly when put at temperature between 30 and 40 °C than at 20 °C Klein and Lurie (1992; 1998). The rate of softening was increased when fruits were stored at 20 °C but still less than non-heated fruit. Fallik et al. (1993) study stated that heated mature green tomato fruit were softer during shelf-life (10 days after harvest) storage than nonheated control. But in comparison to tomatoes in other colour stage such as turning to light red, there was no significant difference between heated and nonheated fruit (McDonald *et al*., 1996); and McDonald et al. (1999) reports also confirmed that there was no significant heat treatment effect on firmness of red fruit.

Polygalacturonase and cellulose enzyme activities were implicated in the studies of Lurie (1998). In tomato mRNA for polygalacturonase was absent when the fruit was heat treated at 40 °C for 4 days, but reappear when the fruit was removed from heat (Lurie, 1998). The length of treatment determines whether the fruit recovers and softens to the same extent or remain firmer than the non-heated fruits. Fallik (2004) reported that polygalacturonase was significantly lower in heat treated tomato and sweet pepper during storage. As a result of this enzyme activity, there was a reduction in fruit softening which may be as a result of the inhibition of peptic hydrolysis, causing a reduced level of cell wall degrading enzyme activity and to the inhibition of ethylene forming enzyme (ACC synthase). Another possibility is that the reduction in softening of heat treated fruit might be due to melting of the wax layer which sealed non visible cracks in the cuticle through which water could be lost. Consequently, this sealing of cracks and natural openings could significantly reduce weight loss, thus maintaining fruit firmness after storage (Fallik, 2004).

The total soluble solids acts as an induction of the amount of sugars present in fruits (Okolie and Sanni, 2012). It is the measurement of the amount of sugar and minerals dissolved in water present in fruits and vegetables. Sugars constitute 80-85 % of soluble solids. Many studies have shown that total soluble solids increased during ripening as a result of degradation of polysaccharides to simple sugars thereby causing an increase in soluble sugars (Okolie and Sanni, 2012).

Many reviews including McDonald and McCollum (1996) have reported that there is no significant heat treatment effect on percent soluble solids, percent titratable acidity and solids/acid ratio in red-ripe tomato. In another study by Lurie (1998) it was reported that in tomatoes hot air heated at 38 °C for 2-3 days, neither titratable acidity nor soluble solids content was affected by heat. Likewise, during hot water dipping for 15 min at 35, 45 or 55 °C for the decay control of strawberries, the heat treatment had no effect on soluble solids content or titratable acidity. Another report stated that the soluble solids concentration in heat treated apples did not differ from that in non-heated fruit; while heat treated tomatoes had higher soluble solids concentrations than non-heated fruit after removal from storage (Klein and Lurie, 1992). In some fruit such as muskmelon the sugar content is favourably affected by heat treatment when dipped for 3 h before cool storage. The heat treatment

prevented the loss of sucrose which occurred in non-heated fruit during storage. As a result the sucrose was transformed and the fruit qualified as a sweeter by a taste panel (Lurie, 1998).

A laboratory experiment was conducted to investigate the effect of post-harvest hot water treatment on the quality of red-ripe tomato. Also included was the effect of heat on weight loss after storage condition. Fallik et al. (1993) reported that there were similarities in weight loss between mature green or pink tomatoes by the end of shelf life regardless of treatment. But the weight loss did not result to shrivelling or cracking of the fruit.

5.2 Materials and Methods

Tomatoes (*Lycopersicon esculentum* Mill. unknown variety) were purchased from a farm shop in Writtle, Chelmsford colour scale (turning pink to light red) with a weight range of 60-80 g. The fruit was put in storage for 24 h at 21-22 ℃ temperature. These tomatoes were used to study the effect of heat treatment on the colour of tomato skin. Fruit were sorted out to eliminate defects and establish uniform size. The fruit were divided into 6 groups having 10 tomatoes in each group as sample treatment and 10 fruit as the control. Weights of 10 fruit were measured at the beginning of the study using the weighing scale for the treatment sample as well as the control. Tomatoes were dipped in hot water bath (Phillip Harris Limited, Lichfield, Staffs England) for the treatment at 39-40 °C or 49-50 °C for 10, 20 and 30 min respectively. After hot water treatment the fruit were stored at 29-30 °C to mimic the average storage temperature in Nigeria; humidity 80-90 % for 1hr or 24 h for each temperature and time period. The weight of the treated fruit was measured at the end of 1h or 24 h storage. After 1 h and 24 h storage, 10 fruits were weighed and a^*/ b^* colour ratio of tomato was measured using a Minolta Chroma meter CR 200 (Minolta Co. Ltd, Japan). After 24 h storage period, five tomatoes were used to measure the fruit firmness and remaining five fruits were used to measure the total soluble solids ($^{\circ}$Brix).

Fruit firmness was determined with `Guss′ fruit texture analyser (FTA), (Atago Pal-1 ACE Industrial Supplies Limited, Staplehurst, Kent England) fitted with a 40 mm diameter, flat-faced aluminium round disc. The food testing instrument was connected to a software which recorded the amount of

force (kg) required to compress the surface of the 3 mm diameter radial pericarp. One tomato fruit was cut into two through the style end of the fruit and two disks (3 mm diameter) of outer pericarp tissue were cut from the equator of each tomato, avoiding radial arms. The recorded values represent the amount of force required for maximum compression for ten tomatoes with two measurements per fruit. The data showed the mean of 3 replicates and each replicate consists of ten tomatoes.

The total soluble solid (TSS) was measured with a refractometer `Atago′ Palette PR-32α (Brix 0-32 %) on five fruit before dipping in hot water at 40 °C for 30 min and at the end of 24 h storage and the values were expressed as degree Brix. Similarly, the same treatment was applied on tomatoes dipped in hot water at 50 °C for 30 min. The experiment was repeated three times. The data was subjected to analysis of variance (ANOVA) with the GenStat program for the effect of treatment temperature on the quality characteristics of tomato in turning-pink colour stage.

Five red tomatoes picked at random from hot water treated fruit at 40 or 50 °C for 30 min were used to determine the total soluble solids (TSS) using `Atago Palette′ hand refractometer PR- 32α (Brix 0-32 %). Two slices of tomato about 2-3 mm diameter were cut from the skin of each fruit avoiding the locule, starting from the top to the bottom of the fruit by the end of 24 h period. A drop of juice was squeezed from each slice to the refractometer and the amount of total soluble solids and values were expressed as degree Brix. Five non hot water treated tomatoes served as the control. Two values were recorded from one fruit.

The experiment for the determination of the physiological loss in weight (PLW) of tomato fruits, the initial weight of the fruits was taken before applying the heat treatment. The tomatoes were divided into six sets, each set comprised of twenty fruits which included ten fruit each for the treatment effect and the control. Ten tomatoes were dipped in hot water at 40 or 50 °C for 10, 20 and 30 min while ten fruits served as the non-treated control for the combination of temperature and period of time. The final weight was recorded for 1 h and 24 h storage and the loss in weight was recorded as the difference between the initial and the final weight and was expressed as a percentage.

5.3 Statistical analysis

The experiment on tomato colour was designed as a complete random design (CRD). The data were subjected to analysis of variance (ANOVA) using a GenStat statistical package. Treatment effects reported were significant according to an F test. Data for the different experiments were analysed separately. The significant differences between the treatments were detected using least significant differences (LSD) at $p \leq 0.05$ (Steal and Torrie, 1987).

5.4. Results

The result of the trial on tomato colour showed no significant difference between the control and treated tomatoes at 40°C for 10 and 30 min in 1 h but in 20 min there was a significant difference. Also after 24 h there was a significant difference between the treated fruit and the control in 10 and 20 except for 30 min (Figure 39). The result of heat treatment at 50 °C showed a significant difference between the control and tomatoes treated for 10, 20 and 30 min respectively, either in storage for 1 h or 24 h (Figure 40). The treatment of tomatoes in 40 or 50 °C water for 10, 20 and 30 min before storage at 1 h and 24 h inhibited the colour development of the skin of tomato. This confirms the report of studies that a short-term heat treatment would be beneficial for maintaining tomato fruit quality. For example, Lurie and Klein (1992) reported that 72 h hot air treatment at 38 °C inhibited ripening of mature green tomatoes; while Lurie (1998), stated that hot water dips at 43-55 °C for up to 10 min delayed yellowing of broccoli. McDonald *et al.* (1996) corroborated these findings that hot water at 42 °C and stored at 13 °C above chilling temperature inhibited tomato ripening.

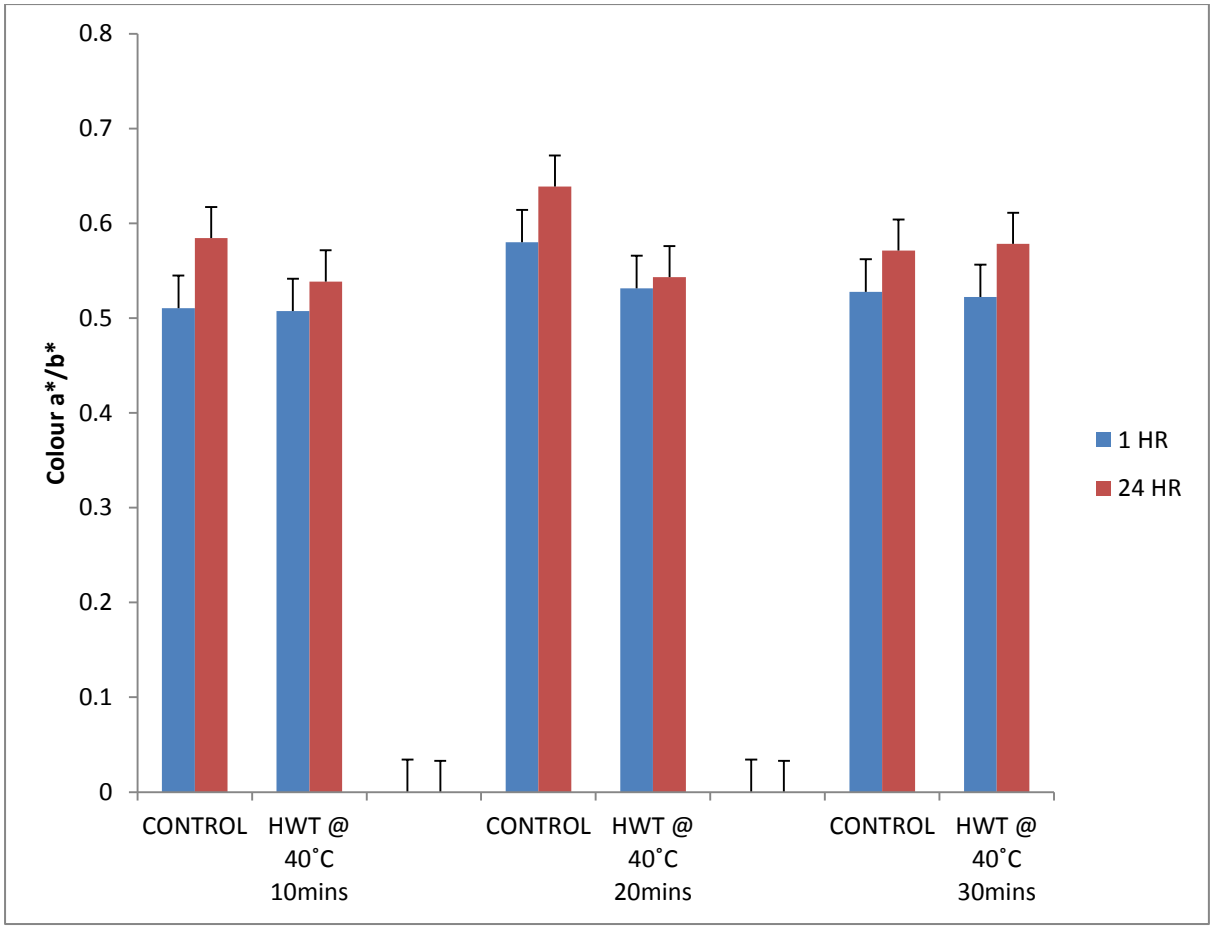

Figure 39: The effect of hot water treatment at 40 °C on the colour of tomato fruit after 24 h storage. Error bars indicated standard error of the mean of 30 fruits for each point.

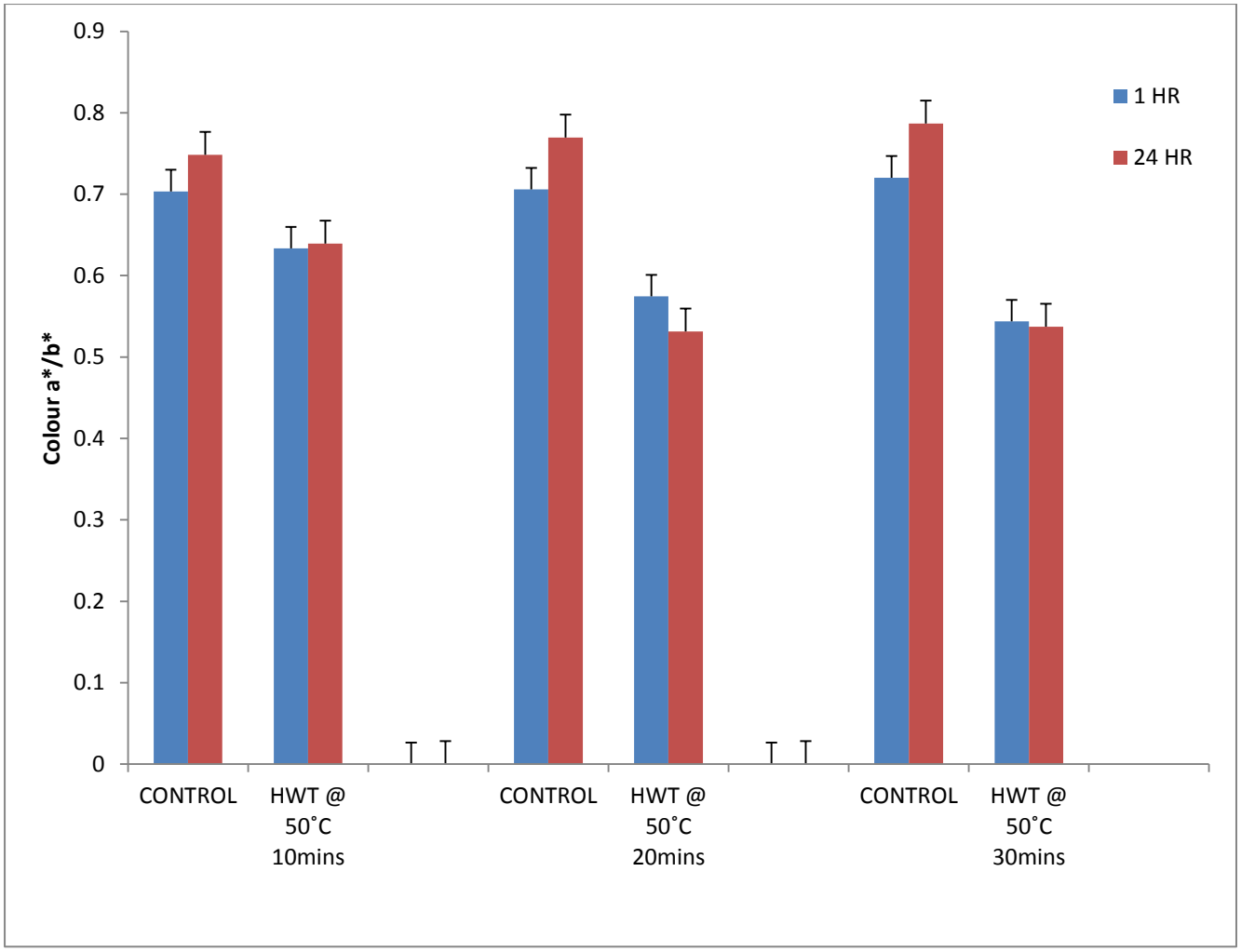

Figure 40 The effect of hot water treatment at 50 °C on the colour of tomato fruit after 24 h storage. Error bars indicated standard error of the mean of 30 fruits for each point.

The results of hot water at 50°C treatment showed a highly significant difference ($P \leq 0.05$) between the control and treated tomato for 10, 20 and 30 min respectively either in 1 h or 24 h storage period (Figure 40).

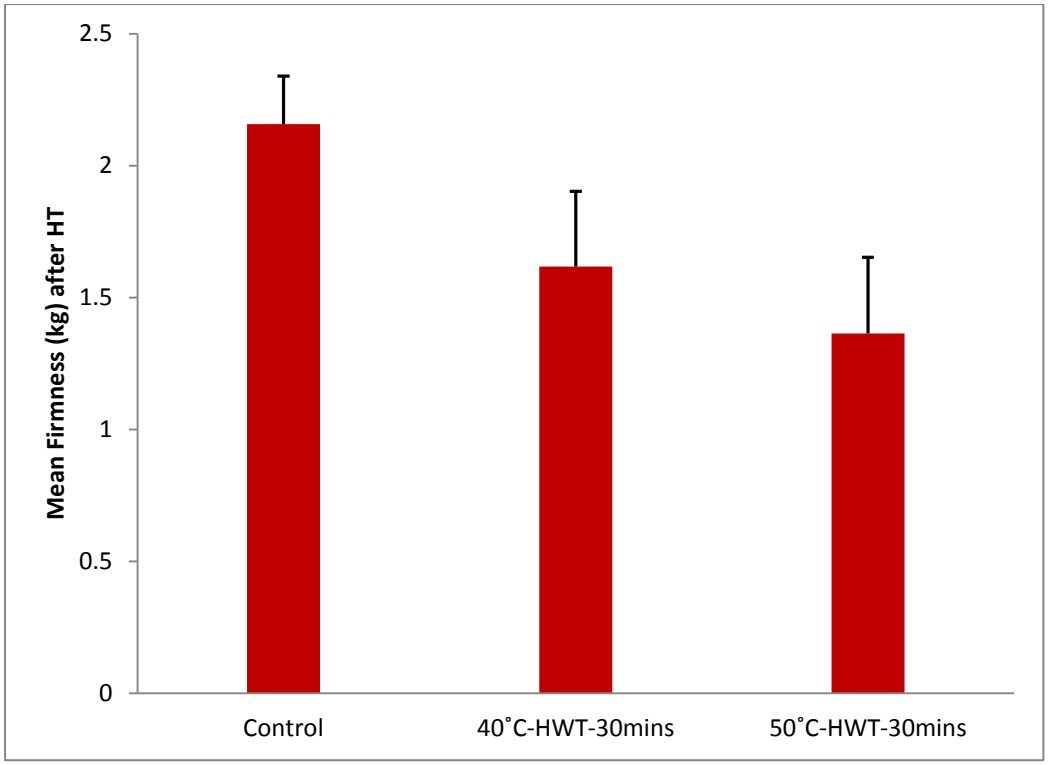

Figure 41 The effect of hot water treatment at 40 and 50 °C for 30 min on firmness of tomato after 24 h storage. Error bars indicate standard error of the mean of 30 fruits for each point.

The results of flat-faced aluminium round disc compression test revealed a significant ($P \leq 0.05$) difference in fruit firmness between hot water at 40 or 50 °C for 30 min and the control after storage for 24 h (Figure 41).

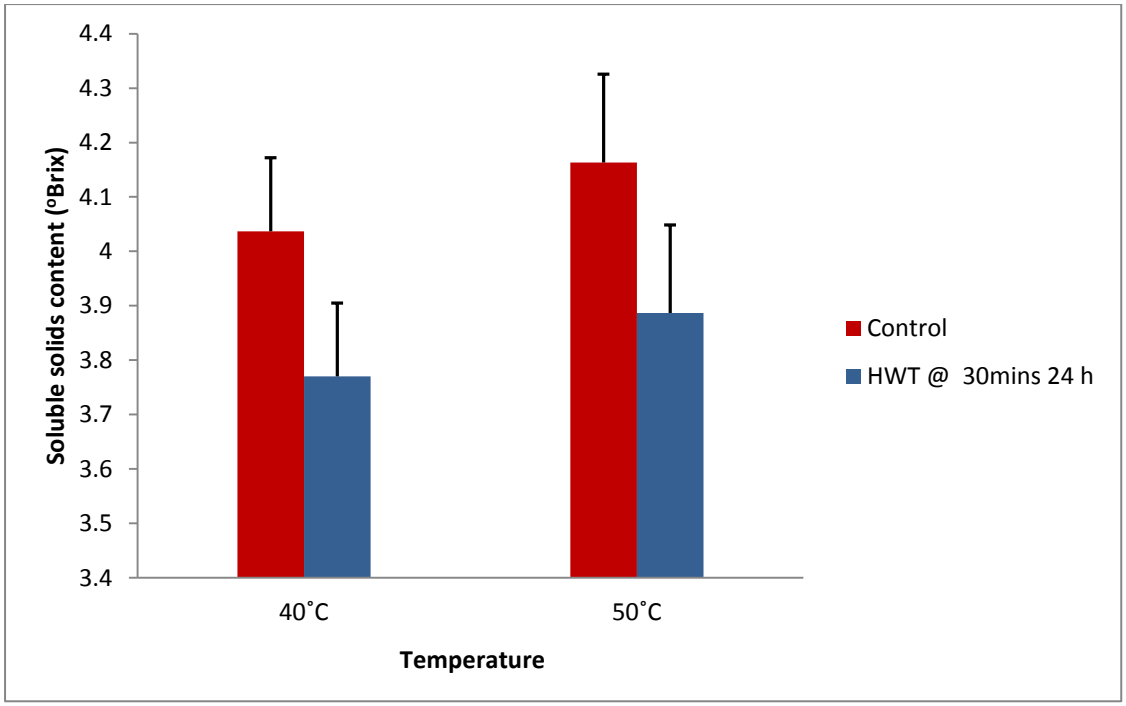

Figure 42: Degree Brix values (0-32 %) of hot water treated tomatoes after 24 h storage. Bars indicated standard error of each point. Error bars indicate standard error of the mean of 30 fruits of each point.

The result showed that the soluble solids concentration in hot water treated tomatoes at 40 or 50 °C showed a significant difference ($P < 0.05$) compared with the control non-treated fruit (Figure 42). However, there was no significant difference in soluble solids concentration of the fruit between the tested temperatures. The data on physiological loss in weight (PLW) as influenced by hot water treatment and the storage conditions showed a significant difference between the hot water temperature treatments and storage conditions at all time period tested (Figure 43). The percentage weight loss progressively increased with an increase in the storage period irrespective of the treatments and the storage conditions. Consequently, the result showed that weight loss by the fruits was similar both at 40 or 50 °C by the end of 24 h period.

Furthermore, the result showed a higher percentage of weight loss in fruit treated at 50°C for 30 min than 40°C after I h storage. Whereas, more water was lost at fruit treated at 40°C for 30 min than 50°C after 24 h. After 24 h storage the percentage weight loss of tomatoes treated at 40 or 50°C for 10 or 20 min showed similar results.

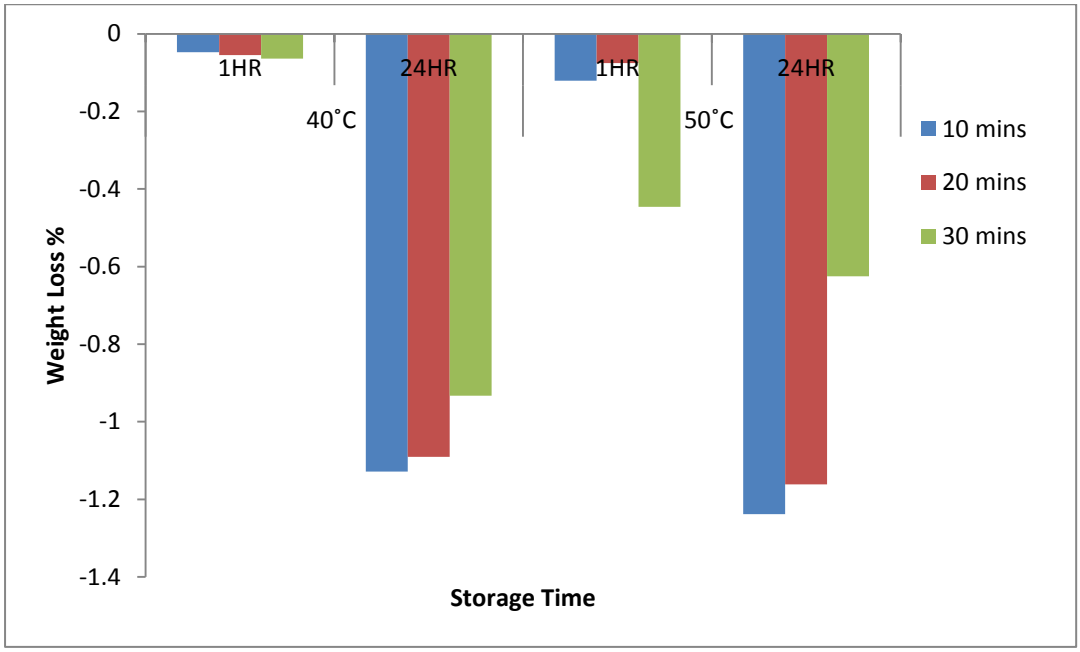

Figure 43: The effect of hot water treatment at 40 and 50 °C for 10, 20, 30 min on tomato weight. The weight loss percentage data was based on the mean of 30 fruits for each point.

5.5. Discussion

The treatment of tomatoes at 40°C for 10 min followed by 24 h storage and either at 50°C hot water for 10 min followed by 1 h storage was sufficient to control the development of skin colour (a^*/b^*) of tomato. Studies have shown that tomato fruit colour was affected by heat treatment, with increasing a^*/ b^* values associated with increasing treatment temperature (McDonald *et al.*, 1999). Whereas, McDonald and McCollum (1996) reported that there was no significant treatment effect on ripeness, lycopene, chlorophyll, percent soluble solids, percent titratable acidity, solids/acid ratio, fruit firmness or C_2H_4 evolution rate in red-ripe fruit. But the result of this trial agrees with the study of McDonald *et al.*, (1999).

The result showed that heat treatment has no effect on the skin firmness of tomato. This result agrees with the study of Mutari and Debbie (2011) which stated that the effect of temperature on fruit firmness was not significant. This might be as a result of sealing the cracks or natural openings which significantly reduce water loss, and thus maintain fruit firmness after storage. In another study by

Fallik (2004) it was reported that the reduction in fruit softening by hot water treatment was due to the inhibition of peptic hydrolysis, showing a reduced level of cell wall degrading enzyme activity and to the inhibition of ethylene production due to a reduction in the activity of the ethylene-forming enzyme.

This process occurred as a result of heat melting the wax layer so that the cracks were sealed and natural openings in the cuticle through which water could escape, thereby maintaining fruit firmness. Fallik *et al.* (1993) reported that heat treatment inhibit ripening processes during storage but did not substantially affect fruit firmness, colour, soluble solid content or acidity by the end of 7 days shelf life at 20 °C. This study agrees with Fallik (1993) and Fallik (2004) findings because there was no significant difference in the firmness of tomato at either 40 °C or 50 °C.

Klein and Lurie (1992) reported that heated tomatoes when removed from storage showed an increase in the concentrations of soluble solids than non-heated fruit; also heated apples e.g. Golden delicious were perceived as sweeter, crisper and more acceptable to the consumer than non-heated fruit. The result of Degree Brix in this study was in agreement with these reports. The increase in soluble solids in the latter report may be due more to decrease in titratable acidity rather than increase in sugar content (Lurie, 1992). Therefore, this result agreed with the reports of Hurtado *et al.* (2009) and McDonald *et al.* (1999) which stated that heated tomatoes soluble solids concentrations were not differently affected by heat treatment at high temperatures and were not differentiated from non-heated fruit by a taste panel.

Weight loss of fresh tomatoes is primarily due to transpiration and respiration; whereas transpiration is defined as a mechanism in which water is lost due to differences in vapour pressure of water in the atmosphere and the transpiring surface (Okolie and Sanni, 2012). Respiration causes a weight reduction because a carbon atom is lost from the fruit each time a carbon dioxide molecule is produced from an absorbed oxygen molecule and released into the atmosphere (Okolie and Sanni, 2012). Therefore, the heat treatment at 40 or 50^0C and storage of red tomatoes for 24 h lowered the respiration rate, caused water loss and subsequently decreased the weight of the fruit (Figure 43).

The result also showed that the heat treated tomato fruits lost weight by evaporation of water from the skin surface irrespective of time of treatment after 24 h storage. Furthermore, the result of heat treatment at 50 °C of tomato in 10, 20 and 30 min were different as compared with one another. This result agreed with the report of Okolie and Sanni, (2012).

5.6 Conclusion

In general, the respiration rate and ethylene evolution of hot water treated tomatoes were lower than that of untreated fruit during storage thereby causing less production of carbon dioxide and ethylene (Fallik, 2004). The effect of heat treatment on tomato fruit increased respiration rate and the production of ethylene, consequently facilitated tomato fruit ripening. This result has shown the relationship between hot water treatment, respiration and biosynthetic products as catalyst to formation of tomato colour. Therefore, the treatment of tomato fruit at 40°C for 10 min followed by 24 h storage, also 50°C hot water treatment for 10 min followed by 1 h storage were sufficient to control the development of the skin colour (a^*/ b^*) of tomato. Hot water treatment of tomato fruits in this study did not have any effect on its firmness. The reason might be due to inadequate time of exposure.

Hot water treatment of tomato fruit in this study did not have any effect on soluble solids (i.e. sucrose) of tomato. The reason might be due to the fact that enzymes are denatured above 40°C as a result contributed to the weakening of enzyme activity of tomato.

Hot water treatment of tomato fruits at 40 and 50 °C caused a loss of water from the fruit beginning from 1 h storage and increased in 24 h period. As the time of storage increases, the weight loss of tomato fruit increases. The hot water treatment of tomato fruits showed no appreciable difference between 40 and 50°C on weight loss of tomato. Therefore, the heat treatment at 40 or 50 °C and storage of red tomatoes for 24 h increased respiration, caused water loss and subsequently decreased the weight of the fruit at the temperature tested. A conclusion can be drawn stating that heat treatment above 40°C of tomato fruit could stay in storage for 1 h without losing much weight.

CHAPTER 6: THE EFFECT OF HOT WATER TREATMENT ON THE AROMA, TEXTURE, JUICINESS, FLAVOUR AND OVERALL ACCEPTABILITY OF TOMATO

6.1 Introduction

Many research studies have reported increased consumer dissatisfaction with fresh tomato (*Lycopersicon esculentum* Mill.) flavour (Maul, *et al*., 2000). This might be due to many reasons including aroma, texture, juiciness and flavour compounds that contribute to the perceived taste of tomato. Some of the reports have suggested ways of improving the inferior flavour quality in fresh tomatoes; some of which include breeding programs on disease resistance, productivity, and fruit firmness in selections at the expense of aroma, taste and overall intensity (Maul, *et al.* 2000). Although reports of the effect of postharvest heat treatment to control fungal pathogens that caused diseases have been exhausted but little is reported on the effect of heat on the aroma, flavour and other quality characteristic of tomato.

Although various biochemical and physiological alterations have been associated with heat treatments (McDonald and McCollum, 1996), which consequently led to an improved quality product, but their effects on aroma and flavour perception have not been properly understood. Studies have shown that postharvest heat treatment controlled the chilling injury of tomato as well as the disease pathogens caused (Fallik *et al*., 1993; Lurie, 1998; McDonald *et al*., 1999) without affecting the sensory attributes such as flavour volatiles and aroma (Maul *et al*., 2000; McDonald *et al*., 1999; Boukobza and Taylor, 2002). Reports of studies have shown that in some commodities sugar content is favourably affected by heat treatment. For example muskmelon dipped in hot water at 45 °C before cool storage prevented the loss in sucrose which occurred in non-heated fruit during storage. The heat treated squash was perceived as sweeter by a taste panel (Lurie, 1998). Likewise, in tomato the highest volatiles levels in ripe fruit were from fruit heated from mature green stage and then stored at 13 °C before ripening at 20 °C (McDonald *et al.*, 1996).

The process of tomato fruit ripening involves many changes both quantitative and qualitative in flavour, aroma volatile compounds, organic compounds, soluble sugars, amino acids that contribute to

characteristic tomato quality (Maul *et al.*, 2000). Because of the diversity of biosynthetic pathways contributing to the formation of volatile compounds as a result of heat treatment the aroma, taste (texture, juiciness, flavour) and overall intensity were deployed as a descriptive quality characteristic of tomato in the perception of a taste panel. This research work is therefore aimed to determine the effect of postharvest hot water treatment on the quality of tomatoes stored at 29-30 °C; 80-90 % relative humidity for 1 h and 24 h.

6.2 Materials and Methods

Mixture of turning and pink tomatoes (unidentified cultivar) was purchased from a farm shop in Writtle, Chelmsford. The fruit were sorted to eliminate defects and establish uniformity of size and colour, and divided into 3 groups of 10 for the taste test. A panel of 10 untrained people (male and female) was formed and the age range is from 18 to 60 years. Ten fruit were treated in hot water at 40 or 50 °C for 30 min, while another ten tomatoes were non-treated and then stored at 29-30 °C for 24 h. The red-ripe fruits were rated by the panelists for the following quality characteristics: aroma, texture, juiciness, flavour and overall acceptability. The experiment was repeated thrice.

6.2.1. Statistical Analysis

The descriptive panelists' scores for aroma, texture, juiciness, flavour and overall acceptability were analysed as complete block design with panelists as blocks and temperatures as treatments. The Kruskal-Wallis post-hoc a non-parametric test ($P \leq 0.05$) was used for the mean separation with Mann-Whitney pair-wise which gave an uncorrected value of the treatment means. These values of the treatments were compared with Kruskal-Wallis P-value figure for test of significance. Furthermore, the data were put into Bonferroni test to convert to corrected values and were compared with the Mann-Whitney values obtained from the data. These attributes were rated from good to poor where good is scored 5 and poor 1.

6.3. Results

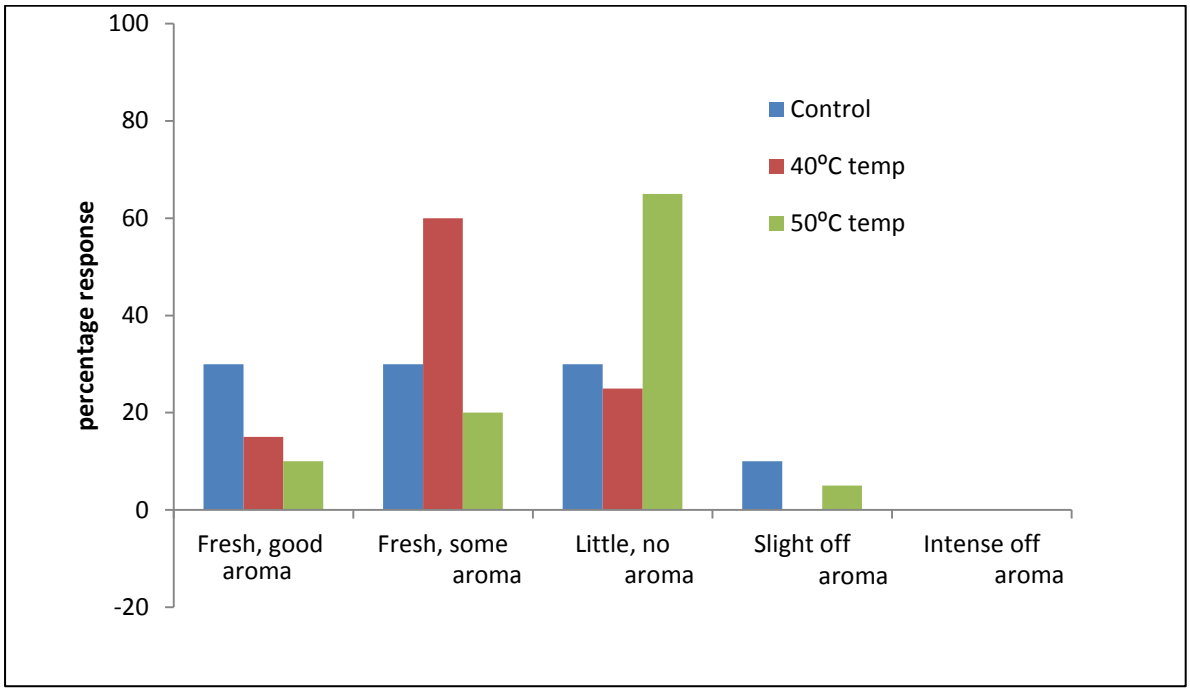

Figure 44: Opinion of taste panel members (n=30) of the aroma of heat treated and untreated tomatoes.

Table 11: Aroma data (n=30)

Uncorrected Mann-Whitney Pairwise		
	40°C	50°C
Control	0.00313	0.03852
40°C		0.8197
Bonferroni Corrected P-value		
	40°C	50°C
Control	0.00939	0.1156 (NS)
40°C		1 (NS)

p =0.0142

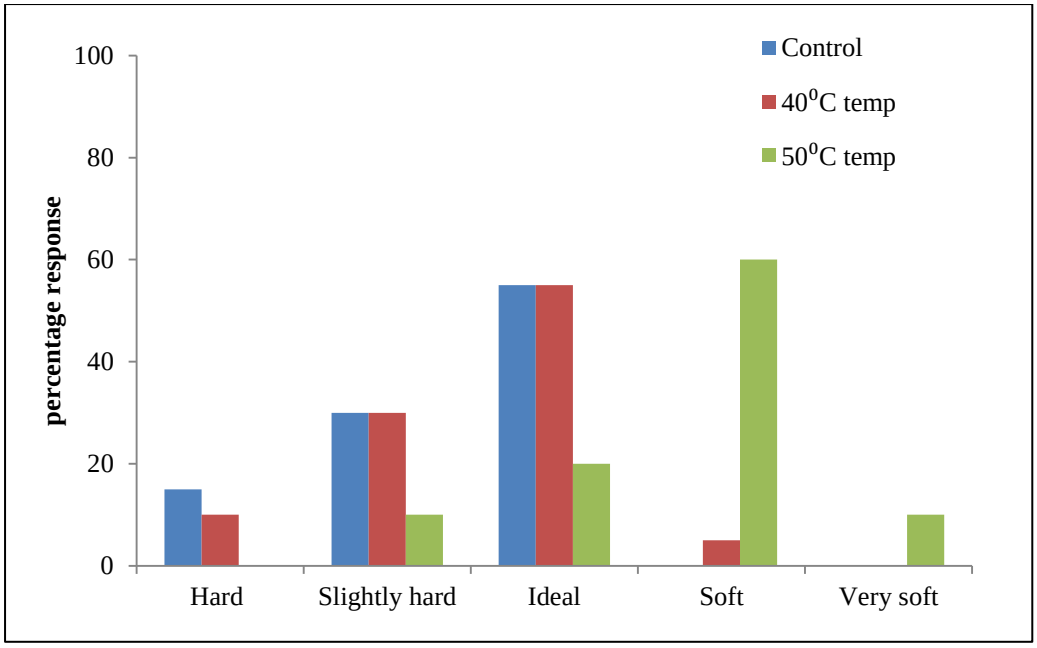

Figure 45: Opinion of taste panel members (n=30) of the texture of heat treated and untreated tomatoes.

Table 12: Texture data (n=30)

Uncorrected Mann-Whitney Pairwise		
	40°C	50°C
Control	0.000399	0.004498
40°C		0.9869
Bonferroni Corrected P-value		
	40°C	50°C
Control	0.001197	0.001349
40°C		1
p =0.0001999		

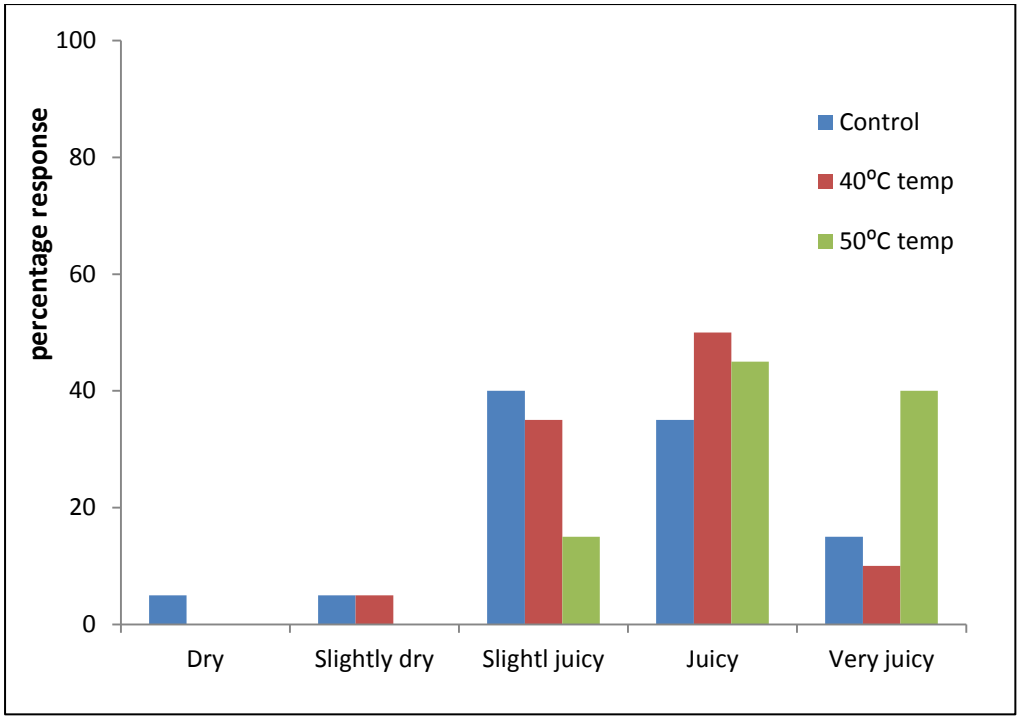

Figure 46: Opinion of taste panel members (n=30) of the juiciness of heat treated and untreated tomatoes.

Table 13: Juiciness data (n=30)

Uncorrected Mann-Whitney Pairwise		
	40°C	50°C
Control	0.0048	0.0039
40°C		0.7124 (NS)
Bonferroni Corrected P-value		
	40°C	50°C
Control	0.0147	0.0116
40°C		1
p =0.004061		

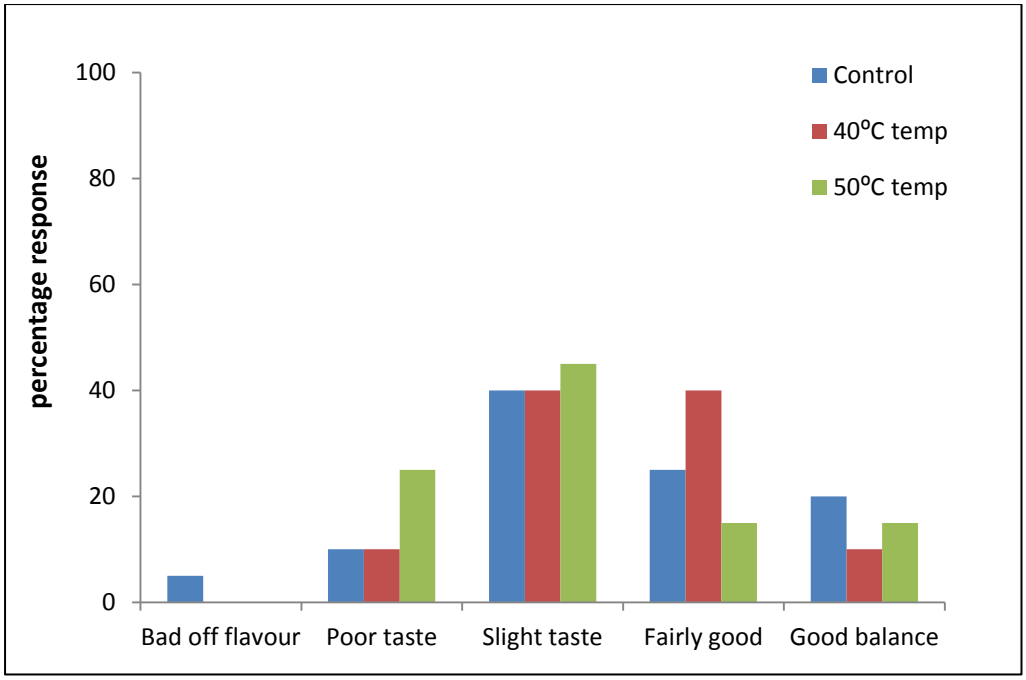

Figure 47: Opinion of taste panel members (n=30) of the flavour of heat treated and untreated tomatoes.

Table 14: Flavour data (n=30)

Uncorrected Mann-Whitney Pairwise		
	40°C	50°C
Control	NS	NS
40°C		NS
Bonferroni Corrected P-value		
	40°C	50°C
Control	NS	NS
40°C		NS
p =0.1003		

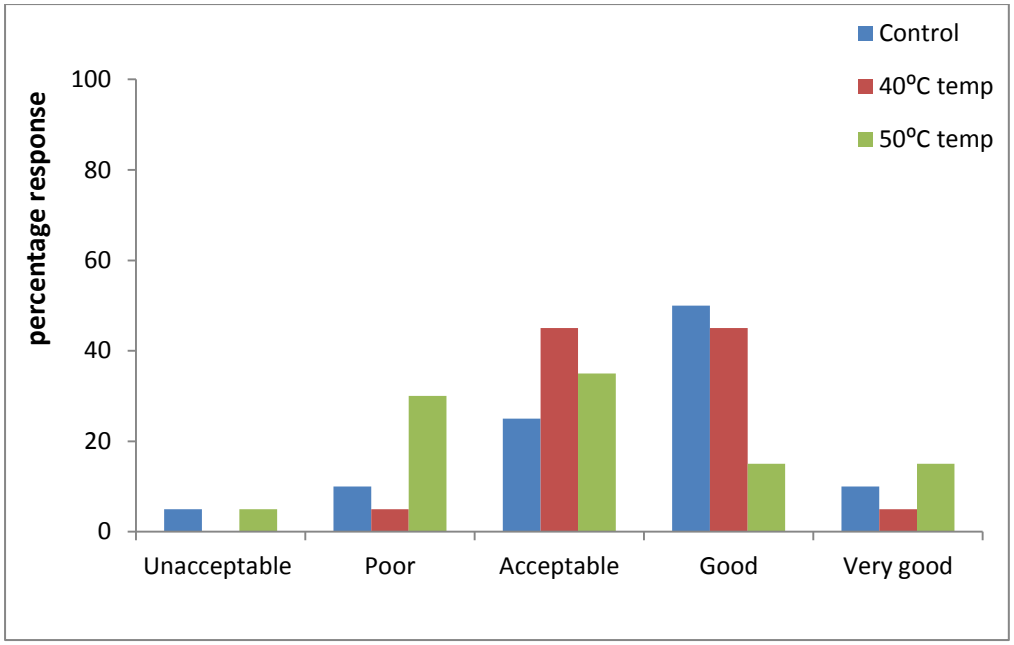

Figure 48: Opinion of taste panel members (n=30) of the overall acceptability of heat treated and untreated tomatoes.

Table 15: Overall acceptability data (n=30)

Uncorrected Mann-Whitney Pairwise		
	40°C	50°C
Control	0.00496	0.00566
40°C		0.5642
Bonferroni Corrected P-value		
	40°	50°
Control	0.01457	0.01697
40°C		1
p =0.00446		

The results showed that 50°C reduced the aroma while 40°C hot water treated fruits were rated fresh and some aroma based on the percentage response of the panelist (Figure 44). For example, the panelists rated about 60% of the heat treated fruit at 50°C to have little or no aroma while about the same percentage rated 40°C heat treated fruit to have fresh and some aroma. The graph showed that the aroma of the treatments decreased from high to low as the temperature increased, there is no treatments in intense off aroma in the figure presented. The untreated fruit maintained its freshness and good aroma quality than the treated fruits (Figure 44). The statistical analysis of the response data of aroma showed a significant difference ($P \leq 0.05$) between the control and the uncorrected Mann-Whitney pairwise values at 40°C and 50°C but there was no significant difference between the treatments. The result showed no significant difference in aroma between 40 and 50°C treated tomatoes. The Bonferroni corrected P-value showed a significant difference between the control and 40^{o}C but no significant difference between 50°C, 40°C and control (Table 11).

The result of the response of panelists showed that the fruit skin became softer when heated at temperature of 50°C compared with 40°C. For example, about 60% of the people rated the tomatoes heat treated at 50°C to be softer while the fruits heated at 40°C had 2% percentage response (Figure 45). The statistical analysis of the scores obtained for texture showed a significant difference ($P \leq 0.05$) between the control, 40 and 50°C for both the uncorrected values (Mann-Whitney pairwise) and the Bonferroni corrected P-value. However, there was no significant difference between 40 and 50°C for both statistical analysis (Table 12).

The percentage response of panelists in this trial showed that heated tomatoes at 40 or 50°C contained more juice than the control. For example, about 50% of the untrained panel members rated tomatoes treated at 40°C to be juicy while 45% of the people reported the fruit treated at 50°C as juicy. Also the heat treated fruit at 50^{o}C was rated as very juicy by about 40% of the people (Figure 46).The statistical analysis showed a significant difference between 40 and 50°C compared with control but no significant difference between 40 and 50^{o}C in the uncorrected Mann-Whitney Pairwise values. The Bonferroni corrected p-value test showed a significant difference ($P \leq 0.05$) between the control, and 40 and 50°C but no difference between 40 and 50^{0}C (Table 13).

The panel members' assessment showed that the treated fruit at 40°C had a fairly good taste compared with 50°C treated tomatoes. For instance the flavour increased from bad off flavour to slight taste for the treatments. A good balance of sugar: acid ratio was reported by the panelists for the two tested temperature regimes. At 50°C the amount of poor taste was about two times more than 40°C treatment. In addition 40°C and the control were given equal rating for poor and slight taste attributes (Figure 47). The statistical analysis of the flavour attributes scores showed no significant difference between 40, 50°C and control of the uncorrected Mann-Whitney pairwise and Bonferroni corrected P-values (Table 14).

On the overall acceptability of the product of this trial, the heat treated tomato at 40 °C was accepted and considered well with the control than the 50°C treated fruit. The sensory panelists also noted that tomato heated at 50 °C was rated poor by about 30% of the panellist than 40 °C heated fruit (Figure 48). There was a significant difference ($P \leq 0.05$) between the control and both $40^{o}C$ and $50^{0}C$ but no significant difference between $40^{0}C$ and $50^{0}C$ temperature (Table 15).

6.4 Discussion

Heat treatment was reported to have a tendency to reduce the level of some flavour volatiles in tomato (McDonald *et al.*, 1996), while some of these volatiles contribute to tomato flavour based on aroma threshold studies. For instance, the fifteen volatiles identified in the study showed decreased levels in fruit exposed to air heat treatment while other volatiles showed decreased level in fruit exposed to water heat treatment e. g. 1-Nitro-2-phenylethane levels were significantly lower in the water heated fruit. Therefore, the effect of heat treatment on fruit flavour depends on the type of heat applied and also may depend on the type and cultivar of the fruit. Flavour characteristics of fruits can be affected by a heat treatment (Lurie, 1998).

Maul *et al*., (2000) studies showed a relationship between flavour volatiles and perceived sweetness or sourness of fruit stored at different temperature. The concentration of some of the volatile compounds correlated negatively with sensory sweetness ratings, while some others are positively correlated with fruit sourness (Maul *et al*., 2000). In another report some fruit sugar content are favourably affected by heat treatment. For example, muskmelons dipped in 45 °C hot water before cool storage prevented the loss of sucrose content. Heated tomatoes were not distinguished from non-heated fruit by a taste panel (Lurie *et al.*, 1998). Golden delicious apple heated in air at 38 °C for 4 days were perceived as sweeter, crisper and more acceptable to the consumer than non-heated fruit (Lurie *et al.*, 1998). In this instance, the sweetness was due to decrease in acidity rather than increase in sugar content the result of which cumulated to a good balance of sugar: acid ratio. In this study, a good balance of sugar: acid ratio was reported at 40 and 50 °C; which made this trial to be in agreement with earlier research studies. There were no significant differences in aroma at 40^{o}C and the flavour at 40 and 50^{o}C compared with the control likewise in the other perceived quality parameters in informal taste tests. Therefore, a suggestion could be made to form a descriptive sensory panel for proper sensory responses in recognition of aroma, flavour volatiles and sucrose solutions of varying concentrations.

6.5 Conclusion

Postharvest heat treatment that controls fungal pathogen infection of tomato can result in altered flavour volatile profiles compared with naturally non treated fruit. Heat treated tomatoes ripened normally, and the changes in flavour volatiles, aroma and other quality characteristic were not detrimental to overall quality as far as could be determined by the informal taste panellists.

CHAPTER 7: GENERAL CONCLUSION

The result of the in vitro trial of this study indicated that 48 h after inoculation of agar plate with hot water at 45 °C treated spore suspension of *A. alternata* showed a reduction in germination rate compared with the control as detailed below. Thus, in 5 min the percentage reduction was 52 % while in 10 min it decreased to 48 % of its control. This represents a statistically significant difference between heat treatment and the control. This result agrees with Lurie *et al*., (1998) findings. At 50 °C hot water treatment, the germination percentage of treated spores was significantly reduced to about 1 % of control in 5 min whereas; in 10 min it was about 10 % compared with the control. However, the report of study on in vitro heat treatment of *Botrytis cinerea* and *Alternaria alternata* showed that percentage spore germination and germ tube elongation of these fungi were inversely proportional to the length of exposure to 45, 50 and 55 °C (Fallik *et al*., 1996). But in this study the reverse condition occurred. This condition might be as a result of heat treatment at 50 °C in 10 min killed some of the spores contained in the suspension; hence they are regarded as being non-germinated.

Many studies have reported that the spore germination of *Alternaria* species becomes noticeable after 48-72 h of inoculation on agar media (Shahin and Shepard, 1979). Therefore, in this trial the observation of heat treatment effect on germination of spores was extended beyond forty-eight hours. Also the effect of heat treatment of inoculated tomato becomes noticeable after 72 h period. Therefore, 72 h after inoculation of treated spores on agar at 45 °C for 5 min, the germination percent was 6 % while in 10 min the germination rate increased to 16 %. This showed a significant difference at this tested temp. It also agrees with the result of Fallik *et al.* (1996) which stated that percentage spore germination and germ tube elongation of *Botrytis cinerea* and *A. Alternaria* were inversely proportional to the length of exposure at 45, 50 and 55 °C. However, 72 h after inoculation of treated spore at 50 °C in 5 min and 10 min on CMA agar plate, the result showed no significant difference in germination rate between the times of exposure. In 96 h after inoculation of treated spores at 45 °C for 5 min, 13 % germination occurred and then increased to 25 % in 10 min treatment period. After 96 h hot water treatment at 50 °C in 5 and 10 min of spores on agar plate, the percent germination was

significantly reduced to nearly zero. The implication of this is that the spores might have died-off during incubation for four days.

The work on a hot water treatment of tomato has shown that disease loading in 24 h after inoculation can be reduced at 40 °C for 10 and 20 min by 40 % and 10 % respectively. At 45 °C for 10 min it was reduced by 30 %, while at 50 °C for 5, 10 and 20 min the percentage reduction was 40, 20 and 10 % respectively. The result of the heat treated fruit at 55^0C for 5 min showed a reduction in the disease emergence by 20 % after 24 h inoculation. After 24 h the mycelia elongation of inoculated tomato was reduced by hot water treatment at 30, 40 and 50 °C for 10 min by 38 %, 46 % and 0 % respectively. Similarly, dipping *Alternaria* infected fruit at 50 °C for 5 min reduced the decay compared to the control fruit. It was observed that heat injury occurred on the surface of the fruit dipped for 5 min. This is in agreement with the study of Lurie *et al.,* (1998) which stated that tomato should not be put in hot water for too long.

The hot water treatment of the tomatoes had the following effects on the attributes of quality: the Brix degrees measurement showed a negligible difference in 40 °C or 50 °C and the control for 30 min heat treatment. This is in agreement with the studies of McDonald *et al.,* (1999) and Hurtado *et al*., (2009), who found no effect on soluble solids after the application of heat on tomato fruit. Furthermore, Fallik *et al*., (1993) reported that heat delayed rise in total soluble solid (TSS) percentage in both mature green and pink tomato, but at the end of storage both colour grade of tomato contained the same level of TSS regardless of heat treatment.

The external appearance of tomato is designated by the colour which constitutes qualitative characteristics that appeal to the consumers. The colour change of tomato fruit is measured by a^*/b^*. In this trial, the change in colour after heat treatment was not statistically significant. This agrees with Whitaker (1994) work where it was suggested to be because of the chlorophyll content of outer pericarp tissue declined slightly in control fruit whereas, there was no change in heat treated tomato.

With the firmness measurement on the flesh of heated tomato no statistically significant difference was found. This is in agreement with Fallik *et al.,* (1993), work which stated that although, heated

mature green tomato were consistently softer during shelf life than non-heated mature fruit. But no such differences were observed between heated and non-heated pink fruit.

Similarly, in the taste test there appears no real difference recorded in the attributes of juiciness, flavour and overall acceptance except that the skin of the tomato was recorded "softer" by some of the taste panel. This does not conflict with the findings on firmness, referred to previously because that was a consideration of the internal texture or flesh.

The results lead to the conclusion that hot water treatment has potentially an important place for low input tomato farmers in countries such as Nigeria. Other factors that would also be favourable are the lack of availability of appropriate agrochemicals, the cost of these chemicals and the almost zero capital cost of using hot water.

Traditionally, postharvest diseases were treated with chemical compounds to control or reduce the microorganism causing the disease. At the moment the application of chemical compound to food is facing a serious threat because of the potential residues left after its application as a postharvest treatment. Also fungi pathogens have developed resistance to some of these chemical compounds. Likewise, the renewal and registration of new chemical compound take a longer time and the effect of them on the environment are some of the issues to contend with in countries where chemical compounds are applied as postharvest treatment for fruit and vegetable diseases. As a result of these difficulties the new paradigm is likely to be towards the use of non-chemical means to control or reduce the incidence of pre or postharvest disease pathogen in fruits and vegetables.

These problems are some of the prevailing situation in developing countries such as Nigeria and they pose a serious concern to our farmers. Also the chemical compounds which are used to treat postharvest diseases are not readily available and the cost of procurement is beyond the reach of small holder farmers. Safety of these chemicals is also a major concern as a result of their application procedure. Stealing of chemicals by farm workers is another problem whereby large amount is stolen and the remaining quantity is diluted thereby reducing the active ingredient and the effectiveness of the postharvest treatment by the chemical compounds. Invariably, other methods of postharvest

disease control are sought which should be simple to apply, readily available and equally inexpensive. Therefore, postharvest hot water treatment of tomato fruit was applied to reduce *Alternaria alternata*, the fungus that cause the black rot disease of tomatoes.

These results have shown that hot water treatment at 50-55 °C was effective in controlling *Alternaria alternata* the disease pathogen of black rot of tomato fruit. Therefore, the use of this non-chemical approach to postharvest disease control offer a promising, safe and effective alternative to fungicides in treatment of postharvest fungal diseases of tomato fruits in Nigeria.

In this study, heat affected the black rot disease pathogen itself, as shown in the result of *in-vitro* inhibition of both spore germination and mycelia growth, it was discovered that spore germination was more susceptible to increasing lengths of exposure to 50 °C. The *in-vivo* experiment of inoculated fruit before hot water treatment at 50 °C for 30 min showed no spore germination (48 h) and mycelia elongation on the inoculated fruits in 96 h.

Again the treatment time period of hot water at 50 °C of the inoculated fruit was reduced to 5 min, the outcome indicated that complete inhibition of *Alternaria* spore germination was not observed. The reduction in fungal growth or decay incidence was attributed to the effect of heat on the spore germination as well as mycelia growth resulting in the reduction of the growth of the fungus on the inoculated fruits. Another experiment was conducted to demonstrate its use by small holder farmers thus; tomato fruits weighing about 5kg were put in hot water (71 litres) at temperature of approximately 55±1 °C for 5 min the results showed that hot water dipping of red tomato at 55 °C for 5 min provided an effective control of germination of *Alternaria alternata* conidiophores.

This study has shown that hot water has reduced spore germination and germ tube elongation in-vitro, as well as inhibited or reduced rotting of artificially inoculated fruit. During the simulation experiment heat damage was observed on fruit dipped for 5 min at 55 °C, for example, the inoculated point on the surface of the fruit became wider when the fruit was treated with hot water. This development might be the effect of heat on the pericarp of tomato fruit. The effect of mechanical damage on the fruit skin and cell wall metabolism consequent to hot water treatment can be studied for future research. However, because this study was carried out under laboratory

conditions therefore it is essential that the same study is carried out on a large scale for future study. For an example, 30 kg which is assumed to be the average weight of tomato in a raffia basket used for packaging to the market. The fruits will be inoculated with *Alternaria* and dipped in hot water at 55 °C for 5 min. As a result heat exchange will be evenly distributed on a larger surface area.

In other to actualise this temperature range 50-55 °C, in a rural farm community, candle wax can be dipped in boiling water, the moment it melts gives the approximate required temperature range. Paraffin wax is the constituent of a candle. The melting point of wax is 58 °C. It is a chemical compound that is malleable at ambient temperatures. Also it is insoluble in water and it usually melts at 55-58 °C, giving a low viscosity liquid (Anonymous, 2013). Therefore, it gives an approximate temperature applicable for hot water treatment to be used to control Alternaria alternata disease pathogen. It is inexpensive and readily available. The hot water method developed does not require any specific resources that the small farmer would not have at present and will provide the farmer with a longer storage life for his family or a stronger position with the merchant as he does not have to sell his crop that instant to a merchant or even on a village stall.

Suggestions of further work

- Studies are required to render this method technically and economically for efficient use on agronomic scale with small farmers. Consequently, there will be a need to look at a larger sample of tomatoes on a commercial scale. For an example, 30 kg is accepted to be the average weight of fruit in a raffia basket used for packaging tomato to the market as a result it is necessary to use this material to test the effect of this method in the village situation.
- Trials need to be carried out to evaluate at the village level how long the tomatoes can be stored for as opposed to a diseased sample.
- The importance of good handling will become more important with lower disease levels and so there is prospect to investigate the potential benefit of appropriate smoother packaging than the traditional raffia basket (Bishop and Ramma, 2012).
- Study efforts should be made to make use of three to four strains of *Alternaria* species so as to overcome the difficulties encountered in sporulation in this study.

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List of Publications

- The use of hot water treatment for the control of *Alternaria alternata*, the cause of black mould disease of tomato (In print with *Acta* Horticulturae – International Society for Horticultural Science publication).

Appendices

Appendix 1

Plate 1: Marketing and sales of tomatoes in Nigerian cities

Appendix 2

The top twenty tomato producing countries of the world (GEOHIVE, 2013)

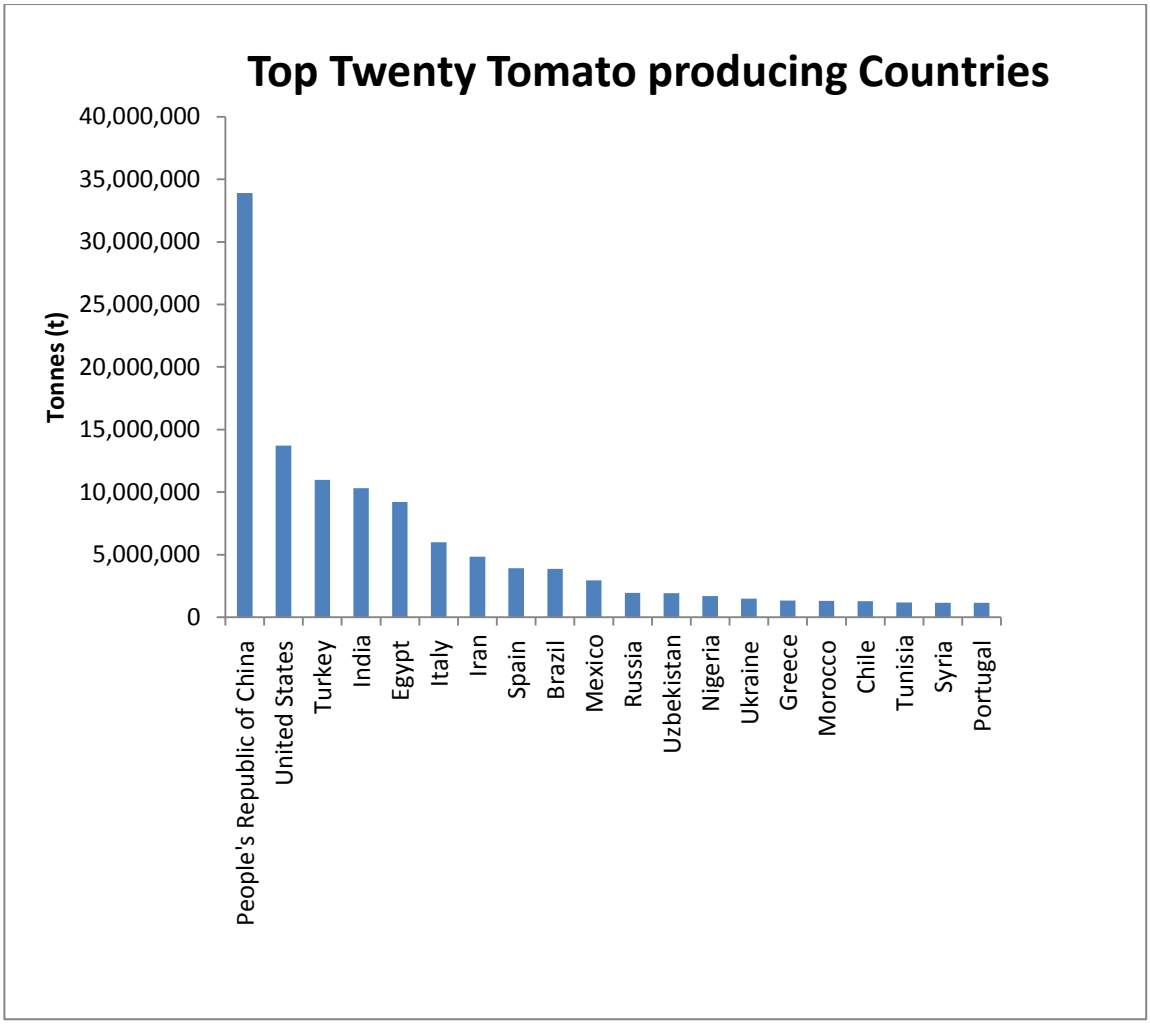

Plate 2: Effects of hot water treatment at 50^{0}C for 30 and 60 min on the germination of *Alternaria alternata* spore suspension using test tubes and petri-dish plates.

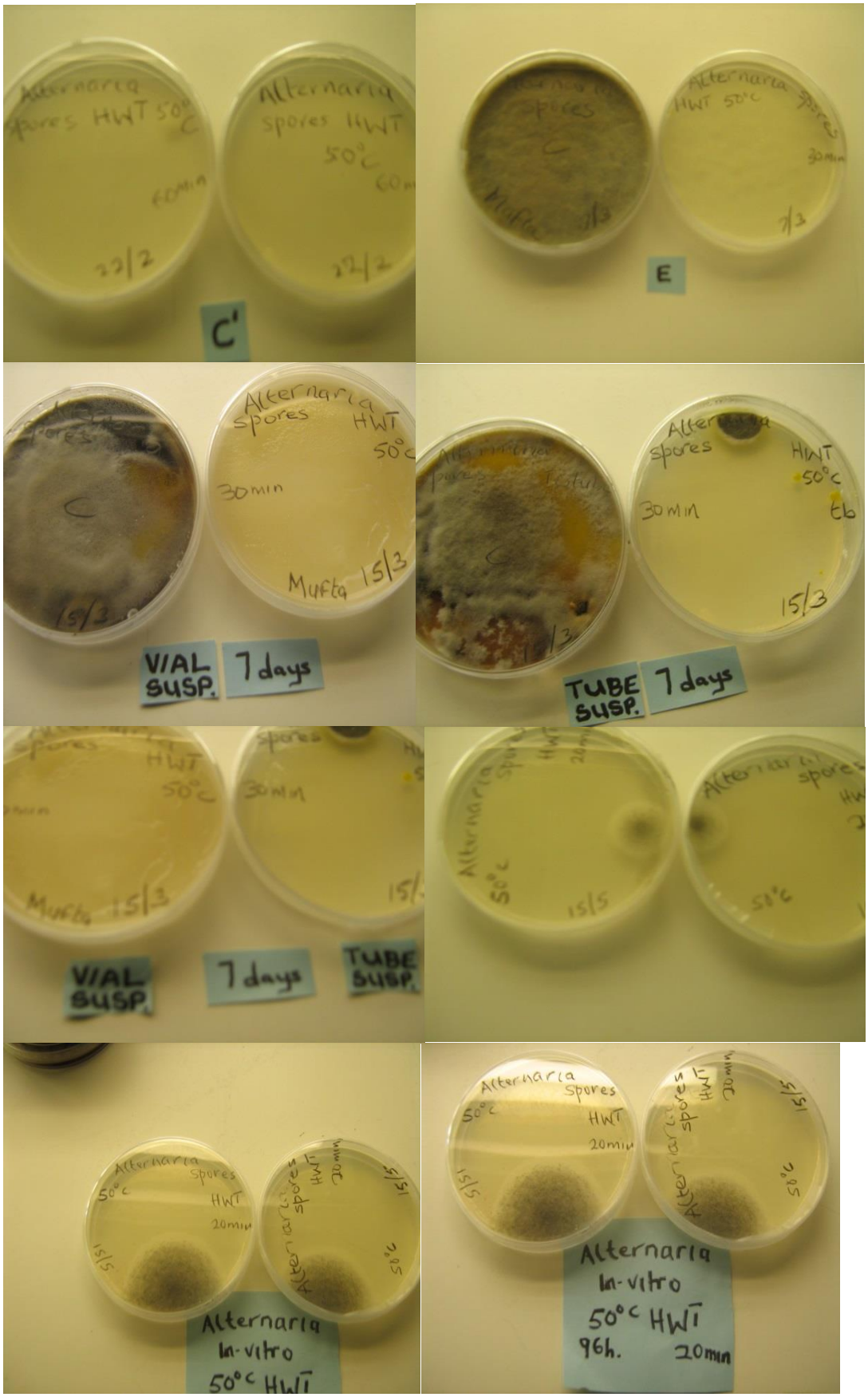

Plate 3: Effect of hot water treatment at 50^{0}C for 20, 30 and 60 min on the germination of *Alternaria alternata* spore suspension using Eppendorf vial, tube suspension and incubated on petri-dish plates.

Appendix 3

Plate 4: Laminar flow cabinet for creating aseptic environment during spore suspension preparation and inoculation.

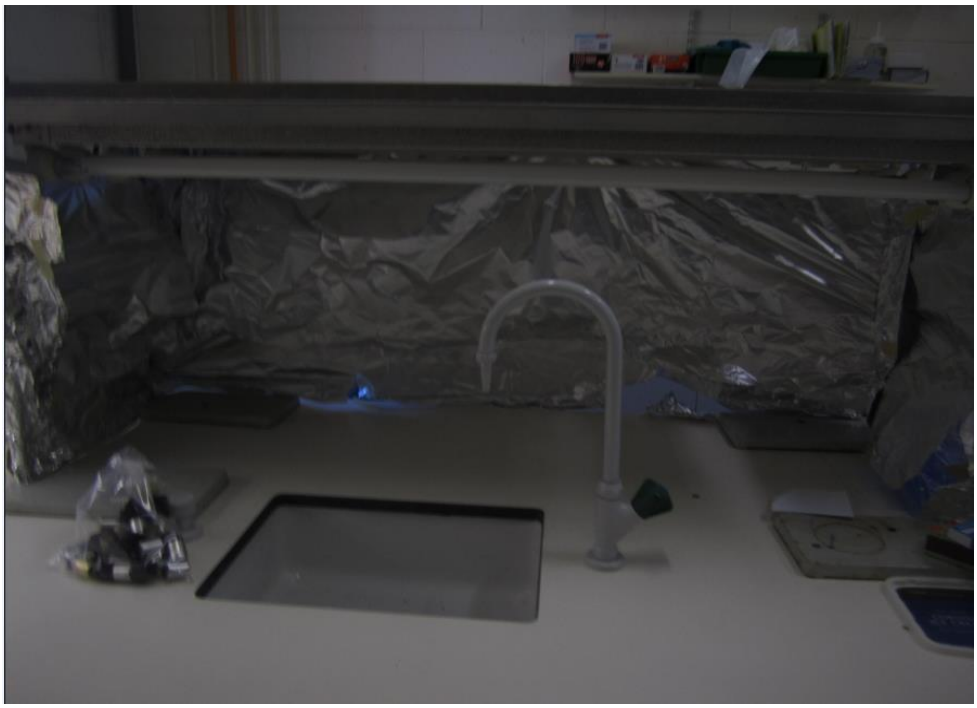

Plate 5: Section of the laboratory used for the incubation of treated specimens under fluorescent light tubes.

Plate 6: JB Grant water bath used for hot water treatment in the laboratory.

Plate 7: Plastic tank used for hot water treatment at 55^0C for 5 min of inoculated tomato fruit in a basket showing the splitting of tomato fruits due to vapour pressure (About 30% split).

Appendix 4

Plate 8 Strain of rotted tomato-cultivar ‘Delycassi’ from Writtle Research Glasshouse

Plate 9 Effect of hot water treatment at 50 °C for 30 min on inoculated tomato fruit

The result of inoculated fruit before hot water treatment at 50C for 30 min showed the germination of spores and mycelia elongation of *Alternaria* significantly inhibited after 48 h (Plate 9 A).

Heat Exchange between Hot Water and Tomato Fruit The heat exchange between the hot water and tomato fruit was calculated to show the efficiency of water heat transfer.

Tomato variety = Cossack

Room temp = 29ºC

Fruit core temp = 23.1ºC

Hot water temp = 54.9ºC

Diameter of plastic tank = 0.75m

Height of water = 0.16m

Thermal capacity tomato = 3.8 KJ/Kg/^{0}C

Thermal capacity water = 4.2KJ/Kg/^{0}C

0.75 diameter = $h \times \pi (d/2)^2$

$= 0.16 \times 3.142 \times (0.75/2)^2 m^3$

$=0.070695 m^3 = 70.7$ litre

1 litre H_20 = 1Kg

Hot water temp @ the end of experiment = 52^{0}C

Room temp = 29^{0}C

Weight of tomatoes = 45kg

Starting temperature = 23.1^0C

End temperature = 29.0^0C

Heat absorbed by tomato: Temp difference x mass x specific heat

$(29\text{-}23.1^0\text{C}) \times 4.5\text{kg} \times 3.8 = 100.89\text{KJ}$

Heat lost by water = $54.9 - 52.0 = 2.9^0$C x moist climate

Water (heat lost) $54.9 - 52.0 = 2.9^0$C

$2.9 \times 70.7 \times 4.2 = 861.126$ KJ

Efficiency in heat transfer $100.89 \times 100 = 11.716 \approx 12\%$

Therefore the water tank can be insulated to conserve heat. A lid or any other device can also serve the same purpose to prevent heat loss to the atmosphere.