CHARACTERIZATION AND MANAGEMENT OF PATHOGENIC FUNGI CONTRIBUTING TO BULB ONION (*Allium cepa* L.) POSTHARVEST ROT IN KENYA

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Characterization and Management of Pathogenic Fungi Contributing to Bulb Onion (*Allium cepa* L.) Postharvest Rot in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this PhD thesis to all actors in the bulb onion value chain internationally. In addition, I dedicate the output of this study to bulb onion stakeholders in Kenya who will benefit from the knowledge and information generated in this thesis to enhance their livelihood and income.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

AlCl	Aluminum Chloride
ANOVA	Analysis of Variance
ANTBC	Average Neck Thickness before Curing
ANTAC	Average Neck Thickness after Curing
AWBC	Average Weight before Curing
AWAC	Average Weight after Curing
BLAST	Basic Local Alignment Search Tool
CLA	Carnation Leaf-Piece Agar
CPE	Cumulative Pan Evaporation
CRD	Completely Randomized Design
DNA	Deoxyribonucleic Acid
DPPH	2, 2-Diphenyl-1-Picryl Hydrazyl
EDTA	Ethylenediamine Tetra-Acetic Acid
FAO	Food and Agriculture Organization of United Nations
FBR	Fusarium Basal Rot
GAE	Gallic Acid Equivalents
GENSTAT	General Statistic Software

GPS	Global Positioning Satellite
HCl	Hydrochloride
HCD	Horticultural Crops Directorate
IW	Irrigation Water
KALRO	Kenya Agricultural and Livestock Research Organization
JKUAT	Jomo Kenyatta of University of Agriculture and Technology
KES	Kenya Shilling
LSD	Least Significant Difference
NCBI	National Centre for Biotechnology Information
OR	Odds Ratio
PDA	Potato Dextrose Agar
PCR	Polymerase Chain Reaction
RGR	Radial Growth Rate
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
TE	Tris-ETDA
TFC	Total Flavonoids Content
TPC	Total Phenol Content

TSS	Total Soluble Solids
USA	United States of America

ABSTRACT

Bulb onion (*Allium cepa L.*) is an excellent source of vitamin C, Vitamin B_6, potassium, magnesium, polyphenols and phytonutrients. Among the aromatic and medicinal crops grown in Kenya bulb onion production was ranked first in 2020. Despite economic importance of bulb onion production in the country, its productivity is low which is attributed to pre- and postharvest handling factors. Furthermore, Kenya's produced bulb onion have poor keeping quality which necessitates the country to import from Tanzania and other neighbouring countries to meet its demands. Currently, scanty information on postharvest handling practices, extent of losses and causes in bulb onions, limits the development of postharvest reduction strategies in Kenya. Postharvest rot in bulb onion has mainly been attributed to losses during handling. However, in Kenya information on fungi contributing to postharvest rot in bulb onions is scanty, thus restricting the development of suitable handling techniques to manage the rot. Curing is one of the recommended practices that can minimize the postharvest losses of bulb onions. Therefore, the purpose of this study was to assess postharvest losses in bulb onions, isolate and characterize fungi that cause postharvest rot in bulb onions, and to determine optimal curing temperature to manage the rot and maintain or improve quality. In both field and market surveys questionnaires were administered through face-to-face interviews with farmers and traders respectively. Data collected during the field survey included postharvest handling techniques, postharvest loss quantity, and their causes at both farm and market levels in Bungoma, Meru, and Kajiado counties which were major growing regions of Kenya. Additionally, from seven major open air markets namely; Nkubu and Meru (Meru County), Kitengela and Kajiado (Kajiado County) and; Chwele, Kimilili and Cheptais (Bungoma County), bulb onions were collected to isolate and identify fungi that cause postharvest rot. Fungal isolates were grown in Potato Dextrose Agar (PDA) and identified using both morphological and molecular methods. Appropriate curing temperature to manage bulb onion postharvest rot was determined by inoculating bulb onions with specific fungal pathogens. Some bulb onions were placed at room temperature and others incubated at different temperatures of 30, 35, 40°C for 14 days and rot severity scores recorded. Effect of different curing temperatures on bulb onions physicochemical and phytochemical quality attributes were assessed. The results on assessment of bulb onion postharvest loss levels indicated that 66% of respondents reported that their farms incurred losses ranging from 5 to 30 percent. Fourty percent of the respondents indicated that bulb onion rot was the main cause of postharvest losses at farm level. Occurrence of postharvest losses in bulb onions at farm level were significantly influenced by harvesting tools and transportation mode. Furthermore, secondary and tertiary education and socio-economic factors showed a significant impact on bulb onion postharvest loss. The study revealed that rot was the primary cause of postharvest losses in bulb onions at both farm (10%) and market (14%) levels. Eighteen fungal pathogens isolated from bulb onions collected from different markets were identified through morphological and molecular methods. Morphologically the pathogenic fungal isolates were categorised into three clusters and identified as *Fusarium* spp. The three *Fusarium* spp. clusters were identified as *Fusarium oxysporum f.sp. cepae* (55%), *F. acutatum* (17%), and *F. solani* (28%)

through molecular analysis. Mainly, *F. oxysporum f.sp. cepae* was obtained from bulb onions collected in Bungoma County, while *F. solani* and *F. acutatum* were mostly found in samples from Kajiado and Meru County, respectively. Bulb onions inoculated with *F. solani* and cured at 24±2°C (room temperature) had significantly ($P \leq 0.05$) higher (2.3±0.08) score in rot severity compared to those cured at 30°C, 35°C and 40°C, 1.7± 0.41, 1.7± 0.35 and 1.3 ±0.13 respectively. Increase in the curing temperature decreased the severity of Fusarium rot in bulb onions. Therefore, the study established that curing bulb onions at 30 to 35°C for 14 days reduced severity of postharvest Fusarium rot. Bulb onions cured at 40°C had significantly higher weight loss (3.9±0.53%) and lowest (2.8±0.26%) at 35°C. The reduction in neck thickness was not significantly different ($P > 0.05$) among bulb onions that were cured at 24±2°C, 30°C, 35°C, and 40°C. Total Soluble Solids (TSS) level in bulb onion was not significantly ($P > 0.05$) influenced by different curing temperatures. Curing improved physical attributes in bulb onion which enhances its marketability and shelf-life. The lowest level (97.5±3.32mg/100g) of total flavonoids content (TFC) was observed after curing bulb onion at 30°C whereas the highest (248.6±2.85mg/100g) level was obtained after curing at 24±2°C. This study concludes that improper postharvest handling of bulb onions in Kenya causes losses ranging from 5 to 30%, with rot accounting for the majority of these losses (10 to 14%) at both the farm and market levels. *Fusarium oxysporum f.sp. cepae, Fusarium solani* and *Fusarium acutatum* are the causes of postharvest rot, and these pathogenic fungi can be controlled by curing bulb onions for 14 days at 30-35°C. The physical and phytochemical qualities of bulb onions were enhanced after curing for 14 days at 30–40°C. Though, bulb onions cured at 40°C developed black sooty rot. Consequently, it is recommended that, to manage postharvest Fusarium rot bulb onions can be cured for 14 days at 30-35°C, while also preserving the quality. In order to reduce postharvest losses, the study further recommends capacity building of farmers and traders on appropriate postharvest management techniques for bulb onions.

CHAPTER ONE

INTRODUCTION

1.1 Study Background

Globally grown for commercial purposes, bulb onions (*Allium cepa* L.) is ranked second in vegetable production after tomatoes (FAO, 2021). Due to its common usage in almost every household diet, there is a constant demand throughout the year. Moreover, bulb onions are high in potassium, magnesium, vitamin B6, C, polyphenols, and phytonutrients, according to Kiura *et al.*, (2021); Rodrigues et al.,(2017). Though bulb onions are considered to be less perishable compared to other horticultural crops, postharvest losses occurs after harvest. Kitinoja and Kader, (2015) reported 5–30% losses occurred during postharvest handling in developing countries which contribute to food and nutrition insecurity (FAO, 2014)

Despite bulb onion production being ranked first among the aromatic and medicinal crops in Kenya, its productivity is low due to pre and postharvest challenges including pest infestations, use of unsuitable agronomic and postharvest handling practices (HCD, 2016). The expansion of agricultural land under irrigation and the adoption of suitable pre- and post-harvest handling techniques are essential for increasing bulb onion productivity. Inappropriate postharvest handling practices such as unsuitable harvesting techniques, limited curing and poor storage facilitates leads to postharvest losses in bulb onion (FAO, 2014). Bulb onion losses occur during harvesting, transportation, marketing and storage and are mainly caused by rotting, sprouting and weight loss (Gorrepati *et al.*, 2018). In Nigeria 21-30% bulb onion postharvest losses were reported (Falola *et al*., 2023) while in Philippines, Calica and Cabanayan, (2018) estimated 31 % losses. Information which would assist in the development of postharvest management strategies for the losses of bulb onions in Kenya is inadequate. Reducing postharvest losses in bulb onions would contribute to food and nutrition security, food safety and quality, and economic growth.

Postharvest loss in bulb onion were mainly caused by diseases and pests, bruises, poor storage facilities, and transportation systems, inadequate agricultural extension and credit services (Falola *et al*., 2023). One of the primary causes of postharvest losses in bulb onions is diseases. Abouzeid *et al.* (2016) estimated that postharvest diseases caused 35–40% of the losses in Egypt, whereas Schroeder *et al.* (2010) reported 16% rots of bulb onions imported in New York. Gitonga *et al.* (2012) conducted a survey in Kenya's major markets and found that one of the main marketing challenges in bulb onion was postharvest rot incidences. Some of the postharvest diseases result from infections that happen just prior to harvest these include; Fusarium rot and neck rot (Le *et al*.,2021). Even though the diseases may not be evident during harvest, these infections remain potentially active and after harvest symptoms could appear, especially if the storage environment encourages the growth of pathogens (Coates and Greg 1997). Postharvest diseases of bulb onions are caused by a number of fungus and bacterial species, which include black mould, neck rot, basal rot, and bacterial rot (Hye *et al*., 2018). Determination of an effective disease control approach depends on accurately identifying the organism causing postharvest disease. *Aspergillus* species, *Penicillium* species, *Alternaria* species, *Fusarium* species, *Rhizopus* species, *Colletotrichum* species and *Botrytis* species are some of the fungal species associated with bulb onion rots (Duduk *et al*., 2017; Kumar *et al.*, 2015; Ghanbarzadeh *et al.*, 2014). While *Erwinia* spp, *Pseudomonas aeruginosa, Serratia marcescens, Bacillus cerus, Klebsiella, enterobactor* and *Escherichia* spp bacteria cause bulb onion rotting (Abouzeid *et al.*, 2016). According to Mahmud and Monjil (2015) postharvest diseases account for 10-15% of losses in Bangladesh and were mainly caused by fungal pathogens.

Cultural postharvest practices such as harvesting at the right maturity indices, use of appropriate harvesting techniques, curing and sorting were reported to reduce bulb onion postharvest losses (Banjaw, 2017). Curing is one of the most important post-harvest management techniques, which helps to minimise losses while allowing bulb onions to be stored for up to six months (Naqash *et al*., 2021). Curing produces tight outer wrapping

scales on the bulbs and is an essential step in preserving the bulbs for long-term storage. This prevents entry of bacterial and fungal infections from the neck into the fleshy bulb scales and reduces moisture loss during storage (Schroeder *et al*., 2012). In addition, application of curing technique as a postharvest management technique in bulb onion reduces occurrence of bacterial and fungal rot during postharvest handling (Panel *et al*., 2022). The conditions under which bulb onions are cured determine how efficiently the infections are controlled. Vahling-Armstrong *et al.* (2016) reported that a significant reduction of bulb onion rot caused by *Pontea agglomerans, P.ananatis* and *P.allii* was observed after curing at ≤35°C for 14 days. In India, 28% of farmers had limited knowledge on curing procedure (Soomro *et al*., 2017) while in Kenya, according to Wepukhulu *et al*. (2011) use of curing technique as a postharvest loss management procedure is limited during handling of bulb onions. Therefore application of appropriate curing conditions would lead to reduction of losses.

Curing reduces postharvest diseases infection, however it also affect bulb onion quality attributes (Gorreapti *et al*., 2017). Bulb onion lose weight as a result of moisture being removed during the curing process. Since this affects their marketability, it is important to establish appropriate curing conditions that would optimize weight loss and change in neck thickness. The marketability of bulb onions cured for ten days at temperatures above 35°C increased by up to 65%, according to Panel *et al*., (2022). Zewdie *et al.* (2019) reported that curing of bulb onions for 36 hours at 30°C resulted in the reduction in 5% of total weight and 46% neck thickness. Bulb onions are one of the main dietary source of flavonoids making up a significant portion of the total amount of flavonoids consumed (Rodrigues *et al*., 2017). According to Kumar *et al*., (2015) curing onion bulbs affect their levels of flavonol and anthocyanin. Eun *et al.* (2016) reported that three days of curing bulb onions at 36°C decreased the content of quercetin, which makes up 80–95% of flavonoids (Rodrigues *et al*., 2017). Applying the correct curing conditions would improve the marketability of bulb onions while preserving their nutritional value which is a great source of flavonoids.

Therefore, this study sought to identify postharvest handling procedures at the farm and market levels, evaluate the extent of postharvest losses of bulb onions, and identify the factors influencing postharvest losses. It also identified the pathogen responsible for the rotting of bulb onions in the markets, determined the optimal curing temperature to reduce the severity of pathogen infection, and assessed how the curing temperature affects quality characteristics.

1.2 Statement of the Problem

Despite Kenya having suitable environmental conditions for the cultivation of bulb onions, its productivity is low (below 10 tons/ha) due to various challenges including; application of poor agronomic practices, pests and postharvest losses (HCD, 2016). According to FAO, (2021) postharvest losses have an impact on food and nutrition security, environment, economic development and food quality and safety. It is essential to quantify the extent of postharvest losses and identify the stage along the value chain where the losses occur to enable development of reduction strategies. Fruits, vegetables and aromatic crops have a short postharvest life due to high moisture content (95%). According to Kitinoja and Kader, (2015) 40% losses has been estimated in horticultural crops caused by wilting and shrivelling, mechanical injury, pathological and biological incidences. Falola *et al*. (2023) reported bulb onion postharvest losses of 21-30% in Nigeria, however information on postharvest losses of bulb onions, its extent and causes in Kenya is scanty.

Various postharvest diseases, such as black mould, blue mould, Fusarium rot, Aspergillus rot, dry rot, soft rot and grey neck rot have been found to cause losses in bulb onions during handling (Yurgel *et al*., 2018; Rasiukevičiute *et al.,* 2016). According to Haapalainen *et al*., (2016) grey mould and Fusarium rot account for 5% of bulb onion losses in Finland, while Mahmud and Monjil (2015) stated that postharvest diseases account for 10-15% of losses in Bangladesh and were mainly caused by fungal pathogens. Various fungal species have been associated with bulb onion rotting in several countries

(Duduk *et al*., 2017; Abouzeid, 2016; Kumar *et al*., 2015), however, despite postharvest bulb onion rot being a major challenge during handling (Gitonga *et al*., 2012) little information is available on diseases causing fungi in Kenya.

One of the most important post-harvest techniques for managing bulb onion rot is curing (Vahling-Armstrong *et al*. 2016; Nega *et al*., 2015). However, Wepukhulu *et al*. (2011) reported that most farmers in Kenya improperly cured bulb onions, which led to rotting during marketing (Gitonga *et al*.,2012). Additionally, the optimum curing temperature to reduce post-harvest rot in bulb onions in Kenya has not been determined.

Although curing is a recommended approach in minimizing postharvest losses, it could have an influence on the physical and phytochemical quality characteristics in bulb onions. Quality attributes of bulb onions has an impact on their marketability and contribute to a healthy diet. The bulb onion's neck thickness, dried outer scales, and bulb weight affect its marketability and postharvest life in Kenya (Gateri *et al*., 2018). While, the flavonols in bulb onions have health benefits for consumers (Sidhu *et al*., 2019), curing conditions may affect its levels (Pane*l et al*., 2022; Naqash *et al*., 2021;Sharma *et al*.,2014).Therefore this study sought to establish appropriate curing conditions to improve or maintain the quality of bulb onions.

1.3 Justification of the Study

One of the main challenges that contributes to low bulb onion productivity in Kenya is postharvest losses, however levels and sources of losses have not been identified at the farm and market stages. Development of bulb onion postharvest reduction strategies is hindered by scarcity of information on level of losses and their causes. Establishment of a postharvest management strategy will therefore be aided by the availability of bulb onion postharvest losses information collected during this study.

Although postharvest rot is one of the main challenge during handling of bulb onions in Kenya, currently there is little information on causative pathogenic organism. Therefore,

identifying the fungal pathogen that cause postharvest disease in bulb onions is essential since it would assist in development of rot management approaches.

One of the most important postharvest cultural practices that has been demonstrated to reduce rot in bulb onions is curing (Gorreapti *et al*., 2017), however currently information on appropriate curing temperature in Kenya is scanty. Determining curing conditions could lead to reduction of postharvest losses caused by rot hence improve bulb onion productivity.

Physical characteristics and phytochemical content of bulb onions are affected by curing temperature (Panel *et al*., 2022; Ko, *et al*., 2015). Bulb weight, neck thickness, and dried outer scales of bulb onion have an impact on its marketability and postharvest life. For this reason, determining suitable curing temperature that maintains or improves bulb onion quality would ensure high returns and provides consumers with the essential health benefits derived from its intake.

Establishment of postharvest practices employed by farmers, the extent of postharvest losses and their causes is critical in developing solutions to reduce losses in bulb onions. Furthermore, identification of fungal pathogen causing postharvest rot in bulb onions ensures application of appropriate handling practices to control the diseases. In addition, use of suitable temperature during bulb onion curing would manage postharvest rot and enhance its quality. Overall, reduction of postharvest losses in bulb onion would contribute to enhanced food and nutrition security and livelihoods of the stakeholders.

1.4 Objectives

1.4.1 Overall Objective

To characterize and manage pathogenic fungi that cause postharvest rot in bulb onion

1.4.2 Specific Objectives

1. To establish level of bulb onion postharvest losses caused by rots in Bungoma, Meru and Kajiado counties
2. To identify fungal pathogens associated with postharvest rot of bulb onion in Bungoma, Meru and Kajiado counties.
3. To determine the optimal curing temperature in management of postharvest rot caused by fungal pathogens in bulb onion.
4. To evaluate the effect of different curing temperatures on physicochemical and phytochemical attributes of bulb onions.

1.5 Hypotheses

1. Rots do not significantly cause postharvest losses of bulb onion in Bungoma, Meru and Kajiado counties
2. Fungal pathogens are not significantly associated with bulb onion postharvest rot in Bungoma, Meru and Kajiado counties
3. Optimizing curing temperature of bulb onion does not significantly manage progression of rot caused by fungi.
4. Optimizing curing temperature does not significantly affect physicochemical and phytochemical attributes of bulb onion.

1.6 Research Questions

1. Do bulb onion postharvest rots mainly contribute to losses in Bungoma, Meru and Kajiado counties?
2. What are the fungal pathogens that cause bulb onions postharvest rots in Bungoma, Meru and Kajiado counties?
3. What is the optimum curing temperature that can significantly manage postharvest rots caused by fungal pathogen in bulb onions?

4. Does curing at different temperatures affect the physicochemical and phytochemical attributes in bulb onions?

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Botany of Bulb Onions

Common onion as frequently referred to is economically the most important Allium crop globally (FAO, 2021) . In addition, it is one of the oldest edible alliums including garlic, leek and Japanese bunching onion. Bulb onion was domesticated more than 5000 years ago in Central Asia (Brewster, 2008). There are over 600 allium species distributed all over the world with Mediterranean Basin, Central Asia and Pakistan having the highest species diversity, followed by Western North America (Fritsch *et al.*, 2002). In Africa, onion was introduced from Southern Egypt through Sudan to Central and West Africa (Grubben *et al.*, 2004).

Allium plants, including onions (*Allium cepa* L.), produce edible leaves and bulbs. It is classified as *Monocotyledons*, order *Asparagales*, family *Alliaceae* and genus *Allium*, species *Cepa* and variety *Cepa* L.(Fritsch *et al.*,2002). In terms of botany, the monocotyledon family *Alliaceae* is related to the *Liliaceae* and the *Amaryllidaceae* (Currah *et al.*, 2012). Onion like most of the *Alliums* are diploid with chromosome number of eight (2n=16) (Grubben *et al.*, 2004). Bulb onion are biannual plants with a well-defined bulb at the base of young leaves. The leaves are joined to form a pseudostem while the true stem is a flattened disc formed at the base of the leaves underneath the soil (Brewster, 2008). As the onion plant continues to grow the base of the leaves thickens, fleshy scales form, developing into bulb. During bulb formation, food is channelled to the leaf scales with the inner scales having the highest storage. When the crop matures the leaves die off and the pseudostem dries off forming the neck of the bulb. Kiura *et al*., (2021) indicated that bulbs are ready for harvest when more than 50% leaves have fallen.

The presence of sulphur-containing chemicals, which produce the typical onion or garlic smell and flavour for which the allium crops are desired, differentiates the majority of

species of the significant *Allium* genus. Saponins and flavonols are significant compounds that contribute to the flavour and health advantages of consuming alliums (Ren and Zhou, 2021). Complex fructans are the primary storage carbohydrates in onions, and they are more prevalent in pungent onions, whereas simple sugars are more prevalent in sweet onions (Vagen and Slimestad, 2008).

2.2 Bulb Onion Production and Benefits

2.2.1 Bulb Onion Production

Globally bulb onion is distributed widely across the temperate and warm temperate zone. In the trropics the crop is grown in mountain regions and moslty under irrigation . Onion vegetative growth and good bulb maturation is obtained under dry and cool conditions with day temperatures ranging between 20-28°C and pH above 5.6 (Brewster, 2008; Grubben *et al*., 2004) . Increased day length and temperatures enhance bulb maturation. Onions require cool condition at early stages of development and a dry, warm period during maturation and harvesting. Onions are harvested when they attain proper maturity stage which is influenced by planting season, cultivar and crop growing conditions (Muhie, 2022). Before being harvested, bulb onions are allowed to dry in the field for two to fourteen days after they attain the appropriate maturity stage. This removes excess moisture from the skin and neck, preventing shrinkage and pathogen entry while also allowing for the development of colour during storage (Ren, 2019).

Bulb onions are grown commercially all over the world. According to FAOSTAT, (2020) bulb onion covered a total area of 50.4 million ha globally in 2018. During the same year 96.8 million tons were produced worldwide. Internationally, China ranks first with over 18 million tons of bulb onion produced, followed by India (11million) and USA (3.2million) tons (FAO, 2020). The highest bulb onion producing African countries were Egypt (1.4million), Sudan (1.3 million), Nigeria (0.93 million) and Uganda (0.22 million) tons as reported by Hanci, (2018).

In Kenya, bulb onion is grown across the regions from Coastal area to the highest altitude of about 2000 m above sea level.

It does well in a wide range of climatic conditions from relatively hot and dry to fairly cool and humid conditions. In dry regions of the country bulb onion is grown under irrigation system. In Kenya, bulb onion is grown in well drained, fertile, sandy loam, non-compacted soils with a pH of 5.8 to 6.8 while optimum temperature range between 15- 30 ºC and rainfall between 500 to 700 mm. Bulb onion varieties commonly grown in Kenya include Bombay red, Jambar F1, Red creole, Red couch F1 and Texas Early grano (Plate 2.1).

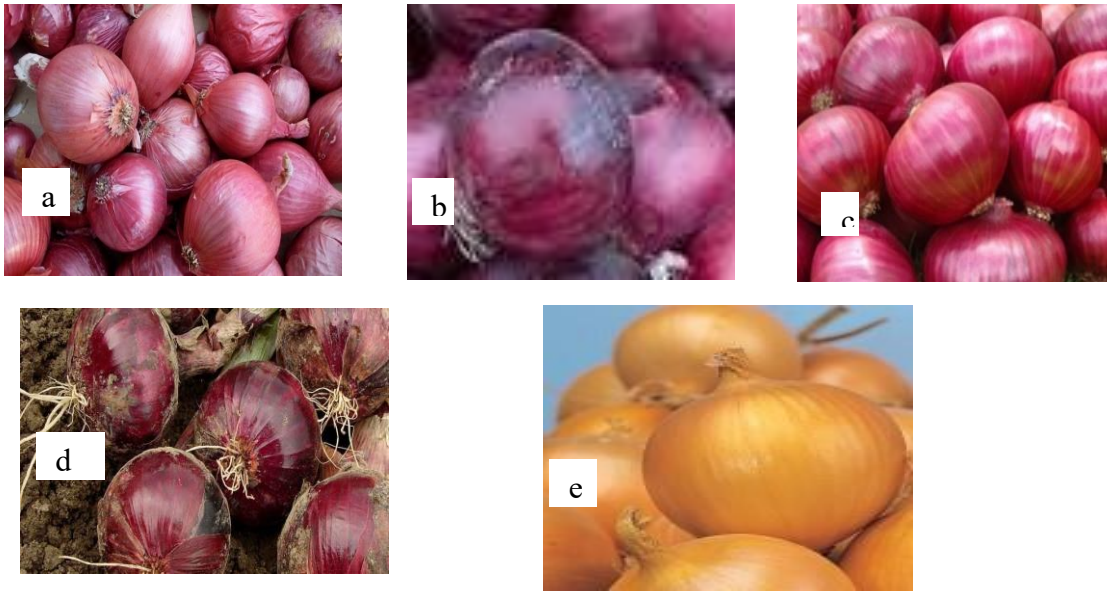

Plate 2.1: Common Bulb Onion Varieties Grown in Kenya (a) Bombay Red, (b) Jamba F1, (c) Red Creole, (d) Red Couch and (e) Texas Early Grano

Source: (Mbaka et al., 2021)

Depending on variety, bulb onions are ready for harvest when leaves dry and top-off, and 3-4 months after planting. In 2020, bulb onion ranked first in terms of area covered,

volume produced and income generated among aromatic crops including spring onions, garlic, chives, leeks and coriander in Kenya (HCD, 2020).

During the same year, bulb onions covered an area of 6,992 ha, 115,113 tons was produced with a value of Kenya Shillings (KES) 4.9 billion.

According to HCD, (2020) the major bulb onion producing counties were Kajiado (26,580 tons), Meru (17,368 tons) and Bungoma (13,814 tons) in 2020.

Bulb onions can be grown from seeds and bulbs from previous production. Although bulb onion can be grown at low soil moisture levels, better yields are achieved where adequate quantity of water is available especially at the initial growing stage (Gateri, 2019).

2.2.2 Bulb Onion Uses and Benefits

Bulb onion is one of the frequently used ingredients in daily household diet, resulting to a constant demand all year round. Use of bulb onion is divided into three main product categories: green salad onions for fresh consumption, dehydrated onions for food processing, and bulbs for the fresh market (which is the most common way of marketing). Bulb onions are considered to be an excellent source of antioxidant-containing foods. These antioxidant components in bulb onions are phenols and flavonoids which protect against various kinds of chronic diseases such as allergies and asthma-related lung congestion (Kim *et al*., 2022). Additionally it is one of the major sources of dietary flavonoids having healthy benefits especially on asthma, allergies and coronary diseases (Rodrigues *et al.,* 2017). Furthermore, bulb onion has been assessed as an excellent supply of compounds that contain sulphur and dietary fibre (Masood *et al*., 2023). It also has a unique flavour which makes it popular in preparation of diverse dishes and it is a good source of vitamin C, Vitamin B_6, potassium, magnesium, polyphenols and phytonutrients (Kiura *et al*., 2021; Rodrigues et al.,2017). Bulbs onions contain 89% water, 7.34 g of carbohydrates, 2.6 g of fiber, 1.83 g of protein, and 276.00 mg of potassium per 100 g (Table 2.1).

Table 2.1: Nutritional Value of Raw Onion per 100g Serving

Nutrient	Content in 100g of raw bulb onion
Water	89.83g
Carbohydrates	7.34g
Fiber	2.6g
Sugars	2.33g
Protein	1.83g
Potassium	276.00mg
Phosphorus	37.00mg
Magnesium	20.00mg
Sodium	16.00mg

Source: (USDA Nutrient Data Base, 2004)

2.3 Bulb Onion Quality and Factors that Influence Their Levels

When marketing, bulb onion quality is important since it affects consumer acceptability. Some of the physical quality characteristics of bulb onions include absence of sprouting, and pathogen infections, bulb size and bulb colour (Petropoulos *et al*., 2017). Other important quality attributes of the bulb onion include phytochemicals, which are source of its health benefits (Rodrigues *et al*., 2017). Quality attributes in bulb onions are influenced by both pre- and post-harvest factors. Type of cultivar, agronomic practices, environmental conditions harvesting maturity and harvesting techniques are some of pre-harvest factors that affect bulb onion quality (Tiwari *et al*., 2013). Postharvest factors such as storage and curing conditions has an influence on levels of phytochemicals and physical characteristics of bulb onions (Ahsanuzzaman *et al*., 2017; Sharma *et al*., 2016; Ko, *et al*., 2016 and Rekha *et al*., 2014).

2.3.1 Physical Quality Attributes in Bulb Onions

Acceptable physical quality of bulb onion is influenced by the peel which should be free from pathogens infestations and have the typical colour depending on variety (Muhie, 2022). Other factors that influence the quality of bulb onion during marketing include

weight, size and the neck thickness (Wanjiku, 2019). These characteristics are obtained by harvesting bulb onions that are fully mature and then maintain the quality using various postharvest techniques such as curing and storage conditions (Kiura *et al*., 2021).

2.3.2 Phytochemicals in Bulb Onions

Phytochemicals are a group of metabolites that are found in fruits and vegetables and are strongly associated with the health benefits of these foods (Petropoulos *et al*.,2018). Bulb onion which is classified as a vegetable has been reported to contain the highest levels of phytochemical compounds that contributes to human diet (Ren and Zhou, 2021).

2.3.2.1 Phenolic Compounds in Bulb Onions

According to Ren and Zhou, (2021) onions as one of the vegetables are a great source of phenolic compounds in human diet. They are significant naturally occurring bioactive chemicals that are present in onions and are widely known for having a beneficial effect on health. Phenolic compounds contribute to bulb onion colour and astringency, and act as defence against parasites and insects. Moreover, these compounds enhance plants' ability to withstand stress (Bibi *et al.*, 2022). There are two categories of phenolics: flavonoids and non-flavonoids (Rodrigues *et al*., 2017) . Red onions usually contain more total phenolic content than white cultivars (Sagar *et al.*, 2020).

2.3.2.2 Total Flavonoids in Bulb Onion

According to Rodrigues *et al*. (2017), onions are one of the main dietary sources of flavonoids globally. In onions, two flavonoid classes predominate: anthocyanins, which gives the red or purple cultivar colour and flavonols, such as quercetin and its related compounds that provide other varieties their characteristic yellow and brown skins.

The flavonoids found in onions most frequently are flavonols. Quercetin aglycone, quercetin monoglucoside, quercetin diglucosides, isorhamnetin, which is a methyl ether

of quercetin, isorhamnetin monoglucoside, rutin, and kaempferol are the seven main flavonol molecules found in onions (Ren and Zhou, 2021). Quercetin 3,4-diglucoside and quercetin 4-glucoside are the main flavonols reported in bulb onions (Sagar *et al*., 2020). Content of quercetin glucosides vary depending on position of scales in bulb onion, outer scales contain 3-5 times higher quercetin glucosides than the inner ones (Ko *et al.,* 2016).

2.3.2.3 Anthocyanins in Bulb Onion

Though anthocyanins are not a significant flavonoid component of onions, red onions have frequently been reported to contain them. According to Rodrigues *et al*. (2017) in red onion cultivars anthocyanin content has been estimated to be between 39 and 240 mg/ kg of fresh weight, and about 10% of the total flavonoid content. Zhang *et al.* (2016) who evaluated the composition of anthocyanins in red, yellow, and white onions found that they had 29.99±1.19, 9.64±1.30, and 0.75±0.40 mg 100 g-1 respectively. According to Ren,(2019) anthocyanins are a source of antioxidant activity and in red onions the pigments are concentrated in the skin or outer layer.

2.4 Pre-Harvest Factors Affecting Bulb Quality

Major pre-harvest factors influencing quality of bulb onion include cultivar type, agronomic practices such irrigation and fertilizer application and stage of maturity during harvesting. These factors are discussed in the following sections.

2.4.1 Influence of Cultivar Type on Bulb Onion Quality

Genetic makeup of commodities defines their different characteristics in terms of quality. In bulb onions number and thickness of tunic layers, pungency of inner layers, bulb colour and pathogenic infections are some of the genetically controlled quality factors (Currah *et al.,* 2012). Ko *et al.* (2002) reported that varietal differences in bulb onion influenced resistance to black mould during storage in bulb onion.

Different onion cultivars have varying amounts of phenolic compounds. Red onions generally contain greater total flavonol concentrations than yellow or white. In a comparative study of 15 different onion cultivars, it was found that red, pink, and yellow onion varieties had the highest levels of quercetin (between 88.40 and 11,885.025 mg/kg), while white onions had low levels (Sagar *et al*., 2020).

2.4.2 Impact of Nutrients and Water Management on Bulb Onion Quality

Bulb onion, in comparison with other crops have relatively shallow roots which are at the depth of 20 cm (Brewster, 2008). This limits accessibility of available soil moisture and nutrients, thus affecting its crop management. Plant nutrition has an impact on the shelf life and postharvest quality in bulb onions. Application of fertilizer during bulb onion growing is important in increasing crop productivity especially in depleted soil fertility. Bekele *et al.* (2018) and Gateri *et al*. (2018) have indicated that optimising nutritional requirements is necessary to improve the postharvest quality and storability of bulb onion. Nitrogen is one of the primary macro-nutrients that influences growth, yield and quality of bulb onions. Bekele *et al*. (2018) reported that during a two-month storage period under ambient conditions, bulb onions grown in nitrogen levels of 150 kg/ha showed increased percentages of bulb sprouts (66%), bulb rots (14%), and weight loss (40%). Gateri *et al*. (2018) reported that application of nitrogen at the rate of 104 kg/ha at six weeks improved quality and marketability of bulb onion. Excess use of nitrogen before harvesting delays bulbs maturation resulting to thick bulb necks which lead to higher water loss and increased susceptibility to infections during storage (Muhie, 2022). Potassium, another important nutrient influences quality and storage of bulb onion. Nabi *et al.*(2010) reported that application of 75-100 kg/ha potassium reduced percentage weight loss (17%), diseases (50%) and sprouting (9.3%) incidences during bulb onion storage.

Irrigation regime especially after bulb initiation is important since it influences bulb yield and quality (Bhagyawant *et al.*, 2016). The authors reported that reduction in shelf-life of bulb onions occurs if they are watered excessively and/or too frequently (twice or more

per week) (Bhagyawant *et al.,* 2016). Nandle *et al.*(2018) recommended that irrigating bulb onion field once a week to a depth of one inch (2.5 cm) increased yield and quality of the produce. Soil moisture content influences loss in weight and sprouting of bulb onion during storage. Kumar *et al*. (2006) reported that bulb onion grown under low soil moisture regime of 0.60 Water to Cumulative Pan Evaporation (IW/CPE) ratio caused physiological loss and 26% sprouting during storage. Petropoulos *et al.* (2017) reported that before harvesting (3-4weeks) water application should cease to allow bulb maturation, drying of the outer scales and hardening of the neck.

2.4.3 Influence of Maturity Indices on Quality of Bulb Onion

Maturity is defined as the stage at which the produce has developed consumers' favorable taste, appearance and exhibits acceptable shelf life (Porat *et al.*,2018). Drying and toppling of leaves in bulb onions are recommended as maturity indicators since they are simple and can easily be identified (Petropoulos *et al*., 2017). Harvesting time affects bulb onion physical quality attributes such as sprouting, disease incidences and number of intact outer scales which influences marketability of bulb onions (Gateri, 2019). On phytochemical quality, Eun *et al*. (2016) reported that bulb onion lifted at appropriate maturity stage contained higher levels of quercetin compared to those harvested early. Harvesting time is influenced by water and nutrients management, onion cultivar and environmental conditions (Gateri *et al.,* 2018). Nonconformity to optimum harvesting stage may result to loss of quality, quantity and monetary returns (Michailides *et al.*, 2009).

2.5 Postharvest Factors Affecting Bulb Onion Quality

Once bulb onions are harvested, in the developmental processes in terms of both chemical and physical quality attributes ceases and hence limited opportunities are available to improve on quality. Therefore, the main focus after harvest is to maintain bulb onion

quality through management of postharvest factors that influence bulb quality namely postharvest handling practices, curing, storage conditions and postharvest diseases.

2.5.1 Influence of Postharvest Handling Practices on Bulb Onion Quality

Bulb onion post-harvest handling practices have not received the same level of attention as other economically important horticultural produce. However there exists challenges during handling which affects bulb onion quality. Quality in bulb onion is affected during harvesting, transportation and marketing which mainly cause rotting, sprouting and weight loss (Gorrepati *et al.*, 2018; Soomro, 2017). Harvesting method in bulb onion affects quality of bulb onion through mechanical damage. Use of hands when harvesting bulb onions ensures quality of bulbs is maintained while use of machetes may cause bruises which lead to quality loss (Banjaw, 2017). After harvesting, onions are trimmed and about 4cm stalk is left (Plate 2.2) and if improperly done the injured part forms an entry point for pathogens (Currah *et al.*,2012).

Plate 2.2: Bulb Onions Indicating a Stalk Left during Harvesting after Trimming the Leaves

Sorting of horticultural crops including bulb onion involves removal of injured and rotting produce. Lack of sorting before packing promoted spread of diseases to healthy produce leading to quality loss.

In addition proper packaging protects produce from compression during handling and transportation. According to Kitinoja *et al.* (2012) mechanical damage occurred in fresh produce when packaged in big, rough and weak packages such as big sacks (0-100%) and baskets (89%) during marketing. Mechanical damage in bulb onions accelerates rate of rotting during storage (Nivedida *et al.*, 2019).

2.5.2 Effect of Curing on Bulb Onion Quality

According to Petropoulos *et al*. (2017), curing can be defined as the elimination of excess moisture from the outer skin and the neck of bulb onions thus minimizing pathogenic infection during storage. Curing is one of the most important post-harvest management techniques, which helps to minimise losses while allowing bulb onions to be stored for up to six months (Naqash *et al*., 2021). Additionally, it minimises shrivelling resulting from moisture loss from inner scales to the outer ones. Bulb onions can be cured either in the field by lifting them to surface and allowing them to dry under the sun and/or artificially using forced air or heated forced air through the storage facility to dry the neck and the outer layers (Gorreapti *et al*., 2017). Wet weather conditions leads to inefficient natural curing, which reduces the storage life of bulb onions. Therefore, artificial curing method which can be done for 14-20 days and it is accurate, safe, and rapid has been recommended in order to obtain quality bulb onion for storage (Naqash *et al.*, 2021). Curing conditions such as duration and temperatures affects incidences and severity of postharvest diseases in bulb onions. According to Vahling-Armstrong *et al.* (2016) a reduction (20-40%) of storage rot caused by *Pantoea agglomerans, P. ananatis* and *P. allii* was observed when curing temperatures are more than 35°C for 2 to 14 days. Curing of bulb onion has been shown to improve chemical quality of bulb onion including

total soluble solids, colour (Nivedida *et al.*, 2019) and total quercetin levels (Ko *et al.*, 2016).

2.5.3 Effect of Storage Conditions on Quality of Bulb Onions

During storage bulb onion quality is affected by water loss, rotting, sprouting and changes in chemical composition. Nabi *et al.* (2013) reported that lower weight loss (6%), sprouting (9.6%) and rotting 1.7% were recorded in cold storage (0-1°C) compared to ambient conditions (27-31°C) where 98% weight loss, 100% sprouting and 70% rotting were registered in bulb onion after four months storage. Temperature and relative humidity are important conditions in storage environment which influence the quality of bulb onion (Islam *et al*., 2019).

Brewster, (2008) recommended that during bulb onion storage relative humidity should be maintained at 65-70% under uncontrolled temperature conditions, this minimises weight loss and skin cracking. Proper ventilation is important during bulb onion storage since it reduced rot. (Dabhi *et al.*, 2017).

2.5.4 Postharvest Diseases in Bulb Onions

Postharvest diseases are categorised according to how an infection occurs. These include quiescent or latent infections where the pathogen initially infects plant during growing. The infection becomes dormant until the physiological status of the plant tissue changes or when the storage conditions are suitable for its development. The other important kind of postharvest diseases are those caused by pathogens developed after the harvest. These pathogens may gain entry through wounds caused by mechanical or insect damage (Coates and Johnson, 1997).

Postharvest diseases are major challenges during bulb onion handling, causing 20-50% losses (Duduk *et al*., 2017). They include black mould, blue mould, neck rot, Fusarium rot and bacterial rots which are caused by diverse pathogen species. *Aspergillus* spp.,

Agaricomycetes spp., *Alternaria* spp., *Fusarium* spp., *Candida* spp., *Clavispora* spp.and *Botrytis* spp. (Yurgel *et al.,* 2018) are some of fungal organisms that cause rotting in bulb onion. In Ghana *Aspergillus niger, A. flavus, Penicillium* spp., *Rhizopus solonifer* and *Fusarium oxysporum* (Adongo *et al.,* 2015) and in Iran *Fusarium* spp. (Ghanbarzadeh *et al*., 2014) were isolated from rotten bulb onions. Bacterial organisms causing rots in bulb onion include *Erwinia*, *Pseudomonas aeruginosa*, *Acetobacter*, *Gluconacetobacter, Citrobacter, Enterobacteriaceae* and *Klebsiella* (Yurgel *et al.*, 2018). Bacteria pathogens have been reported to cause bulb onion rots in several countries, *Pantoea ananatis* in Morocco (Achbani *et al.*, 2016).

2.5.4.1 Black Mould

Black mould caused by *Aspergillus niger,* is a common postharvest disease in bulb onion which occurs under hot and humid conditions. The disease causes black discolouration on bulb neck, lesions on the outer scales and streaks of black mycelia and spores beneath the outer dry scales (Ko *et al.,* 2002). *Aspergillus niger* in bulb onions has been reported in Egypt (Khalifa, 2016), in Ghana (Adongo *et al.,* 2015) and Canada (Yurgel *et al.*, 2018). *Aspergillus niger* is considered a saprophyte and its spores are found in air and soil. It infects the bulb onion through wounds in the neck, bruised outer scales and basal stem plate (Polderdijk, 2000). *Aspergillus niger* is commonly isolated from water, buildings, air and diseased plant parts using various selected natural and artificial agar. Romero-Cortes *et al.* (2019) stated that *Aspergillus* strains cultured on PDA media can be distinguished morphologically by having colonies with a diameter of 66–70 mm, a black colony colour, and globose-shaped conidia with biseriate, dark brown to black heads. *Aspergillus niger* optimal growth occurs between 30 to 35°C and relative humidity ranging from 75-80% and therefore black mould diseases may be easily managed by storing bulb onion in a cold storage facility between 0 and 2 °C (El-Nagerabi *et al.*, 2003).

Plate 2.3: Symptom of Black Mould (Aspergillus niger) on Bulb Onion (CABI, 2023)

2.5.4.2 Blue Mould

Blue mould is a postharvest bulb onion disease caused by *Penicillium* species. Various species namely *P. polonicum, P. glabrum* and *P. expansum* were reported by *Duduk et al*. 2017) in Serbia and *P. aurantiogriseum* in Pakistan (Khokhar and Bajwa, 2015). The species produce air-borne conidia that contaminate floors and walls in storage facilities. Symptoms of blue mould include soft watery rot with a blue green mould on scales of bulb onion. Using potato dextrose agar (PDA) or malt extract agar, *Penicillium* spp. are commonly isolated from part of a diseased bulb onion. The basis for morphological identification is the existence of two-staged conidiophores, which are distinguished by smooth, globose to subglobose, 3–4 μm in diameter conidia (Çakır and Maden, 2015).

Proper storage, choice of variety and curing minimise incidences of *Pencillium* spp. infections in bulb onion (Ahsanuzzaman *et al*.,2017).

Plate 2.4: Symptom of Blue Mould (*Pencillium polonicum*) on Bulb Onion

Source: (Lazarevic et al., 2014)

2.5.4.3 Neck Rot

Neck rot caused by *Botrytis spp. Botrytis allii, B. aclada* and *B. byssoidea* have been associated with neck rot in bulb onion during postharvest handling (Bertolini *et al.,* 1997). Botrytis rot typically affects onion plants while they are still growing, however symptoms often appear after the bulbs have been harvested and are in storage. Bulb onion affected by *Botrytis* spp. exhibit water-soaked neck tissues and yellow discolouration from the neck into scales (Chilvers *et al*., 2006). Neck rot disease may be identified by observing presence of conidiophores and conidia on infected plant tissues, however growing the fungus on agar media and measuring conidial diameters under a compound microscope are necessary for differentiating *Botrytis* species associated with onion bulb (Chilvers and Toit, 2006). In order to reduce the chances of neck rot infection during bulb storage, cultural control techniques are employed. Bulbs are either naturally or artificially cured to prevent infection during storage (Steentjes *et al*., 2021)

Plate 2.5: Symptom of Neck Rot (Botrytis allii) on Bulb Onion

Source: (Chilvers and Toit, 2006)

2.5.4.4 Fusarium Rot

Fusarium rot in bulb onion is associated with several species of *Fusarium* spp. Ghanbarzadeh *et al.* (2014) and Haapalainen *et al*. (2016) reported *F. oxysporum, F .proliferatum and F. redolens* as species causing bulb onion rots in Finland and Iran. In addition to these species Kalman *et al*. (2020) isolated *F. acutatum* and *F. anthophilium* from bulb onion rots in Israeli.

Fusarium spp. primary infection occurs when the fungus enter the roots of the bulbs through the lesions or on the base of bulb scales. Brown discolouration that extend from the bulb onion base to the scales are the symptoms of the infection. Despite the fact that the infection begins in the field, symptoms do not develop until during storage (Le *et al*., 2021). Cultured on PDA media *Fusarium* species are differentiated morphologically by distinctive characteristics such as colony colour, colony development rate, conidia size and shape, and the presence or absence of macro- and micro-conidia (Bayraktar *et al*., 2010). Chemical management is one way to control Fusarium rot, but maintaining field hygiene and disinfecting field equipment on a regular basis are additional critical factors in preventing infection sources. It is recommended that postharvest treatments be applied and bulb onion should be properly stored to manage Fusarium rot (Le *et al*., 2021).

Plate 2.6: Symptoms of Fusarium Rot (Fusarium spp.) in Bulb Onion

Source: (Shin *et al*., 2023)

2.5.4.5 Bacterial Rots

Several bacteria pathogens are associated with bulb onion rots. Yurgel *et al.* 2018 reported *Klebsiella, Pantoea*, *Entrobacteriaceae*, *Erwinia,, carotovora* and *Pseudomonas* bacteria pathogens that caused rotting during storage of bulb onion in Nova Scotia while *Pseudomonas auruginosa* was reported in Egypt (Abd-alla *et al.*, 2011). Onions are most frequently affected by bacterial soft rot when they are being stored or transported, but the infection can also appear in the field prior to harvest, following a period of heavy rain, or during the drying stage of the leaves. Crop residue and contaminated soil are the main sources of inoculum (Calle-Bellido *et al*., 2013). Bacterial rot can be isolated from diseased plant tissue and cultured on yeast peptone dextrose agar (YPDA) as a growing medium and morphologically characterized based on colony distinctive features such as colour (Rahman *et al*., 2012). Application of appropriate postharvest practices such as curing would manage bacterial rot infection in bulb onion. Vahling-Armstrong *et al.* (2016) reported that bulb onion cured at more than 35˚C for 2 or 14 days reduced incidence of *Pantoea* spp.

Plate 2.7: Bacterial Rot (Pectobacterium caratovorum) in Bulb Onion

CHAPTER THREE

ESTIMATION OF BULB ONIONS POSTHARVEST LOSSES IN SELECTED SUB-COUNTIES OF KAJIADO, MERU AND BUNGOMA COUNTIES, KENYA

Abstract

Bulb onion (*Allium cepa L.*) is a good source of vitamin C, Vitamin B_6, potassium, magnesium, polyphenols and phytonutrients. Kenya has suitable conditions for bulb onion production. However, its productivity is low which is attributed to pre- and postharvest handling factors. Information on bulb onion postharvest losses and their causes in the country is scanty which limits development of postharvest reduction strategies. This study was carried out in three major bulb onion growing counties of Kenya namely Bungoma, Meru and Kajiado to determine the extent and factors influencing postharvest losses of this crop at farm and market levels. Face-to-face interviews were conducted using structured questionnaires to collect information on postharvest handling practices, postharvest loss levels and their causes at farm and market levels. A total of 169 and 72 respondents at farm and market levels respectively were interviewed to obtain the data. The respondents were randomly selected using a multi-stage sampling method. Data was subjected to descriptive and logistic regression analysis methods. The results indicated that 48% and 25% of the respondents used toppling and drying of upper leaves respectively as maturity indices when harvesting bulb onions. Fourty two (42%) of the respondents used machete as a harvesting tool which significantly ($P \leq 0.05$) influenced postharvest losses. Sixty six percent (76%) and 88% level of the respondents indicated that 5-30% postharvest losses occurred at farm and market level respectively. Majority of respondents (40% and 68%) indicated that bulb onion rots mainly caused losses at farm and market level respectively. Rotting of bulb onions caused 10% of the total losses at farm level and 14% at market stage. The results indicated that occurrence of bulb onion postharvest loss was significantly ($P \leq 0.05$) influenced by secondary and tertiary education levels. Transportation of bulb onion using

bicycles and donkeys significantly influenced postharvest losses at P≤.05. From the study it was concluded that at farm level postharvest losses (5-30%) that occurred and were mainly caused by rotting (10-14%). Secondly socio-economic characteristics and postharvest handling practices influenced bulb onion losses. Therefore, development of postharvest reduction strategies on bulb onions should focus on alleviating rot during handling practices.

3.1 Introduction

Postharvest losses can be defined as degradation in both quantity and quality of food structure from harvest to consumption. Postharvest losses that occurs during postharvest handling and processing operations can also be referred to as food losses (Meyer *et.al.*, 2017). Postharvest losses contribute to food insecurity, mismanagement of human effort, farm input, livelihood, farming investments and also scarce natural resources such as water (Hailu and Derbew, 2015). Fruits, vegetables and aromatic crops have a short postharvest life due to high moisture content (75-95%) (Raghuvanshi *et al*., 2018). Wilting and shrivelling, mechanical injury, pathological and biological incidences are major causes of postharvest losses in horticultural crops (Raghuvanshi et al., 2018).

Compared to other horticultural crops bulb onion are less perishable however postharvest losses are expected. The postharvest losses in bulb onion occur during harvesting, transportation, marketing and storage which are mainly caused by rotting, sprouting and weight (Gorrepati *et al*., 2018). It has been estimated that 5-35% postharvest losses of fresh produce occurs in developed countries while in developing countries 20 to 50% which mainly occurred at farm and market levels (Kasso and Bekele, 2018).

In Sub Saharan Africa according to FAO,(2014), postharvest losses of fresh produce were estimated at 56% with net production of 230 million tons per annum. In bulb onion about 35-40 % in both quality and quantity losses were estimated during various postharvest

operations including handling and storage according to Calica and Cabanayan, (2018). In Kenya, information on bulb onion postharvest losses is limited.

Quantifying postharvest losses and determining their causes would assist in developing postharvest management strategies thus improving bulb onion productivity and enhance farmers' income. Therefore, this study was carried out to quantify bulb onion postharvest losses and to determine their causes at farm and market levels in major growing regions of Kenya namely Kajiado, Bungoma and Meru counties.

3.2 Materials and Methods

3.2.1 Preparation of Data Collection Tool

Two structured questionnaires (Appendix 1 and 2) were developed to collect data at both farm and market levels respectively. Data collected included: socio-economic characteristics (gender, age and primary level of education (8 years), secondary level of education (12 years) and tertiary level of education (>12years) and land size), bulb onion harvesting techniques, postharvest handling practices and causes of postharvest losses. In addition, the market level questionnaire was designed to collect information on postharvest handling practices such as sorting, grading and causes of bulb onion postharvest losses. Both market and farm level questionnaires were pre-tested using an expert review methodology (Ikart, 2019). Three experts including a sociologist, statistician and postharvest physiologist revised the questionnaires and their comments were included. The questionnaires were then administered through face-to-face interviews with farmers and traders.

3.2.2 Study Sites Selection

A multi-stage sampling design was used to select the study areas according to Basavaraja *et al.* (2007) with slight modification. Two stage sampling design was used in selection of study sites. The first stage involved selection of three major bulb onion growing counties in Kenya and the second one, a Sub-County was chosen in each county. In the

first stage Bungoma, Meru and Kajiado counties with annual bulb onion production of 5,682, 4,421 and 2,415 tons respectively were selected (HCD, 2016). In the second stage, one Sub-County with the highest bulb onion production level per the chosen counties was selected using information provided by County Agriculture Officers. The three Sub-counties selected as study sites were Kajiado East in Kajiado County, Buuri in Meru County and Mt. Elgon in Bungoma County.

3.2.3 Description of Study Sites

3.2.3.1 Kajiado County

Kajiado County is situated in Southern part of Kenya between altitude of 500 m and 2000 m above sea level. It covers an area of 21,900.9 Km^2 and lies between 360 5' and 370 5' longitudes and latitudes of 10 0' and 30 0'. The county has three types of soils namely quaternary volcanic, pleistocene and basement rock soils. It receives bi-modal rainfall pattern ranging from 300 mm to 1250 mm annually, while temperatures range from 10 to 34°C. The short rains occur from October to December and long rains occur between March to May (Kajiado County Government, 2013). Most parts of the Kajiado County are semi-arid areas with livestock production being a major agricultural economic activity. However, horticulture such as bulb onions production is gaining popularity especially in Kajiado East Sub-County. The map below indicates the areas where the data was collected (Figure 3.1).

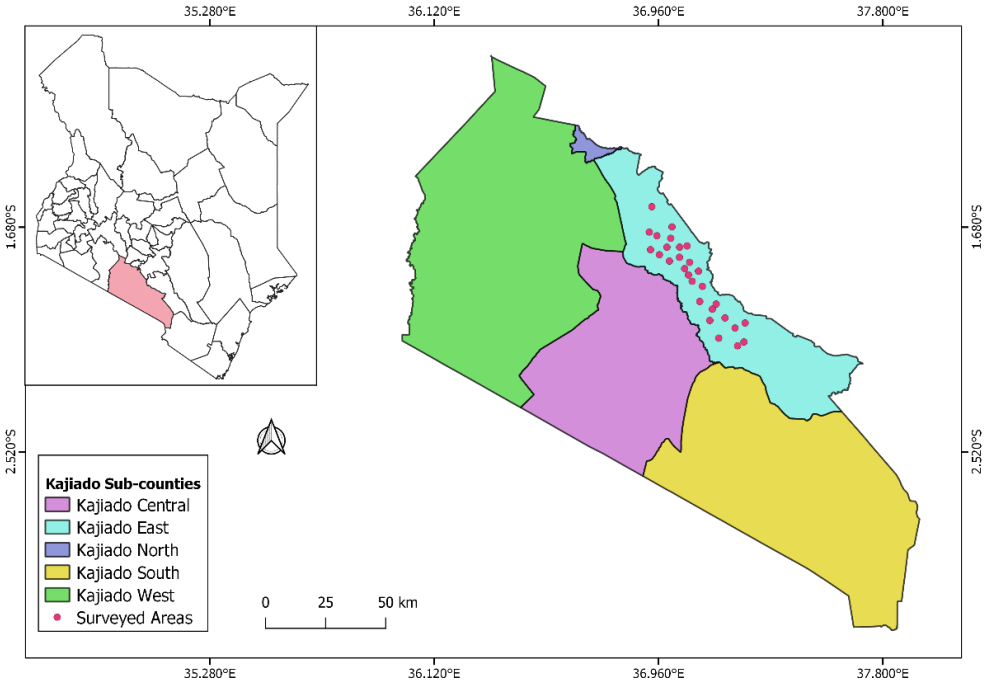

Figure 3.1: The Location of Farms (Dots) Drawn Using QGIS Geographical Information System (version 3.14) with GPS data Collected in Kajiado County, Kajiado East Sub-County

3.2.3.2 Meru County

Meru County is located in Eastern part of Kenya and lies along the equator within 0˚ 6' North and 0˚ 1' South, and latitudes 37˚ West and 38˚ East. It is situated between altitudes of 300 to 5199m above sea level. The County covers an area of 6936.2Km^2. It has diverse ecological zones stretching from upper Highlands, lower highlands, upper midlands and lower midlands. The county has a variety of soil types, including lithosols, andosols, nitosols, and cambisols. Daily temperatures range from 8 to 32˚C during the cold and hot season respectively. On average Meru County receives moderate amount of rainfall between 300 mm to 2500 mm per annum. It has bi-modal rainfall pattern with short rains being received between March and May while long rains are received between October and December (County Government of Meru, 2013). Agriculture is a major economic

activity in the County, with both food and horticultural crops enterprises being undertaken.

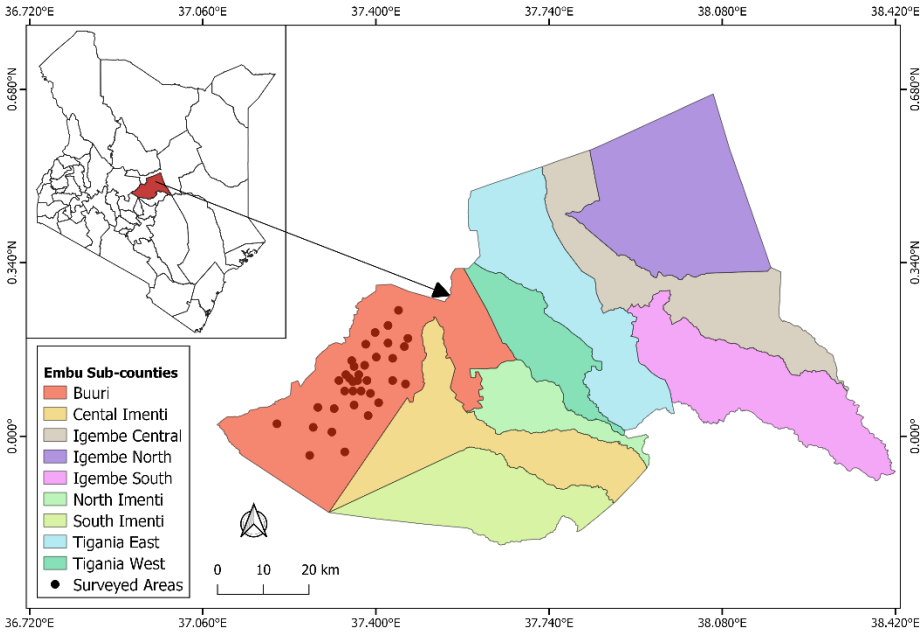

Figure 3.2: The Location of Farms (Dots) Drawn Using QGIS Geographical Information System (Version 3.14) with GPS Data Collected in Meru County, Buuri Sub-County

3.2.3.3 Bungoma County

Bungoma County lies between longitudes 340 20' East and 350 15' East of the Greenwich Meridian, and latitudes 00 28' and 10 30' North of the Equator. The altitude ranges from 1200 to 4000m above sea level covering an area of 3032.4 Km^2. The county is has a variety of soil types, including foot slopes (F1), bottom land (B11), upland (U115), foot slopes (M9), and volcanic foot ridges (R1). Bungoma County has diverse of ecological zones namely Upper Highlands, Lower Highlands, Upper Midlands and Lower Midlands. Annual rainfall ranges from 400 mm to 1800 mm with two seasons, long rains which occur between March and July while short rains are from August to October. The County

temperatures ranges from 0 to 32°C (County Government of Bungoma, 2018). The map below (Figure 3.3) indicates location of the farms where the data was collected.

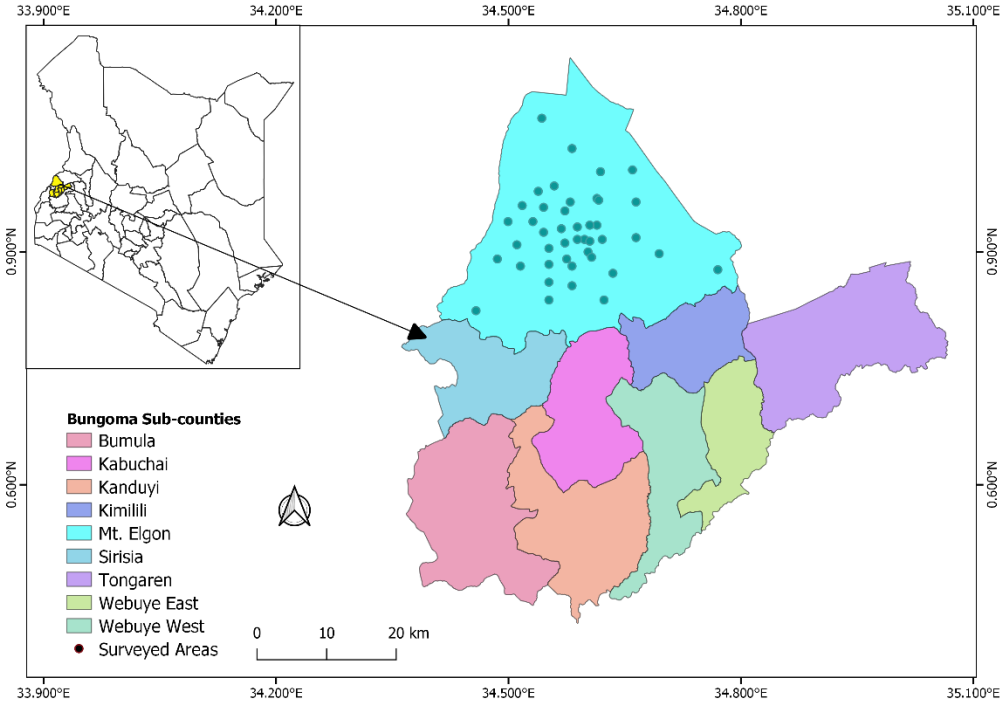

Figure 3.3: The Location of Farms (Dots) Drawn Using QGIS Geographical Information System (Version 3.14) with Data Collected Bungoma County, Mt. Elgon Sub-County

3.2.4 Selection of Respondents and Data Collection at Farm and Market Level

At farm level lists of bulb onion farmers from the selected Sub-Counties namely Kajiado East in Kajiado County, Buuri in Meru County and Mt. Elgon in Bungoma County were generated with the assistance of County Agriculture Officers. From the lists, farmers were randomly selected. A sample size (n) was determined based on the formula by Israel, (1992) which assumes 95% confidence level and P = 0.05.

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

Where:

n = the sample size,

N = the population size and

e = the level of precision

From the Sub-Counties a total of 169 respondents were interviewed; 47 in Kajiado East, Kajiado County 56 in Buuri, Meru County and 66 in Mt. Elgon, Bungoma County. The survey was conducted during the months of June, July and September 2018 through face-to-face interviews with an adult person present in the farm during the time of the visit.

The market survey was conducted in seven markets in: Kajiado, and Kitengela markets within Kajiado County; Nkubu and Meru (Gakoromone) markets within Meru County; and Kimilili, Cheptais, and Chwele markets within Bungoma County. These markets were selected according to the quantity of traded bulb onions, and County Agricultural officers provided the information. Snowball sampling approach was used to select 72 traders comprising of wholesalers and retailers in the three counties. Forty four percent of the total respondents were in Bungoma County, 28% in Kajiado, and 28% in Meru County. Market survey was conducted from June to September 2018 through face-to-face interviews with the traders.

3.2.5 Data Analysis

Data obtained from farm and market surveys were coded and analyzed using Statistical Package for the Social Sciences (SPSS) software version 20 for descriptive analysis and the results were expressed as percentages in charts. Logistic regression analysis was employed to determine factors influencing postharvest losses of bulb onion at farm level. The model used was statistically significant at Chi-square = 46.619 and $P<0.05$ as given:

Logit (p) = β0 + β1 X1 + β 2 X2 + β 3 X3 + β 4 X4 + β 5 X5 + β 6 X6 + β7 X7+ β8 X8+ β9 X9+ β10 X10.

Where logit (p) is postharvest losses (dependent variable), β is population regression coefficient,

X1 is secondary level of education, X2 is tertiary level education, X3 is land size under bulb onion, X4 is use of machete as harvesting tool, X5 is curing, X6 is sorting, X7 is toppling of leaves as maturity indices, X8 is use of bicycle as transport mode, X9 is use of donkey as transport mode, and X10 is distance to market > 5km.

3.3 Results

3.3.1 Postharvest Losses in Bulb Onions at Farm Level

3.3.1.1 Socio-Economic Characteristics of Respondents

Majority of respondents in Kajiado, Meru and Bungoma counties, interviewed were males 83, 68 and 57% respectively with an average age of 40 years. Fourty five percent of the respondents had attained primary education level while 37 % were educated up to secondary education level, with their main business being farming (88%). Nine point four (9.4) ha in Kajiado, 1.9 ha in Meru, and 2.3 ha in Bungoma counties were the average farm sizes owned by the respondents, with 1.9, 0.7, and 0.8 ha under bulb onion cultivation, respectively. The average size of the farms owned by the respondents in the three counties of Kajiado, Bungoma, and Meru was 1.6 hectares, of which 0.8 ha were used for the cultivation of bulb onions.

3.3.1.2 Postharvest Management Practices of Bulb Onion

3.3.1.2.1 Bulb Onion Maturity Indices

As indicators of harvesting time, the respondents in the counties of Kajiado, Meru, and Bungoma employed various bulb onion maturity indices. In Kajiado County, majority of respondents indicated the number of days after planting (30%) and the drying of the

leaves (38%) as indices of bulb onion maturity (Plate 3.1). While in Meru and Bungoma counties majority of respondents, 57% and 45% respectively used toppling of upper leaves as maturity indices in bulb onions (Table 3.1).

Table 3.1: Proportion of Respondents in Counties of Kajiado, Meru and Bungoma who Used Various Maturity Indices to Harvest Bulb Onion

County	Percentage of respondents (%)				Number of respondents (n)
	Size (%)	Number of days (%)	Drying of leaves (%)	Toppling of leaves (%)	
Kajiado	15	30	38	17	47
Meru	14	21	7	57	56
Bungoma	13	8	33	45	66
Overall	**14**	**19**	**26**	**41**	**169**

Across all the three counties, toppling and drying of upper leaves were used by a large proportion of respondents 48 and 25% respectively, as indication of maturity in bulb onion (Fig. 3.4). Only 11% of the repondents used size as maturity indicator while 16% used growing period as an idicator to harvest bulb onions across the three counties

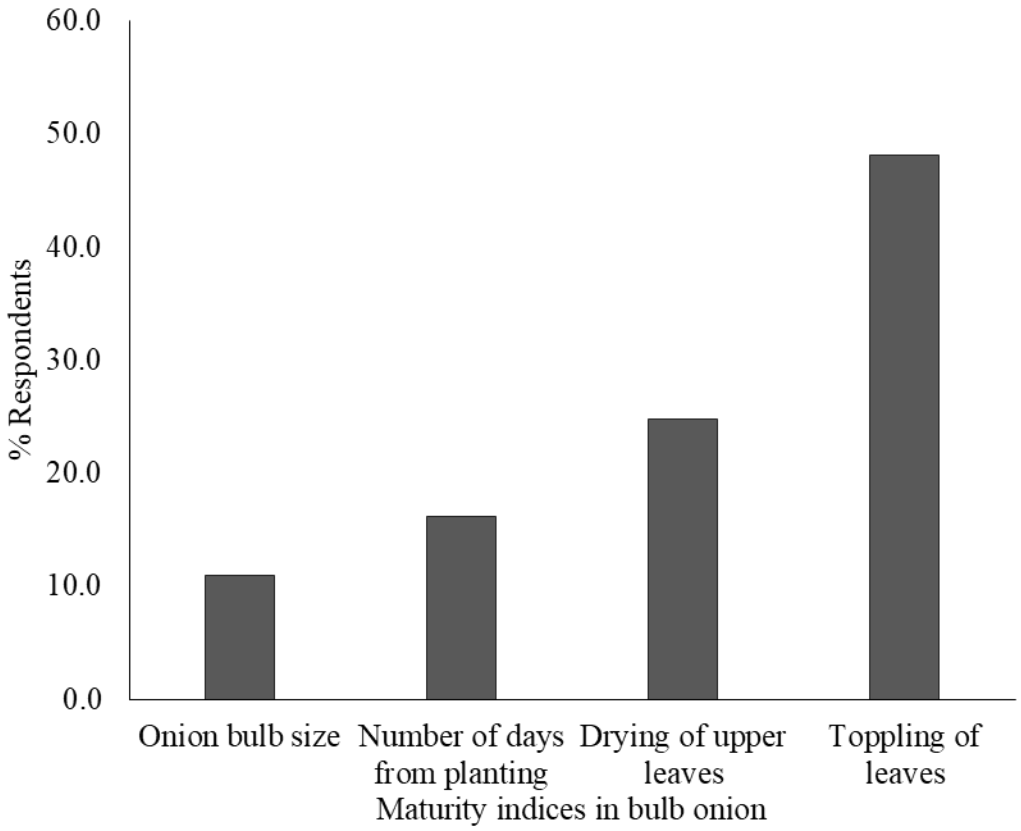

Figure 3.4: Overall Proportion of Respondents Across Counties of Kajiado, Meru and Bungoma who Used Various Maturity Indices to Harvest Bulb Onion (n=169)

Plate 3.1: A Field of Bulb Onions Showing Drying of Upper Leaves an Indicator of Maturity (Kajiado County)

3.3.1.2.2 Harvesting Tools for Bulb Onion

In Kajiado and Bungoma counties majority of respondents, 96 and 57% respectively did not use any tool during harvesting , while in Meru County a large proportion of respondents (68%) used machete. A larger proportion of respondents (59%) across the three counties did not use any harvesting tools while 41% of repondents used the machete for harvesting.

3.3.1.2.3 Postharvest Handling Practices of Bulb Onion

The respondents in Kajiado, Meru and Bungoma counties who were interviewed carried out various postharvest handling practices at farm level. In Kajiado County majority of respondents (57%) cured the bulb onions, while in Meru County only 5% carried out the practice. Majority of the reposndents sorted and graded bulb onions in Kajiado (62 and

60% respectively), Meru (50 and 89% respectively) and Bungoma (90 and 92% respectively) counties. Overall sixty nine percent of the respondents carried out sorting at farm level across the three counties. Overall in the counties of Kajiado, Bungoma and Meru counties a greater proportion (82%) of the respondents indicated that they were grading bulb onions at farm level (Table 3.2). Majority of the respondents (66%) graded according to size while 31% used colour. The results indicated that a small proportion of respondents (25%) across the three counties (Table 3.2) cured bulb onions while 59% of those who cured did it for less than ten days.

Table 3.2: Proportion of Respondents who Employed Different Bulb Onions Postharvest Handling Practices in Counties of Kajiado, Meru and Bungoma at Farm Level

Counties	Percentage of respondents			Number of respondents (n)
	Curing (%)	Sorting (%)	Grading(%)	
Kajiado	57	62	60	47
Meru	5	50	89	56
Bungoma	19	90	92	66
Overall	25	69	82	169

3.3.1.3 Level of Postharvest Losses

Majority of respondents, 95% in Bungoma County and 72% in Kajiado County stated that they experienced postharvest losses and only 5% in Meru County reported the same. During handling bulb onions at the farm level, 59% of respondents from all the counties indicated they encountered postharvest losses.

A significant number of respondents (85 and 71%) in the counties of Kajiado and Bungoma respectively stated that bulb onions handling at the farm level caused 5–30% of postharvest losses. A higher percentage of respondents (77%) in all three counties collectively stated that 5–30% losses were experienced during postharvest handling of bulb onions at the farm level (Fig.3.5).

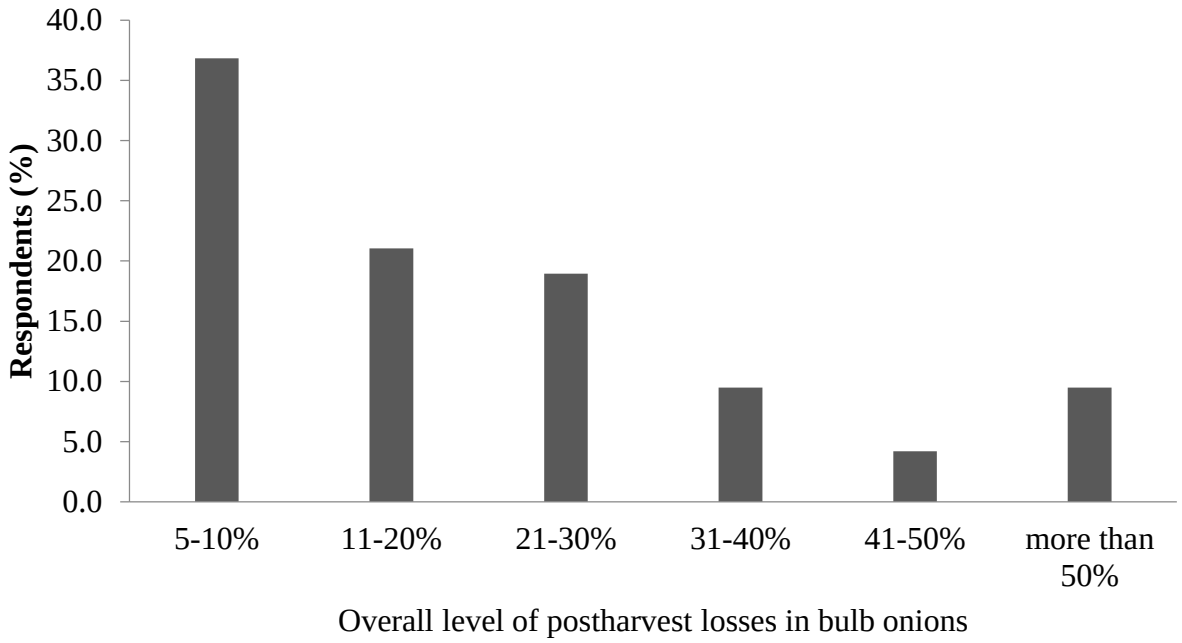

Figure 3.5: Percentage of Respondents Indicating Different Levels of Bulb Onion Postharvest Losses at Farm Level (N=169) Across the Three Counties

3.3.1.4 Causes of Postharvest Losses in Bulb Onion

During handling of bulb onions at farm level various causes of postharvest losses were identified by the respondents in Kajiado, Meru and Bungoma counties. Forty percent of the respondents indicated that postharvest losses in bulb onions were caused by rotting (40%), sprouting (37%), and 14 % showed shrivelling, while only 3% registered theft as (Fig. 3.6).

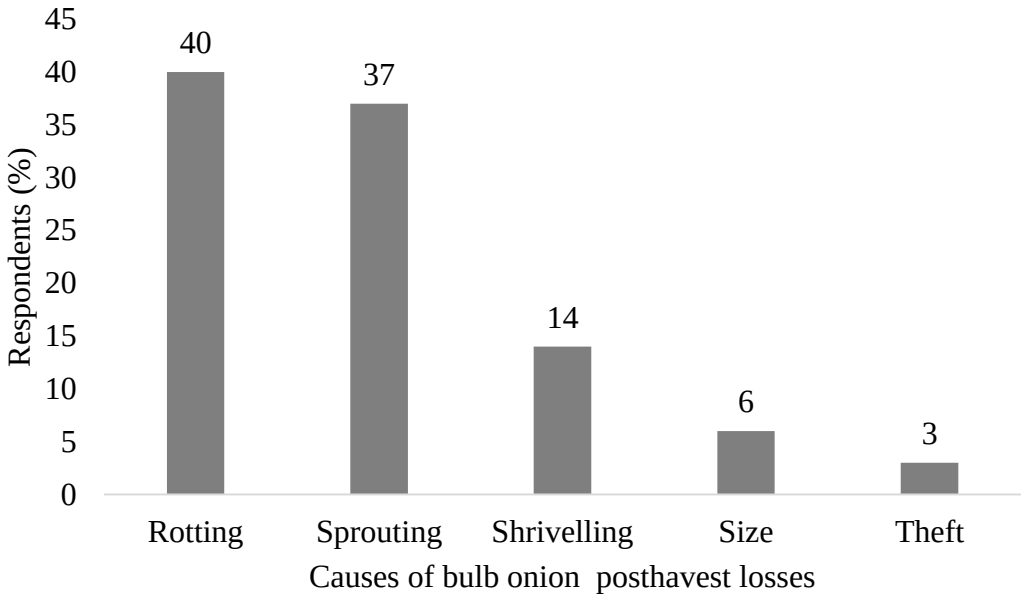

Figure 3.6: Overall of Proportion of Respondents Indicating Different Causes of Bulb Onion Postharvest Losses Across Kajiado, meru and Bungoma Counties (n=169)

According to the findings of this study, rots were the cause of 14% of the total postharvest losses in Kajiado County, 17% in Bungoma, and 2% in Meru. Overall, rotting accounted for 10% of all postharvest losses at the farm level across the three counties.

3.3.1.5 Factors Influencing Bulb Onion Postharvest Losses at Farm Level

Logistic regression modelling results indicated that secondary and tertiary education levels were significant at P≤0.05 to influence postharvest losses of bulb onion at farm level (Table 3.3). Farmers who had secondary and tertiary education levels were less likely to encounter postharvest loss in bulb onions (OR: 0.057 and 0.079) respectively. Land size under bulb onion production could significantly (P≤0.05) influence postharvest losses at farm level. Farmers with large land size under bulb onion production were more likely to encounter postharvest losses (OR: 1.636) (Table 3.3). The results also indicated

that use of machete as harvesting tool, curing and sorting significantly ($P \leq 0.05$) influenced postharvest losses of bulb onions at farm level. Farmers using machete during harvesting were likely to encounter postharvest losses with a positive coefficient 2.633 and Odd Ratio (OR) of 13.922 (Table 3.3). Farmers practicing curing and sorting were less likely to encounter losses (OR. 0.283 and OR 0.133 respectively).

Use of bicycles and donkeys (Plate 3.2) as transport mode from the farm to the market significantly influenced postharvest losses in bulb onions at $P \leq 0.05$. Farmers who used bicycles as a mode of transport were more likely to encounter postharvest losses than the ones using donkey (OR: 48.53, 0.024) respectively. Distance from farm to the market significantly ($P \leq 0.05$) influenced postharvest losses in bulb onion. Bulb onion transported for more than 5 Km to the market were more likely to encounter losses (OR: 68.94) (Table 3.3).

Table 3.3: Logistic Regression for Factors Influencing Bulb Onion Postharvest Losses at Farm Level (n=169)

Variable	Coefficient	Standard error (S.E)	Wald Stat.	P Value	Odds ratio (OR)
Gender	-1.006	.897	1.258	0.262	0.366
Age	-.028	.034	0.697	0.404	0.972
Primary education	-1.196	2.018	0.351	0.553	0.302
Secondary education	-2.867	1.185	5.855	0.016*	0.057
Tertiary education	-2.536	1.270	3.983	0.046*	0.079
Land size	-0.016	0.055	0.087	0.768	0.984
land size under onion production	0.492	0.299	2.714	0.099***	1.636
Skilled labour	1.461	1.114	1.720	0.190	4.310
Family labour	.943	1.539	0.375	0.540	2.567
Use of machete as a harvesting tool	2.633	1.042	6.382	0.012*	13.922
Curing	-1.267	0.417	9.243	0.002*	0.282
Sorting	-2.017	0.997	4.094	0.043*	0.133
Grading	0.654	1.133	0.334	0.563	1.924
Storage	-1.109	1.008	1.210	0.271	0.330
Toppling of leaves as maturity indices	1.902	0.521	13.322	<.001**	6.699
Human as a mode transport	.698	2.122	0.108	0.742	2.009
Bicycle as a mode transport	3.882	1.594	5.933	0.015*	48.534
Motor bike as a mode transport	-.522	1.185	.194	0.660	0.593
Donkey as a mode transport	-3.746	1.797	4.348	0.037*	0.024
Motor vehicle as a mode transport	.960	1.026	.876	0.349	2.612
Distance of the farm to the market (≥5km)	4.233	1.041	16.549	<.001**	68.940

Significant at *** 1 %, **5 %,* 10% level

2 Log likelihood=70.748, Cox &Snell R Square=0.601, Nagelkerke R Square=0.806, Power of correct prediction =56.7%, Chi-square value = <.001

Plate 3.2: Donkeys Transporting Bulb Onions to the Market in Bungoma County

3.3.2 Postharvest Losses at Market Level

3.3.2.1 Socio-Economic Characteristics of Respondents

Traders in the counties of Kajiado, Meru, and Bungoma revealed a range of socioeconomic characteristics. The average age of the traders who were interviewed was 38 years and 71% of the respondents were female. Majority of the respondents (59%) had completed their primary education, 35% their secondary education, and only 6% their higher education. The results of this study indicated that while some of the traders (51%) were farmers and operated side businesses including hair salons, farming, and security, 60% of the traders earned their livelihoods mostly by selling farm produce.

3.3.2.2 Postharvest Handling Practices of Bulb Onion

According to results of market survey 73% of the traders sourced their bulb onions from within the County where the markets were located, 25% from outside the County, and only 2% from outside the County. Results of this study reported that to transport bulb onions from farms to markets, 53% of traders used motor vehicles, 32% motor cycles and 15% used alternative modes of transportation like donkeys and tuktuks. The market

survey results indicated that bulb onions were packaged by majority of traders (61%) in nets, 35% in bags, and only 3% in cartons during transportation.

At the market level, 31% of traders did not sort their onions before selling. Fourty three percent of the traders carried out sorting due to rotting symptoms, with 46% indicating it was because of size while 7% indicating they did so because of sprouting while 4% due to maturity (Fig.3.7).

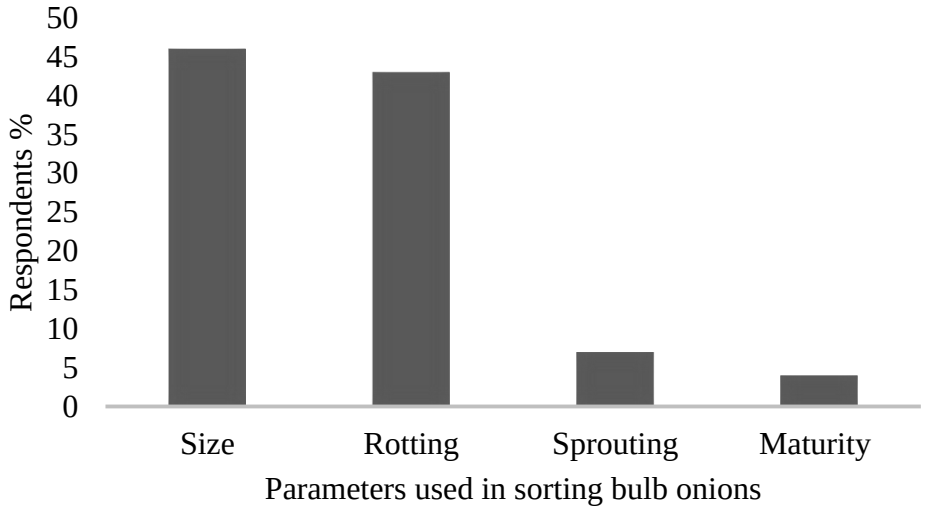

Figure 3.7: Proportion of Traders Showing Different Parameters Considered when Sorting (n=72)

Overall in the three counties of Bungoma, Kajiado and Meru grading of bulb onion was carried out by 88% of the traders. This was done according to size 57%, colour 34% and 9% other characteristics such as rotting, dryness and maturity. Eighty six percent of the traders stored the produce during their marketing period. Majority of the traders (52%), stored in open air while only 48% of the traders had stores. Fifty two percent of traders kept the bulb onions for 2-4 weeks while 47% stored for 0-1 weeks, only 1% of traders stored the bulb onions for more than five months.

3.3.2.3 Level of Postharvest Losses in Bulb Onion

A large proportion of traders (88%) experienced 5-30% overall postharvest losses while only 12% of the traders experienced more than 31%. Sixty eight percent of the traders indicated that rotting caused losses, 17% indicated sprouting, 13% shrivelling while only 2 % indicated size (Fig.3.8).

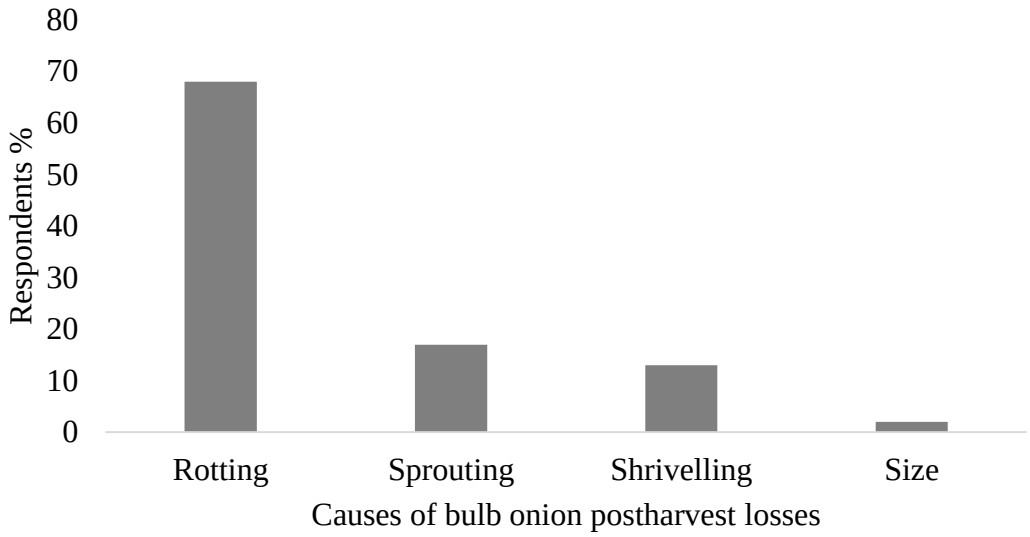

Figure 3.8: Percentage of Respondent Indicating Different Causes of Postharvest Losses at the Market Level (n=72)

The results indicated that 14% of overall postharvest losses experienced during marketing was through rotting.

3.4 Discussion

Demographic data indicate the characteristics of a population which includes age, sex, education level that may affect agriculture negatively or positively. The average age of Kenyan farmers was 60 years as reported by KNBS, (2018) however, the results indicated the average age for onion bulb grower was 40 years.

Onion bulb enterprise requires intensive labour and other farm management techniques (Calica and Cabanayan, 2018) therefore relatively young farmers were involved in bulb onion production. Education level influenced postharvest losses incurred at farm level. Having no education or primary education level limits farmers to gain knowledge and skills on postharvest handling during trainings. In developing world two-thirds of population live in rural areas with about 475 million farmers in small holder farms of about two hectares according to Rapsomanikis, (2015). The author also reported that in Kenya farmers owned an average farm size of 1.2ha, however according to the results bulb onion producers in Kajiado East, Buuri and Mt. Elgon Sub- Counties owned on average 1.6 ha with 0.8ha under bulb onion production. Onion production has gained popularity over the years due to its low supply and high demand in domestic markets and HCD, (2018) ranked it first under aromatic crops production profile. This resulted to farmers opening up vast areas previously not engaged in onion bulb production to meet market demand, case in point were Kajiado East and Mt. Elgon Sub-Counties. Farmers with large land sizes were likely to harvest more bulb onion than farmers with smaller land size leading to increased postharvest losses. Therefore proper postharvest techniques such as curing, sorting and grading should be employed to minimize losses.

According to Kader, (2005) maturity is defined as the stage at which the produce has developed consumers' favourable taste, appearance and exhibit acceptable shelf life. Bulb onions were ready to harvest when the top leaves start to dry and fall (Petropoulos *et.al*., 2017). The study by Wright *et al*. (2016) indicated that bulb onions harvested when 60-80% of foliage had dried and topped down led to good post-harvest quality and enhanced storage life. Bulb onion harvested when less than 50% leaves had topped down have poor physical quality and a short shelf life (Kiura *et al.,* 2021). Majority of farmers 60-70% in the study were aware on bulb onions maturity indices. Maturity indices should be used in combination, since if only one maturity index is used it is likely to influence postharvest losses positively during postharvest handling of bulb onions. However the exact time to harvest bulb onion depend on agronomic practices, onion variety and

climatic conditions according to Petropoulos *et al.* (2017). Nonconformity to optimum harvesting stage may result to loss of quality, quantity and monetary returns as stated by Michailides *et.al.* 2009. Harvesting of bulb onion should be done appropriately to minimize mechanical injuries such as bruises and cuts. The results of this study indicated that postharvest losses are influenced by harvesting techniques, such as use of a machete. The study findings correlate with those of Banjaw (2017), who noted that employing inappropriate harvesting tools could result in cuts and bruises which influence postharvest losses in horticultural crops.

To ensure reduction of postharvest losses proper harvesting methods such as hand lifting in bulb onions should be employed since it reduces bruising on bulb onion (Banjaw, 2017). Postharvest handling practices such as curing, sorting and grading reduces postharvest losses in crops (Kitinoja and AlHassan, 2012). After harvesting bullb onion should be subjected to curing to dry their outer scales, reduce skin cracks and to narrow the neck, thus reducing pathogenic rot (Petropoulos *et al*., 2017). According to Eshel *et al.* (2014) curing bulb onion for nine days at 30° C narrowed bulb onion neck and reduced rotting by 80%. Curing as one of the postharvest handling practices in bulb onion contribute to maximum bulb quality, reduce losses through water loss and pathogenic infections (Eshel *et al.,* 2014). Sorting of bulb onion involves removal of injured and rotten bulb onions, lack of sorting before packing promoted spread of diseases to healthy produce leading to losses (Kitinoja and Kader, 2015). A study carried out by Gorrepati *et al.*(2018) showed that weight loss, sprouting and rotting which are physical losses were major causes of bulb onion postharvest losses at farm level. Rotting was a major cause of postharvest losses both at farm level as also reported by Calica and Cabanayan, (2018). Physical losses in bulb onion could be influenced by immature harvesting, inappropriate harvesting techniques such use of machetes as a harvesting tool, limited curing and poor handling.

In developing countries market location is far from farms and majority of roads are inaccessible, thus traders use various mode of transport in order to reach the markets in

good time (Arah *et al*., 2016). Taking bulb onions to markets situated away from farms, that is, at more than 5 Km exposed the produce to postharvest losses due to market delays. Farmers who used bicycles to transport bulb onion from farms to markets were more likely to cause postharvest losses since they piled them on top of each other compressing the produce resulting to mechanical injury. While those using donkeys hang the packs on both sides and therefore the produce is protected from compression. A study by Calica and Cabanayan, (2018) reported that 3% postharvest losses occurred during transportation of bulb onion therefore use of appropriate transport mode is important in minimizing postharvest losses.

Packaging during transportation protect bulb onions from mechanical injuries, contamination from physical, biological and chemical sources. Packaging also provides sizeable units that can be used in marketing. Since majority of traders used nets in packaging, this would provide the bulb onions with good aeration thus reducing build-up of heat resulting from respiration.

3.5 Conclusion

From the current study it was concluded that up to 5 to 30% losses in bulb onions occurred both at farm and market level. In Bungoma County majority of farmers experienced higher losses compared to Kajiado and Meru counties. In addition, at market and farm levels postharvest losses caused by rotting were 14 and 10% respectively of the total losses. In Bungoma County 17% of the total postharvest losses in bulb onions are caused by rots, in Kajiado 14% and in Meru 2%. At farm level the losses were influenced by various factors such as respondent's education level, size of land under bulb onion production, use of machete as a harvesting tools, limited curing and sorting, use of bicycles and donkeys as transportation mode. At farm level the major causes of postharvest losses in bulb onions were mainly caused by rotting followed by sprouting and finally shrivelling. Therefore, based on results of this study it is recommended that

postharvest reduction strategies in bulb onion should focus on decreasing occurrence of rotting both at farm and market level.

CHAPTER FOUR

IDENTIFICATION OF FUNGAL PATHOGENS CAUSING BULB ONIONS (*ALLIUM CEPA* L.) POSTHARVEST ROT IN SELECTED MAJOR GROWING REGIONS OF KENYA

Abstract

Rot is a major cause of bulb onion losses in Kenya, accounting for about 14 % of total postharvest losses. In Kenya, the fungi associated with bulb onion postharvest rot of onion postharvest rots are not well known. Therefore, this study aimed at identifying the fungal pathogens contributing to bulb onion postharvest rot in major growing regions of Kenya. Bulb onion sample was collected from seven major markets and isolates were obtained by cutting 3 mm tissue segments from the edges of rotten lesions. These were then plated on water agar and incubated for seven days at room temperature (23 ± 3°C). After seven days, mycelial plugs from the growing edge of each colony were sub-cultured in potato dextrose agar and incubated for ten days. Fifty fungal isolates were obtained from the isolations and *in vitro* pathogenicity test was done on bulb onions. Eighteen fungal isolates that turned out to be pathogenic were inoculated in bulb onions to assess their level of virulence by measuring size of the lesions after 21 days of incubation at room temperature (23 ± 3°C). The fungal isolates caused ($P \leq 0.05$) significant different sized lesions, ranging from 0.4±0.1 to 2.6±0.5 cm. Based on morphological characteristics the 18 fungal pathogenic isolates were identified as *Fusarium* spp. and were grouped into three clusters. Molecular technique confirmed the three *Fusarium* spp. clusters as *Fusarium oxysporum* f.sp. *cepae* (55%), *F. acutatum* (17%) *and F. solani* (28%). The *Fusarium oxysporum* f.sp. *cepae* was predominantly isolated from bulb onions collected in Bungoma County, while *F. solani* was mainly obtained from samples in Kajiado County and *F. acutatum* on bulb onions from Meru County. The results of this study indicated that the three *Fusarium* species are the main fungal species causing postharvest rot in the major bulb onion growing regions of Kenya.

Application of appropriate postharvest technology such as curing before storage would minimize postharvest rot in bulb onion.

4.1 Introduction

Postharvest diseases are the main contributor to bulb onion losses during postharvest handling. In Asia 20-30% losses occurs due to postharvest diseases while in Serbia 20-50% losses are often reported (Duduk *et al*., 2017). In addition, in Kenya bulb onion postharvest rot contributes 14% of the total postharvest losses at market level (Gathambiri *et al*., 2021). Bulb onion rot can be associated with several pre-and postharvest factors such as variety, soil properties, climatic conditions, agronomic management practices and storage conditions (Yurgel *et al.,* 2018). Postharvest diseases in bulb onion mostly begin in the field and become severe during handling. Rasiukevičiute *et al.,* (2016) reported that *Aspergillus* spp., *Botrytis* spp. and *Fusarium* spp. which are known to be seed-borne fungi can contribute to rot during postharvest handling. Fungal infections are the main cause of postharvest harvest diseases in bulb onions according to Hye *et al*. (2018).

It is essential to isolate and identify fungal pathogens in order to understand the biology of the organism and develop strategies for postharvest disease management techniques. According to Yurgel *et al.* (2018), *Aspergillus* spp., *Agaricomycetes* spp., *Alternaria* spp., *Fusarium* spp., *Candida* spp., *Clavispora* spp. and *Botrytis* spp. are some of the fungal pathogens contributing to postharvest diseases in bulb onion. Various countries have reported diverse fungal pathogens as causative agents of postharvest rot. In Ghana, Adongo *et al.* (2015) highlighted *Aspergillus niger, A. flavus, Penicillium* spp., *Rhizopus solonifer* and *Fusarium oxysporum* while in Iran *Fusarium oxysporum* f.sp. *cepae, F. solani, F. redolens* and *F. proliferatum* were identified (Ghanbarzadeh *et al*., 2014). In Sudan, *Aspergillus niger* was reported to contribute to 80% of bulb onion postharvest rot (El-Nagerabi *et al*., 2003).

In Kenya, several fungal pathogens; *Lasiodiplodia thebromae, Neofusicoccum parvum, Nectria pseudotrichia, Fusarium solani, F.oxysporum, F.equiseti* and *G. candidum* have been identified to cause postharvest diseases in avocado fruit (Wanjiku *et al*., 2020).

Due to their diversity in species and potential for including both pathogenic and non-pathogenic microorganisms, identification of fungi can be complicated (Taylor *et al*., 2016). Therefore, careful consideration must be used while choosing isolation and identification techniques. Fungal isolates are obtained from the tissues of bulb onions and grown in a variety of synthetic and/or natural growth media, such as yeast malt extract agar, potato dextrose agar, and malt extract (Al-Enazi *et al*., 2018). After growing in media, the initial stage in identifying fungal pathogens is morphological identification based on colony characteristics, as well as macroscopic and microscopic assessment (Matuo, 1973). However, morphological identification of fungal pathogens has been a challenge even to mycologist. This is due to the inconsistent categorization of fungi that has been presented by different researchers, primarily based on microscopic characteristics that can vary greatly depending on the surroundings and growing media used. Furthermore, the identification and diagnosis of fungi may become more difficult due to the deterioration of the cultures and the development of mutations (Ghanbarzadeh *et al*., 2014). Recently, the use of species-specific primers that has been developed based on DNA sequence polymorphism has made it possible to quickly, accurately, and reliably identify fungal pathogens (Lager, 2011). Therefore, accurate fungal pathogens identification is obtained by combining the molecular and morphological methods.

Although postharvest rot is a major constraint in enhancing bulb onion productivity in Kenya, information on pathogenic fungi associated with postharvest diseases is limited. Therefore, this study was carried out to identify the pathogenic fungi contributing to postharvest bulb onion rot in the major growing regions of Kenya using morphological and molecular techniques.

4.2 Materials and Methods

4.2.1 Study Sites and Bulb Onions Sample Collection

Onion samples were collected from major markets in Kajiado and Kitengela (Kajiado County), Meru and Nkubu (Meru County), and Kimilili, Cheptais and Chwele markets (Bungoma County) (Fig 4.1).

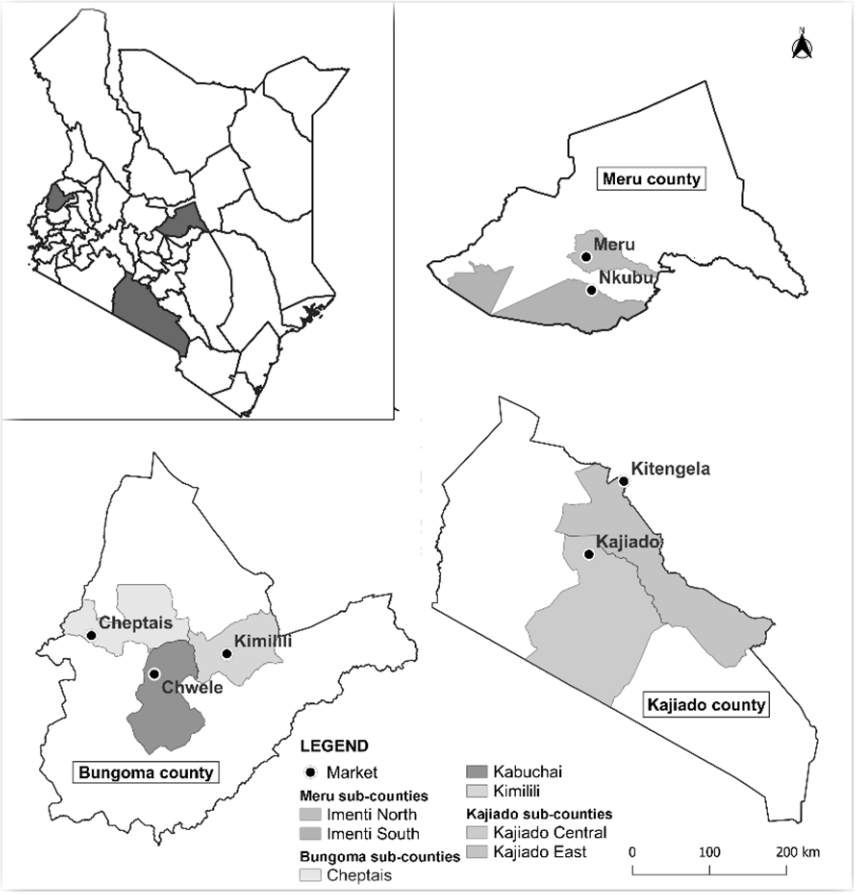

Figure 4.1: Locations of the Seven Markets (Dots) Drawn Using QGIS Geographical Information System (Version 3.14) with GPS Data from Bungoma, Meru and Kajiado counties

In each market, a list of all bulb onion wholesalers was compiled and a simple random sampling procedure was used to select the traders selling at least 100 kg or more per week.

A formula to determine the number of bulb onion samples to be taken per wholesaler was based on 15% of the total postharvest losses occurring at market level and 10% of the total losses caused by rot (Sharma, 2016).

Number of samples = (0.15 QT)×0.01)

Where;

QT is quantity sold per week

Bulb onions that were soft and gave in slightly under finger pressure at the base and neck were collected from the markets. In total, a sample of 524 bulb onions was collected from the seven markets (Appendix 3). The bulb onions were coded based on the market they were collected from; KJ (Kajiado market), KT (Kitengela market), MR (Meru Market), NK (Nkubu market), KM (Kimilili market), CP (Cheptais market) and CL (Chwele markets). The sample was collected in brown bags, placed in cool boxes and transported to the Kenya Agricultural and Livestock Research Organization (KALRO) Kandara Postharvest Laboratory. They bulb onions were placed on the laboratory bench at room temperature (23 ± 3℃) and after one week, those with visible signs of rot were used for fungal isolation

4.2.2 Fungal Isolation from Bulb Onions

A total of 300 bulb onions with clear symptoms of rot were prepared for fungal isolation by stripping the outer dry scales and washing under running water to remove soils and any debris. Fungal isolation was done a as described by Li *et al.* (2017) with slight modification. The bulb onions were sterilized in 70% alcohol for 60 seconds and rinsed three times in a row with sterile distilled water and blot-dried with cotton wool. Using a

sterile scalpel blade, the bulb onions were cut open at the neck to expose the infected inner scales. Two-3 mm segments per bulb onion were cut out from the edges of the lesions with a sterile scalpel. Each segment was plated in Petri dishes containing water agar (Oxoid LP0013 Agar Technical No. 3), and placed in a laboratory growth chamber at 23 ± 3℃ under natural light for seven days. After this time, the mycelia plugs from the growing edge of each colony were sub-cultured in Potato Dextrose Agar (PDA) (Oxoid CMO-139) containing an antibiotic (Aminobenzylpenicillin) at a concentration of 0.05g/litre to prevent the growth of bacteria.The Petri dishes were sealed with Para-film® and incubated at room temperature (23 ± 3℃) for ten days to enhance sporulation. From each culture some mycelia were cut-off and observed under microscope (B-350 Optika) to confirm sporulation.

4.2.3 Isolation of Pure Fungal Cultures through Hyphal-Tip Method

To obtain pure fungal isolates hyphal-tip procedure was used according to Rahman *et al*. (2018); Niemeyer and Andrade, (2016) with slight modification. Spore suspension was prepared by soaking ten-day-old fungal colonies in five millilitre sterilised water and carefully spread with a sterile L-shaped plastic rod. The fungal suspension was sieved into sterile 30 ml universal plastic tubes using a sterile muslin cloth, to remove mycelia. From each isolate, 10µl of spore suspension was placed into each of the four haemocytometer (Marienfeld, German) chambers (A, B, C and D). The spores in each chamber were counted and the average number of spores per isolate was calculated.

The spores per ml was calculated using the formula of Gilchrist-Saavedra *et al.* (2006) :

$$\text{Spores/ml} = (n) \times 10^4$$

Where: n = the average number of spores counted.

The spore suspension concentration was adjusted to the desired concentration of 1.0×10^5 spores/ml using sterilised water. One millilitre of 1.0×10^5 spores/ml was drawn from the

spore suspension, then spread on water agar using a sterile plastic L-shaped plastic rod. The plates were left open for 20 minutes under laminar flow hood to allow drying. They were then sealed with Para-film® and incubated at 23 ± 3℃ for 24 hours. A single strand of one day old mycelia observed under a microscope was cut from the edge and placed on PDA covered with small pieces of sterile filter papers (Whatman medium fast qualitative circle 90 mm). This was incubated at room temperature (23 ± 3℃) under natural light for seven days. Seven-day old isolates grown on pieces of sterile filter papers were peeled off, placed in clean Petri dishes and dried in silicon dioxide (SiO_2) (silica gel self-indicating-course blue) for seven days (Fong *et al*., 2000). Fifty dried pure fungal isolates on sterile filter papers were placed in small brown envelopes and stored at 4° C in a refrigerator.

4.2.4 Fungal Isolates Pathogenicity Evaluation on Bulb Onions

Fifty preserved pure fungal isolates obtained from bulb onion were evaluated for pathogenicity *in vitro* according to Li *et al*. (2017) with slight modification. Three millimetre of filter paper containing pure preserved fungal isolates were placed on PDA and incubated for 10 days at room temperature (23 ± 3℃) under natural light. Spores were obtained by flooding the colonies with two millilitre gelatin solution (0.2%) to ensure the suspension would adhere on bulb onions, and was spread carefully using a sterile L-shaped plastic rod. Using a muslin cloth the fungal suspension was sieved in 30 ml plastic universal tube to remove mycelia. The spore suspension concentration was calculated as previously described and then adjusted to 1.0 x 10^5 spores/ml using sterile water.

Using a sterile cork-borer, a wound of about 5 mm in diameter and 3 mm deep was punctured in healthy bulb onions (*Red couch* variety) after surface sterilization with 70% ethanol. The punctured bulb onions were inoculated with 40 µl of 1.0 x 10^5 spores/ml suspension. Each isolate was inoculated in six bulb onions and other six bulb onions inoculated with 2% gelatin solution were used as control. An experiment of three bulb

onions per replicate in two replications for each isolate was set in a completely randomized design (CRD). After 21 days of storage at room temperatures (23 ± 3°C), the infected bulb onions were dissected with a sterile scalpel blade through the point of inoculation. Rotting symptoms were compared to bulb onions initially collected from markets.

In addition, one three-millimetre of the inoculated bulb onion segment was cut from the rotten lesion, plated on PDA and incubated at room temperature (23 ± 3°C) for seven days. Fungal colony colour of re-isolated fungi were noted and compared to the original fungal isolate.

4.2.5 Virulence Evaluation of Pathogenic Fungal Isolates on Bulb Onions

Virulence assessment of eighteen preserved pathogenic isolates was done as described by Duduk *et al*. (2017), using healthy bulb onions (Red couch variety) that were purchased from a commercial farm in Kajiado County. Spore suspension of the isolates was prepared as previously described in section 4.2.3. Using a sterile cork-borer a wound of about 5 mm in diameter and 3 mm deep was punctured in healthy bulb onions. Forty microliter of 1.0 x 10^5 spores/ml suspension was inoculated in the wound. Nine bulb onions per isolate were inoculated while nine bulb onions were inoculated with 2% gelatin solution and used as control. Each treatment had three bulb onions replicated three times. These were arranged on trays in a CRD and placed on laboratory benches at room temperature (23 ± 3°C) for 21 days. Using a sterile blade, the bulbs were dissected into half and from the point of inoculation the diameter of the lesions was measured using a ruler.

4.2.6 Fungal Pathogen Morphological Identification

4.2.6.1 Assessment of Fungal Isolates Colony Radial Growth Rate

Colony radial growth rate was determined by tabulating Radial Growth Rate (RGR) for each isolate according to Pal *et al.* (2019) with slight modification. Eighteen isolates found to be pathogenic were grown on PDA at room temperature (23 ± 3°C) in triplicates.

From the point of initial inoculum, two diameter readings perpendicular to each other (Plate 4.1) were recorded from the underside of the petri-dish on day four after inoculation.

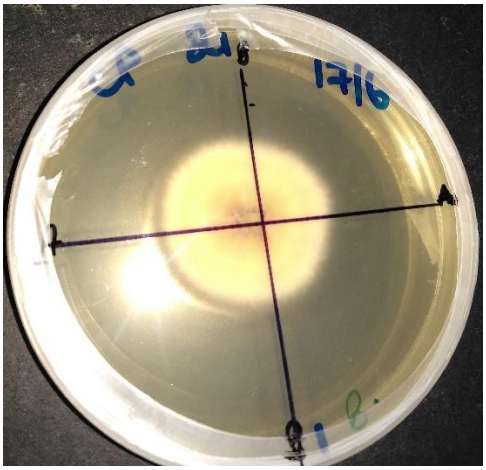

Plate 4.1: Perpendicular Lines A and B on the Underside of the Petri-Dish Indicating Where the Diameter of the Fungal Colony was Measured

Colony radial growth rate (cm/day) of each isolate was calculated using the formula:

RGR (cm/day) = (D/2)/ d

Where;

RGR= Radial Growth Rate per day (cm/day)

D = Average colony diameter

d = Number of days incubated

4.2.6.2 Colony and Conidia Description

For morphological identification, eighteen pathogenic isolates were grown on Carnation Leaf-Piece Agar (CLA) for 10 days at room temperature (23 ± 3℃). Carnation Leaf-Piece Agar was prepared by placing sterile carnation leaf pieces into a petri dish and adding 2% water agar (20 g agar in one Litre of water). Culture characteristics of each isolate were identified by describing their colony appearance and pigmentation and conidia structures (Duduk *et al.*, 2017; Manoj *et al*., 2016). Using a DP72 digital camera, Japan and an Olympus BX51 powered microscope with DIC Nomarski view, conidia photos in magnification of 400X were taken. In addition, 10 micro and macro-conidia of each isolate were randomly selected, and their sizes measured from stained slides using the microscope.

4.2.7 Molecular Identification of Pathogenic Fungal Isolates

4.2.7.1 Fungal Isolates DNA Extraction

DNA extraction was done on 18 pathogenic isolates as described by Aamir (2018) and Cenis (1992). Five-day old culture mycelia of each isolate grown on PDA were scraped using a sterile scalpel and placed in sterilised Eppendorf micro-centrifuge tubes containing ceramic bead after which 500 µl of extraction buffer (1M Tris-HCL, 5M NaCl, 0.5M EDTA, 0.5% SDS) was added and the mixture placed in faststep® -24 genogrinder at 4 m/s for 1 minute to grind the mycelia. A 200 µl of 3M sodium acetate pH 5.5 was added and the samples kept at -20˚C for 10 minutes, then centrifuged at 13,000 rpm for five minutes. Seventy microliter of the supernatant was pipetted into sterilised tubes and an equal amount of iso-propanol added and left at room temperature (23 ± 3℃) for five minutes. The samples were centrifuged at 13,000 rpm for 10 minutes and the supernatant was poured out to obtain the DNA pellets. A 500 µl of 70% ethanol was added to wash the DNA pellets and centrifuged at 13,000 rpm for five minutes. Ethanol as supernatant was decanted and the samples were air dried under the laminar flow hood for 30 minutes. Fourty microliter of Tris-EDTA (TE) buffer was added to solubilize the DNA pellets and

left at room temperature for 30 minutes. The quality of DNA was assessed using a Thermo scientific Nano Drop 2000c spectrophotometer, USA.

4.2.7.2 Fungal Isolates DNA Amplification

Amplification of the translation elongation factor (TEF) 1α gene and internal transcribed space (ITS) regions was done using primer pair of ef1 (5'ATGGGTAAGGA(A/G)GACAAGAC-3' and ef2 (5'GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') (Chehri *et al*., 2012), and ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) *(*Whit*e et al*.,1990) respectively. ITS are the universal fungi primers while ef1 primers are *Fusarium* species-specific (Kalman *et al*., 2020). DNA amplification was carried out according to Karlsson *et al.* (2016) with slight modification. It was done using GeneAMP 9700 DNA Thermal Cycler (Perkin-Elmaer) with a reaction volume of 20 µl containing: 10 µl of one-taq quick load 2X master mix with standard buffer, 0.4 µl of 10 µM of each primer, 2 µl of 50ng/ml template DNA and 7.2 µl nuclease free water. The amplification process involved initial denaturing step at 94˚C for 30s, followed by 35 cycles at 94˚C for 30s and annealing process for ITS4 and ITS5 primers at 56 for 45s while ef1 and ef2 primers were done at 60˚C for 45s. Extension was done at 68˚C for one minute and the final extension at 68˚C for five minutes for both primer pairs. The quality of PCR products was confirmed on 1 % agarose gel and visualised under UV light using EDURO^{TM} GDS, UK. The amplified rDNA was submitted for Sangar sequencing with ef1 and ef2 forward and reverse primers at Inqaba Africa Genomic platform, South Africa. The 18 obtained sequences were subjected to Basic Local Alignment Search Tool (BLAST) in Fusarium-ID database to enable identification of eighteen pathogenic isolates. The 18 sequences (Accession Numbers OL631163 to OL631180) were submitted to National Centre for Biotechnology Information (NCBI) databases.

4.2.8 Data Analysis

Mean of colony radial growth rate and lesion size were calculated and subjected to one-way ANOVA using GENSTAT statistical software (15th Edition). The means were separated using Tukey's test at 5% probability level. The mean spore size for each isolate was calculated using Microsoft excel program.

4.3 Results

4.3.1 Isolated Fungal Microorganisms and Pathogenicity

Out of a sample of 524 bulb onions collected from major markets in Bungoma, Kajiado and Meru Counties, 300 bulb onions developed symptoms that were clearly visible in the inner scales (Plate 4.2). They included 8% bulb onions from Kitengela market, 12% from Kajiado market, and 17% from Meru market, 13% from Nkubu market, 25% from Chwele market, 16% from Cheptais market and 9% from Kimilili market.

Plate 4.2: Onion Bulb from Bungoma County Showing Symptoms of Rot in the Inner Scales

A total of 50 fungal isolates were obtained from 300 diseased bulb onions. Upon inoculating healthy bulb onions of Red couch variety with 50 fungal isolates, 18 of them exhibited symptoms similar to those initially observed in infected bulb onions collected

from various markets. This study did not take into account the remaining 32 fungal isolates since they did not cause rot on healthy bulb onions.

The symptoms observed for the eighteen fungal isolates included brown discolourations extending to the neck or base in the inner scales of the bulb onion (Plate 4.3) and were identified to satisfy Koch's postulates. Disease symptoms were not observed in control treatments. The re-isolated fungal isolates showed similar colony characteristics on PDA as observed for original fungi isolated from bulb onions collected from different markets.

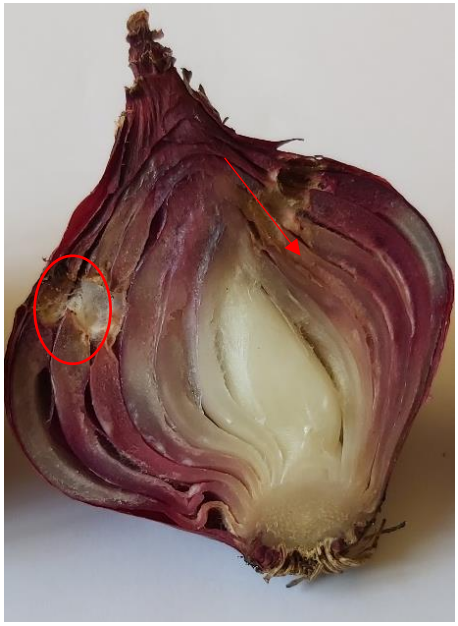

Plate 4.3: Bulb Onion Indicating Rot Symptoms after Inoculating with a Fungal Isolate and Incubated at Room Temperature (23 ± 3◦C) for 21 Days

Out of the eighteen pathogenic isolates 67% were obtained from Bungoma County samples, 22% from Meru County and 11% from Kajiado County.

4.3.2 Virulence of Pathogenic Fungal Isolates

The isolates caused mean lesion size ranging from 0.4±0.1 to 2.6±0.5 cm (Fig. 4.2). KTA isolate caused the largest mean lesion size (2.6±0.5cm) while KM26 caused the smallest (0.4±0.1cm) lesions on bulb onion. Mean lesion size (2.6±0.5cm) formed by KTA isolate was significantly ($P \leq 0.05$) larger compared to lesions caused by KTB (1.1±0.23 cm), NK (1.0±0.36 cm), CP110 (1.0±0.19cm), CP19 (0.8±0.23cm), CL110 (1.0±0.12cm), CP12 (0.8±0.19cm) and KM26 (0.4±0.10cm) on bulb onions. Fungal isolate KM26 caused significantly ($P \leq 0.05$) smaller lesion size (0.4±0.1cm) compared to lesions caused by CL14 (1.5±0.13cm), CPF2 (1.9±0.33cm), FMR2 (1.9±0.03cm), MR31 (2.0±0.19cm), FMR1 (2.2±0.08cm) and KTA (2.6±0.53cm) isolates on bulb onion.

Fungal isolates; FMR1 (2.2±0.08cm), MR31 (2.0±0.19cm), FMR2 (1.9±0.03cm) and NK (1.0±0.36 cm) collected from Meru County developed lesions on bulb onions that were not significantly ($P > 0.05$) different from each other in size. Among the isolates collected from Bungoma County, CPF2 isolate formed significantly ($P \leq 0.05$) larger lesion (1.9±0.3cm) compared to lesion (0.4±0.1cm) produced by KM26 isolate collected from the same County. In addition, CPF2 isolate caused lesion that was not significantly different ($P > 0.05$) in size compared to those formed by CL41 (1.5±0.13cm), CL13 (1.3±0.20cm), KM29 (1.3±0.42cm), KM28 (1.3±0.11cm), CP17 (1.2±0.41cm), CP21 (1.2±0.31cm), CP110 (1.0±0.19cm), CP19 (0.8±0.23cm), CL110 (0.8±0.12cm) and CP12 (0.8±0.19cm), isolates collected from the same County of Bungoma. KTA fungal isolate obtained from Kajiado County caused statistically ($P \leq 0.05$) larger lesion (2.6 ±0.53) compared to lesion (1.1±0.23cm) caused by KTB isolate from the same County.

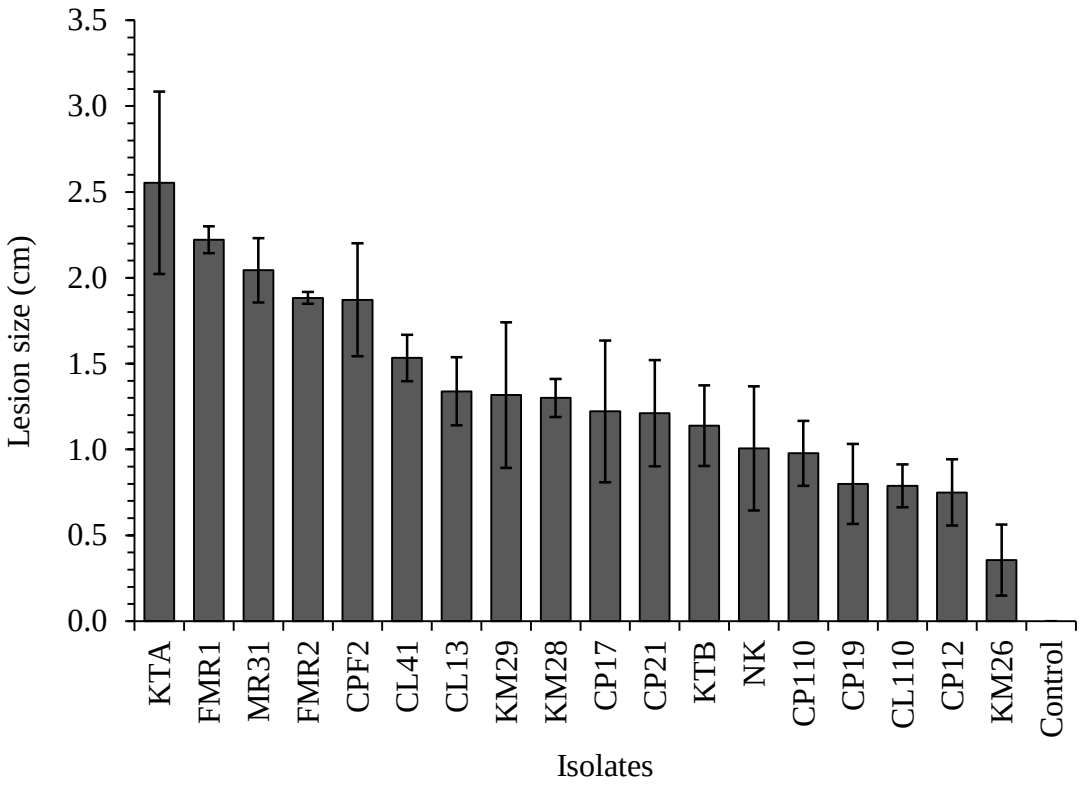

Figure 4.2: Mean Lesion Size (Cm) On Bulb Onion 21 Days after Inoculation with Different Fungal Isolates

Error bars represents standard error (SE) of the mean. The isolates codes relate to the market where bulb onion samples were collected

4.3.3 Fungal Isolates Cultural and Morphological Characteristics

4.3.3.1 Fungal Colony Growth Rate

Colony radial growth rate for the eighteen fungal pathogenic isolates ranged from 0.6 ±0.078 to 1.0±0.004 cm per day. An isolate collected from Meru market (FMR2) had the highest colony growth rate (1.0±0.004 cm cm/day) while KTB isolate collected from Kajiado market had the least colony growth rate of 0.6 ±0.078 cm/day (Fig.4.3). FMR2 isolate grew significantly ($P \leq 0.05$) faster (1.0±0.004cm/day) compared to FMR1 (0.6

±0.005 cm/day), CP110 (0.8 ±0.002 cm/day), KTA (0.8 ±0.044 cm/day), CL13 (0.8 ±0.046 cm/day), CP19 (0.7 ±0.108 cm/day), KM26 (0.7 ±0.057 cm/day),

CP17 (0.7 ±0.073 cm/day), CP21 (0.7 ±0.068 cm/day), CL110 (0.7 ±0.022 cm/day) and KTB (0.6 ±0.078 cm/day), isolates (Fig.4.3). Though FMR2 had the highest colony growth rate among the isolates (1.0±0.004cm/day), the growth rate was not significantly ($P>0.05$) different compared to that of CL41 (0.9±0.017cm/day), NK (0.9±0.019cm/day), KM28 (0.9±0.045cm/day), MR31 (0.9±0.031cm/day), CP12 (0.9±0.047cm/day), CPF2 (0.9±0.072cm/day) and KM29 (0.9±0.042cm/day) isolates. In addition, KTB isolate had the lowest colony growth rate (0.6 ±0.08 cm/day) but it was not statistically ($P>0.05$) different to growth rates of CL110 (0.7 ±0.022 cm/day), CP21 (0.7 ±0.068 cm/day), CP17 (0.7 ±0.073 cm/day), KM26 (0.7 ±0.057 cm/day), CP19 (0.7 ±0.108 cm/day), CL13 (0.8 ±0.046 cm/day) and KTA (0.8 ±0.044 cm/day), fungal isolates as indicate in Figure 4.3. Colony growth rate of FMR2 (1.0±0.004 cm/day), NK (0.9±0.02cm/day) and MR31 (0.9±0.03cm/day) isolates obtained from Meru County were not significantly different ($P>0.05$) from each other. However, FMR2 (1.0±0.004cm/day) grew significantly ($P\leq0.05$) faster compared to FMR1 (0.8±0.005 cm/day) isolate obtained from the same County of Meru. Isolates from Kajiado County; KTA (0.8±0.044 cm/day) and KTB (0.6 ±0.08 cm/day) exhibited growth rate that were not significantly ($P>0.05$) different from each other. Among the isolates from Bungoma County, CL41 isolate exhibited statistically ($P\leq0.05$) higher colony growth rate (1.0±0.004 cm/day) compared to CP110 (0.8 ±0.002 cm/day), CL13 (0.8 ±0.046 cm/day), CP19 (0.7 ±0.108 cm/day), KM26 (0.7 ±0.057 cm/day), CP17 (0.7 ±0.073cm/day), CP21 (0.7 ±0.068 cm/day) and CL110 (0.7 ±0.022 cm/day) isolates from the same County of Bungoma. However, CL41 colony growth rate (0.9±0.017 cm/day) was not statistically ($P\geq0.05$) different from that of KM28 (0.9±0.045cm/day), CP12 (0.9±0.045cm/day), CPF2 (0.9±0.072cm/day), KM29 (0.9±0.042cm/day) fungal isolates collected from the same Bungoma County.

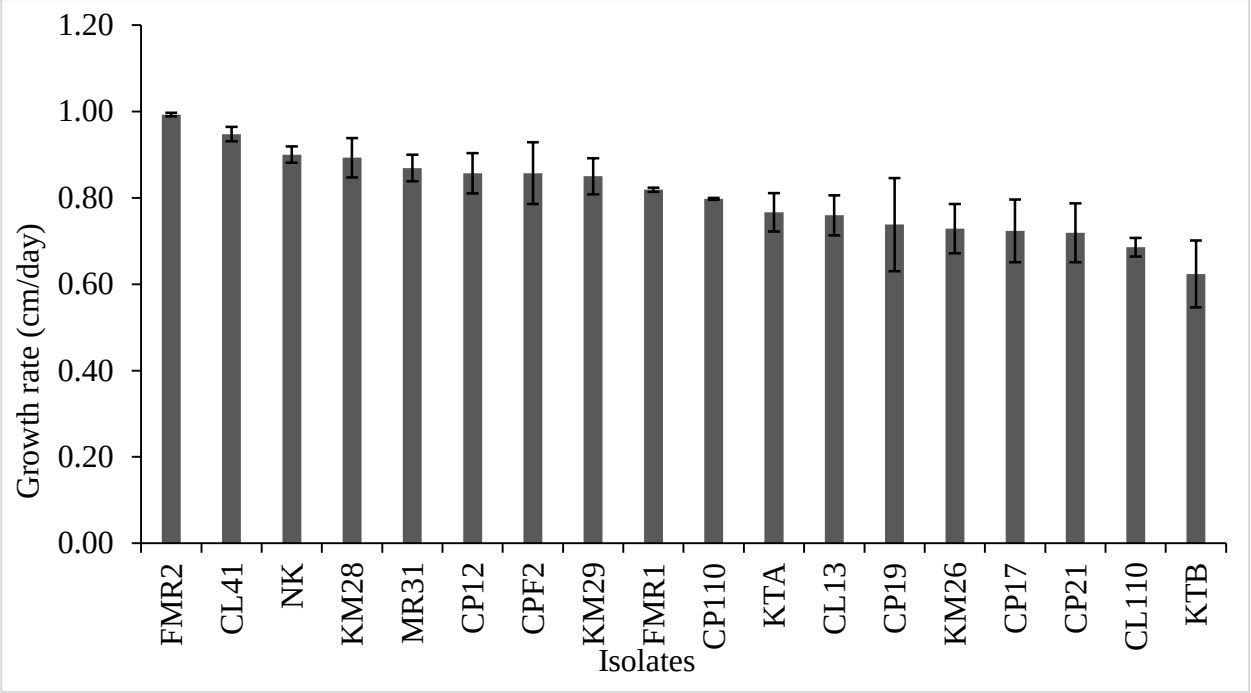

Figure 4.3: Mean Colony Growth Rate (cm/day) of Pathogenic Fungal Isolates Obtained from Bulb Onions Collected from Different Markets

Error bars represents standard error (SE) of the mean. The isolate codes relate to markets where bulb onions were collected.

4.3.3.2 Colony and Conidia Characteristics of Fungal Isolates

The 18 pathogenic fungal isolates showed varied characteristics on the upper side of the colony that included white dense to sparse aerial mycelia. The pigmentation on PDA varied from white, violet, brown while on CLA conidia were categorized as oval, straight, and curved in shape and were either aseptate or septate. The length of microconidia ranged from 0.50±0.41 µm to 0.84±0.61 µm while that of macroconidia was between 19.48±1.43 µm to 34.60±1.57 µm (Table 4.1).

Table 4.1: Morphological Features of Pathogenic Fungal Isolates Obtained from Bulb Onions Collected from Kajiado, Meru and Bungoma Counties (n=10)

				Macroconidia			Microconidia		
Isolate code	**Market**	**County**	**Cluster**	**Septation**	**Shape**	**Mean size (µm)**	**Septation**	**Shape**	**Mean size (µm)**
CL13	Chwele	Bungoma	1	4	Straight	31.9±2.31	1	Oval	0.84±0.61
CPF2	Cheptais	Bungoma	1	3	Straight	30.2±1.98	0-1	Oval	0.61±0.67
CP17	Cheptais	Bungoma	1	4	Straight	27.8±2.72	0-1	Oval	0.87±0.65
CL41	Chwele	Bungoma	1	3	Straight Pointed	30.6±3.30	0-1	Oval	0.81±0.51
CP12	Cheptais	Bungoma	1	3-4	Straight	31.2±1.56	0-1	Oval	0.83±0.65
NK	Nkubu	Meru	1	3-4	Straight	34.7±1.82	0-1	Oval	0.50±0.41
KM29	Kimilili	Bungoma	1	4	Straight	31.3±2.30	1	Oval	0.62±0.49
KM28	Kimilili	Bungoma	1	3	Straight	32.2±2.12	0	Oval	0.50±0.41
CP110	Cheptais	Bungoma	1	3	Straight	31.0±1.72	0-1	Oval	0.87±0.63
CP21	Cheptais	Bungoma	1		Straight	31.9±1.60	0-1	Oval	0.59±0.51
KM26	Kimilili	Bungoma	2	3	Straight		0	Oval	0.79±0.49
FMR2	Meru	Meru	2	3	Straight slender		0	Oval	0.72±0.32
MR31	Meru	Meru	2	3	Straight	19.8±1.43	0	Oval	0.78±0.71
FMR1	Meru	Meru	2	3	Straight	20.3±1.13	0	Oval	0.79±0.61
CL110	Chwele	Bungoma	2	3	Straight	20.2±1.23	0	Oval	0.82±0.51
CP19	Cheptais	Bungoma	3	4-5	Straight	34.6±1.57	2	Oval	0.84±0.61
KTB	Kitengela	Kajiado	3	3-5	Straight	32.5±1.43	2	Oval	0.73±0.81
KTA	Kitengela	Kajiado	3	3-4	Straight	28.3±2.13	2	Oval	0.82±0.54

Based on colony and conidial features the isolates were grouped into three clusters as follows; cluster one (CL13, CPF2, CP17, CL41, CP12, NK, KM29, KM28, CP110 and CP21), cluster two (KM26, FMR2, MR31, FMR1 and CL110) and cluster three (CP19, KTB and KTA).

On PDA, cluster one isolates developed colonies with dense white cottony mycelia on the upper side and on reverse side there was brown colour at the centre of the colony (Plate 4.4). On CLA, macroconidia were observed which had falcate to almost straight shape, and pointed at the end with three to four septa (Plate 4.5). They were short to moderate in length ranging from 27.8±2.72 to 34.7±1.82µm. Cluster one isolates formed microconidia that were oval or kidney shaped (Plate 4.5).

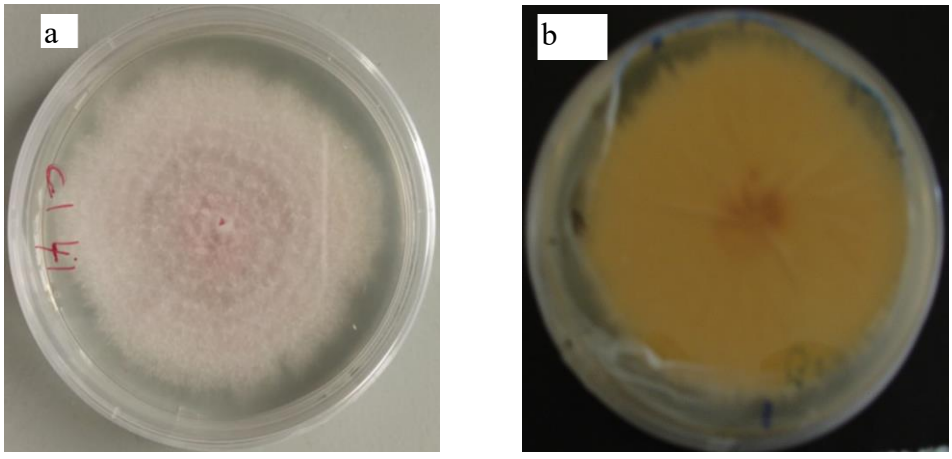

Plate 4.4: Representative Colony (CL41) of Cluster One Isolates; (a) Top Side and (b) Underside

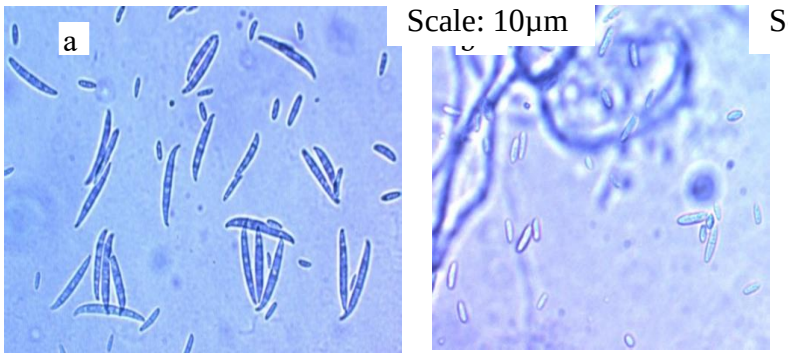

Plate 4.5: Spores (CL41) of Cluster One Isolates; (a) Macroconidia and (b) Microconidia

Cluster two isolates formed sparsely white colony on the upper side while on reverse side the colony was white with pale yellow pigmentation spreading throughout the colony area (Plate 4.6). On CLA, macroconidia were sparsely produced, had bent apical and were foot shaped with three septa. The cluster two isolates had numerous microconidia that were oval in shape and aseptate (Plate 4.7).

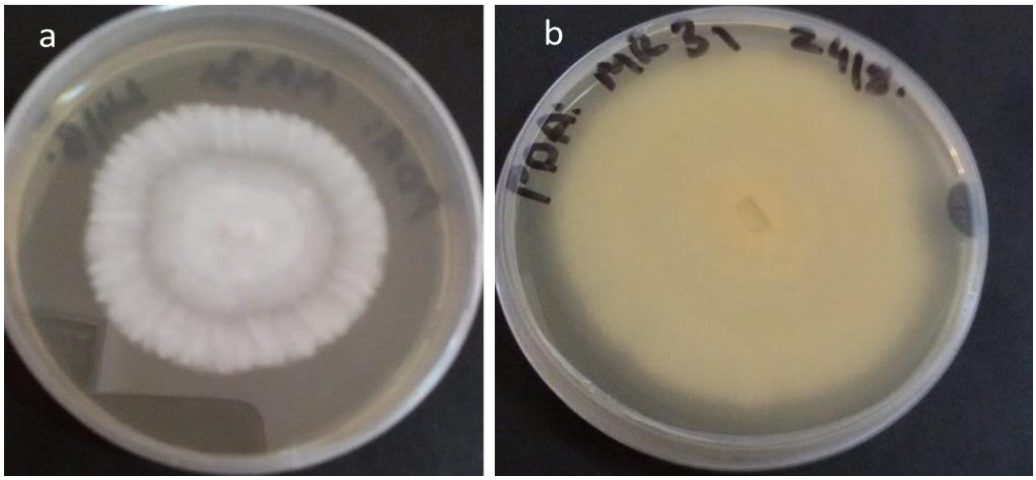

Plate 4.6: Representative Colony (MR31) of Cluster One Isolates; (a) Top Side and (b) Underside

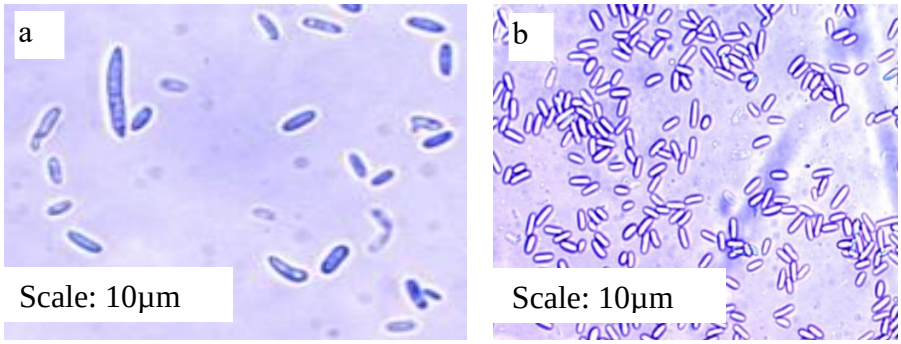

Plate 4.7: Fungal Spores (MR31) of Cluster Two Isolates; (a) Macroconidia and (b) Microconidia

On PDA, cluster three fungal isolates formed white to violet, sparse floccose mycelia on the colony upper side and the reverse side had violet pigmentation (Plate 4.8). On CLA, the cluster three isolates produced long (28.1±2.13 to 34.6±1.57 µm) macroconidia that were slightly curved with relatively wide three to five septate (Plate 4.9). Microconidia were formed in false heads on long monophialides, oval in shape and two septate.

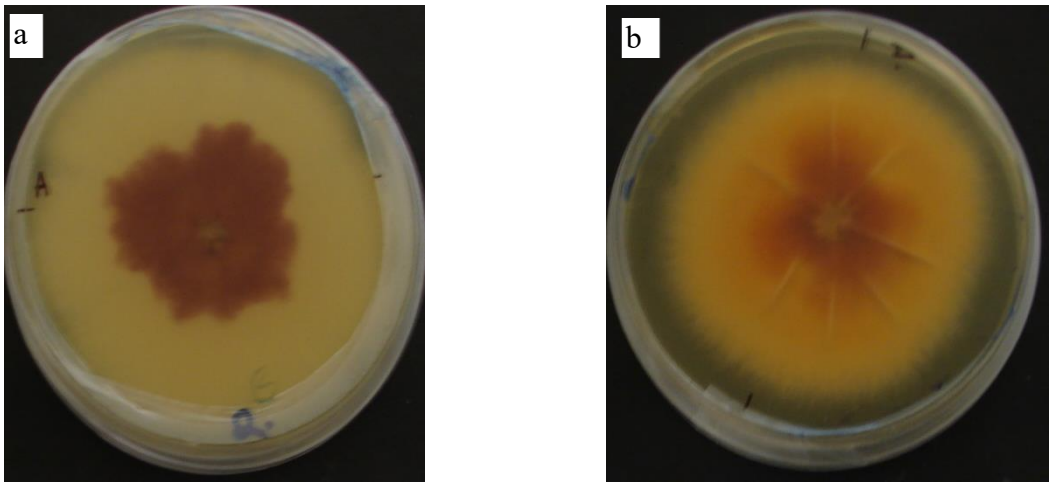

Plate 4.8: Representative Colony (KTB) of Cluster Three Isolates (a) Top Side and (b) Underside

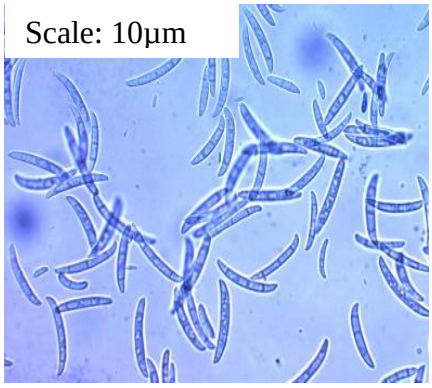

Plate 4.9: Macroconidia Spores (KTB) of Cluster Three Fungal Isolates

4.3.4 Molecular Identification of Pathogenic Isolates from Bulb Onion

Molecular identification technique was used to confirm pathogenic isolates described using morphological characterisation. Three *Fusarium* species were identified and clustered as follows: cluster one; *F. oxysporum* (OL631180, OL631166, OL631165, OL631169, OL631174, OL631175, OL631173, OL631170, OL631179, and OL631167), cluster two; *F. acutatum* (OL631178, OL631171, OL631172, OL631164 and OL631177) and cluster three; *F. solani* (OL631176, OL631163 and OL631168) (Fig. 4.4).

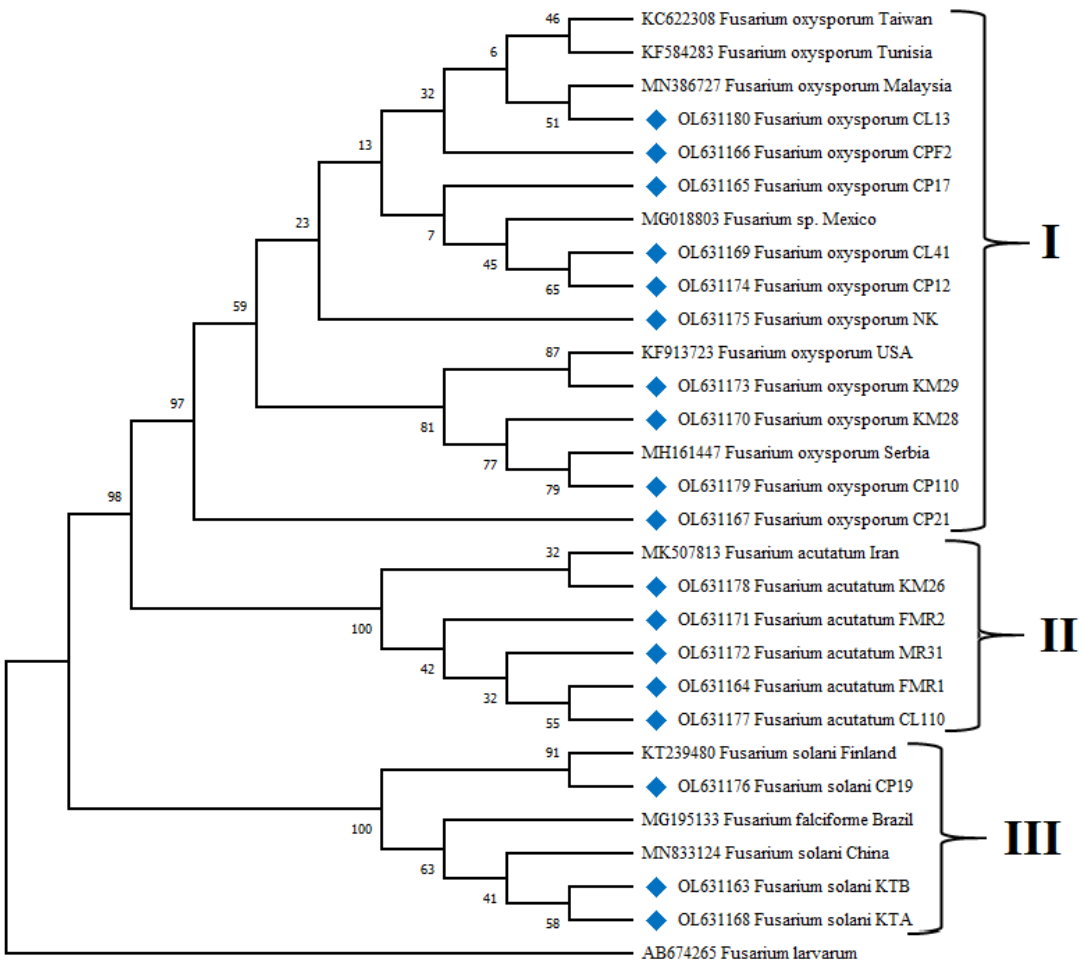

Figure 4.4: Phylogenetic Tree of Pathogenic Fusarium Species Isolated from Bulb Onions Collected from Different Markets

The percentage query coverage aligned to isolates sequences from this study and those obtained from the Fusarium_ID Gene bank had a range of 99 to 100 percent (Table 4.2). Fifty five percent of the isolates were *F. oxysporum*, 28% were *F. acutatum*, while *F. solani* were 17%. *Fusarium solani* was mainly isolated from Kajiado County, *F. acutatum* from Meru while *F. oxysporum* was predominately from Bungoma County.

Table 4.2: Molecular Identification of Fusarium Species Isolated from Bulb Onions Collected from Different Markets

Isolate code	Cluster	*Fusarium* spp.	Isolated *Fusarium* spp Genebank accession number	Genebank accession with best match	Percentage query coverage	Percentage similarity
CL13	1	*Fusarium oxysporum*	OL631180	MN386727	100	98.34
CPF2	1	*Fusarium oxysporum*	OL631166	CP052043	100	99.02
CP17	1	*Fusarium oxysporum*	OL631165	KP964904	100	92.73
CL41	1	*Fusarium oxysporum*	OL631169	KP964890	100	99.15
CP12	1	*Fusarium oxysporum*	OL631174	KP964890	99	98.88
NK	1	*Fusarium oxysporum*	OL631175	KP964904	99	99.72
KM29	1	*Fusarium oxysporum*	OL631173	MT305189	100	99.12
KM28	1	*Fusarium oxysporum*	OL631170	MH161447	100	98.89
CP110	1	*Fusarium oxysporum*	OL631179	KP964881	100	96.16
CP21	1	*Fusarium oxysporum*	OL631167	MK172059	100	99.15
KM26	2	*Fusarium acutatum*	OL631178	MT010989	100	98.88
FMR2	2	*Fusarium acutatum*	OL631171	MT010989	100	99.02
MR31	2	*Fusarium acutatum*	OL631172	MT01098	100	98.88
FMR1	2	*Fusarium acutatum*	OL631164	MT010989	100	99.16
CL110	2	*Fusarium acutatum*	OL631177	MK507814	100	98.92
CP19	3	*Fusarium solani*	OL631176	KT313615	99	99.05
KTB	3	*Fusarium solani*	OL631163	MN833124	99	99.17
KTA	3	*Fusarium solani*	OL631168	MN833124	100	99.04

4.4 Discussion

Rot caused about 14% losses during bulb onions postharvest handling in Kenya (Gathambiri *et al*., 2021),though pathogens causing the decay have not been identified. However, globally mainly fungal genera are associated with bulb onion rot, namely *Aspergillus*, *Penicillium*, *Alternaria, Fusarium, Rhizopus* and *Botrytis* (Yurgel *et al*., 2018). To successfully control postharvest rot in bulb onions, it is important to identify the pathogens contributing to the rot (Klokocar-Smit *et al*., 2008).

Fifty fungal isolates were obtained from 300 rotting bulb onion samples that were collected from different markets; this indicated that some of the diseased bulb onion samples may have been infected by different microorganisms. According to Ghanbarzadeh *et al.* (2014), a number of fungal and bacterial infections may be responsible for postharvest bulb onion rot. However, Mahmud and Monjil, (2015); Yurgel *et al*. (2018) reported that fungi mainly cause postharvest diseases in bulb onions.

From this study, 18 fungal isolates developed similar symptoms to those observed in bulb onions collected from study sites of Bungoma, Kajiado and Meru Counties, therefore the fungal pathogens were found to cause postharvest rot. Fungal pathogens had been reported to cause postharvest rot in tomato and avocado fruits in Kenya (Mugao and Birgen, 2021; Wanjiku *et al.,* 2020), respectively. More pathogenic fungi (67%) were isolated from Bungoma County compared to Meru (22%) and Kajiado (11%) counties. Hence, the estimation of bulb onion postharvest loss levels due to rot which showed that Bungoma County experienced higher (17%) losses due to rot than Kajiado (14%) and Meru (2%) counties, validated the results. According to Tischner *et al*. (2022) climatic conditions have an effect on fungal pathogens prevalence and distributions, and in addition postharvest practices employed by bulb onion value chain actors affect occurrence of postharvest rot (Gathambiri *et al*., 2021).The pathogenic fungal isolates were able to cause rotting in bulb onions 21 days after incubation at room temperature (23 ± 3°C). Similar results were reported by Bektast and Kusek, (2019) who indicated

that *F. oxysporum* f.sp. *cepae* inoculated in bulb onion developed symptoms 21 days after incubation at 24 ± 3℃.

The 18 pathogenic isolates were grouped into three clusters based on colony and conidia characteristics. The colony and conidia characteristics described in this study for the three clusters defined *Fusarium* spp.as described by Keith, (1996). Thus, this is the first report which indicated that *Fusarium* spp. fungal pathogens contribute to bulb onion postharvest rot in Kenya. The results of this study corroborates with other researchers who observed similar colony and conidia features; Wanjiku *et al*. (2020) described cluster one isolates' features, Chehri *et al.* (2015) observed cluster two isolates and Kalman *et al*.(2020) stated cluster three isolates. Consequently, molecular identification method confirmed the three *Fusarium* spp. clusters which were identified as *F. oxysporum* (cluster one), *F. acutatum* (cluster two) *and F. solani* (cluster three). Therefore, *F. oxysporum* f.sp. *cepae, F. acutatum and F. solani* were identified as fungal causative agents of postharvest rot in bulb onion in Bungoma, Meru and Kajiado Counties of Kenya. Majority of pathogenic fungal isolates (55%) obtained during this study were identified as *Fusarium oxysporum* f.sp. *cepae*. Thus bulb onion postharvest rot in major growing regions of Kenya is mainly caused by *Fusarium oxysporum* f.sp. *cepae*. In addition, from the results it was observed that *F. oxysporum* f.sp. *cepae* was mainly isolated from Bungoma, *F. solani* from Kajiado and *F. acutatum* from Meru, therefore the isolated *Fusarium* spp. may be regional specific. According to Le *et al.* (2021) factors such as soil and environmental conditions, and plant host may affect the distribution of *Fusarium* spp.

Eighteen pathogenic fungal isolates obtained from the study sites of Bungoma, Meru and Kajiado Counties caused different lesion sizes after inoculation in bulb onions. The study findings indicated that *Fusarium solani* was most virulent, followed by *Fusarium acutatum* and *Fusarium oxysporum* f.sp. *cepae*. Despite the fact that Kajiado County had a lower percentage (11%) of isolated fungi than Meru County (22%), more rot-related losses (14%) were incurred due to the prevalence of *F. solani*, which was found to be more virulent than other species.

The results did not corroborate with Ghanbarzadeh *et al.* (2014) who reported that *Fusarium oxysporum* f.sp. *cepae* was more virulent than *F. solani* on bulb onion. However, the results of this study were in agreement with Kalman *et al.* (2020) who reported that *Fusarium acutatum* was more virulent compared to *F. oxysporum* f.sp. *cepae* on bulb onion. In addition, based on the results of this study *Fusarium acutatum* had higher colony growth rate compared to *Fusarium oxysporum* f.sp. *cepae.* This could be an indication that colony growth rate and virulence of fungal isolates may have a positive correlation.

Fusarium oxysporum f.sp. *cepae, F. acutatum and F. solani* isolated and identified from this study are known to cause Fusarium basal rot (FBR) disease in bulb onions according to Le *et al*. (2021). The disease can be caused by single *Fusarium* spp.; *F. falciforme* (Tirado-Ramírez *et al*., 2018) or a complex of different *Fusarium* spp.*; F. solani, F, acuminatum, F. oxysporum, F.verticilliodes* and *F.proliferatum* (Delgado-Ortiz *et al*., 2016). Fusarium basal rot has been reported in Asia to contribute about 30 to 40% bulb onion loss during storage (Gupta and Gupta, 2013). The infection starts in the field where the symptoms include damping-off, stunting, chlorotic leaves, roots, bulb discolouration and eventually death of the roots. However, the infected onions do not always exhibit disease symptoms in the field, the infection remains latent until storage where the signs of rotting are observed thus causing postharvest rotting (Lager, 2011). Postharvest rots occur when the infection happens late in the season during bulb onion growing (Le *et al*., 2021). Therefore based on results of this study, FBR disease may contribute to postharvest loss of bulb onion during postharvest handling in Kenya.

4.5 Conclusion

This study indicates that fungal pathogens associated with postharvest rots in major growing regions of Bungoma, Kajiado and Meru Counties of Kenya were *Fusarium* species namely; *Fusarium oxysporum* f.sp. *cepae, Fusarium solani* and *Fusarium acutatum*. The isolated *Fusarium* species were mainly regional specific, thus future

studies should be done on effect of geographical variations in bulb onion postharvest Fusarium rot. *Fusarium solani* was the most virulent among the three pathogens, however *F. oxysporum* f.sp. *cepae* was frequently isolated. All the three *Fusarium* species isolated are associated with FBR which is an economically important disease of bulb onion postharvest rot. This is the first report of *Fusarium* spp. which contributes to bulb onion postharvest loss in Kenya. Pre- and postharvest management practices such as use of pesticides, biological methods and curing of bulb onions can be employed to manage postharvest rot caused by *Fusarium oxysporum* f.sp. *cepae, Fusarium solani* and *Fusarium acutatum*.

CHAPTER FIVE

DETERMINATION OF OPTIMAL CURING TEMPERATURES IN MANAGEMENT OF *FUSARIUM* SPP. CAUSING BULB ONION POSTHARVEST ROT

Abstract

Fusarium species are casual agents of postharvest rots in bulb onions which significantly contribute to postharvest losses. To reduce bulb onion postharvest rot, one of the recommended management practices is curing. Therefore, the objective of this study was to determine optimal curing temperature to manage Fusarium rot in bulb onions.

Two experiments were carried out at room temperature (24± 2°C), and in incubators set at 30°C, 35°C and 40°C under relative humidity ranging from 60-85%. Bulb onions were inoculated with *Fusarium solani* and cured for 14 days after which they were stored at room temperature for 21 days and rot severity score recorded. For the second experiment, colony radial growth rate of *Fusarium* spp grown on potato dextrose agar and incubated at 24±2°C (room temperature), 30°C, 35°C and 40°C were recorded on the fourth day. The results indicated that bulb onion inoculated with *F. solani* and cured at 24±2°C developed rot that was significantly ($P \leq 0.05$) higher in severity score (2.3±0.08) compared to those cured at 30°C, 35°C and 40°C. From the results it was evident that high curing temperature of Fusarium rot in bulb onion reduced. However, in bulb onion samples cured at 40°C black sooty rot occurred. The findings on mean colony growth rate indicated that as the temperature increased from room temperature to 40°C, *Fuasrium* spp rate of colony development was reduced. Based on results of this study it was concluded that the optimal temperature for curing bulb onion of red couch variety to manage progression of Fusarium rot is between 30 to 35°C for 14 days.

5.1 Introduction

Bulb onion (*Allium cepa* L.) is termed as a semi-perishable commodity (Rana and Sinija, 2014). However postharvest losses may occur through weight loss, sprouting and rotting (Gorrepat *et al*., 2018; Bhagyawant, 2016) thus reducing its storage period. According to the results in chapter three of this thesis, in Kenya rots contribute to about 14% of total postharvest losses in bulb onion.

Fusarium spp are some of the major fungal pathogens that contributes to bulb onions postharvest losses (Rasiukevičiute *et al.,* 2016). In Ethiopia, Sintayehu *et al.* (2011) reported that 20% postharvest losses were caused by Fusarium rot , while in Asia 30 to 40% losses were estimated (Gupta and Gupta, 2013). Similarly, according to Gathambiri *et al.* (2023) Fusarium rot cause bulb onion postharvest losses in Kenya. It affects bulb onion at all stages of development, however it is severe during postharvest stage (Mandal and Cramer, 2020). According to Lager, (2011), the infection normally starts in the field towards the end of the bulb onion growing season, remains dormant, and eventually manifests its symptoms during storage. Fusarium rots mainly occur in latently infected bulb onions since secondary infection or its spread during postharvest handling is limited (Le *et al*., 2021).

Chemical, biological and crop rotation management methods have been reported to control Fusarium rot in bulb onion in the field (Afzal *et al*., 2021; Degani and Kalman, 2021; Alemu, 2015). However, information on management of Fusarium rot during postharvest handling in bulb onion is scanty (Le *et al*., 2021). Cultural postharvest practices such as harvesting at the right maturity indices, use of appropriate harvesting techniques, curing and sorting has been reported to reduce bulb onion postharvest rots (Banjaw, 2017).

Curing is one of the postharvest management practices, recommended for the postharvest losses in bulb onion. Curing reduces excess moisture from bulb onion especially in one

or two outer leaves layers and neck tissues (Rana and Sinija, 2014). Curing dries the neck tissues and outer leaf scales resulting in a tight, dry wrapper around the bulb onion hence reducing incidences of postharvest disease infection and water loss during storage (Gorreapti *et al*., 2017). In addition, curing has been reported to decrease postharvest rots development according to Adel, (2016) bulb onion cured at 36-38˚C for five days reduced *Botrytis allii* growth. Similarly, a significant reduction of bulb onion rot caused by *Pontea agglomerans, P.ananatis* and *P.allii* (Vahling-Armstrong *et al.,*2016) and enterobacter decay (Schroeder *et al.,* 2012) was observed after curing at more than 35°C for14 days. Two bulb onion curing techniques are employed, they include natural and artificial methods. In the artificial method, the conditions (temperature and time) are controlled. While natural technique involves drying bulb onions under ambient conditions and its efficiency is completely dependent on climatic conditions (Gorreapti *et al*., 2017).

In Kenya, majority of farmers do not subject their bulb onions to curing process which contributes to postharvest rots (Gathambiri *et al*., 2021). Currently there is limited information on management of bulb onion postharvest Fusarium rot through curing in Kenya. Therefore, this study aimed to determine optimum curing temperatures in management of postharvest Fusarium rot in bulb onion.

5.2 Materials and Methods

5.2.1 Sourcing of Bulb Onions Sample

Red couch bulb onion variety was chosen due to its popularity and was obtained from a commercial farm in Kajiado County, Kenya. The farm was situated at latitude 1˚37'44"S, longitude 36˚46'31"E and 1674 meters above sea level as located using a Global Positioning Satellite (GPS) instrument (Magellan Triton, China). Samples of bulb onions were harvested at the maturity stage, when over 50% of the leaves had dried and topped off. Immediately after harvesting bulb onions samples were packaged in plastic crates and transported to Kenya Agricultural and Livestock Research Organization, Thika Postharvest Laboratory. The samples ranged in weight from 57.2 to 67.4 g.

The following day, an experiment was designed to assess the effectiveness of curing bulb onions at different temperatures to manage Fusarium rot.

5.2.2 Evaluation of Optimum Curing Temperature on Fusarium Rot Management in Bulb Onion

5.2.2.1 Preparation of *Fusarium solani* Inoculum

Fusarium solani which was found to be the most virulent among the *Fusarium* species identified to cause bulb onion postharvest rot in Kenya (Gathambiri *et al*., 2023) was used as the inoculum in this study. Three millimetre of filter paper containing pure preserved *Fusarium solani* (KTB) were placed on Potato Dextrose Agar (PDA) in Petri dishes and incubated at room temperature of 24±2°C under natural light for ten days to enhance sporulation. The ten- day old fungal colonies were soaked in 5 ml 0.2% gelatin solution and spread carefully using a sterile L-shaped plastic rod. Using sterile muslin cloth, the fungal suspension was sieved in 30 ml plastic universal tube to remove mycelia. Ten microliter of fungal spore suspension was placed into each of the four haemocytometer (Marienfeld, German) chambers (A, B, C and D). Spores in every chamber were counted and the average number was calculated per isolate.

Spores per/ ml was calculated using the formula:

$$\text{Spores/ml} = (n) \times 10^4$$

Where: n = the average spores counted.

The spore suspension concentration was calculated using the following formula according to Gilchrist-Saavedra *et al.* (2006) and adjusted to desired concentration of $1x10^5$ spores/ml using 0.2% gelatin solution.

$$C_1V_1=C_2V_2$$

Where C_1= Initial concentration

V_1= Initial volume

C_2= Desired concentration

V_2= Final volume

5.2.2.2 Development of Fusarium Rot Severity Scale on Bulb Onion

Twenty bulb onions were inoculated with microliter of *Fusarium solani* spore suspension and placed on a laboratory bench. Fusarium rot severity scale in pictorial form was developed using disease symptoms progression as described by Chiang, (2020) with slight modification (Table 5.1).

Table 5.1: Description of Bulb Onion Rot Severity Scale Ranging from 1 To 6

Score	Description
1	No rotting symptoms
2	Slight rotting with up to 5% area with lesions
3	Slight moderate rotting with 6- 20% area with lesions
4	Moderate rotting with 21- 30% area with lesion
5	Severe rotting 31-50% area with lesion
6	Very severe rotting with ≥ 50% area with lesion

Source: Chiang *et al*. (2020)

The pictorial disease severity scale description was developed as shown in Plate 5.1.

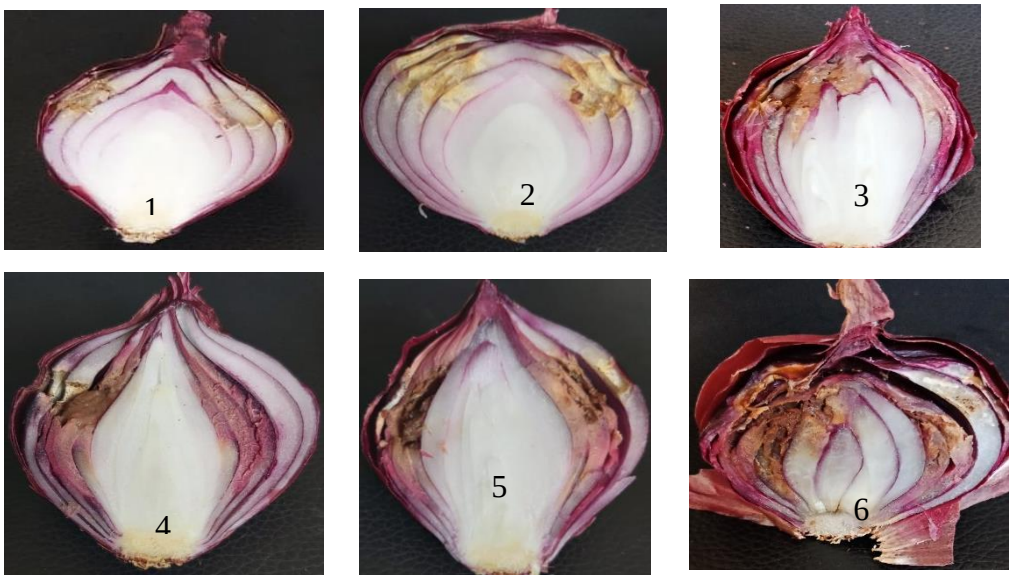

Plate 5.1: Pictorial Fusarium Rot Progression Indicating Severity Scores from 1 to 6

5.2.2.3 Determination of Fusarium Rot Severity in Bulb Onion Inoculated with *F. solani* Cured under Different Temperature

Fouty microliter of $1x10^5$ spores/ml *F. solani* (KTB) spore suspension was introduced into the shoulder region of each bulb onion as described by Vahling-Armstrong *et al*., (2016) with slight modification. After inoculation four replicates with a set of five bulb onions were arranged on sterilized trays in a completely randomized design (CRD). The inoculated bulb onions were placed in incubators (LBI-250E, Labtech, Korea) set at 30°C, 35°C, 40°C, and on laboratory benches (room temperature) for 14 days. Using a digital thermo-hygrometer (Brannan, England), the relative humidity (RH) was recorded daily and maintained between 65 and 80% by placing a bowl of water inside the incubators.

Then after curing for 14 days the bulb onions were stored on laboratory benches for three weeks to simulate storage. After 21 days storage at room temperature (24±2°C), cured inoculated bulb onions were cut into halves with a sterile scalpel and the fleshy scales

exhibiting Fusarium rot symptoms were assessed and recorded using rot severity scale that was developed (Plate 5.1).

5.2.3 Determination of *Fusarium* spp. Colony Radial Growth Rate under Different Temperatures

Fusurium rot is caused by a single or complex of different *Fusarium* species (Tirado-Ramírez *et al*., 2018; Delgado-Ortiz *et al*., 2016) and according to Gowda *et al*. (2020) temperature influences their growth rate. Therefore, a second experiment was set aimed to assess growth of *Fusarium oxysporum* f.sp. *cepae*, *F. solani,* and *F. acutatum* at different temperatures of room temperature, 30℃, 35℃ and 40°C. Colony radial growth rate was used as a parameter to investigate *Fusarium* spp. development in view of virulence.

5.2.3.1 Assessment of *Fusarium* spp. Colony Radial Growth Rate

Three-millimeter filter paper containing pure preserved *Fusarium oxysporum* f.sp. *cepae* (CL41), *F. solani* (KTB), and *F. acutatum* (MR31) spores were placed on Petri dishes containing PDA (Oxoid CMO-139) and sealed with Para-film®. For four days, a set of three Petri dishes, each containing one *Fusarium* species in triplicate, were placed on laboratory benches (room temperature) and in incubators (LBI-250E, Labtech, Korea) with temperature settings of 30°C, 35°C, and 40°C. On the fourth day, two colony diameter readings perpendicular to each other from the point of initial inoculum were recorded. Colony radial growth rates (mm/day) of *Fusarium oxysporum* f.sp. *cepae, F. solani* and *F. acutatum* were calculated using the formula :

Colony radial growth rate (mm/day) = (D/2)/ 4 where

D = Average diameter of the colony

4 = Number of days incubated

5.3 Statistical Data Analysis

Fusarium rot severity scores data were subjected to a one-way ANOVA, whilst the colony radial growth rate (mm/d) data were subjected to a two-way ANOVA using the 15th edition of GENSTAT statistical software. The Tukey test was used to separate the means of the Fusarium rot severity ratings and the colony radial growth rate. The comparison was made using the Fishers protected least significant difference (LSD) at a 5% significant level.

5.4 Results

5.4.1 Effect of Different Curing Temperatures on Bulb Onion Rot Severity Caused by *F. solani*

Bulb onion inoculated with *F. solani* and cured at room temperature (24±2℃) developed rot that was significantly (P≤0.05) higher in severity score (2.3±0.08) compared to those cured at 30℃ (1.3 ±0.13), 35℃ (1.7± 0.41) and 40℃ (1.7± 0.35) (Fig.5.1). In addition, there was no significant difference (P>0.05) in rot severity score between bulb onion samples cured at 30°C, 35°C and 40°C. However, based on results obtained from this study rot severity generally seemed to decrease when the curing temperature was higher than room temperature (24±2℃).

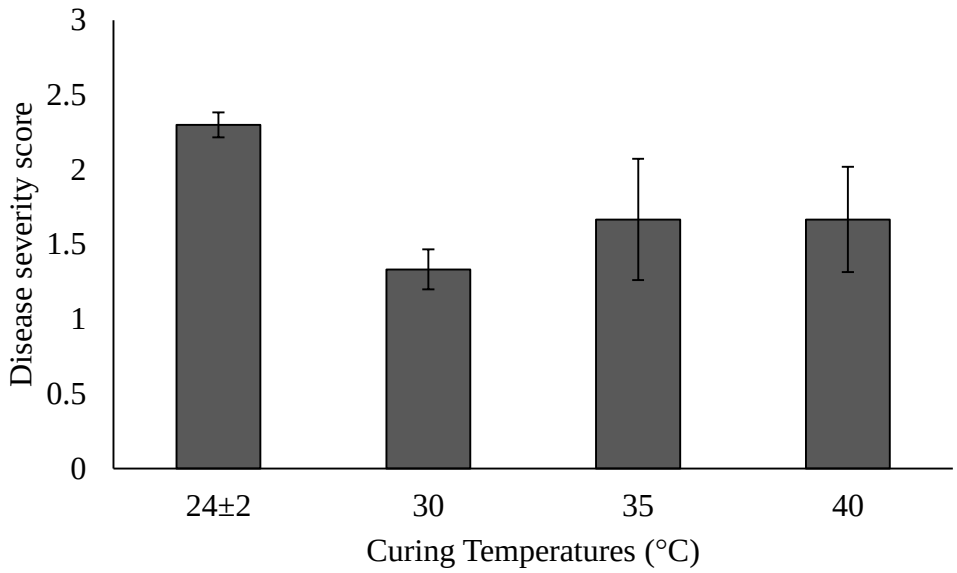

Figure 5.1: Mean Rot Severity Score of Bulb Onion Inoculated with F. solani, Cured at Different Temperatures 24±2, 30, 35 and 40◦C for 14 Days, and Placing on Laboratory Benches for 21 Days

Error bars represents standard error of the mean (n=15)

Plate 5.2: Bulb Onion Showing Black Sooty Rot Observed at Curing Temperature of 40◦C

5.4.2 *Fusarium* spp. Colony Growth Rate at Different Temperatures

Increase in temperature had a significant (P≤0.05) effect on colony development of *Fusarium solani, F. acutatum* and *F. oxysporum* f.sp. *cepae*. However, at 40°C colony growth was not observed in *Fusarium* spp. *Fusarium solani* incubated at 24±2℃ grew significantly (P≤0.05) faster (9.1±0.16mm/d) compared to growth rate at 30˚C (6.2±0.04 mm/d) and 35˚C (3.7±0.04 mm/d). In addition, there was significant difference (P≤0.05) in colony growth rate of *F. solani* incubated at 24±2℃, 30˚C, and 35˚C. Colony development of *F. acutatum* at 24±2℃ was statistically (P≤0.05) faster (6.9±0.52 mm/d) compared to growth rate at 30˚C (4.1±0.09 mm/d) and 35˚C (2.3±0.10 mm/d). Subsequently, at 30˚C *F. acutatum* colony grew statistically (P≤0.05) at a higher rate (6.3±0.15 mm/d) compared to growth rate at 35˚C mm/d. *Fusarium oxysporum* f.sp. *cepae* incubated at 24±2℃ grew significantly (P≤0.05) faster (6.6±0.03mm/d) compared to growth rate at 30˚C (4.1±0.09 mm/d) and 35˚C (2.3±0.10 mm/d). In addition, there was significant difference (P≤0.05) in colony growth rate of *Fusarium oxysporum* f.sp. *cepae* incubated at 30°C (4.1±0.0 mm/d) and 35°C (2.3±0.10 mm/d). From the results it was evident that as the temperature increased from 24±2℃ to 40˚C the colony radial growth rate of *Fusarium* spp. reduced (Table 5.2).

5.4. 3 Comparison of the Effects of Various Curing Temperatures on the Colony Growth Rate among the *Fusarium* spp.

Fusarium solani grew significantly (P≤0.05) faster (9.1±0.16 mm/d) compared to *F. acutatum* (6.9±0.52 mm/d) and *Fusarium oxysporum* f.sp. *cepae* (6.6±0.028 mm/d) at 24±2°C (Table 5.2). However, at the same temperature there was no significant difference (P>0.05) in colony growth rate between *Fusarium acutatum* and *Fusarium oxysporum* f.sp. *cepae.* At incubation temperature of 30˚C, *Fusaraium acutatum* exhibited faster growth rate (6.3±0.15 mm/d) followed by *F. solani* (6.2±0.043 mm/d) and *Fusarium oxysporum* f.sp. *cepae* (4.1±0.087mm/d) was the slowest in development. However, there was no significant difference (P>0.05) in rate of colony development between *F.*

*acutatum (*6.3±0.15 mm/d) and *F. solani (*6.2±0.04 mm/d) at 30˚C. At 35°C *Fusarium oxysporum* f.sp. *cepae* grew statistically (P≤0.05) slower (2.3±0.10mm/d) compared to *F. acutatum* (5.0±0.17mm/d) and *F. solani* (3.7±0.04mm/d). There was significant difference (P≤0.05) among the *Fusarium* spp in colony growth rate at 35°C.

The results of this study indicated that as the temperature increased from 24±2 to 35˚C, *F. oxysporum* f.sp *cepae* rate of colony development was reduced by 65%, *F. solani* by 59% while *F. acutatum* by 27%. Overall, colony growth rate of *Fusarium* spp reduced as the temperature increased, however the rate of decrease was dependent on species.

Table 5.2: Mean Colony Growth Rate (mm/d) of Three Fusarium Species Incubated at Different Temperatures of 24±2°C, 30°C, 35°C and 40°C

Temperature (◦C)	Colony growth rate (mm/d)		
	F. solani	***F. acutatum***	***Fusarium oxysporum* f.sp. *cepae***
24±2	9.1 ± 0.16^{a}	6.9 ± 0.52^{b}	6.6 ± 0.03^{bc}
30	6.2 ± 0.04^{c}	6.3 ± 0.15^{c}	4.1 ± 0.09^{e}
35	3.7 ± 0.04^{e}	5.0 ± 0.17^{d}	2.3 ± 0.10^{f}
40	0 ± 0^{g}	0 ± 0^{g}	0 ± 0^{g}

When the same superscript letter is present in the same column, based on the Tukey test there are significant (P≤0.05) differences in the mean colony growth rates (mm/d) of the same *Fusarium* species incubated at different temperatures. According to the Tukey test, there are significant differences (P≤0.05) in the mean colony growth rates (mm/d) among F. solani, *F. acutatum* and *F. oxysporum* f.sp. *cepae* when different superscripts are present in the same row.

5.4 Discussion

This study demonstrated that exposing the fungus to temperatures of 30˚C, 35˚C and 40˚C for 14 days reduced progression of Fusarium rot in bulb onions. Though, curing bulb onions at 40˚C for 14 days reduced Fusarium rot severity, development of fungal pathogens (black sooty) was observed. According to (Polderdijk *et al*., 2000) fungal

pathogens such as *Aspergillus niger* that thrives well at temperatures of 40°C and above occurs. The reduction of rot severity could be attributed to chemical changes that occur during curing of bulb onion where antifungal agent, 3, 4-dihydroxybenzoic acid is formed through oxidation of quercetin by peroxidase (POX) (Takahama and Hirota, 2000). The reason for the lower rot severity at 30°C (1.3 ±0.13) compared to 35°C (1.7± 0.41) and 40°C (1.7± 0.35) could be attributed to the optimal temperature for POX activity which could be at 30°C.

The results obtained from this study were in agreement with Goktepe *et al*. (2007) who reported that curing bulb onions at 30°C and above minimized postharvest rot development caused by *Botrytis allii*. In addition, Vahling-Armstrong *et al.* (2016) established that curing bulb onion at 35°C for 14 days reduced postharvest rot caused by *Pontea agglomerans, P.ananatis* and *P. allii*.

As curing temperatures were increased from room temperature (24±2°C) to 35-40°C Fusarium rot severity was reduced in bulb onions, the effects were further evidenced by results obtained from evaluation of colony growth rates of *Fusarium* species where the growth was reduced as the temperature increased. The *Fusarium* species colony development rate and the progression of rot may be linked; as growth increases, so does the severity of the rot. The findings were in agreement with Mbaka, (2011) who reported that that *P. cinnamomi* colony growth rate is positively related to severity. However at 40°C growth was not observed and these results corroborates the study of Gowda *et al*. (2020) who reported that *Fusarium solani* growth on PDA declined at 30°C and above and there was no growth at 40°C. Similarly, the results of this study were in agreement with Saleh *et. al*., (2021); Yan and Nelson, (2020) who established that *Fusarium* spp. optimum growth on PDA occurred below 30°C and at 40°C the growth did not happen. Tho *et al*., (2019) stated that temperatures below 30°C are favourable for *Fusarium* spp. fungal development, spore germination, and sporulation which results in the development of disease incidences. Hence, based on results of this study fungal development was inhibited at curing temperatures between 30 and 35°C for 14 days, which minimised the

progression of bulb onion rot. Further evidence supporting these results was obtained from reduction of colony radial growth rate as the temperature increased from 24±2 to 40˚C.

The colony growth rate of *F. oxysporum* f.sp *cepae* and *F. solani*, though *F. solani* varied at different temperatures thus indicating that each *Fusarium* spp had an ability to respond differently in specific curing temperatures. *Fusarium acutatum* colony growth is less affected by increase in temperature compared to *F. oxysporum* f.sp *cepae* and *F. solani*, though *F. solani* developed faster at room temperature. According to Yan and Nelson, (2020) *Fusarium* spp are thermal-specific pathogens. Therefore, in future strategies to manage postharvest Fusarium rot progression through curing of bulb onions should target each *Fusarium* spp since they are thermal specific.

5.5 Conclusion

Curing bulb onions for 14 days at 30 to 35°C would reduce (26-45%) the development of Fusarium rot. *Fusarium oxysporum f.sp cepae, Fusarium acutatum* and *F. solani*, which cause Fusarium rot in Kenya, are a thermal-specific pathogens. *Fusarium acutatum* is more heat stable compared to *Fusarium oxysporum f.sp cepae* and *F. solani*. In order to reduce Fusarium rot, a curing strategy should be developed depending on the species that cause the disease.

CHAPTER SIX

EFFECT OF CURING TEMPERATURES ON PHYSICAL AND PHYTOCHEMICAL QUALITY ATTRIBUTES OF BULB ONION (*Allium cepa* L.)

Abstract

Though curing is one of the recommended postharvest techniques employed to reduce postharvest losses, it may affect both physical and phytochemical quality attributes in bulb onions. Thus, this study evaluated the effect of different curing temperatures on attributes which included weight loss, neck thickness and total soluble solids (TSS), and phytochemical characteristics; total phenol content (TPC), total flavonoids content (TFC) and anthocyanins. To evaluate influence of different curing temperatures on quality attributes, a sample of 180 bulb onions was randomly selected and clustered into four sets for each curing temperature. Each cluster of 45 bulb onions was put on a tray in a complete randomized design (CRD), and separately placed on laboratory benches (room temperature 24±2°C) as control temperature and in incubators set at 30°C, 35°C, and 40°C for fourteen days. All study parameters were taken before and after curing. The results indicated that weight loss was highest (3.9±0.53%) for bulb onions cured at 40°C and least (2.8±0.26%) at 35°C. The reduction in neck thickness among bulb onions cured at 24±2°C, 30°C, 35°C, and 40°C did not differ significantly ($P>0.05$). Total soluble solids (TSS) level was not significantly ($P>0.05$) influenced by the curing temperatures used in the study. While bulb onions cured at 24±2°C, 30°C, 35°C, and 40°C for 14 days resulted in significantly ($P\leq 0.05$) higher amounts of total phenolic content (TPC) compared to fresh bulb onions. Those that were cured at 24±2°C had significantly ($P\leq 0.05$) more total flavonoids content (TFC) (248.6±2.85 mg/100g) than those that were cured at 30°C, 35°C, and 40°C. Curing bulb onions at 24±2°C, 30°C, 35°C, and 40°C statistically ($P\leq 0.05$) increased anthocyanins content compared to uncured ones.

Based on the results of this study, it can be concluded that curing bulb onions at 35°C and 40°C improved their physicochemical and phytochemical attributes which contribute to enhanced quality.

6.1 Introduction

Based on results obtained in chapter three of this thesis, 30% postharvest losses that occur during bulb onion handling were mainly attributed to rotting in Kenya. Various postharvest practices such as harvesting stages, method of harvesting and curing are employed to reduce losses (Muhie, 2022; Naqash *et al.*, 2021; Getahun, 2019; Nivedida *et al*., 2019). Curing bulb onion is one of the most important postharvest management practices employed during handling in order to prolong their shelf life and minimize postharvest losses (Gorreapti *et al.*, 2017). During bulb onion curing, metabolic activities and respiration rates are reduced (Nabi *et al*., 2013). According to Downes *et al.* (2009) flavonols and anthocyanins content in bulb onions was reduced when bulb onions were cured at 28°C. Bulb onions quality influences marketability and contribution to healthy diet, therefore curing should aim to maintain both physical and chemical attributes of the commodity.

During curing, a substantial moisture loss occurs that leads to drying of outer scales and neck tightening which improves the physical characteristics of bulb onion (Naqash *et al*.,2021; Eshel *et al*., 2014). Zewdie *et al*. (2022) assessed optimal curing conditions of bulb onion and found that ideal curing process leads to weight loss ranging from 2.8 to 3.9% which depends on curing temperature (Gorreapti et al., 2017). Eshel *et al*., (2014), evaluated fast curing of bulb onions in Israel and demonstrated that at 30°C for nine days it caused weight change ranging between 7-8%. During bulb onion curing, neck diameter reduction is critical since it prevents entry of pathogens and reduces water loss from the inner scales. Narrowing of bulb onion neck indicates efficiency in curing according to Zewdie *et al*. (2022).

Zewdie *et al*. (2019) evaluated effect of curing conditions on bulb onion physical characteristics in Ethiopia and reported that curing at 30°C, 40°C and 50°C for 36 hours caused 46% decrease in neck diameter while weight loss ranged from 5 to 8.5%.

Bulb onions are rich in polyphenol compounds and act as source of flavonoids in diets (Rodrigues *et al*., (2017). Polyphenol compounds in bulb onion are responsible for pigmentations and provide defensive mechanism against biotic and abiotic stress (Crozier *et al*., 2007). According to Nile and Park (2013), flavonoids accounts for high percentage of total phenolic compounds in bulb onions. Flavonoids contents in bulb onion contribute majorly to its pigmentation, disease resistance among others (Panel *et al*., 2022). Two subclasses of flavonoids are found in bulb onion; anthocyanins which are responsible for red or purple colour, and flavonols that contribute to yellow and brown colour (Ren and Zhou, 2021). Red onions contains the highest level of anthocyanins of about 10% of total flavonoid while quercetin 4′-glucoside and quercetin 3, 4′-diglucoside are major (80-90%) derivatives of flavonols (Rodrigues *et al*., (2017). In bulb onions, polyphenol compound, especially flavonoids contribute a key function in antioxidants activity. Use of natural antioxidants in diet may prevents non-communicable human diseases such as cardiovascular diseases, inflammation and various types of cancer among others (Nusrat *et al*., 2022). According to Zhang *et al*. (2016), antioxidant activity is directly related to levels of polyphenol content in bulb onions.

Flavonol compounds are responsible for yellow and brown colour, however red bulb onions contain both anthocyanins and flavonol thus exhibiting high level of total flavonoids. Sagar *et al*. (2020) who evaluated levels of phytochemical compounds in different varieties of bulb onions, demonstrated that 11,888 mg/kg total flavonoids was quantified in red onion types while 1669.9 mg/kg was quantified in the yellow variety. In addition, Zhang *et al*. (2016) evaluated the level of anthocyanins in different types of bulb onions and reported that higher contents (29.99 mg/g) were obtained from red onions compared to yellow (9.64 mg/g), while white onion had the least (0.75 mg/g).

Other than bulb onions cultivar (red, yellow and white), various factors such as environmental conditions, agronomic practices, harvesting stage and postharvest practices affects the level of total phenols, total flavonoid and anthocyanins in bulb onions (Bibi *et al*., 2022; Naqash *et al*., 2021; Zhang *et al*., 2016; Feiyue Ren, 2019) . While curing bulb onions is a common postharvest technique used to extend shelf life, the duration and temperature at which it is done can have an impact on the quantity of phytochemical compounds. Panel *et al*.(2022) demonstrated that bulb onions cured at 28°C for five days increased levels of quercetin by 8%. In addition, Sharma *et al*. (2015) who studied the effect of artificial curing in a growth chamber at 30°C for six days, reported that the level of total quercetin content reduced from 0.968mg/100g to 0.858mg/100g.

In Kenya, curing of bulb onion is scarcely practised (Gathambiri *et al.*, 2021), therefore this study aimed to determine the effect of different curing temperatures on the postharvest quality of bulb onions.

6.2 Materials and Method

6.2.1 Sourcing of Bulb Onion Samples

Mature bulb onion of cultivar Red couch, were sourced from the same farm as those used in chapter five. Bulb onions grown using recommended agronomic practices according to Mbaka *et al*. (2021) and more than 50% to 70% of the crop had topped down were harvested. The roots and tops of bulb onions were cut off as soon as they were harvested, put in plastic crates then taken to KALRO-Thika postharvest laboratory. Bulb onions were placed on laboratory bench at room temperature until the following day when the experiment was set up. The bulb onions used in this study weighed between 45 and 80 grams.

6.2.2 Establishment of the Effect of Different Curing Temperatures on Bulb Onion Quality Characteristics

One hundred and eighty bulb onions were selected at random depending on size and level of maturity to determine both physical and phytochemical quality attributes levels after curing. The sample was initially separated into four sets of 45 bulbs and placed on a tray in a completely randomized design (CRD). In each tray the sample of 45 bulb onions was randomly divided into three replicates of 15 pieces. Five of the 15 bulb onions were for phytochemical analysis and the other ten were assigned serial numbers ranging from one to ten for the purpose of evaluating their physiochemical attributes. The four clusters with 45 bulb onions were separately placed for 14 days on laboratory benches (room temperature) as well as in incubators (LBI-250E, Labtech, Korea) set at 30, 35 and 40°C.

6.2.2.1 Determination of Weight Loss in Bulb Onion

Weight loss was calculated as described by Gorrepati *et al*. (2018) with minor modification. An electronic scientific balance (UW2200H, (0.01g) Shimandzu, Japan) was used to measure the weight of each 120 bulb onions before and after curing at different temperatures. The formula below was used to calculate weight loss and expressed as a percentage.

$$Y = ((AWBC-AWAC) / AWBC) \times 100$$

Where:

Y= weight loss (%)

AWBC = average weight before curing

AWAC = average weight after curing

6.2.2.2 Determination of Bulb Onion Change in Neck Thickness

Using a digital calliper (inGCO, China), neck thickness was measured at the base where it emerges from the bulb according to Zewdie *et al.* (2019). Each of the 120 bulb onions had its neck thickness measured both before and after curing, and the change was expressed as a percentage as shown:

$$P = (ANTBC-ANTAC)/ ANTBC\times100$$

Where:

P= Percent change in bulb neck thickness

ANTBC = average neck thickness before curing

ANTAC =average neck thickness after curing

6.2.2.3 Determination of Total Soluble Solids Content in Bulb Onion

Total soluble solids value was measured as °Brix using a digital refractometer (HI96801, Woonsocket, USA) as described by Zewdie *et al*. (2019) with slight modification. Thirty fresh bulb onions were grouped into three replicates having 10 pieces per replicate. They were cut into halves using a sterile scalpel, two drops of sap were squeezed from middle leaf scales, placed on the hand refractometer and TSS readings taken twice per piece. Total soluble solids readings of fresh (uncured) bulb onions was used as an initial readings. The TSS readings of 10 bulb onions in triplicate, cured at respective temperatures of 24±2°C (room temperature) and in incubators (LBI-250E, Labtech, Korea) set at 30°C, 35°C and 40°C for 14 days were recorded twice per piece.

6.2.3 Determination of Phytochemicals in Bulb Onion

Determination of total flavonoids, total phenols and anthocyanins were done on fresh bulb onions and those cured at different temperatures. Ten fresh bulb onions were placed in Ziploc bags and frozen for 14 days in a deep freezer (-20°C) awaiting phytochemical analysis. After curing for 14 days, 10 bulb onions in each temperatures of 24±2 (room temperature), 30, 35 and 40°C were placed in Ziploc bags and transported to Jomo Kenyatta University of Agriculture and Technology, Food Science department laboratory for analysis.

6.2.3.1 Preparation of Bulb Onion Sample Extract for Phytochemical Content Determination

Whole fresh bulb onions and those cured at 24±2°C, 30°C, 35°C and 40°C for 14 days were chopped into small pieces separately. The chopped bulb onions were dried in an oven (Memmet UF110, German) at 45°C for 48 hrs. Into a 250 ml conical flask, 2.5 g of grounded samples were weighed, then 25 ml methanol added, closed securely using parafilm and covered with aluminum foil. The mixture was put in a shaker (KS250basic, IKALARBOTECHNIK, German) for 30 minutes at the rate of 250 motions/minute then kept in the dark to extract for 72 hours. After 72 hours, the samples were filtered through Whatman No. 4 filter paper, the filtrate was topped up to 25 ml using methanol. Bulb onion extract was transferred into vial bottles and securely closed. The bulb onion extract was used for total flavonoids and total phenolic content analysis.

6.2.3.2 Determination of Total Flavonoid Content in Bulb Onion

Aluminium chloride ($AlCl_3$) colourimetric method was used in determination of total flavonoids content according to Sharma *et al*. (2014) with slight modification. One milliliter of bulb onion extract was mixed with four milliliter of distilled water into a 10 ml volumetric flask. Three minutes later 0.3 ml of 5 % sodium nitrite solution was added followed by 0.3 ml of 10 % aluminum chloride after three minutes. Two milliliter of 1M

sodium hydroxide was added after five minutes to stop the reaction and the volume was made up to 10 ml using distilled water. For each sample, the absorbance was determined at 415 nm using a UV-Vis spectrophotometer (Shimadzu UV-VIS 180, Kyoto, Japan), in three readings. Quercetin was used as standard calibration curve and total flavonoid content were expressed as milligram quercetin equivalent per hundred grams of samples (mg/100g) dry weight (dw).

6.2.3.3 Determination of Total Phenolic Content in Bulb Onion

Total phenolic content of bulb onion extract was determined spectrophotometrically following the Folin–Ciocalteu method described by Sharma *et al*. (2015) with a minor modification. Methanol was used to make a standard gallic in concentrations of 0, 10, 20, 30, 40, and 50µg/ml. A 0.5 ml was taken from each standard gallic concentration and placed in a test tube. In each test tube two milliliter of diluted Folin-Ciocalteu reagent (10%) was added, the same was put in 0.5 ml bulb onion extract and incubated at room temperature for 3 min. Four milliliters sodium carbonate (0.7N sodium carbonate) was added and placed on laboratory bench for two hours at room temperature and the absorbance was then read at 765 nm using a spectrophotometer (Shimadzu UV-VIS 180, Kyoto, Japan). Total phenolic content was determined as gallic acid equivalents (GAE) using the linear equation based on the calibration curve:

$$C = (c \times V)/m,$$

Where C = total content of phenolic compounds (mg/100g plant extract in GAE)

c = concentration of gallic acid obtained from calibration curve (mg/ml)

V = the volume of the sample solution (ml)

m = weight of the sample (g).

All tests were conducted in triplicate.

6.2.3.4 Determination of Total Anthocyanins in Bulb Onion

Anthocyanins in bulb onion was determined using the procedure described by Zhang *et al*. (2016) with slight modification. Twenty-five grams of chopped onion bulb were weighed and the sample was extracted using ethanol and 0.1M HCl mixtures (85:15) in 1:2 ratio between puree and solvent and mixed together for 1hr using magnetic stirrer. The mixture was filtered through vacuum filtration and supernatant solution collected. The extraction procedure was done in triplicate and the extract was used for total anthocyanins determination. One ml of the extract was diluted to 50 mL volumetric flask with pH 1.0 buffer (0.025 M potassium chloride). The diluted sample test absorbance was measured using spectrophotometer (Shimadzu UV-VIS 180, Kyoto, Japan) at 520nm and 700nm. The second test portion in which one milliliter ml of the extract was diluted to 50 mL volumetric flask with pH 4.5 buffer (0.4M sodium acetate) The diluted sample test absorbance was measured using spectrophotometer (Shimadzu UV-VIS 180, Kyoto, Japan) at 520nm and 700nm. The results were calculated using the formula:

$$\text{Anthocyanin} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

Where:

A (Absorbance) = $(A_{520nm}-A_{700nm})$ pH1.0 - $(A_{520nm}-A_{700nm})$ pH4.5

MW (Molecular Weight) = 449.2 gMol-1 for cyaniding-3-glucoside

DF=dilution Factor

I= Path-Length (cm)

ε= 26,900Molar extinction co-efficient in l/Mol cm for cyaniding-3-glucoside

6.2.4 Statistical Data Analysis

Using GENSTAT statistical software (15th edition), data on weight loss, and change in neck thickness, total soluble solids, total flavonoid content, total phenolic content, and total anthocyanins were subjected to one-way ANOVA. Tukey test was used to separate the means after they were compared using Fisher's protected LSD (least significant difference) at 5% significance level.

6.3 Results

6.3.1 Physicochemical Characteristics of Bulb Onions Cured at Various Temperatures

The initial average weight of bulb onions cured at 24±2°C was 58.70±13.4 g, and an average neck thickness of 0.99±0.43 cm. The original neck thickness of bulb onions cured at 30°C was 1.03 ±0.44 cm, and the weight was 67.41 ±12.50g. Bulb onions cured at 35°C averaged 0.82±0.35cm for the initial neck thickness, whereas it was 0.94±0.35cm for those cured at 40°C. For bulb onions cured at 35°C, the average initial weight was 65.20±12.72g, and those cured at 40°C, it was 62.02±10.92g (Table 6.1).

Table 6.1: Average Level of Weight and Neck Thickness of Bulb Onions before Curing at Different Temperatures (n=30)

Quality attributes levels	Curing Temperature (°C)			
	24±2	**30**	**35**	**40**
Weight (g)	58.70±13.4	67.41±12.50	65.20±12.72	62.02±10.92
Neck thickness (cm)	0.99 ±0.43	1.03 ±0.44	0.82±0.35	0.94±0.35

Prior to curing at 24±2, 30, 35, and 40°C, the bulb onions had an average total soluble solids level of 11.94±0.46 °Brix, anthocyanins was 24.32±0.55 mg/100g, total phenol

content was 65.20±1.19 mg/100g and 96.31±2.7 mg/100g, total flavonoid content (Table 6.2).

Table 6.2: Average Level of Total Soluble Solids (n=30) and Phytochemical (Three Readings per Parameter) Quality Attributes of Bulb Onions before Curing

Quality attributes levels	
Total soluble solids (°Brix)	11.94±0.46
Anthocyanins (mg/100g)	24.32±0.55
Total phenol content (mg/100g)	65.20±1.19
Total flavonoids content (mg/100g)	96.31±2.79

6.3.2.1 Effect of Curing Temperature on Bulb Onion Weight Loss

There was no significant difference ($P>0.05$) in weight loss between samples cured at 30°C and 40°C (Fig.6.1). However, weight loss in bulb onions that were cured at 40°C was significantly higher ($P\leq0.05$) than those cured at 24±2°C and 35°C. Overall, weight loss was highest for bulb onions cured at 40°C (3.9±0.53%) and lowest for those at 35°C (2.8±0.26%).

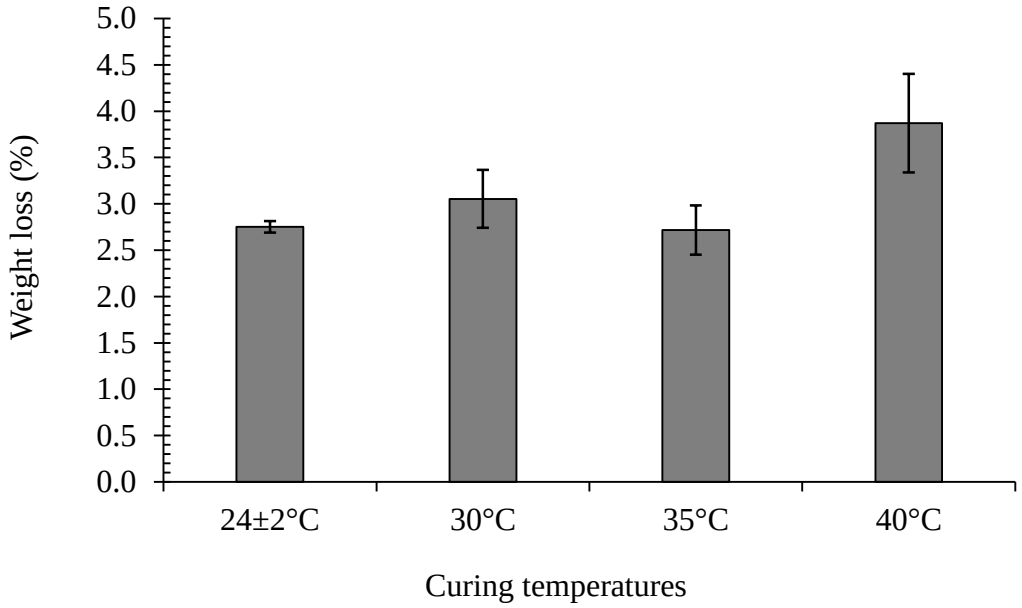

Figure 6.1: Mean Weight Loss (%) of Bulb Onions Cured at 24±2°C, 30°C, 35°C, and 40°C for 14 Days

Vertical bars represent the mean's standard error (SE).

6.3.1.2 Effect of Curing Temperature on Bulb Onion Neck Thickness

The reduction in neck thickness among bulb onions that were cured at 24±2°C, 30°C, 35°C, and 40°C did not differ significantly (P>0.05) (Fig.6.2). The highest decrease (48.7±3%) was observed on bulb onions that were cured at 35°C, whereas those that were cured at 24±2°C had the least reduction (46±3%) in neck thickness. Generally, neck thickness was decreased in a range of 46±3 to 48.7±3% when bulb onions were cured at 24±2°C, 30°C, 35°C, and 40°C.

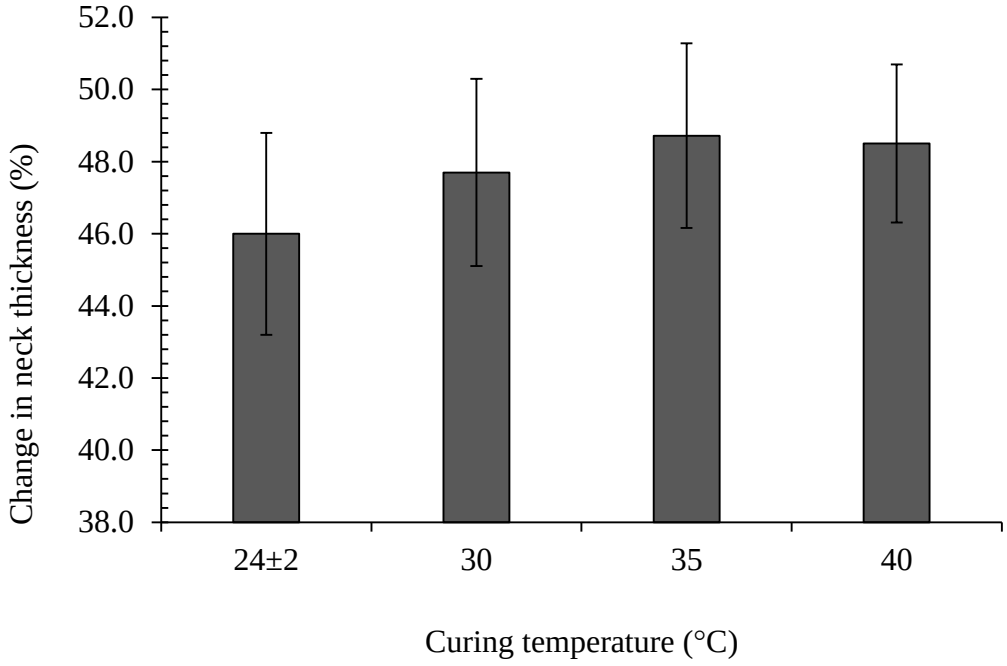

Figure 6.2: Mean *Reduction in Neck Thickness* (%) of *Bulb Onions Cured* at 24±2°C, 30°C, 35°C, and 40°C for 14 *Days*

Vertical bars represent the mean's standard error (SE).

6.3.1.3 Effect of Curing Temperature on Total Soluble Solids (TSS) in Bulb Onion

The findings of this study indicated that bulb onion cured at 24±2°C, 30°C, 35°C, and 40°C for 14 days had no significant (P>0.05) in TSS levels (Fig.6.3). In addition, it was evident that bulb onions cured at 24±2°C, 30°C, 35°C and 40°C for 14 days had no significant (P>0.05) difference in TSS content. Overall, the highest (12.7±0.24°Brix) and lowest (11.9±0.51°Brix) levels of TSS were found in bulb onions that were cured at 35°C and 24°C, respectively.

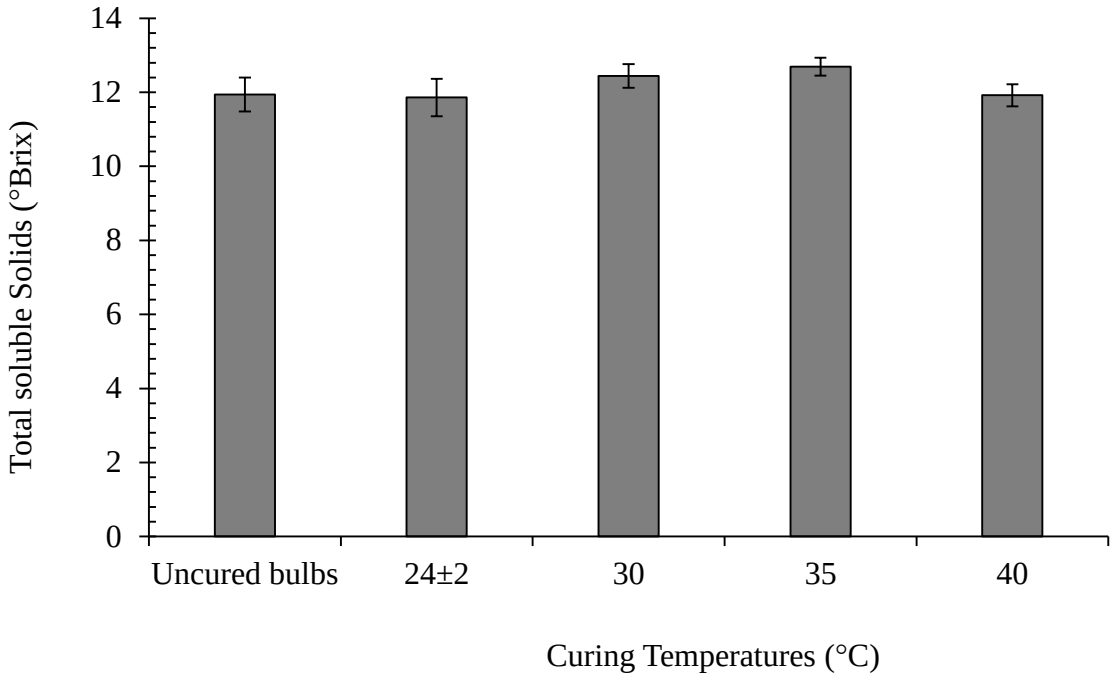

Figure 6.3: Mean Total Soluble Solids Concentrations in Bulb Onions that were Cured at 24±2°C, 30°C, 35°C, and 40°C for 14 Days

Vertical bars represent the mean standard deviation.

6.3.2 Effect of Curing Bulb Onions at Different Temperatures on Phytochemicals

6.3.2.1 Total Phenolic Content in Bulb Onion

Curing bulb onions at 24±2°C, 30°C, 35°C, and 40°C for 14 days resulted in significantly ($P \leq 0.05$) higher amounts of total phenolic content (TPC) compared to fresh bulb onions (Fig.6.4). Bulb onions that were cured at 30°C had significantly greater TPC (148.4±0.31mg/100g) compared to those at 24±2°C, 35°C, and 40°C. However, there was no significant ($P>0.05$) difference of TPC in bulb onions that were cured at 24±2°C and 35°C. Bulb onions cured at 24±2°C had the lowest level of TPC (76.5±0.95 mg/100g), while those at 30°C was greatest (148.4±0.31mg/100g). Major change in TPC was

generally obtained in bulb onions that were cured at 30°C (128%), compared to 17%, 20%, and 25% for those that were cured at 24±2°C, 35°C, and 40°C respectively.

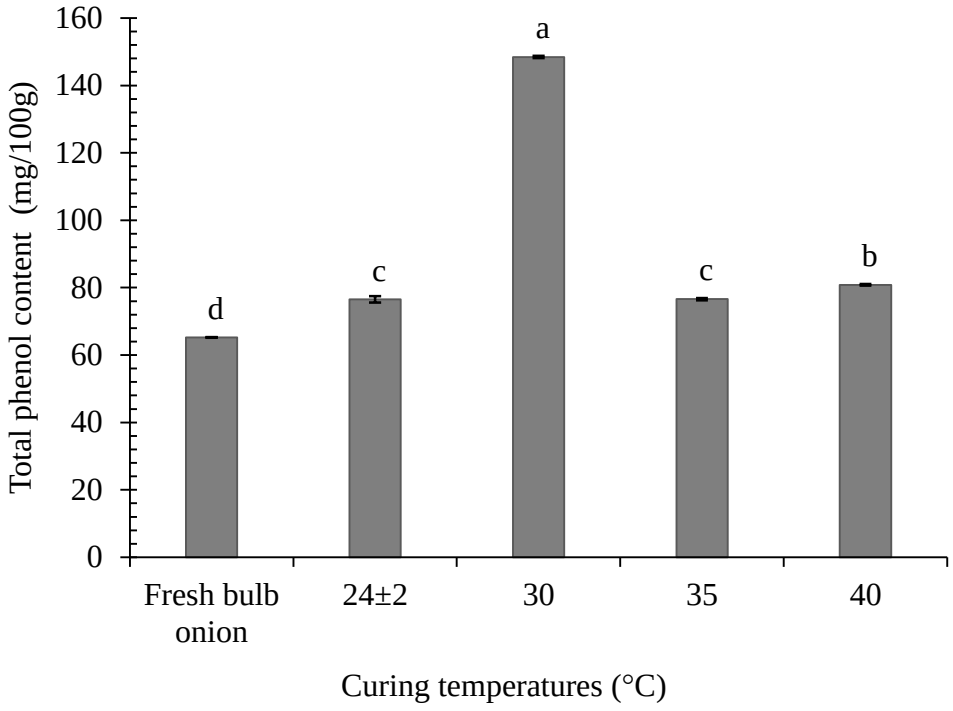

Figure 6.4: Mean Total Phenolic Content (TPC) of Bulb Onions Cured at 24±2°C, 30°C, 35°C, and 40°C for 14 Days

SE of the mean is indicated by vertical bars.

6.3.2.2 Total Flavonoids Content in Bulb Onion

Curing bulb onions for 14 days at 24±2°C, 30°C, 35°C, and 40°C generally increased total flavonoids (TFC) (Fig.6.5). Bulb onions that were cured at 24±2°C had significantly ($P \leq 0.05$) more TFC (248.6±2.85 mg/100g) than those that were cured at 30°C, 35°C, and 40°C. Bulb onions that were cured at 30°C resulted in significantly ($P \leq 0.05$) lower TFC (97.5±3.32 mg/100g) than those cured at 24±2°C, 35°C, and 40°C. However, there was

no significant (P>0.05) difference in TFC between bulb onions that were cured at 35°C and 40°C, resulting in 166.5±8.86 and 154.8±7.37 mg/100g, respectively. The TFC of 14-day-cured bulb onions at 30°C was lowest (97.5±3.32mg/100g), while the levels in those at 24±2°C were greatest (248.6±2.85mg/100g). The highest rate (158%) of TFC increase was observed in bulb onions that were cured at room temperature (24±2°C).

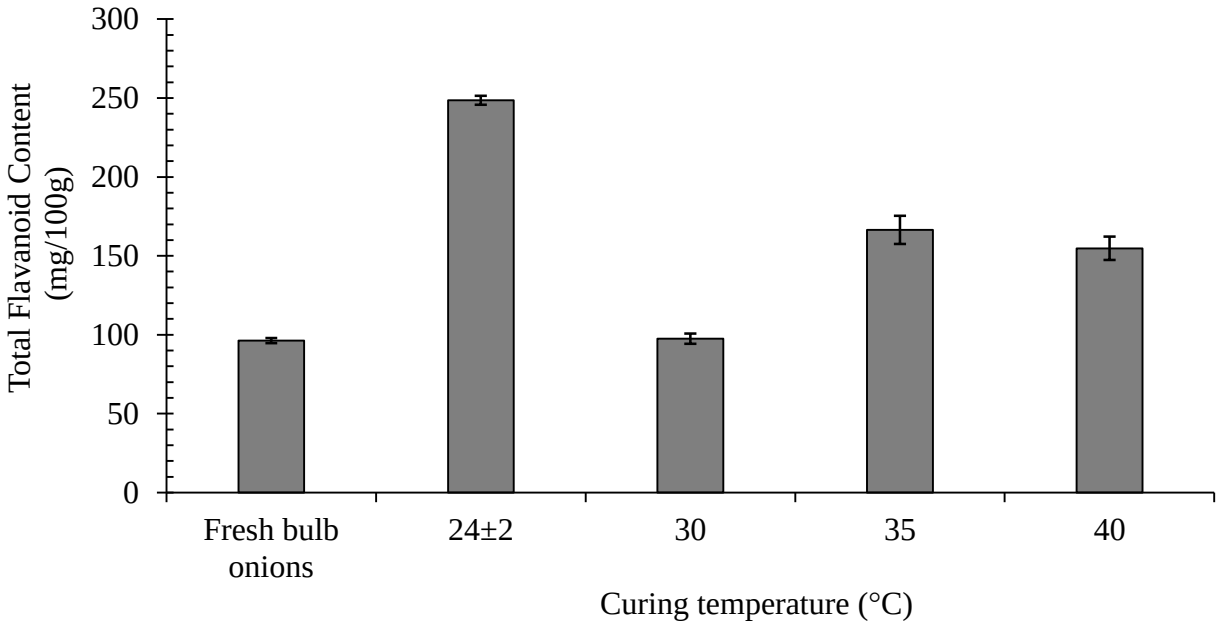

Figure 6.5: Mean Flavonoid (mg/100g) of Bulb Onions Cured at 24±2°C, 30°C, 35°C and 40°C for 14 Days

Error bars represents standard error (SE) of the mean.

6.3.2.3 Anthocyanins Content in Bulb Onion

Curing bulb onions at 24±2°C, 30°C, 35°C, and 40°C statistically (P≤0.05) increased anthocyanins content compared to uncured ones (Fig.6.6). Bulb onions that were cured for 14 days at 24±2°C had anthocyanins content that was significantly (P≤0.05) higher (42.9±0.21mg/100g) than those cured at 30°C, 35°C, and 40°C. Additionally, bulb onions cured at 30°C exhibited statistically (P≤0.05) the lowest concentration of anthocyanins

(37.6±3.32 mg/100g)) compared to those cured at 24±2°C, 35°C, and 40°C. The anthocyanins content of 14-day-cured bulb onions at 35°C and 40°C was 37.6±0.84 mg/100g and 38.3±0.66 mg/100g, respectively, with no significant (P>0.05) difference between the two temperatures. The largest change (79%) in anthocyanins content was observed in bulb onions that were cured for 14 days at 24±2°C (room temperature), whereas the lowest change (33%) was observed in bulbs that were cured at 30°C.

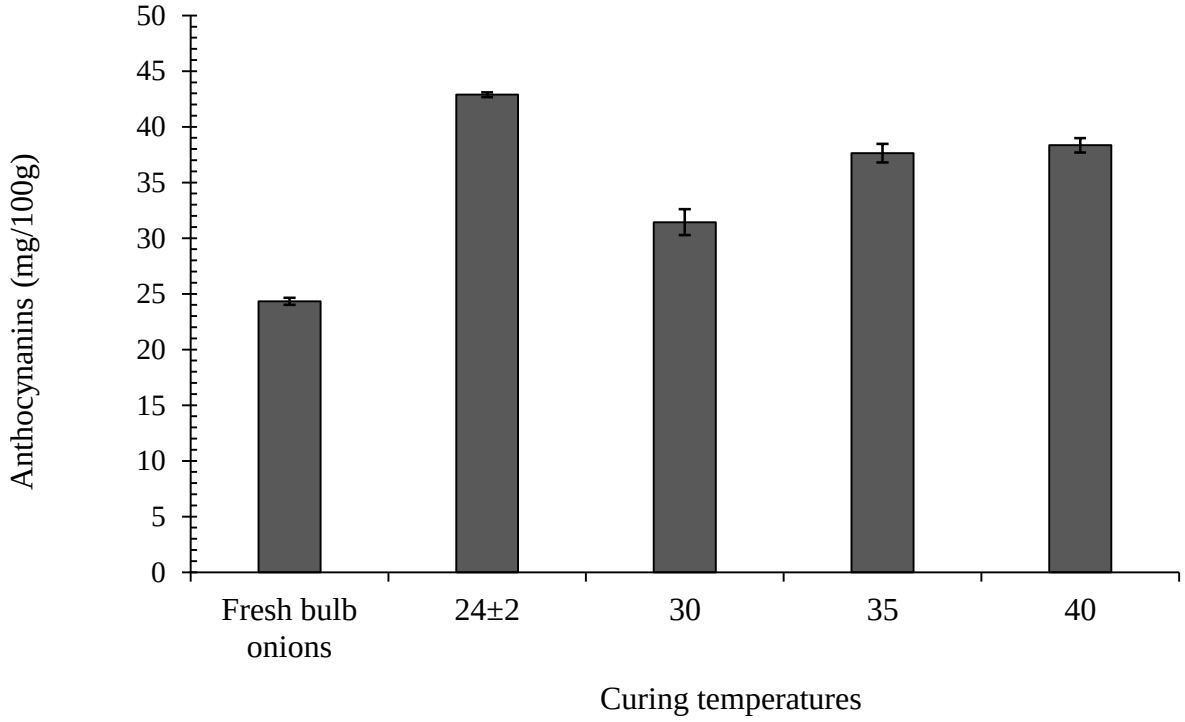

Figure 6.6: Mean Anthocyanins Content of Bulb Onion Cured at 24±2°C, 30°C, 35°C, and 40°C for 14 Days

The vertical bar displays the mean standard error.

6.4 Discussion

One of the recommended postharvest practices for bulb onions is curing, which prolongs storage life and preserves quality (Eshel *et al*., 2014; Gorreapti *et al*., 2017). Since bulb

onions are sold by weight, minimizing weight loss during curing is essential to ensuring higher returns. Bulb onions cured at 24±2, 30, 35, and 40°C for 14 days resulted in completely dry outer scales thus preventing loss of water from the inner scales which led to minimal weight loss. Additionally, there was no evidence of outer bulb onion scales cracking (results not shown) a condition that reduces the percent weight loss during curing according to Rekha *et al*. (2014). The results of this study concur with those of Gorreapti *et al*., (2017), who reported that bulb onions cured under controlled conditions may lose on average 3-5% of their weight. However, the findings did not support the report by Eshel *et al*. (2014) who stated that bulb onions cured at 30°C for nine days caused 7-8% of weight reduction. Zewdie *et al*. (2022) found that a minimum weight drop of 2.8-3.9% was sufficient to effectively cure bulb onions. Therefore, bulb onions were sufficiently cured for 14 days at 24±2, 30, 35, and 40°C based on the findings of this study, where a minimal weight loss of 2.7 to 3.9% was achieved.

One of the most important indication on effectiveness in curing process is decrease in bulb onion neck thickness (Zewdie *et al*., 2022). The results of this study showed that neck thickness was decreased by 46 to 49% in bulb onions that were cured at 24±2°C, 30°C, 35°C, and 40°C for 14 days. Narrowing of bulb onion neck during curing seals it, prevents moisture loss from inner scales thus minimising weight loss and entry of pathogens. The results were consistent with those published by Zewdie *et al.* (2019), who found that 36 hours of curing bulb onions at 30°C, 40°C, and 50°C decreased neck thickness by 46%. Hence, narrowing of bulb onion neck thickness during curing (30°C, 35°C, and 40°C for 14 days) was sufficiently achieved to maintain the quality.

This study findings revealed that levels of TSS were not significantly affected by curing bulb onions at 24±2°C, 30°C, 35°C and 40°C. According to Eshel *et al.* (2014); Nabi *et al.* (2013), curing bulb onions reduces moisture from the outer scales and neck, therefore the inner scales where TSS measurements were taken during this study were not affected by curing at 24±2°C, 30°C, 35°C and 40°C for 14 days. Additionally, based on findings of this study bulb onions cured at 24±2°C, 30°C, 35°C and 40°C for 14 days did not

affect chemical composition in the inner scales. The results corroborated with Zewdie *et al*., (2019) who demonstrated that levels of TSS remained constant over the 36 hours curing of bulb onions at 30°C, 40°C, and 50°C.

Different methods of bulb onion curing affect phenolic concentration levels (Gorreapti *et al*., 2017). The research findings of this study generally demonstrated that bulb onions cured at 30- 40°C for 14 days increased levels of total phenolic content (TPC) attribute. This could have been due to phenylalanine ammonia lyase (PAL) activity, which is associated with phenolic metabolism (Ren, 2019). Benkeblia (2000), stated that PAL activity is influenced by temperature, as the temperature increased PAL activities is reduced and the level of phenolic content increased. The results of the study indicated that TFC increased more at 30°C (128%) than it did at 35°C (25%) and 40°C (25%). This could be that PAL activity was optimal at that temperature (30°C). The results of this study did not agree with those of Ko *et al*. (2016) who indicated that bulb onions cured at 30°C for six days lowered the level of phenolic compounds. Instead, the findings showed that bulb onions cured at 24±2°C, 30°C, 35°C, and 40°C resulted in increased level of TPC.

During curing outer bulb onion scales turn into dry brown colour (Gorreapti *et al*., 2017), this is as a result of oxidation of quercetin by peroxidase (POX) to form 3,4-dihydroxy benzoic acid (Takahama and Hirota, 2000). The results of this study indicated that when bulb onions were cured at room temperature (24±2°C), the TFC level was higher than those they were cured at 30, 35, and 40°C. However, the concentrations were higher compared with fresh bulb onions. This was a as result of POX activity which is influenced by temperature and also thermal instability of quercetin (Zotta *et al.*, 2013). The results were in agreement with Eun *et al*. (2016) reported that curing bulb onions at 36°C for three days reduced level of quercetin concentration which is the main component (80-95%) of TFC (Rodrigues *et al.*, 2017). In addition, the results of these study agreed with Panel *et al. (*2022) who stated that increased curing temperature reduce level of TFC.

Generally, this study findings showed that polyphenols in bulb onion improved during curing and that is directly related to antioxidant activity, these results were in corroboration with Ouyang *et al*. (2018); Ko, *et al*. (2015) who demonstrated a positive correlation between polyphenol concentrations and antioxidant activity in bulb onions.

6.5 Conclusion

From this study, it can be concluded that curing bulb onions at 30-40°C enhanced their physicochemical characteristics and phytochemical content. Based on the results of this study optimal weight loss and reduction in neck thickness were obtained on bulb onions cured between 30-40°C. Additionally, when bulb onions were cured at 30-40°C, the levels of phytochemicals were enhanced. However, more investigation could be conducted to determine how curing temperature at 30 to 40°C affects bulb onions quality during storage.

CHAPTER SEVEN

GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

One of the main challenge causing low bulb onions productivity in major growing counties of Kajiado and Bungoma is postharvest loss where majority of growers experience 5-30% losses. However, in Meru County only 5% experience losses during postharvest handling of bulb onion at farm level. In Bungoma County 17% of the total postharvest losses in bulb onions are caused by rots, in Kajiado 14% and in Meru 2%. Overall rots at the farm level accounted for 10% of total postharvest losses and 14% during marketing. Therefore bulb onion postharvest rots is one of the main cause of losses during handling especially in Bungoma and Kajiado counties. Post-harvest losses in bulb onions were influenced by a range of factors, including education level, size of land under bulb onion cultivation, use of machete during harvesting, limited curing procedure, inadequate sorting, and use of bicycles and donkeys for transportation.

Bulb onion postharvest rot was primarily found to contribute to postharvest losses in Kajiado, Bungoma and Meru counties. Therefore, identification of fungal causative agent was critical to develop postharvest reduction strategies. Three Fusarium species namely; *Fusarium oxysporum* f.sp. *cepae, Fusarium solani* and *Fusarium acutatum* were isolated and identified to cause postharvest rot in bulb onions in the three counties. *Fusarium solani* was mainly isolated from Kajiado County, *F. acutatum* from Meru while *F. oxysporum* was predominately from Bungoma County. Therefore *Fusarium* spp. fungal pathogen are mainly associated with bulb onions postharvest rot in Kajiado, Bungoma and Meru counties.

Curing is one of the recommended postharvest management strategy to reduce incidences of rot in bulb onion. Therefore temperature optimization is fundamental during bulb onion curing to manage rot caused by *Fusarium oxysporum f.sp. cepae, Fusarium solani,* and

Fusarium acutatum in Kajiado, Bungoma and Meru counties. Hence, this study established that Fusarium rot severity decreased when bulb onions were cured for 14 days at a temperature range of 30 to 35°C. Therefore curing bulb onions at temperature range of 30-35°C for 14 days significantly reduce progression of Fusarium rot (26-45%) during postharvest handling.

According to the results of this study, curing bulb onions at 30 to 40°C optimized weight loss and reduction in neck thickness. These quality attributes contribute to reduced occurrences of bulb onion rot, which minimizes postharvest losses and improve its marketability. In addition, curing bulb onions at 30 to 40°C for 14 days improved their phytochemical content (20-75%). Therefore, optimizing bulb onion curing temperature at a range of 30-35°C significantly maintains and improves physical and chemical quality attributes. It is recommended that further research should be conducted to determine impact of curing bulb onions at temperatures between 30 and 40°C for 14 days on their quality during storage.

Overall, it can be concluded from this study that during postharvest handling of bulb onions in Bungoma and Kajiado counties 5 to 30% losses occur which is majorly caused by rot. *Fusarium oxysporum f.sp. cepae, Fusarium solani, and Fusarium acutatum* are the cause of postharvest rot and its progression is reduced by curing bulb onions at 30-35°C for 14 days. Bulb onions cured at 30-40°C for 14 days enhanced their physical quality and phytochemical content, however at 40°C other fungal pathogens are likely to develop. Therefore curing bulb onions at 30-35°C would significantly reduce (26-45%) postharvest rot caused by *Fusarium* spp. and at the same time improve their quality.

7.2 Results Recommendations

Based on the results of this study bulb onion rot contribute mainly to postharvest loss at farm and market level. Therefore it is recommended that:

- Bulb onion value chain actors should be trained to employ appropriate handling practices such use of proper harvesting tools and transportation mode that influences postharvest losses especially those caused by rot.
- Fusarium rot majorly contribute to postharvest losses in bulb onions.
- Bulb onions should be cured at 30 to 35°C for 14 days in order to reduce incidences of postharvest rot and enhance quality

7.3 Recommendation for Future Research

- Further research should be conducted to assess the influence of regional and environmental factors on postharvest Fusarium rot in bulb onions.
- Further research should be done to investigate effect of curing at 30 to 35°C for 14 days on effect of bulb onion storage life.

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APPENDICES

Appendix I: Farm Level Survey Questionnaire

A survey to determine level of postharvest losses in bulb onions at farm level

The specific objectives will be to:

i) Quantify losses of bulb onion at farm level
ii) Quantify losses incurred through rotting in bulb onions
iii) Identify and assess factors influencing PHL
iv) Determine PHL economic losses

Enumerator's introduction note

The enumerator start with greetings preferably in the local language or Swahili and introduces himself or herself. The purpose of the visit and the project are then stated by him/her as follows: "**Postharvest losses in bulb onions"** is the topic of my research project. Your farm was chosen at random for this investigation. The data collected will be stored securely and used exclusively for the objectives of this study. It is voluntary to participate in this study. You have the freedom to end the interview at any moment and to decline to answer any question, without repercussions, if you decide to participate in the study. To be clear, all of the information you submit will be kept private and confidential. Answering the questions should take roughly one and a half hours. Would you be interested in taking part in our research? We appreciate your cooperation in advance. We will gather information and complete a form with the responses.

SECTION 1: BACKGROUND INFORMATION

1. Enumerator's name ----------
2. Enumerator's phone number ----------

3. Name of respondent ----------
4. Relationship of respondent to house hold head ----------
5. Mobile phone of respondent ----------
6. County ----------
7. Sub County ----------
8. Ward ----------
9. Location ----------
10. Village ----------
11. GPS readings ----------Altitude----------M

SECTION 2: HOUSEHOLD CHARACTERISTICS

12. Sex of Respondent [_____] (Codes) 1= Male 2= Female
13. Age of respondent (in years) [_____]
14. Education level of the household head 1=None 2=Primary 3= Secondary 4= Tertiary
15. Main occupation of respondent ----------

SECTION 3: ONION FARM CHARACTERISTICS

16. Size of owned farm land (Acres) [_____]
17. Farm size under onion season in acres [_____]
18. What onion varieties do you grow and why?

No.	Variety Grown	Reasons for growing

19. Total bulb onion produced last season [_____] Kgs

20. Quantity of bulb onion sold last season [_____] Kgs
21. Quantity of bulb onion consumed at home or given out [_____] Kgs
22. How many seasons do you plant per year [_____]
23. Other cultivated crops in terms of priority
    a. ----------
    b. ----------
    c. ----------
    d. ----------
    e. ----------

24. What diseases do you encounter in your onion crop?
    a. ----------
    b. ----------
    c. ----------
    d. ----------
    e. ----------

Bulb onion marketing and transport

25. Who is the main buyer of your bulb onions? (Tick one only) [_____] (Codes) 1=none (Home consumption) 2= Individual Households 3= Trader 4= Hotels 5= Schools/hospitals 6= Supermarkets
26. Average selling price per kg [_____] (kshs)
27. What form of transport do you use to get bulb onion to the buyer or nearest market? [_____] (Codes) 1= human 2= Bicycle 3 = Motor bike 4= Donkey 5= vehicle 6= other, specify----------
28. How far is the market from the farm [_____] (Codes)? 1= <5km, 2=<10km, 3=<20km

SECTION 4: FARM FACTORS ASSOCIATED WITH ONION HANDLING PRACTICES

29. How did you know the onions are ready for harvesting [_____] (Codes) 1=Size 2= Number of days 3= Drying of upper leaves 4= toppling of the leaves
30. Who harvests the bulb onion [____] (Codes)? 1=Hired skilled labour, 2=Hired unskilled labor, 3=Family members
31. What tools do you use for harvesting [_____]? 1= Panga 2=Jembe 3=None
32. When do you harvest? [____] 1=Rainy season 2= Dry season 3= Both
33. Do you cure the onions 1= Yes 2= No
    a. If yes how long do you cure them 1= 1-5 days 2= 6-10 days 3= 11-15 days
34. Do you sort onions after harvesting? [_____] (Codes) **1**= Yes **2**= No
    a. If yes what do you sort for? [____] (codes); 1= small size 2= rotting 3= pest damage 4= sprouted 5=other (specify)
    b. If no, why don't you sort? [___] (codes) 1=no need, 2=the buyer buys all, 3=others (specify)--------
35. Do you grade your onions [_____] (Codes) **1**=Yes **2**=No
    a. If yes what do you grade for? [____] (codes) **1**= Size; **2**=Colour; **3**= Other (specify) _____
    b. If no why don't you grade? [_____] (codes) 1=No need, 2=The buyer buys all, 3=Others
36. Do you store the onions? [_____] 1=Yes 2=No
    a. If yes where do you store? [_____] (Codes)? 1=in the house, 2=Granary, 3=under a shade

    b. How long do you store? [_____] 1=0-1wk, 2= 2-4wks, 3=More than five months
37. Do you encounter any losses after harvest? [____] 1= Yes, 2=No

38. If yes what caused the losses [_____] (Codes). 1= Sprouting, 2= Rotting, 3=Shriveling, 4=Size
39. If rotting caused the losses what percentages [_____] %
40. Overall what percentage of postharvest loss do you incur [_____] (Codes): 1=5-10%, 2=11-20% 3= 21-30%, 4=31-40%, 5=41-50%, 6= more than 50%
41. Name ONE most important factor on your farm causing bulb onion spoilage? ----------
42. ONE solution which can be done to reduce the problem of bulb onion spoilage on your farm

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Thank you for sparing your time to participate in this important exercise.

Appendix II: Market Survey Questionnaire

A survey to determine level of postharvest losses in bulb onions at market level

The specific objectives will be to:

v) Quantify bulb onion losses at market level
vi) Quantify losses incurred through rotting
vii) Identify and assess factors influencing PHL
viii) Determine PHL economic losses

Enumerator's introduction note

The enumerator start with greetings preferably in the local language or Swahili and introduces himself or herself. The purpose of the visit and the project are then stated by him/her as follows: "**Postharvest losses in bulb onions"** is the topic of my research project. Your business was chosen at random for this investigation. The data collected will be stored securely and used exclusively for the objectives of this study. It is voluntary to participate in this study. You have the freedom to end the interview at any moment and to decline to answer any question, without repercussions, if you decide to participate in the study. To be clear, all of the information you submit will be kept private and confidential. Answering the questions should take roughly one and a half hours. Would you be interested in taking part in our research? We appreciate your cooperation in advance. We will gather information and complete a form with the responses.

SECTION 1: BACKGROUND

1. Enumerator's name ----------
2. Enumerator's phone number ----------
3. Name of respondent ----------

4. Mobile phone of respondent ----------
5. County ----------
6. Sub County ----------
7. Market ----------
8. GPS reading ---------- Altitude--------- ----M

SECTION 2: MARKETING CHARACTERISTICS

9. Sex of Respondent [_____] (Codes) 1= Male 2= Female
10. Age of respondent (in years) [_____]
11. Respondent level of education 1=None 2=Primary 3= Secondary 4= Tertiary
12. Main occupation of respondent----------
13. Bulb onion marketing and transport
14. Total bulb onion bought last season [_____] Kgs
15. Total bulb onion sold last season [_____] Kgs
16. Where do you source the bulb onions? [_____] (Codes) 1=within the County 2= outside the County (Specify----------) 3= outside the Country (Specify ----------)
17. Average buying price per Kg [_____] (kshs
18. Average selling price per kg [_____] (kshs)
19. What varieties do you sell and why

No.	Variety sold	Reasons for selling

20. What form of transport do you use to get bulb onion from the farm to the market? [_____] (Codes) 1= Human 2= Bicycle 3 = Motor bike 4= motor vehicle 5= other, specify----------
21. Where do you package the onions? 1=sacks 2= carton, 3=nets 4=no packing

SECTION 4: MARKET FACTORS ASSOCIATED WITH ONION HANDLING PRACTICES

22. Do you sort before selling? [_____] (Codes) **1**= Yes **2**= No
    a. If yes what do you sort for? [____] (codes); 1= small size 2= rotten ones 3= pest damaged 4=sprouted 5=other (specify)
    b. If no, why don't you sort? [___] (codes) 1=no need, 2=the buyer buys all, 3=others (specify)--------
23. Do you grade your onions [_____] (Codes) **1**=Yes **2**=No
    a. If yes what do you grade for? [____] (codes) **1**= Size; **2**=Colour; **3**= Other (specify) _____
    b. If no why don't you grade? [_____] (codes) 1=No need, 2=The buyer buys all, 3=Others
24. Do you store the onions? [_____] 1=Yes 2=No
25. If yes where do you store [_____] (Codes)? 1=store, 2=open air, 3=under a shade
26. How long do you store? [_____] 1=0-1wk, 2= 2-4wks, 3=More than five months
27. Do you encounter any losses during marketing? [____] 1= Yes, 2=No
28. If yes what caused the losses [_____] (Codes). 1= Sprouting, 2= Rotting, 3=Shriveling, 4=Size
29. If rotting caused the losses what percentages [_____] %
30. Overall what percentage of postharvest loss do you incur [_____] (Codes): 1=5-10%, 2=11-20% 3= 21-30%, 4=31-40%, 5=41-50%, 6= more than 50% 23.

31. Name **ONE** most important factor during marketing that causes bulb onion spoilage?

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32. Name **ONE** solution which can be done to reduce the problem of bulb onion spoilage on your farm

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Thank you for sparing your time to participate in this important exercise.

Appendix III: Number of Bulb Onion Samples Collected from Major Markets of Kajiado, Meru and Bungoma Counties

Market	County	Traders' code	Quantity sold per week (kg)	Number of bulb onion samples collected (one bulb @ 0.06kg)
Kitengela	Kajiado	A	80	20
		B	90	22
		C	95	23
		Sub-total		**65**
Kajiado		A	90	22
		B	110	24
		C	85	21
		Sub- total		**75**
Meru	Meru	A	120	30
		B	145	36
		C	130	32
		Sub-total		**96**
Nkubu		A	120	30
		B	80	20
		Sub-total		**50**
Chwele	Bungoma	A	180	45
		B	140	35
		C	115	28
		Sub-total		**108**
Cheptais		A	120	30
		B	120	30
		C	125	30
		Sub-total		**90**
Kimilili		A	75	18
		B	90	22
		Sub-total		**40**