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PRE AND POST-HARVEST CALCIUM AND ETHEPHON TREATMENT ON THE PHYSIOLOGY, QUALITY AND SHELF-LIFE OF ORANGE-FLESHED SWEETPOTATO

(*Ipomoea batatas Lam.)*

BY

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(10532130)

A THESIS SUBMITTED TO THE COLLEGE OF BASIC AND APPLIED SCIENCES, UNIVERSITY OF GHANA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY IN CROP SCIENCE (POST-HARVEST TECHNOLOGY).

DEPARTMENT OF CROP SCIENCE,

UNIVERSITY OF GHANA

LEGON

DECEMBER, 2021.

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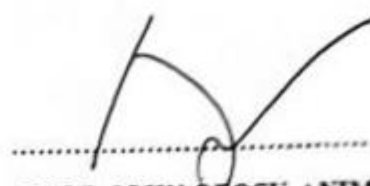
DECLARATION

I, Albertina Naa Adorkor Allotey, do hereby declare that except for references to the work of other researchers which have been duly cited. This work submitted as a thesis for the award of Master of Philosophy in Crop Science (Post-harvest Technology) is the result of my own research and findings and that this thesis has neither in whole or in part been presented elsewhere for another degree.



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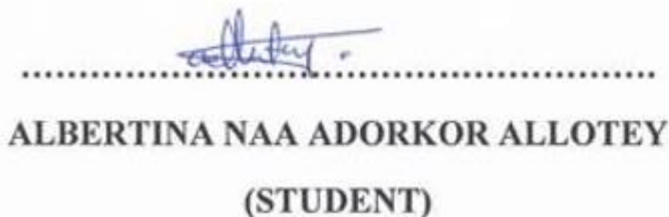

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ABSTRACT

The storage stability and shelf-life of agricultural produce is of great concern to both producers and consumers. Due to the harsh climatic conditions of the tropics, sweetpotato roots deteriorate at an increasing rate posing a challenge to its post-harvest storage life. The pre-harvest and post-harvest application of calcium salts has been successful in slowing down the ripening process of agricultural produce and reducing produce loss of firmness. The firmness of crops can be maintained through the application of calcium salts (calcium chloride) by stabilizing cell membranes and enhancing cell turgor. Ethylene, a naturally occurring plant growth hormone is also used in improving the growth of plants and has diverse effects on the development, growth and storage life of agricultural produce. In this research, calcium chloride was applied at two different concentrations at the sixth and twelfth week after Orange-Fleshed Sweetpotato cultivation and ethephon, seven days before harvest as foliar spray. Untreated roots were also dipped in the same calcium chloride and ethephon treatment concentrations a day after harvest. The changes in the physiological, biochemical and overall quality and shelf-life (weight loss, sprouting, decay, shrinkage) was studied. The efficacy of calcium chloride in inhibiting the growth of certain pathogens was also assessed and the anti-fungal activity of the calcium chloride was determined by measuring the mycelia growth of the pathogens. The pathogens isolated from the orange-fleshed sweetpotato included *Botryodiplodia theobromae* and *Colletotricum* spp. The results showed that calcium chloride at 2% and 4% concentration was not able to inhibit the growth of the pathogens. Both pre- and post-harvest treatment applications inhibited sprouting of the sweetpotato roots. However, the pre- and post-harvest calcium and ethephon treatments were not able to reduce the physiological weight loss, shrinkage and decay of the orange-fleshed sweetpotato roots. The pre- and post-harvest calcium chloride and ethephon treatments had no significant effect on the biochemical properties of the roots as compared to the control.

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DEDICATION

I dedicate this thesis to my grandmother, Virgilian Allotey of blessed memory, my father, Festus Allotey, my mother, Margaret Allotey and my sister, Dr. Grace Indirah Adoley Allotey.

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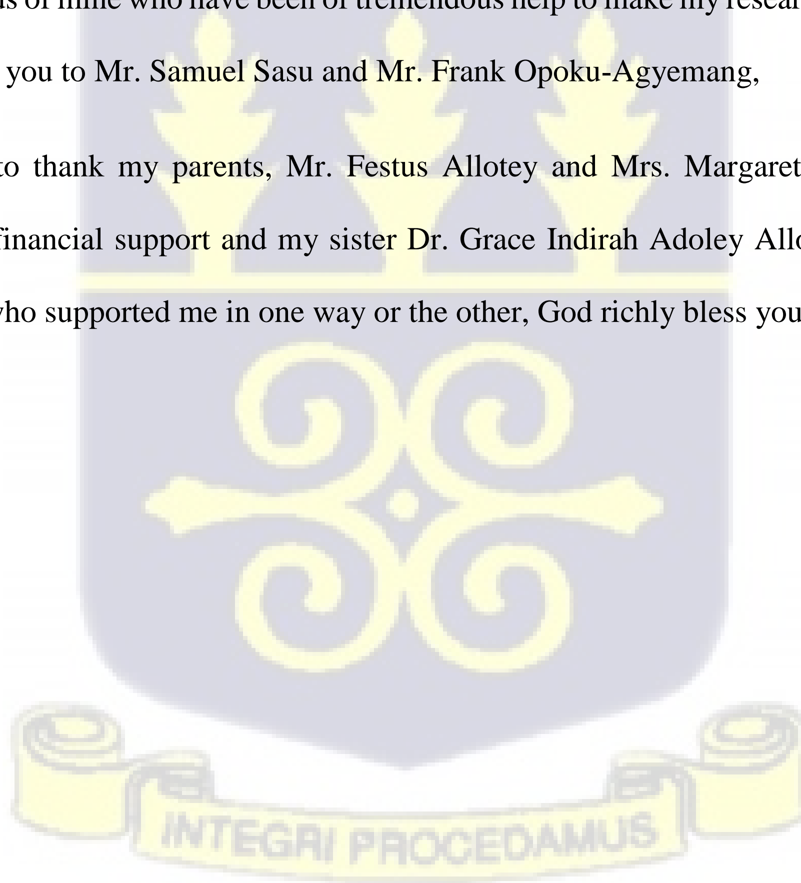
ACKNOWLEDGEMENTS

My sincere gratitude first goes to the Almighty God for his grace, mercy, protection and the opportunity to go through my two years of study in the University, especially, the period of this study. My profound gratitude to my supervisors, Dr. Gloria L. Essilfie and Prof. John Ofosu-Anim for their guidance, encouragement and criticisms towards this achievement.

My heartfelt thanks goes to Dr. Seloame Nyaku, Dr. Eric Cornelius, Mr. Prosper (Farm manager), Mr. Atsu (Assistant Farm manager) and all Technicians at the University Farms for assisting me with my field work. Many thanks to Mr. Duke, Madam Matilda (Crop Science Post-harvest Laboratory), Mr, Richard Otoo (Crop Science Pathology Laboratory) and Mr. Asante (Crop Science Physiology Laboratory) for assisting me in my laboratory aspects. My sincere thanks also go to some friends of mine who have been of tremendous help to make my research work a success. I say a big thank you to Mr. Samuel Sasu and Mr. Frank Opoku-Agyemang,

Finally, I wish to thank my parents, Mr. Festus Allotey and Mrs. Margaret Allotey for their motivation and financial support and my sister Dr. Grace Indirah Adoley Allotey for her moral support. To all who supported me in one way or the other, God richly bless you.



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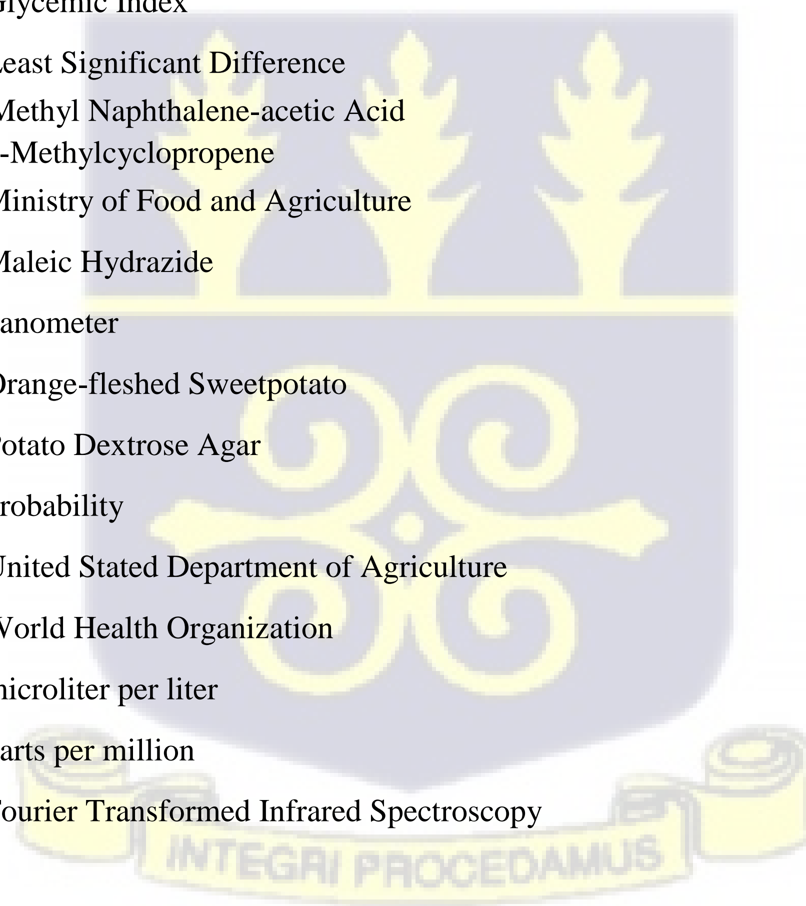
ABBREVIATIONS



ANOVA	Analysis of Variance
AVG	Aminoethoxyvinylglycine
CRD	Completely Randomized Design
CIP	International Patato, Lima, Peru
CRI	Crops Research Institute
CIPC	Chlorophenylcarbamate
C_2H_4	Ethylene
DW	Dry Weight
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
FW	Fresh Weight
GI	Glycemic Index
LSD	Least Significant Difference
MENA	Methyl Naphthalene-acetic Acid
1-MCP	1-Methylcyclopropene
MOFA	Ministry of Food and Agriculture
MH	Maleic Hydrazide
nm	nanometer
OFSP	Orange-fleshed Sweetpotato
PDA	Potato Dextrose Agar
p	probability
USDA	United Stated Department of Agriculture
WHO	World Health Organization
µl L-1	microliter per liter
ppm	parts per million
FTIR	Fourier Transformed Infrared Spectroscopy

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CHAPTER ONE

1.0 INTRODUCTION

Sweetpotato (*Ipomoea batatas Lam.*) is a dicotyledonous plant which belongs to the *Convolvulaceae* family. It is an important food security crop in many developing countries including Ghana (Essilfie *et al*., 2015).

Sweetpotato is currently ranked as the sixth most important crop in the world having a total production of about 105 million tonnes in 2016 with Asia accounting for close to 76% of world's production, followed by the African continent with about 19.5% (FAO, 2015; Mu & Zhang, 2019). It is one of the vegetables most cultivated throughout the world. However, in 2018 the global production of sweetpotato was around 92 million tons which also contributed to the world's food production (FAOSTAT, 2020). It is an important crop grown in almost all the agro-ecological zones in West Africa which covered about 1.82 million hectares of land with an estimated production of 5.48 million tons of storage roots in 2016. In Ghana, sweetpotato is grown widely by peasant and small-holder farmers in the Upper East, Northern, Upper West, Volta and Central Regions where about 0.132 million tonnes of sweetpotato is cultivated annually on 9,622 hectares of land (Bidzakin *et al*., 2014; SRID, 2013).

Sweetpotato is easily handled, and widely adaptable to different climatic conditions and soils, resisting drought and showing low production costs (Burri, 2011). It is considered to be a basic food source for the populations of various developing countries, since it is an energy-rich carbohydrate food (Bovel-Benjamin, 2007; Burri, 2013; Mosta, *et al*., 2015). The storage roots are the main food product although the leaves can also be consumed and provide essential minerals, vitamins and proteins (Bovell-Benjamin, 2010). The storage roots are rich in starch, sugar, vitamin C, β-carotene, iron and several other minerals (Laurie *et al*., 2012; Oloo *et al*., 2014; Sanoussi *et al*., 2016). Sweetpotato has a large number of cultivars which differ from one another in the storage

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root skin colour, flesh colour, the sizes and shape of storage roots and leaves, the depth of rooting, the time to maturity, resistance to diseases and pests, and in the texture of cooked storage roots (Bovell-Benjamin, 2007). White or yellow-fleshed varieties are the most cultivated in Ghana, but a few orange-fleshed varieties are available. Orange-fleshed sweetpotatoes enriches the diets of consumers due to its extreme levels of bio-available pro-vitamin A and also improves food availability and livelihoods.

The national average fresh storage roots yield has been reported as 25 metric tons per hectare but due to low productivity in crop production, low adoption of existing technologies due to poor market incentives and inaccessibility to relevant inputs, the national yield obtained has declined to about 11.5 tonnes per hectare (MoFA, 2016). Also, the lack of appropriate technologies for processing, transportation, handling and storage of sweetpotato and the limited knowledge in postharvest management has resulted in higher post-harvest losses (MoFA, 2010). Sweetpotato roots have a very short shelf-life of about four weeks or less under tropical ambient conditions (Nedunchezhiyan & Ray, 2010; Teye, 2010). The short shelf-life is attributed to the higher moisture content of the sweetpotato storage roots (about 80%) as compared to other staple foods like legumes and cereals (Alvarez-Jubete *et al*., 2010). However, in the temperate areas, under controlled temperature (13-15^{0}C) and relative humidity (90%), it can be stored for several days before consumption and secondary processing depending on the type of cultivar and the storage conditions (Wang *et al*., 2016; Ray *et al*., 2010). The storage conditions play a major role in the storage life of sweetpotato. In Ghana sweetpotato is stored by leaving it in the mounds even when matured, which ties the soils down to the crop and lead to fibrous roots and high weevil infestation (Mariga, 2000). Unlike potato tubers, sweetpotato root does not undergo dormancy and can sprout any time after harvest if conditions are conducive (Afek & Kays, 2004). Some farmers store their sweetpotatoes in lined pits and on floors of dark airy rooms with higher temperature and relative

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humidity which induces sprout. Sprouting is one of the factors limiting the post-harvest life of sweetpotato roots especially at elevated temperatures and high relative humidity. Sprouted sweetpotato roots have a higher respiration rate, leading to greater loss and shriveled roots (Edmunds *et al*., 2008).

Different pre- and post-harvest treatments have been employed to improve product quality and minimize post-harvest losses. Some of the commonly used and proven methods include ultraviolet radiation for protecting fruits against pathogenic fungal infections (Guo *et al*. 2015), the use of polyamines to counteract the destructive effects of ethylene to extend the shelf-life of fruits (Sharma *et al*. 2017), storage in a controlled or modified atmosphere, and dipping agricultural produce in different protective solutions (Moradinezhad *et al*. 2013).

Pre- and post-harvest application of calcium has been used to reduce incidence of different disorders, maintain quality and extend post-harvest life of agricultural produce. Post-harvest calcium dips have been shown to improve the shelf-life of agricultural produce (Hernandez-Munoz *et al*., 2006). Calcium is an essential macronutrient involved in numerous biochemical and physiological processes in plants. It acts as intracellular messenger and is responsible for maintaining the plant cell wall integrity. These properties instigate significant impact on the post-harvest fruit quality, ripening, senescence, decay, and physiological disorders by maintaining cell turgor, membrane integrity, tissue firmness and delays membrane lipid catabolism, enhancing resistance to attack by fungi and bacteria hence, extending storage life of fresh produce (Picchioni *et al*., 1998; Hernandez-Munoz *et al*., 2006). Ethylene is also an important commercial hormone used by farmers or crop producers to preserve climacteric and non-climacteric produce by either extending or reducing the shelf-life of such agricultural produce (Abeles *et al*., 2012). It has the ability to regulate dormancy, sprouting and senescence in potatoes and onions and also maintain the overall quality of sweetpotato and other agricultural crops (Cheema *et al*., 2008; Amoah, 2014).

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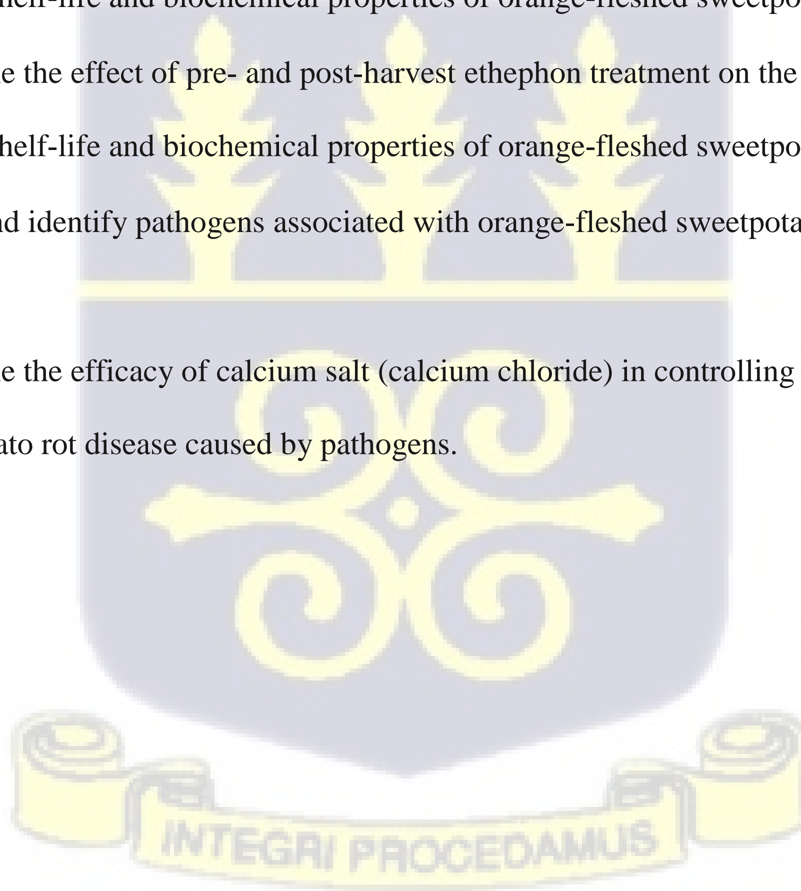
Studies involving the use of ethylene gas to reduce sprouting and to improve the overall storage of crops like potato and onion has been conducted (Daniels-Lakes *et al.*, 2005; Buffler, 2009). Ethephon, an ethylene precursor can be used to generate ethylene which is capable of regulating the growth and development of plants and crops. It is therefore imperative to evaluate the effect of calcium chloride and ethephon used as a pre- and post-harvest treatment to inhibit storage root rot, sprouting, and extend the storage life of sweetpotato. It is also important to isolate and identify pathogens associated with sweetpotato decay in storage and to investigate the efficacy of calcium chloride in controlling sweetpotato decay caused by pathogens.

The objectives of this study were to:

- Determine the effect of pre- and post-harvest calcium treatment on the physiology, quality, shelf-life and biochemical properties of orange-fleshed sweetpotatoes.
- Determine the effect of pre- and post-harvest ethephon treatment on the physiology, quality, shelf-life and biochemical properties of orange-fleshed sweetpotatoes.
- Isolate and identify pathogens associated with orange-fleshed sweetpotato decay in storage.
- Determine the efficacy of calcium salt (calcium chloride) in controlling orange-fleshed sweetpotato rot disease caused by pathogens.



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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and History of sweetpotatoes.

Sweetpotato (*Ipomoea batatas L.*), one of the most cultivated vegetables in the world is an energy-rich food source for the populations of various developing countries (BovelBenjamin, 2007; Burri, 2013; Mosta *et al*., 2015). It is able to adapt widely to different climatic conditions and soils, drought resistant, has low production costs and can be handled easily (Burri, 2011).

Sweetpotato originated from the tropical regions in the South and Central America (Austin, 1988). Sweetpotato was domesticated 5000 years ago in Central America (Bovell-Benjamin, 2014; Nishiyama, 2006) and is now extensively cultivated in the tropical, subtropical and some temperate areas with diverse agro climatic conditions (Chandrasekara & Josheph Kumar, 2016). It is cultivated in over 100 developing countries, within which it is ranked among the first five most important food crops grown in about 50 of those countries (FAOSTAT, 2012). It is considered in most eastern and southern African countries such as Uganda, Rwanda, Kenya, Tanzania, Ethiopia, Zambia, Mozambique and South Africa as a major food crop (FAOSTAT, 2009; Shonga *et al*., 2013).

2.2 World production of sweetpotato

Globally, roots and tubers form part of the majority of food consumed by many populations with an average per capita consumption of 19.4 kg/year from 2013 to 2015 (OCED-FAO, 2016). This consumption estimate is projected to be 21.0 kg/year by the year 2025 (OCED-FAO, 2016). In 2017, a total of about 494.6 million tonnes of roots and tubers was produced globally (FAOSTAT, 2019). Sweetpotato is one of the world's most important, versatile and under-exploited food crops

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with more than 90 million tonnes annual production, mostly contributed by Asian and African countries, especially China (FAOSTAT, 2020). Sweetpotato is the seventh most consumed crop worldwide, with an average land producing area of about 8.2 million hectares and an average yield of 12.1 tonnes per hectare (Chueyen & Eun, 2013; Warammboi *et al*., 2011; FAOSTAT, 2016).

Asia and Africa contributes 85% and 10% of the total world production respectively and the remaining 5% is contributed by other countries, with China alone producing over 80% of the global production (FAOSTAT, 2015). According to FAOSTAT, Bangladesh is the second leading producer of sweet potato after India in South Asia, accounting for about 27% and 68% respectively of their total production (FAOSTAT, 2020). As of 2020, Malawi, Tanzania, Nigeria, and Angola are the top four global producers of sweetpotato ranking after China (Tridge Intelligence Data, 2020). About 105 million tonnes of sweetpotato was produced in the year 2016 with China producing 71 million tonnes making them the world's largest producing country (FAOSTAT, 2016). In that same year, Papua New Guinea produced about 700 thousand tonnes making them the only Pacific Island Country among the top 20 largest sweetpotato producers globally (FAOSTAT, 2016). In the year 2017, the total world production was around 113 million tonnes in about 115 countries, with China still having the highest production of about 72 million tonnes, followed by Nigeria, Tanzania, Indonesia, and Uganda (FAOSTAT, 2019). In 2020, the production of sweetpotato in China was around 48.95 million metric tonnes, producing about 54.75% of the total world production (Tridge Intelligence Data, 2020). In 2017, it was reported that sweetpotato was the third most important food crop in seven Central and Eastern African countries, fourth crop in six South African countries, and eighth in four West African countries (Vollmer *et al*., 2017).

Sweetpotato is the main staple crop in Rwanda, Burundi, Malawi and Uganda and a secondary staple crop in Eastern and Southern Africa (Low *et al*., 2017). In the Pacific Islands, sweetpotato

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is the most important staple food crop (McGregor *et al*., 2016a, 2016b) and a supplement in the diets of the citizens of Fiji, New Caledonia and Vanuatu (Thaman, 1990). It is also an important cultural crop in New Zealand, Easter Island and Hawaii (Ladefoged *et al*., 2005; Roullier *et al*., 2013). And because of sweetpotatoes climatic and ecological adaptations, it is also well known in Polynesia and the low-lying islands (Iese *et al*., 2016; McGregor *et al*., 2016a, 2016b).

2.3 Sweetpotato production in Ghana

In Ghana, sweetpotato is ranked the fourth most important root and tuber crop after cassava, yam and cocoyam in terms of its production (MoFA, 2016). About 96000 hectares of farmland is used for the cultivation of the crop (MoFA, 2016). It has the ability to adapt to various environmental conditions and it is mainly cultivated in the Guinea savannah, Coastal savannah and Forest Transition ecological zones of Ghana (Bidzakin, 2014). Sweetpotato is grown in all ecozones in Ghana but it is widely cultivated by peasant farmers in the Northern, Upper East, Upper West, Central, and Volta Regions by smallholder farmers (Bidzakin, 2014). According to the Food and Agricultural Organization, about 73,400 hectares of land was used in the cultivation of the root crop (FAO, 2010). Ghana is ranked 35th among the sweetpotato producing countries with an estimated production of about 135,000 metric tonnes in 2013 (FAO, 2013). In 2016, the total production was around 143,111 tonnes with 76,594 hectares under cultivation (FAOSTAT, 2016).

2.4 Nutritional benefits and uses of sweetpotato

Sweetpotato is now a root crop well known to a majority of people and is been consumed on a large scale because of the numerous benefits it has on the health and wellbeing of people (Su *et al*., 2017a; Mohanraj & Sivasankar, 2014). It plays a massive role in human diets since it has a very high nutritive value (Scruggs & Quesada-Ocampo, 2016; Da Silva & Clark, 2013). It is a root crop of approximately 25-30% carbohydrate content, with about 98% of its carbohydrate being

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easily broken down to obtain energy when consumed in the body (Clark *et al*., 2013). About 58-72% of the entire sweetpotato root is composed of water. In vivo studies has shown that the carbohydrate from sweetpotato has certain antidiabetic properties which has the ability to stabilize blood sugar levels and decrease insulin resistance (Mohanraj & Sivasankar, 2014; Anbuselvi *et al*., 2012). It also serves as an important source of proteins (Benjamin, 2007).

Sweetpotato contains several micronutrients like vitamin C, thiamin, riboflavin, niacin, panthothenic acid, pyridoxine, vitamin E and folic acid (Hernández Suárez *et al*., 2016). It also contains some essential minerals and trace elements like iron, potassium, calcium, zinc, sodium, magnesium and manganese (Luis *et al*., 2013; Antia, 2006). According to Hernández Suárez *et al*. (2016), sweetpotato provides several bioactive compounds like phenolic acids and anthocyanins which are found to inhibit the growth of human colon cancers, stomach cancers and leukemia. It is also highly rich in polyphenols like chlorogenic, isochlorogenic, caffeic, cinnamic, and hydroxycinnamic acids which helps in the prevention of diabetes, inflammation and cancers (Kurata *et al*., 2007). The leaves and roots of sweetpotato is capable of warming up the body, due to the sugar and other nutrients influencing temperature present in it (Nagai *et al.,* 2011). It also helps in impacting on congestion which is beneficial for people suffering from bronchitis. (Nagai *et al.,* 2011).

Sweetpotato leaves, shoots and vines are all edible and are a very good source of dietary fiber with the roots being low in fats and cholesterol (Anderson & Gugerty, 2013; Odongo *et al*., 2002). In tropical and subtropical countries, both the roots and the tender shoots are used as food (Chai *et al*., 2015). They are also used in feeding livestock and domestic pets in China and the USA (Scott, 1992). According to Collado *et al*., (2001), about 33% of the starch used in the industries in China was derived from sweetpotato. It has also been used extensively as a raw material in the industrial

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field for the production of alcohol (Collins, 1984) and the young leaves have also been used to manufacture teas and powder drinks for human consumption. Sweetpotato is also used in the baking industries for the manufacturing of bread, noodles and cake, for the preparation of adhesives, textile and paper sizing. The crop is also made into flour and the roots, used in the production of sweetpotato chips (Sebben *et al*., 2016).

The nutritional benefits, medicinal benefits of sweetpotato gives it great potential in combating the food shortage and malnutrition that might occur as a result of the increase in the rate of population (Zhang *et al*., 2018). This crop is considered the single most successful example of bio-fortification of a staple crop (The World Food Prize, 2016).

2.4.1 Dry Matter Content

Sweetpotato like other root crops has high moisture content but low in the dry matter (Woolfe, 1992). The high dry matter varieties of sweetpotato are known to be preferred by consumers in Africa. However, varieties that have high carotenoid contents have lesser dry matter contents (Kapinga & Carey, 2003). Orange-fleshed sweetpotato varieties consisting of both high carotenoid and dry matter levels are been bred by the International Potato Center (CIP, 2016). The dry matter content of sweetpotato is mostly 30% but varies, depending on certain factors like the agronomic practice, type of cultivar and soil, the location and its climatic condition and the occurrence of diseases and pest (Bradbury & Holloway, 1988). Tomlins *et al*. (2012) conducted a research to assess the bio-fortified sweet potatoes sensory attribute in Africa over a wide range of dry matter contents (26.8–39.4%) and carotenoid (0.4–72.5 μg/g fresh weight) and the result showed that the sweet potatoes dry matter content reduced by 1.2% with a doubling of the carotenoid content. The yield and dry matter content of the roots is likely to reduce by 30-40 % when the planting time of sweetpotato is delayed (Sajedi *et al*., 2009). According to Aidoo & Tetteh (2004) the dry matter

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content of five different varieties grown in Ghana was within the ranges 34.4 to 37.4 %. On the other hand, a range of 20 to 40 % dry weight content was obtained by Ghana Crops Research Institute for eight varieties (Asafu-Agyei, 2010).

2.4.2 Total Carbohydrates

The dry matter of sweetpotato is about 80% to 90% carbohydrate consisting of several quantities of starch, pectin, cellulose, soluble sugars and hemicelluloses (Woolfe, 1992). The longevity of the roots is determined by these compounds and the storage is determined by its carbohydrate content. And the particular carbohydrates it contains. The carbohydrates may not be the same depending on the type of cultivar, the environment in which the root is grown, growth stage and the storage environment. A study by Reddy & Sistrunk, (1980) and Zhang *et al*., (2002) on the reduction in the carbohydrate composition in sweetpotato during storage shows that prolong storage of raw sweetpotato root before processing reduced the sweetpotato roots firmness and the viscosity of the flour paste with an increase in the sucrose and glucose content (Zhitian *et al*., 2002). Storing sweet potatoes at low temperatures for more than three months causes a reduction in starch content and a rise in alpha-amylase (Zhitian *et al*., 2002). Storing of the sweet potatoes also affects both cooking and sensory properties.

2.4.2.1 Starch

The major carbohydrate in the sweetpotato storage roots is starch, and its formation ends as soon as the storage roots are harvested. For this reason, the quantity of stored starch is very necessary for longer storage life of the storage roots the living tissues since it depends on the stored reserves for biochemical activities. The stored starch under goes enzymatic conversion to glucose (Aiyer, 2005). When the sweetpotato is cooked, the majority of the starch is converted into maltose, which

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gives a sugary taste to the cooked storage roots (Onwueme & Charles, 1994). There are so many health and nutritional gains from consuming sweetpotato starch because of its low glycemic index (GI) grade. However, there is slight increase in blood sugar due to the slow breakdown of the starch when consumed (FosterPowell *et al*., 2002). Consequently, for patients with type-2 diabetes, sweetpotato can be a good diet in curbing obesity. According to Zuraida (2003), in order to make starch for ethanol production the roots can either be baked, boiled, fried or processed. It is a substitute flour for cake or bread production. Most food products use sweetpotato as one of their ingredients. The use of sweetpotatoes for the production of such food products could be potential reasons for increase in the demand for sweetpotatoes. There are known genetic variations for starch content of sweetpotato which acts differently in the stored form or when used as food (Collado & Corke, 1997). Starch composition varies with location, season, growth conditions and maturity (Tian *et al*., 1991). Noda *et al*., (1997), proposed that early planting time of sweetpotato helps in raising gelatin materials and starch present in the crop. In China about 40% of annual sweetpotato produced is used for animal feed mainly for pigs (Scott & Suarez, 1992). The roots and leaves of sweet potatoes can be used as feed in many forms, but due to the low break down of raw starch its usage is limited to some extent. According to Noda *et al*. (1992), different sweetpotato genotypes have different rates of starch digestibility. It has been made clear that sweetpotato flour and starch digestibility have very strong positive correlation (Zhang *et al*., 1993). Zhang *et al*. (2002), conducted a study on six genotypes of sweetpotato roots during storage in relation to the changes in the amount of carbohydrate, α-amylase, digestibility trypsin inhibitor activity and the pasting abilities. After 0–180 days of storage, there was a slight decrease in the starch content of the genotypes. However, in the genotype Hi-dry, it showed a significantly decreased. In the first two months of storage, alpha-amylase activity increased, but later reduced to a level similar to time of harvest (Zhang *et al*., 2002).

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2.4.2.2. Sugars

Sucrose, glucose and fructose are the main forms of sugars in sweetpotato. The comparative amounts of sugars vary amongst genotypes (Zhang *et al*., 2002). The bulk of sweetpotato sugar is sucrose. The concentration of sucrose is higher than other sugars when cultivars are analyzed in raw form, (Van Den *et al*., 1986). According to La Bonte *et al*., (2000), sucrose makes up 68% of total sugar and the rest account for 22- 32% depending on the variety. However, when sweetpotatoes are cooked, they tend to have maltose which results from hydrolysis of the stored starch (Van Den *et al*., 1986). Again, storage increases the amount of sugar in sweetpotato storage roots (Zhitian *et al*., 2002). The sugar content in the fresh storage roots of different American varieties showed by Picha (1985) ranged from 2.9% to 3.2% whilst Puerto Rico varieties fluctuated from 6.3 to 23.6 mg/100 g of dry weight as determined by Martin & Deshpande (1985). The profile for fructose content differs within and among cultivars. Beauregard showed a regular rise in fructose through the development while there was a constant decrease with regards to 'Whitestar'. During the evaluation, inconsistencies were shown in the fructose content of the other cultivars. The glucose content was similar to that of fructose changes during development. Zhang *et al*. (2002) found that the concentration of glucose and sucrose increased in the early stage of storage and remained relatively constant.

2.4.2.3 Total Proteins

According to Maeshima *et al*. (1985), large amounts of two proteins which are more than 80% of the entire proteins are contained in the storage roots of sweetpotato and every 100 g of the fresh storage roots of sweetpotato contain 1.0-1.7 g of protein (Ishida *et al.,* 2006). The two proteins in monomeric forms are sporamins A and B. During the dormant period, the two forms were present, but a reduction was noticed during sprouting (Chokchaichamnankit *et al*., 2009). The proteins were

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separated from each other by electrophoresis on polyacrylamide gels in a non-denaturing buffer. With respect to amino acid the two proteins are found to be similar to each other by composition, peptide map and immunological properties. With sporamin A, the amino acid sequencing of the amino terminal part showed that it is made up of more than two molecular species with varied combinations of a few amino acids. According to Hattori *et al.* (1990), there is an uneven distribution of the protein in the storage root. There is a greater concentration at the proximal end than the distal end. Significantly, it has been noted that there is a higher sprouting at the proximal end than the distal end. This could be as a result of the high protein concentration in the proximal end.

2.5 Variability of sweetpotato roots

Sweetpotato has a large number of varieties which differ from one another in the storage root skin colour (white, cream, yellow, brown, orange or purple), flesh colour (beige to white, red, pink, violet, yellow, orange, and purple), sizes and shape of storage roots and leaves, depth of rooting, time to maturity, the resistance to disease and in the texture of cooked storage roots (Purseglove, 1991). Some varieties of sweetpotatoes for instance, the orange-fleshed sweetpotato contains a very high amount of β-carotene (Low & Van Jaarsveld, 2008). The consumption of this variety by humans, converts it into vitamin A to help prevent the epidemic of vitamin A deficiency in most developing countries (Girard *et al*., 2017). The white, orange and purple-fleshed sweetpotato varieties are well known for their antioxidant and radical scavenging activities (Bovell-Benjamin, 2007; Furata *et al*., 1998; Cevallos-Casal & Cisneros-Zevallos, 2004). The purple-fleshed sweetpotato varieties have been reported to have certain anti-mutagenic properties (Yoshimoto *et al*., 1999) and are very good sources of anthocyanins which are usually concentrated in the starchy core and outer skins (Bae *et al*., 2006). Some sweetpotato varieties have phenolic extracts which

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has chemo-preventive properties required to tackle and prevent anaemia, hypertension and diabetes (Shimozono *et al*., 1996; Rabah *et al*., 2004; Ludvik *et al*., 2004).

2.5.1 Flesh colour

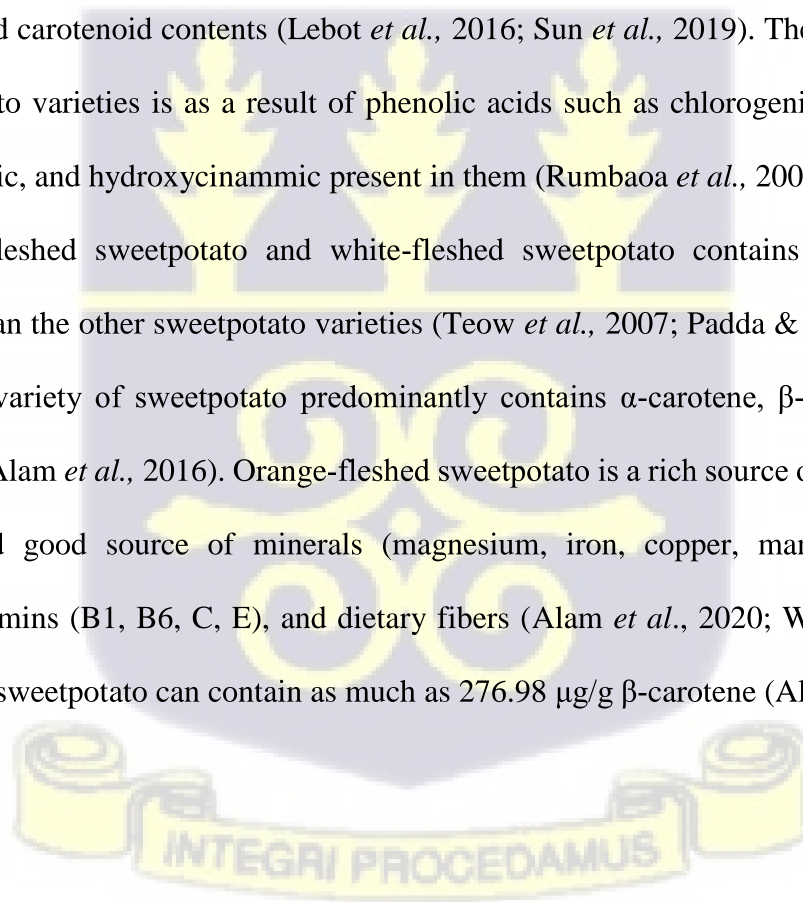
Sweetpotato flesh ranges in colour from beige to white, white or cream, purple, red, pink, violet, yellow to pale orange and deep orange (Alam *et al.,* 2016).

2.5.2 Skin colour

The storage root skin colour also ranges from white, cream, yellow, brown, orange and purple (Alam *et al.,* 2016).

2.5.3 β-carotene content

The antioxidant activities of sweetpotato have mostly been attributed to their phenolic compounds, anthocyanin, and carotenoid contents (Lebot *et al.,* 2016; Sun *et al.,* 2019). The sensory qualities in all sweetpotato varieties is as a result of phenolic acids such as chlorogenic, isochlorogenic, caffeic, cinammic, and hydroxycinammic present in them (Rumbaoa *et al.,* 2009; Musilová *et al.,* 2017). Purple-fleshed sweetpotato and white-fleshed sweetpotato contains higher levels of anthocyanins than the other sweetpotato varieties (Teow *et al.,* 2007; Padda & Picha, 2008). The orange-fleshed variety of sweetpotato predominantly contains α-carotene, β-carotene, and β-5 cryptoxanthin (Alam *et al.,* 2016). Orange-fleshed sweetpotato is a rich source of β-carotene (pro-vitamin A) and good source of minerals (magnesium, iron, copper, manganese, calcium, potassium), vitamins (B1, B6, C, E), and dietary fibers (Alam *et al*., 2020; Wang *et al.,* 2016). Orange-fleshed sweetpotato can contain as much as 276.98 μg/g β-carotene (Alam *et al.,* 2020).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2.6 Orange-fleshed Sweetpotato

Orange-fleshed sweetpotato is one of the varieties of sweetpotatoes easily cultivated and can serve as a source of energy, nutrients, color, flavor and natural sweetener (Woolfe, 1992). Unlike the other varieties of sweetpotato, orange-fleshed sweetpotato is a bio-fortified crop. It is highly rich in β-carotene, which when consumed serves as a good source of vitamin A (Burri, 2011). This helps to prevent vision impairments in most especially children under the ages of five, who are the most vulnerable to vitamin A deficiencies (Meenakshi *et al*., 2010; Bovell-Benjamin, 2010; Andrade *et al*., 2009). Unlike the white-fleshed sweetpotato, orange-fleshed sweetpotato has a very sweet taste and the yellow to orange color which is pleasing to the eyes of children, making it possible for them to consume when incorporated in their diets (Kaguongo, 2012). Orange-fleshed sweetpotato is rich in pro-vitamin A, which contributes to normal eyesight, healthy skin and mucous membranes, healthy cell growth, reproduction, and immunity to diseases such as malaria, measles, and respiratory diseases (Stathers *et al.,* 2005). Orange-fleshed sweetpotato comprises of non-digestible dietary fiber, minerals, vitamins, and antioxidants (Endrias *et al*., 2016; Rodrigues *et al*., 2016). The antioxidant property is as a result of the presence of phenolic compounds and carotenoids (Steed & Truong, 2008). Due to the nutritional components, orange-fleshed sweetpotato contributes tremendously in wellbeing of humans by preventing carcinogenic and cardiovascular disease (Chandrasekara & Josheph Kumar, 2016; Jung *et al*., 2011).

2.7 Pre and post-harvest handling of sweetpotato

The storage life of sweetpotato shelf-life is dependent on all the field practices during production, the handling practices after harvest, and the type of postharvest treatments applied to the crop (Ray & Nedunchezhiyan, 2012; da Silva & Clark, 2013; Adu-Kwarteng *et al*., 2014). This is because,

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the root is affected by pre-harvest factors like the type of variety, soil type, time of planting and harvesting, stage of maturity, fertilizer application and the pest and diseases. These factors if not well taken into consideration can predispose the roots to physiological disorders and microbial attack (da Silva & Clark, 2013). Postharvest factors like poor handling, transportation and storage may also cause decay losses. The use of chemical sprays on the field are used to modify the ripening process and the time of maturity which has an influence on the storage of the storage roots and the quality of the storage roots on the market (Siddiqui *et al*., 2014).

2.7.1 Sweetpotato Storage

Due to the lack of appropriate storage facilities and the unfavorable environmental conditions, sweetpotato growers and sellers in the sub-tropics and the tropics face lots of challenges in the storage of the storage roots. In most tropical countries, sweetpotato storage roots are not able to remain wholesome for longer periods because they are kept in structures traditionally created. A typical example is the sweetpotato storage roots in the East Africa market chain which are able to last for less than a week after harvest (Van Oirschot *et al*., 2003). The storage structure used determines how long the roots can be stored. A lot of methods have been developed regarding the structures used in storing the roots within the tropics but their effect on the compositional changes, nutritive value and shelf-life of the roots have not been extensively assessed (Amoah *et al*., 2011; Agbemafle *et al*., 2013). These methods developed barely create the optimum environmental conditions for prolonged storage. The longest time of storage that can be achieved by some of those storage methods is four (4) weeks or shorter (Amoah, 2014). Due to this, innovative storage methods of pre-treating sweetpotato storage roots with botanical plant extracts repellants from neem and *lantana camara* in pits and evaporative barn have been tried in some parts of Ghana (Abano *et al*., 2011).

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2.7.2 Storage Requirements to Improve Sweetpotato Quality

Prolonged shelf-life and storage of sweetpotato storage roots requires that the harvesting, curing and storage are carefully conducted. In order to achieve that, care must be taken to minimize bruising and injuries on the storage roots during digging, collection, grading and packaging into storage containers. The storage room must at all times be kept clean and able to provide favorable conditions of relative humidity, temperature and gas composition. Injured roots and diseased roots must at all times be removed from the storage room.

2.7.2.1 Temperature and Relative Humidity

In order to preserve agricultural produce for a longer period, they must be properly stored (Watada, 1999). Temperature plays a vital role in maintaining the quality of fresh vegetables after harvest. This is because, depending on the temperature in the storage environment, elements like respiration, produce softening, senescence, colour changes, moisture loss, wilting, sprouting and microbial damage can either be enhanced or reduced (Hardenburg *et al*., 1990). The temperature during harvesting must be taken into consideration and must be done during the coolest times of the day (Thompson, 1996). The shelf-life of sweetpotato storage roots can be extended to a year when stored under optimal temperatures of 12-15°C, below 12°C can also cause chilling injury and therefore should be avoided at all cost (Woolfe, 1992). Such low temperature storage can be achieved by the use of air-cooled stores, refrigeration and commercial cold storage. High relative humidity of about 85-90% is also required in storage to reduce moisture loss from the root skin in order to slow down wilting of the sweetpotato storage roots (Paull, 1999).

2.7.2.2 Physiological loss of weight

Physiological weight loss is a means of deterioration of the roots which can also cause rotting. It is attributed primarily to the loss of water by the storage roots through respiration and the

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transformation of dry matter to energy. The storage roots also loose water through transpiration when there is the difference between the vapor pressure in the interior part of the root and the outside environment. All these phenomenon and processes leads to loss in weight of the roots during storage (Kushman & Pope, 1972; Picha, 1986). The variety of the sweetpotato and the type of storage systems utilized can contribute to weight losses in sweetpotato. Ray *et al*. (1994) conducted an experiment to find out the improvement of shelf-life of sweetpotato by storing them in locally available materials including sand, soil and saw dust, and to evaluate the percentage of weight loss, rotting and weevil infestation associated with storage. At the end of the experiment, it was clearly shown that the losses in fresh weight were mainly due to moisture loss and respiration. According to Amoah *et al*., (2011), a weight loss of about 5% when stored for two (2) weeks was observed in a comparative storage performance study of two varieties of sweetpotato. The two varieties of sweetpotatoes were stored in an evaporative cooling barn and a pit structure. After twelve (12) weeks of storage, the physiological loss in weight amounted to 32.5% (Amoah *et al*., 2011).

2.8 Post-harvest losses of sweetpotato

Despite all efforts made in promoting the usage of both the fresh and processed sweetpotato (*Ipomoea batatas*) products in order to fight food insecurity and malnutrition problems, sweetpotato is faced with high postharvest losses in most developing countries (Dandago & Gungula, 2011; Abidin *et al*., 2016; Sugri *et al*., 2017). The harvested storage roots have a very short shelf-life of about four weeks or less under tropical ambient conditions (Nedunchezhiyan & Ray, 2010; Teye, 2010). Due to this, countries like China, Japan, Indonesia and the Phillipines mostly process the sweetpotato storage roots into dry products (Woolfe, 1992). The short shelf-

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life can also be attributed to the higher moisture content of sweetpotato (about 80%) as compared to other staple foods like legumes and cereals (Alvarez-Jubete *et al*., 2010). Sweetpotato is likely to lose some of the moisture during storage which can cause the degradation of both carotene and ascorbic acid and can also change the porous structure of the storage roots through shrinking which eventually alters the taste of any product made from the storage roots (Haralampu & Karel, 1983; Yamsaengsung & Moreira, 2002; Koc *et al*., 2008). The storage roots are also likely to sprout during storage which reduces the quality of the storage roots reserved for sale. Orange-fleshed sweetpotato roots are subjected to several forms of postharvest losses after harvest. As a result of high moisture content (60-75%), and high respiratory rate of the storage roots, they are very perishable in storage. The heat produced during storage softens the textures resulting in it being susceptible to damage (Okporie *et al.,* 2022).

Sweetpotato storage roots are biologically active. Due to transpiration and respiration during storage (Picha, 1986; Rees, 2001), sweetpotato storage roots undergo physical and chemical changes which in the end affects carbohydrate content of the storage roots (Zhang, 2002). Sweetpotato production is also constrained by pre- and post-harvest losses caused by weeds, pest (sweetpotato weevils), diseases and root knot nematodes, just to mention a few (NRCRI, 2010). The damages caused by these macro and microorganisms during storage and marketing of the sweetpotato storage roots cause an increase in the total weight loss (Rees, 2003; Afek, 2003).

Low productivity in crop production in Ghana can be attributed to several factors such as poor soil conditions, low and poor distribution of rainfall, diseases and pests, inadequate clean planting materials, lack of appropriate technologies and low adoption of those technologies for processing, transporting, handling and storage of produce, and the limited knowledge in post-harvest management (MoFA, 2010). A survey conducted in the Cape Coast Metropolis by Birago (2005)

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revealed that sweetpotato farmers in that area do not store their harvested storage roots at all because of high damages due to inappropriate storage technology.

2.8.1 Skinning

Orange-fleshed sweetpotato (Beauregard for instance) is the most common sweetpotato variety that is highly susceptible to skinning (surface abrasion) during harvest and postharvest handling (Boudreaux 2012; Meyers *et al*., 2013). Since pathogens are mostly able to get inside crops through the wounds created on them, the skinned areas become highly susceptible to pathogen infections (Clark *et al*., 2013). The wounds created on sweetpotato storage roots during harvesting and handling can be healed through a process known as curing, by coating the roots with suberin in order to protect them from any pathogenic invasion (Walter *et al*., 1989). The skinned regions also cause severe moisture loss, which makes them unattractive to consumers on the market (Clark *et al*., 2013). It is therefore important to reduce or avoid skinning and wounding at harvest in order to obtain good quality roots (Clark *et al*., 2013).

2.8.2 Sprouting

Storage roots/Tubers are the thickened underground part of a stem or rhizome which acts as food reserves and can be used in the propagation of new plants (Chandrasekara & Josheph Kumar, 2016). During propagation time, vigorous sprouts production is required (Hall, 1993) but during storage and marketing the sprout decreases the quality and value of the sweetpotato storage roots for fresh market sales (Lewthwaite & Triggs, 1995). In order for these tubers to last for longer periods, the roots undergo a state known as dormancy for a certain period of time. Within that particular period, a phenomenon known as sprouting is inhibited. But towards the end of the dormant period, the buds begin to grow and sprouting exhibited (Mani & Hannachi, 2015). Sprouting of these roots is a major concern, since certain essential nutrients might be lost in the

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process and the quality of the roots/tubers reduced (Go´mez-Castillo *et al*., 2013). Sweetpotato storage roots sprout easily in the tropics. In the tropics, sweetpotato storage roots sprout very fast, have a short period of dormancy of about two weeks when kept in the ambient storage and because of the high moisture content of the roots, metabolism occurs rapidly. All the sprouts arise at the proximal sections of the storage root as the buds in the distal and middle segments go through paradormancy but as they stay for longer periods in storage, the sprouts appear at other segments of the roots (Onwueme & Charles, 1994). Several researches are been conducted to find a solution to this problem and traditional chemicals like Chlorpropham are been applied to prevent sprouting (Huang *et al*., 2014; Cools *et al*., 2014). Endogenous growth hormones like abscisic acid, giberillins and cytokinins control sprout factors and dormancy (Cheema, 2010). And can be controlled by manipulating the relative humidity and temperature of the stored roots (Cheema, 2010). Spraying with chemicals like maleic hydrazide and methyl naphthalene-acetic acid (MENA) in acetone has been found to prevent sprouting and also prolonging the storage period from four weeks to eight weeks but these chemicals have adverse effect of the environment and living organisms (Kay,1987).

2.8.3 Diseases and pests of sweetpotato

Pathogenic infections cause spoilage and reduces the shelf-life of agricultural produce during post-harvest handling, distribution and storage. They cause damage to the sweetpotato storage root in the field and in storage. About 60% post-harvest losses may be incurred as a result of pest and disease for a single growing season (Mariga, 2000). Fungal diseases, viral diseases, nematodes, insect pests, mites and rodents together cause high damages to the storage roots after harvest.

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2.8.3.1 Fungal diseases of sweetpotato

Sclerotium blight, cercospora leaf spots, wilting and storage root surface rot, leaf distortion, black rot and Fusarium root rot are fungal diseases known to affect the production of sweetpotato, its storage and marketing (Thottappilly, 2009).

2.8.3.1.1 Black rot

Black rot is the most important fungal disease of sweetpotato. Sweetpotato storage roots infected with black rot shows symptoms of dark circular depressions which spreads through the entire root.

The infected roots produce toxins which may be harmful for human consumption (Wilson *et al*., 1970).

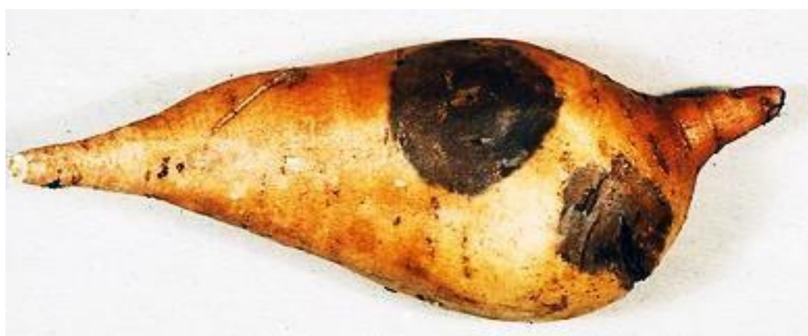

Figure 2.1: Sweetpotato infected with the black rot disease.

2.8.3.1.2 Charcoal Rot

Charcoal rot, a post-harvest disease caused by the fungus *Macrophomina phaseolina* causes great damage to the sweetpotato storage roots. The organism causing the disease is able to survive and proliferate rapidly in warm conditions with an extensive host range. It forms a reddish-brown coloration on the tissues of the sweetpotato at the initial stages and later turns black behind the active decay portions which finally progresses towards the center of the sweetpotato roots following massive decay (Bartz & Brecht, 2002).

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Fig 2.2: sweetpotato infected with the charcoal rot disease.

2.8.3.1.3 Java Black Rot

Java black rot is also one of the major post-harvest diseases affecting sweetpotato. The disease is caused by the fungus *Diplodia tubericola* which starts at one or both ends of the storage root. The initial stage of the decay on the sweetpotato storage root is firm and moist but becomes completely black and shriveled after a short period of time during storage. The disease can fully be recognized when the pathogen forms black stromatic lesions which erupt from the periderm of the storage roots (Skoglund & Smit, 1994).

Fig 2.3: sweetpotato infected with the java black rot disease.

2.8.3.1.4 Scurf

Scurf is also a fungal disease affecting sweetpotato. According to Dewayani, *et al*. (2021), the spread of the disease is very slow and it causes brownish blotches on the sweetpotato storage roots.

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Figure 2.4: Sweetpotato infected with the scurf disease.

2.8.3.1.5 *Fusarium* wilt

Fusarium wilt or stem rot caused by *Fusarium oxysporum f. batatis* is another fungal disease of sweetpotato. It destroys the vascular tissues especially the xylem of the plant causing the leaves to become yellow and wrinkled.

Figure 2.5: *Fusarium* wilt on the leaves and vines of sweetpotato.

2.8.3.1.6 *Fusarium* root rot

The disease is caused by *Fusarium* spp. and do not only affect the produce in the field but are also a major threat to stored storage roots. In China and the USA, storage root rot also known as dry rot

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or end rot caused by *Fusarium solani* is one of the most important post-harvest diseases of sweetpotato (Clark *et al*., 2013). Wang *et al*. (2014) recorded a disease incidence of about 10–20% in China of *Fusarium* root rot on sweetpotato. *Fusarium solani* is a soil-borne pathogen which is only able to penetrate the sweetpotato storage roots through wounds created on them during harvesting to cause the infection (Clark *et al*., 2013). The disease is able to progress into the stems to cause stem cankers and rotting on the sweetpotato storage roots which in the end leads to end rot disease (Clark *et al*., 2013).

Figure 2.6: *Fusarium* root rot disease of sweetpotato.

2.8.3.1.7 Soft rot

Soft rot is a serious post-harvest fungal disease of sweetpotato, commonly known as *Rhizopus* soft rot. It is mainly controlled by the use of resistant varieties and disease-free planting materials, fungicide application before planting and burning of all infected crop residues.

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Figure 2.7: Soft rot disease of sweetpotato.

According to da Silva & Clark (2012) and Stokes *et al*., (2012), sweetpotato pathogens include *Fusarium solani, Macrophomina phaseolina, Lasiodiplodia theobromae*, and *Diaporthe batatatis*. However, the most pathogenic fungi include *Fusarium oxysporum*, *Aspergillus niger*, soft rot (*Rhizopus stolonifer, Rhizopus oryzae*), Java black rot (*Botryodiplodia theobromae*), Black rot (*Ceratocystis fimbriata*), *Sclerotium* rot (*Sclerotium rolfsii*), charcoal rot (*Macrophomina phaseolina*), *Curvularia lunata* (*Cochliobulus lunutus*), *Rhizoctonia solani*, *Plenodomus destruens*, and *Penicillium* spp. (Ray & Nedunchezhiyan, 2012; Olaitan, 2012). But then, previous studies conducted on sweetpotato in Ghana has shown that *Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer, Trichoderma viride, Fusarium oxysporum, Penicellium digitatum, Cladosporium herbarum* and *Aspergillus ochraceus* were the most virulent (Tortoe *et al*., 2010).

2.8.3.2 Viral diseases of sweetpotato

Sweetpotato virus disease (SPVD) is one of the most important diseases affecting sweetpotato (Geddes, 1990). The mosaic is another virus disease of sweetpotato in both USA and Africa causing malformed, small and mottled leaves which eventually affects the roots of the sweetpotato

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crop (Onwueme, 1978). Plants infected with such disease must be removed and burnt to prevent the spread of the disease on the entire field. Another virus disease of sweetpotato is the feathery mottle complex which comprises the leaf spot virus, the yellow dwarf virus (transmitted by the white fly) and the internal cork virus (transmitted by aphids and develops corky patterns within the flesh of the roots leading to a bitter taste when cooked) (Campbell *et al*., 1974). The feathery mottle complex causes stunting of the plants, yellowing spots of the adult leaves and yellowing veins of the younger leaves. Its strains has been the causal agent to various virus diseases of sweetpotato (Campbell *et al*., 1974).

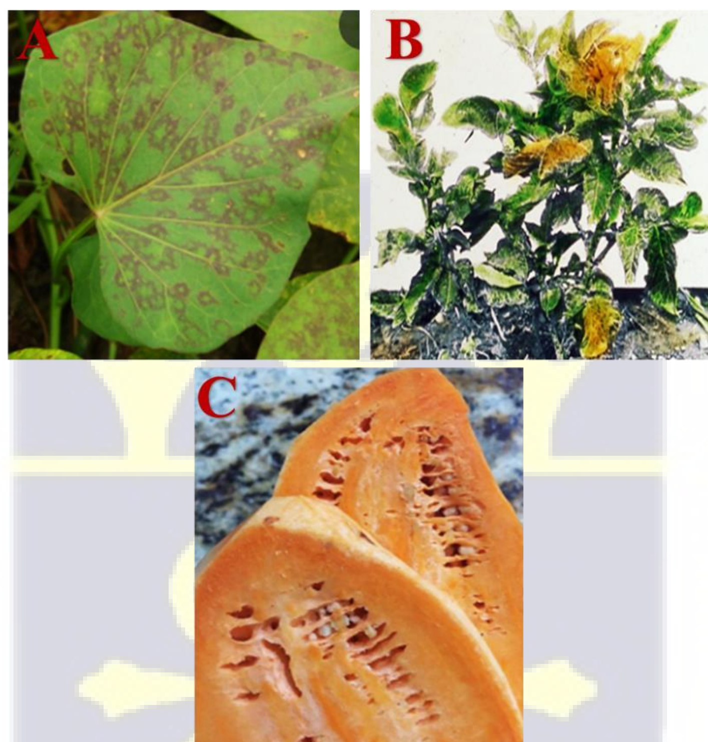

Figure 2.8: (**A**) Feathery mottle complex of sweetpotato leaves, (**B**) yellow dwarf viral disease of sweetpotato, (**C**) internal cork viral disease of sweetpotato.

2.8.3.3 Nematodes affecting sweetpotato

Three major types of nematodes attack the sweetpotato on the field which causes poor growth of the crop, cracked roots and eventually a very low crop yield at the end of production (Giamalva *et*

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al., 1963). The three major types include the root knot nematode (*Meloidogyne*), the sting nematode (*Belonolaimus gracilis*) and the lesion nematode (*Pratylenchrus*) which can be controlled by the use of resistant varieties and crop rotation (Giamalva *et al*., 1963).

Figure 2.9: Sweetpotato affected with root knot nematode (*Meloidogyne*).

2.8.3.4 Pests affecting sweetpotato

The sweetpotato weevil of the genus *Cylas* has been reported as the most serious pest feeding on the sweetpotato crop (Lema, 1992). In Africa, three different species of the sweetpotato weevil (*Cylas formicarius, Cylas puncticollis* and *Cylas brunneus*) have been found to attack the crop. The adult weevil feeds on both the stored roots and the leaves and vines. The larvae of the weevil feeds on the roots and creates tunnels within them, reducing the quality and wholesomeness of the root (Horton, 1989).

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Figure 2.10: Weevil (*Cylas*) attack on sweet potato.

Several methods have been employed to curb microbial decay of which curing have been found to be the most appropriate technique to reduce microbial rot by stimulating wound healing. Meanwhile, the use of fungicides, irradiation, bio-control and storage in ash and saw dust have been found to have moderate effects in controlling decay and improving shelf-life of sweetpotato storage roots. Diseases and pests of sweetpotato has been traditionally controlled by the use of chemicals and cultural methods but lately due to the emergence of pathogen resistance, development of new pathogen biotypes, and an increase of toxic chemical residues in food and the potential dangers posed to both the farmer and the environment, an alternative which is the exogenous use of plant defense hormones have been effectively utilized in the reduction of pathogen (Tian *et al*., 2016; Dukare *et al*., 2019).

2.9 Calcium

The adequate supply of plant nutrients in the right quantity is essential for the proper growth of plants, higher yield and better quality of fruit. The soil is the main source or reservoir of nutrients to plants. It constitutes most or all of the basic nutrients which is required to improve upon the nutritional status of the plant (Lanauskas *et al*., 2006). However, despite the fact that the elements may be present in their right quantities in the soil, uptake by the plant might be a problem

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(Lanauskas *et al*., 2006). This problem is especially important in terms of calcium uptake by plants, which is required for the firmness of the fruit tissues and the quality and shelf-life of agricultural product (Fallahi *et al*., 1997).

As part of the production of agricultural produce, pre-harvest foliar sprays or dipping of harvested produce in calcium salt solutions has been one of the methods used in order to improve upon agricultural produce quality and to minimize the use of fungicides. Soil fertilization with calcium can be an alternative for increasing the concentration of calcium in crop produce. However, foliar application has been proven by previous studies for being the most effective way to increase the amount of calcium in the produce (Tzoutzoukou & Bouranis 1997). The external use of calcium that is by pre-harvest spraying or post-harvest dipping significantly increases the concentration of calcium in agricultural produce and overall affects the maturity and quality of the produce (Poovaiah 1986).

Calcium plays an important role in the plants growth and development (Conway *et al*., 2002). It helps in the stabilization of the cell wall and plasma membrane and also maintains several cellular functions (Conway *et al*., 2002; Palta, 1996). It also alters both intracellular and extracellular processes of the produce by reducing the respiration rate which reduces ethylene production and eventually delays the ripening process and the incidence of postharvest decay (Conway, 1987; Manganaris *et al*., 2005; Raese & Drake, 1993). Alternatively, calcium deficient in the produce increases the respiration rate which at the end reduces the shelf-life of the produce. Therefore, calcium present in the produce can ultimately improve upon the quality, increase shelf-life and reduce losses (Salem & Khoreiby 1991). Lara, (2013) also confirmed in an experiment conducted that significant changes has been realized in the content of antioxidant compounds such as phenols and ascorbic acid in some fruits treated with calcium. Due to this effect, the pre-harvest and postharvest application of calcium salts has been successful in slowing down the ripening process

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of agricultural produce and reducing produce loss of firmness (Floros *et al*., 1992; Holb *et al*., 2012; Mohammed *et al*., 1991; Saftner *et al*., 1998; Souty *et al*., 1995).

2.10 Calcium chloride application

Generally, recognized safe chemicals like calcium chloride, calcium carbonate, calcium biocarbonate, calcium proprionate, and ethylene diamine tetra acetic acid are not injurious to human health (Sewalt *et al*., 2016). These chemicals are found to control post-harvest diseases of fruits and vegetables. Firmness of produce can be maintained through the application of calcium salts (Diaz-Corona, *et al.,* 2020), which can be achieved by stabilizing cell membranes (Picchioni *et al*., 1996) and enhancing cell turgor (Mignani *et al*., 1995). For instance, an experiment conducted by Bhalerao *et al*. (2010), showed that the pre-harvest application of calcium chloride (1% concentration) reduced the percentage physiological loss in weight during storage and also improved upon the physicochemical qualities of sapota fruits. The application of calcium chloride (2% concentration) to pome fruits also increased the protective effect of some antagonistic yeasts. The combination of calcium chloride (2% concentration) and yeast antagonist candida spp. was also able to control gray and bluemolds on apples and pears (McLaughlin *et al*., 1990; Wisniewski *et al*., 1995; Singh, 2005; Zhang *et al*., 2005). The application of an aqueous suspension (108 CFU/ml) of Kloeckera apiculate in calcium chloride (2% concentration) has been used to control *Rhizopus* rot of peaches.

A number of experiments have been conducted to assess the efficacy of calcium chloride on mycelial growth of various fungus. For instance, *Fusarium oxysporum* f.sp. cepae (the causal agent of onion rot) (Turkkan, 2013), *Alternaria alternata, Alternaria solani, Aspergillus niger, Botrytis cinerea, Fusarium solani* isolated from guava fruits (Hassanein *et al*., 2018) and *Lasiodiplodia theobromae* isolated from mango (Nur Fatimma *et al*., 2018). A study by Eryani-Raqeeb *et al*.

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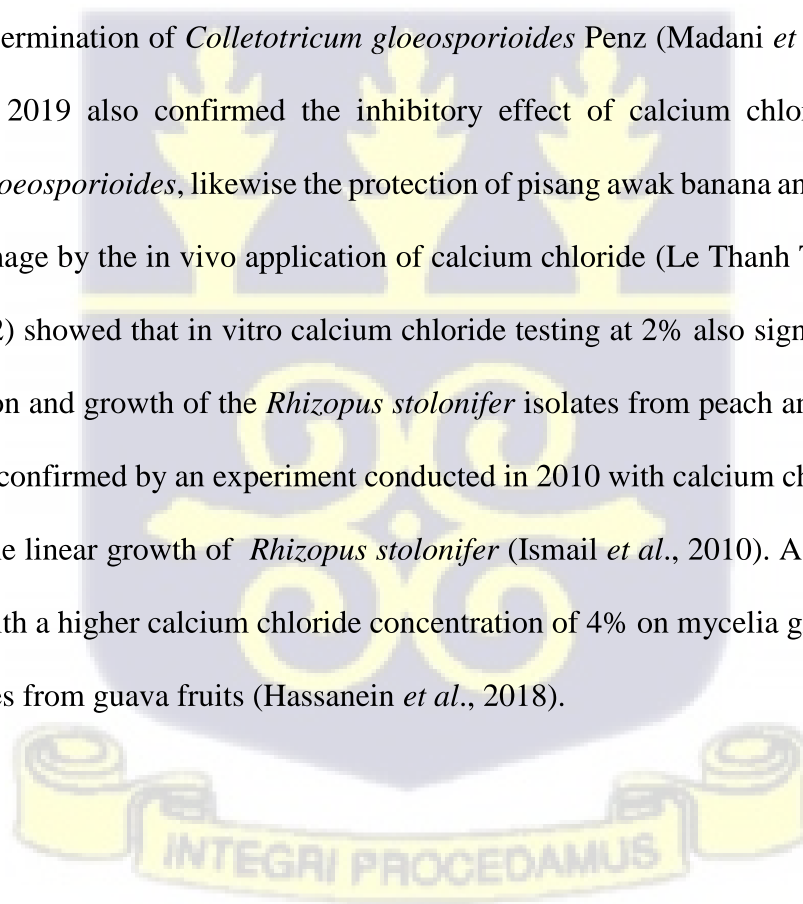
(2009) showed that spore germination of *Colletotricum gloeosporiodes* was effectively inhibited by the postharvest application of calcium (calcium dip) on papaya, likewise that on red-flesh dragon fruit (*Hylocereus polyhizus*) (Awang *et al*., 2011). This finding was later shown in other studies and confirmed the reduction of anthracnose lesion diameters in infected papaya fruits (Madani *et al*., 2016; Ayon-Reyna *et al*., 2017). Similar effect of Calcium in inhibiting the growth of *Botryosphaeria dothidea* and *Botrytis cinerea* on apple
(Biggs *et al*., 1997; El-Gali, 2008) and *Monilinia fructicola* on peaches was reported (Biggs, 2004). Due to the inhibitory effect of calcium, a study conducted in 2014 showed that calcium chloride at 1.5% and 2% significantly inhibited spore germination and hyphal growth of *Colletotrichum acutatum* and *Colletotricum gloeosporioides* isolated from apple anthracnose lesions (Stosic *et al*., 2014). Moreover, a lower concentration of 1%, calcium chloride was also able to significantly decrease spore germination of *Colletotricum gloeosporioides* Penz (Madani *et al.,* 2014). Also, a recent study in 2019 also confirmed the inhibitory effect of calcium chloride (in vitro) on *Colletotricum gloeosporioides*, likewise the protection of pisang awak banana and chilli fruits from anthracnose damage by the in vivo application of calcium chloride (Le Thanh Toan *et al*., 2019). Tian *et al*. (2002) showed that in vitro calcium chloride testing at 2% also significantly inhibited spore germination and growth of the *Rhizopus stolonifer* isolates from peach and nectarine fruits, which was later confirmed by an experiment conducted in 2010 with calcium chloride (2%) being able to reduce the linear growth of *Rhizopus stolonifer* (Ismail *et al*., 2010). A similar result was reported even with a higher calcium chloride concentration of 4% on mycelia growth of *Rhizopus stolonifer* isolates from guava fruits (Hassanein *et al*., 2018).



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2.11 Ethylene

Ethylene is a naturally occurring plant growth hormone used to boost agricultural production through plant growth improvement and has diverse effects on the development, growth and storage life of agricultural produce (Yaseen *et al*., 2010). Harvested produce may be exposed to natural levels of endogenous and exogenous sources of ethylene intentionally or un-intentionally (Saltveit, 1999). Depending on the type of produce, physiological stage of development and the rate of ethylene application, it has both beneficial and detrimental effect on crops (Abeles *et al.,* 2012). A research conducted by Cheema *et al*. (2013) on two varieties of sweetpotato found that there was a decrease in the amounts of fructose and glucose when applied with 10 µl L-1 of ethylene at 25 °C for four weeks in storage. This further stated was associated with higher respiration rates of the ethylene treated storage roots and the activation of other ethylene processes which uses energy through sugar metabolism (Cheema *et al*., 2013). This was further confirmed by Amoah (2014) in another research. However, a study conducted before showed that exogenous application of ethylene rather increased the concentration of sugars in potato (Foukaraki *et al*., 2012).

Ethylene also has sprout inhibition effects that is capable of providing a viable solution to minimize sweetpotato sprouting in developing countries (Cheema *et al*., 2013). This implies that the shelf-life of sweetpotato can be extended and quality preserved when exposed continuously to ethylene in order to reduce sprouting. The continuous application of ethylene effectively inhibits sprout growth of potatoes during storage, but it also leads to darkening of the roots when fried (DanielsLake *et al*., 2005). A study conducted by Bufler (2009) to assess the effect of ethylene on onion sprouting showed that exogenous application of ethylene suppressed the growth of dormant onion bulbs and as well as already sprouting onion bulbs by inhibiting leaf blade elongation. Due to the nature of ethylene at room temperature, handling it mostly in the tropics is difficult for the

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treatment of agricultural produce. However, chemical compounds such as ethephon and calcium carbide which serve as precursors to ethylene generation exists.

2.12 Ethephon

Ethephon (2-chloroethylphosphonic acid) is a systemic plant growth regulator used in agriculture for the generation of ethylene responses (Abeles *et al*., 2012). According to Bremer *et al*., (2008), ethephon decomposes at a pH of 4 or higher. Ethylene is an important commercial hormone used by farmers or crop producers to preserve climacteric and non-climacteric produce by either extending or reducing the shelf-life of such agricultural produce (Abeles *et al.,* 2012). Amoah (2014) conducted a study and concluded that the exogenous application of ethylene on low ethylene producing crops has the ability to regulate dormancy, sprouting and senescence in potatoes and onions. Also, the quality of sweetpotato and other agricultural crops can be maintained with ethylene application (Cheema *et al*., 2008). According to Sitienei (2016) ethephon, an ethylene precursor was applied to orange-fleshed sweetpotato to ascertain its effect on storage decay, weight loss, shrinkage and sprouting. Although ethephon has not been registered for use on sweetpotato, an attempt to get it registered is being made because of the potential effect ethephon has on sweet potatoes (Wang *et al*., 2013).

A research conducted by Wang *et al*. (2013) on sweetpotato showed that the pre-harvest application of ethephon to the leaves and vines of the sweetpotato reduced the skinning effect of sweetpotato (Beauregard) storage roots. However, a negative effect of pre-harvest ethephon application was realized in an experiment conducted by Arancibia *et al*. (2013). The experiment was to determine the possible relationship between pre-harvest foliar ethephon application and storage root tip rot. It was shown that after 1-2 months of root storage, tip rot occurred rather in the stored storage roots

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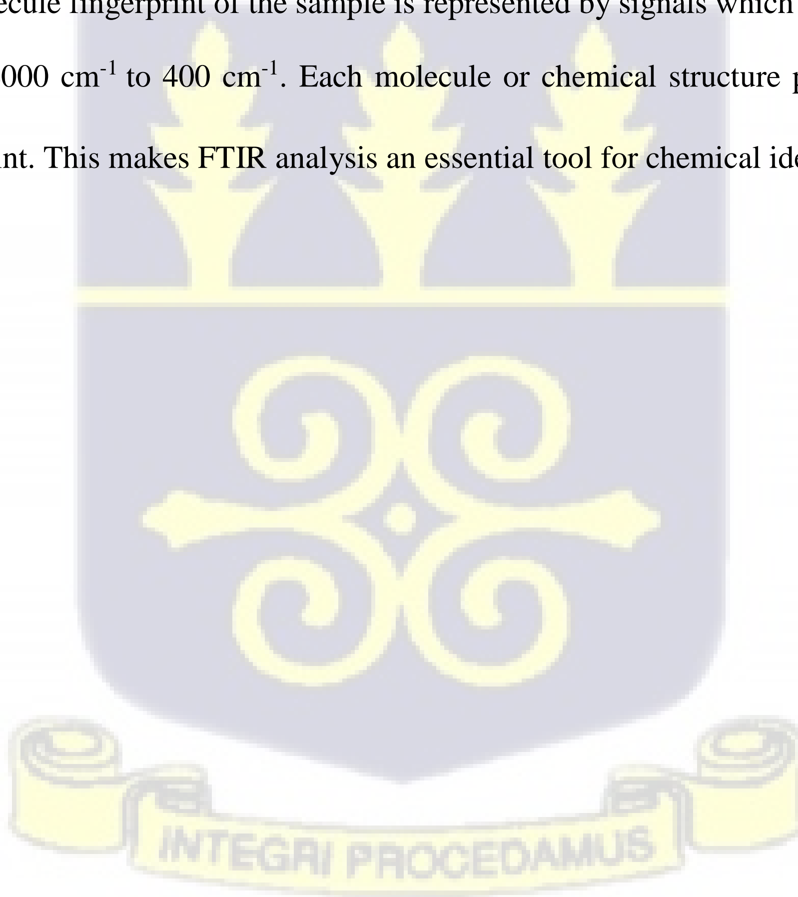
treated with the ethephon. There was an increase in the storage root tip rot in regards to the rate of ethephon applied (Arancibia *et al*., 2013).

2.13 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy is an analytical technique used to identify organic, polymeric, and at times inorganic materials. It is commonly referred to as FTIR Analysis or FTIR Spectroscopy. Chemical properties are observed and test samples are scanned by the use of infrared light. Analyzing a sample using the FTIR instrument, infrared radiation of about 10,000 to 100 cm^{-1} is passed through the sample. Those radiations either pass through the sample or are absorbed by the sample. The sample molecule converts the absorbed radiation into rotational and/or vibrational energy. The molecule fingerprint of the sample is represented by signals which are present on the spectrum from 4000 cm^{-1} to 400 cm^{-1}. Each molecule or chemical structure produces a unique spectral fingerprint. This makes FTIR analysis an essential tool for chemical identification.



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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was carried out at the University of Ghana Farms from February, 2021 to May, 2021. The experimental site lies within the coastal savannah with an estimated mean annual rainfall of 112 mm and a temperature of about 32°C. The Adenta series which has a sandy loam topsoil is the type of soil present on the experimental site. Its colour is pale brown to brown and has a very weak and loose granular structure and consistency (FAO/UNESCO, 1999).

3.2 Experimental design and layout

The field experiment was a Randomized Complete Block Design (RCBD). The area of 111.1 m^2 was divided into three blocks (5.4 m x 3 m) for the pre-harvest application purpose and an area of 2.7 m × 11 m for the cultivation of orange-fleshed sweetpotato roots for the postharvest application purposes.

3.3 Experimental field preparation

Before planting, the experimental site was ploughed and harrowed in order to break the large soil clods to a fine tilth. The debris were also removed from the field. This was as a result of the previous cultivation of maize on the field. The field was then lined, pegged and the individual experimental plots demarcated. Ridges were finally created and spaced at an interval of 90 cm (30 cm high and 45 cm at the base) and vine cuttings (10 cm in length) planted at 30 cm intervals.

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3.4 Planting material

Freshly cut orange-fleshed sweetpotato vines (Apomuden) were obtained from a sweetpotato producer at Samsam in the Greater Accra Region and was used for the cultivation of the storage roots at the University of Ghana farms. Disease and pests free vines were used for the cultivation. They were planted at an angle of 45^0 in the rows at a depth of about 5 cm, leaving at least 2 leaves above the ground.

The treatments used for the experiment were calcium 2% (T1), calcium 4% (T2), ethephon 500 ppm, (T3),calcium 2% + Ethephon 500 ppm (T4), calcium 4% + Ethephon 500 ppm (T5) and control, (distilled water) (T6).

The calcium salt (Calcium chloride) was obtained from a licensed chemical dealer (Fregeosco) and concentrations of 2% and 4% was prepared in the laboratory and used. Chemophon was also obtained from a licensed chemical dealer (Chemico Co. Ltd, Ghana) and a concentration of 500 ppm was prepared in the laboratory and used for the study

3.5 Pre-harvest application of calcium salt

Calcium chloride at two concentration (2% and 4%) was used for the field application. The calcium chloride (2%) solution was prepared by dissolving 120 g of calcium chloride powder in 6 L of distilled water. The calcium chloride (4%) solution was prepared by dissolving 240 g of calcium chloride powder in 6 L of distilled water. The mixture was poured into a knapsack sprayer and sprayed uniformly at the vegetative stage onto the leaves. The application was done after sundown to prevent the heat from the field from interacting with the chemical applied.

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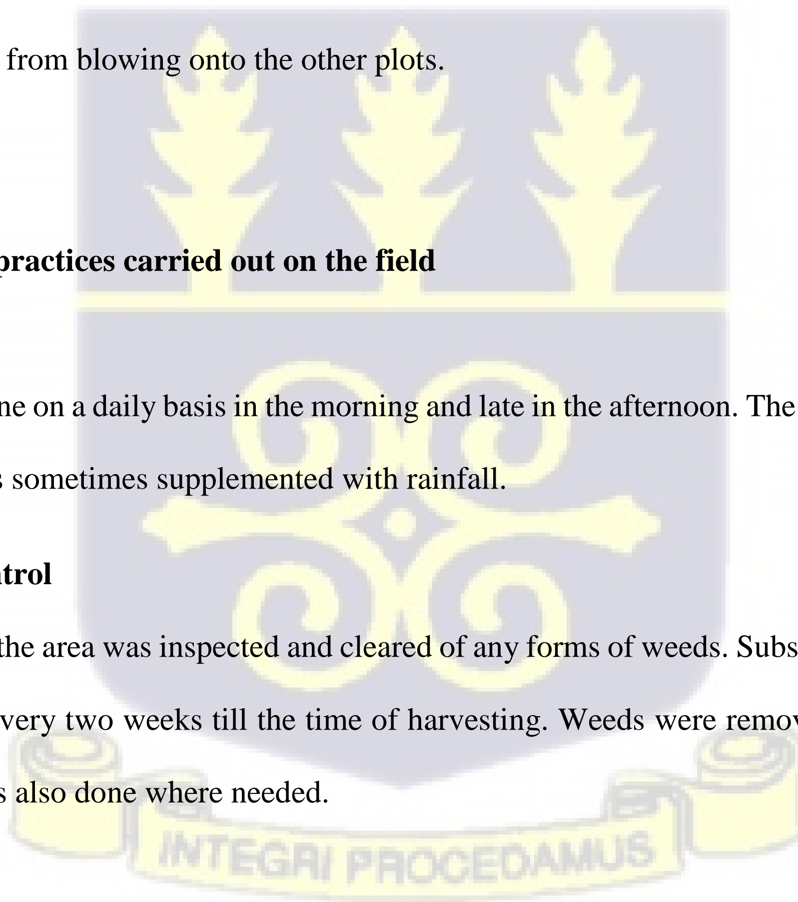
3.6 Foliar (Pre-harvest) application of ethephon

Ethephon, commercially known as Chemophon was obtained from Chemico Company Limited, Ghana, and was used for the foliar application. It is a systemic plant growth regulator which can be used for the generation of ethylene in order to regulate growth processes in crops like pineapples, rubber, cotton and tobacco.

Ethephon at the selected concentration (500 ppm) was applied uniformly on the selected plots seven (7) days before harvesting. The ethephon mixture was prepared by pouring 10ml of 480 SL Chemophon stock solution in 15 L of water in a knapsack sprayer and mixed thoroughly. The mixture was sprayed, ensuring that each leaf was fully soaked in the mixture. The other plots not receiving the ethephon mixture was covered with a polythene sheet to prevent the spray from spilling onto them. The direction of the wind was taken into consideration during spraying to prevent the wind from blowing onto the other plots.



3.7 Agronomic practices carried out on the field

3.7.1 Irrigation

Watering was done on a daily basis in the morning and late in the afternoon. The field was irrigated properly and was sometimes supplemented with rainfall.

3.7.2 Weeds control

Before planting, the area was inspected and cleared of any forms of weeds. Subsequently, weeding was performed every two weeks till the time of harvesting. Weeds were removed by hoeing and hand picking was also done where needed.

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3.7.3 Pest and disease control

The application of an organic pesticide (Attack) was used during the incidence of pests on the field which was on the tenth week after planting. The pesticide was sprayed late in the afternoon, onto the leaves.

3.8 Harvesting

Harvesting of the storage roots was done exactly three and half months after cultivation. The field was irrigated to soften the soil before harvesting. This was done to prevent or reduce surface injuries, wounds and cuts from being created on the sweetpotato roots. The storage roots were finally harvested early in the morning to reduce field heat using hand hoes and a cutlass. The storage roots were immediately washed off to prevent contamination by soil-borne microorganisms, and sorted into wholesome and healthy storage roots. The cleaned roots were packed into white polythene bags and sent to the crop science postharvest laboratory for storage.

3.9 Storage

For the storage of the orange-fleshed sweetpotato storage roots, the five storage roots were each packed in plastic containers. A total of fifteen (15) orange-fleshed sweetpotato roots were used for each treatment, distributed in three (3) separate plastic containers. The roots were later stored at room temperature.

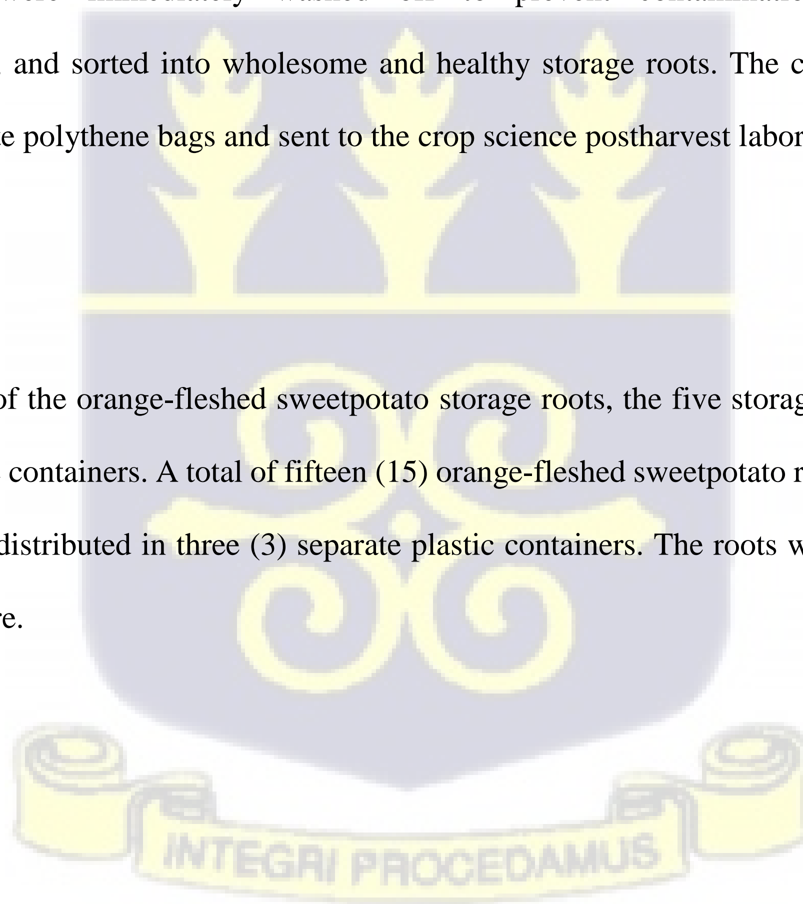

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Figure 3.1: The orange-fleshed sweetpotato storage roots packed in plastic containers and stored in a cool, dry room.

3.10 Post-harvest application of calcium chloride and ethephon

Healthy and uniform sweetpotato storage roots were used for this experiment. The treatments included dipping in aqueous solution of calcium chloride ($CaCl_2$) at concentrations 2% and 4%, ethephon at 500 ppm, a combination of $CaCl_2$ (2%) and ethephon 500 ppm, a combination of $CaCl_2$ (4%) and ethephon 500 ppm, and distilled water (control). The Calcium chloride and ethephon was prepared as described for the pre-harvest foliar spray and applied after harvesting. The sweetpotato storage roots were cleaned with water, air-dried and dipped in the calcium chloride solutions for 10 minutes and the ethephon solution for 2 minutes to prevent the sweetpotato storage roots from staying too long in the chemical solutions prepared. The combination of calcium chloride and ethephon was done by dipping fifteen sweetpotato storage roots first in the calcium chloride solution for 10 minutes, airdried and later dipped in the ethephon solution for 2 minutes. Thereafter, the roots were air-dried to remove the surface moisture, packed in baskets and stored at room temperature for 28 days. The post-harvest treatment was applied 24 hrs after harvesting.

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Each treatment was replicated three times with each replicate containing five sweetpotato storage roots. The experiment was conducted in a completely randomized design (CRD).

3.11 Data collection and analysis

During the storage period, data was taken once a week for four weeks on the weight of the individual roots, number of decayed, sprouted and shriveled roots.

3.11.1 Weight loss

The sweetpotatoes were weighed initially and at the end of the storage time, the weight loss percentage was calculated using the following equation.

Weight loss (%) = (initial weight – final weight) / (initial weight) × 100

3.11.2 Decay

The roots were assessed for the percentage of surface showing visible rotting every week. Decay was calculated based on the over 10% of the surface showing visible rotting of each root. Roots that showed extensive rotting (over 50% surface) was removed from the experiment (Wenzhang *et al*., 2004). Decay was determined as scores, 1 = none, 2 = slight, 3 = moderate, 4 = moderately severe and 5 = severe.

3.11.3 Shrinkage

Shrinkage of the storage roots was determined by measuring the mean diameter of the storage roots with a Vernier caliper at the start of the study and later at weekly interval for the entire storage period. The diameter measuring points at the start was marked with a permanent marker and used as the subsequent measuring point. The difference in the initial and final diameter was used to calculate the percentage root shrinkage.

Root shrinkage (%) = $\frac{D_0 - D_1}{D_0} \times 100$

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Where; D_0 is initial diameter and D_1 is the subsequent diameters at different time of storage.

3.11.4 Sprouting index

Sprouting was also a parameter used to assess the physiological quality of harvested produce. In post-harvest, a sprout is a defect, and a lot is considered as having sprouted if 10% of the roots have sprouts greater than 19 mm (EAC, 2010). The sweetpotato storage roots were assessed for the number of sprouted storage roots, the mean number of sprouted buds per storage root and the maximum sprout length (mm) per storage root. Any bud growth up to 1 mm or more was counted as a sprout. The sprouting index was then calculated using the formula by Obetta *et al*., (2007)

$$\text{Sprouting index } (\%) = \frac{number\ of\ sprouted\ roots}{total\ number\ of\ roots} \times 100$$

The data obtained were subjected to Analysis of Variance (ANOVA) using the Genstat statistical package and the individual means were separated using the least significant difference (LSD).

3.12 Biochemical analysis

Sample orange-fleshed sweetpotato roots were selected after the storage period from each experimental unit for biochemical analysis. The biochemical changes in the sweetpotato storage roots over the storage period was determined using the Fourier Transform Infrared Spectroscopy analyzer (FTIR).

3.13 Preparation of Potato Dextrose Agar (PDA) and sterilization of petri dishes

A 1.2 g of potato dextrose agar was weighed into eight small bottles and mixed with 30 ml of distilled water. The bottles were covered with a metallic foil and autoclaved for 2 hrs. They were

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finally placed in a water bath. Twenty-four petri dishes were sterilized in an oven at 175ºC for 2 hrs and 30 mins and allowed to cool for 30 mins.

3.14 Isolation and identification of pathogens associated with the spoilage of sweetpotato storage roots

Two separate experiments were conducted. For the first experiment, the storage roots were washed under clean running water and cut into 2mm^2 segments at the junction between healthy and infected parts of the storage root using a scalpel. The cut tissues were surface sterilized for 1 min in 1% sodium hypochlorite (NaOCL) solution to get rid of contaminants on the surface and rinsed two times with sterile distilled water (Okigbo *et al*., 2009). Four segments of the tissue pieces were plated out on water agar right after sterilization. The plates were left in the lamina chamber to incubate at 28°C for five days and observations were made on a daily basis for the growth and development of the pathogen. After five days, the pathogen which grew from them were sub-cultured on PDA and incubated at 28°C. The pure cultures obtained were used in the identification of the pathogen with the help of a compound microscope and guide. The second experiment involved the direct picking of the microbial spores onto Potato Dextrose Agar (PDA) in petri dishes and the preparation of slides using the microbial spores growing directly on the storage roots. This was later viewed under the compound microscope and the type of pathogen ascertained.

3.15 Pathogenicity test

The fungal isolate (*Colletotrichum spp*) obtained from the rotten storage roots was inoculated into healthy orange-fleshed sweetpotato storage roots. Three healthy orange-fleshed sweetpotato storage roots were washed under running tap water and surface-sterilized in 1% sodium hypochlorite (NaOCL) solution for 1 min after which they were rinsed twice with distilled water.

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One cylindrical disc each was removed from the three roots with a sterile cork borer. Mycelial discs (4 mm diameter) were made from the cultures and a fungal disc was put into the holes created in the roots. The orange-fleshed sweetpotato discs were replaced and inoculation sites wrapped around with a paraffin wax. This was done to seal the wound created and to prevent contamination. The storage roots were properly labelled and incubated at 28°C for 14 days. Disease symptoms produced by artificial inoculation after the incubation period were compared with those observed on the naturally infected storage roots collected from storage. The fungi was re-isolated from the inoculated diseased sweetpotato storage roots and cultured on PDA plates. The morphology of each pathogenic fungus was compared with that of the original culture.

Figure 3.2: Orange-fleshed sweetpotato inoculated with fungal isolate (*Colletotrichum spp*)

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3.16 Anti-pathogenic susceptibility test

For the 4% concentration, 1.2 grams (g) of the calcium chloride was poured into two (2) small bottles each containing 30 mL of molten PDA and anti-biotics added to prevent the growth of bacteria. Each small bottle containing the 4% concentration of the calcium chloride and 30 ml of molten PDA, was dispensed into six (6) petri dishes. For the 2% concentration, 0.6 grams (g) of the calcium chloride was poured into two (2) small bottles each containing 30 mL of molten PDA and anti-biotics added to prevent the growth of bacteria. Each small bottle containing the 2% concentration of the calcium chloride and 30 ml of molten PDA, was also dispensed into six (6) petri dishes. The petri dishes were swirled gently on the work bench to enhance even dispersion of the chemical. The mixture was allowed to solidify and used for the inhibition of mycelia growth of the two fungi identified (*Botryodiplodia theobromae and Colletotrichum spp*). Plugs of the mycelia was inoculated centrally onto the medium. Three (3) petri dishes containing 0.08 g of a fungicide (Mancozeb) and molten PDA was used as the standard for *Botryodiplodia theobromae* and an extra three (3) containing 0.08 g of Mancozeb, 0.12 g of Carbendazim and molten PDA was used as the standard for *Colletotrichum spp*. Controls were six (6) petri dishes containing PDA with no chemical. The treatments were replicated three (3) times in a completely randomized design (CRD). They were then incubated at 28^{0}C for fourteen (14) days and the diameter of the radial mycelia measured using a meter rule at an interval of 24 hrs. Inhibition of fungal growth was calculated using the formula

$$\frac{R_1 - R_2}{R_1} \times 100$$

Where R_1= radial growth of the pathogen in the control plates.

R_2= growth of the pathogen with treatment.

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CHAPTER FOUR

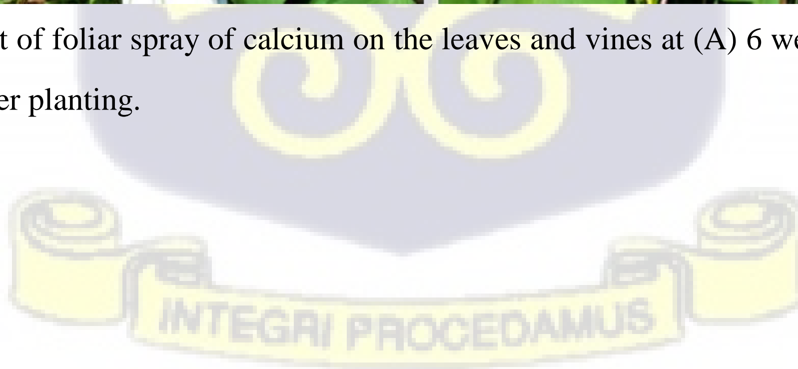
4.0 RESULTS

4.1 Effect of pre-harvest application of calcium and ethephon treatment on the physiology of orange-fleshed sweetpotato

4.1.1 Effect of pre-harvest calcium treatment application on the physiology of orange-fleshed sweetpotato

Calcium chloride application to the orange-fleshed sweetpotato at pre-harvest had no major effect on the leaves and vines. It was observed that, applying calcium chloride at 6 and 12 weeks did not result in any colour change of the leaves and vines. (**Figure 4.1**).

Figure 4.1 Effect of foliar spray of calcium on the leaves and vines at (A) 6 weeks after planting (B) 12 weeks after planting.



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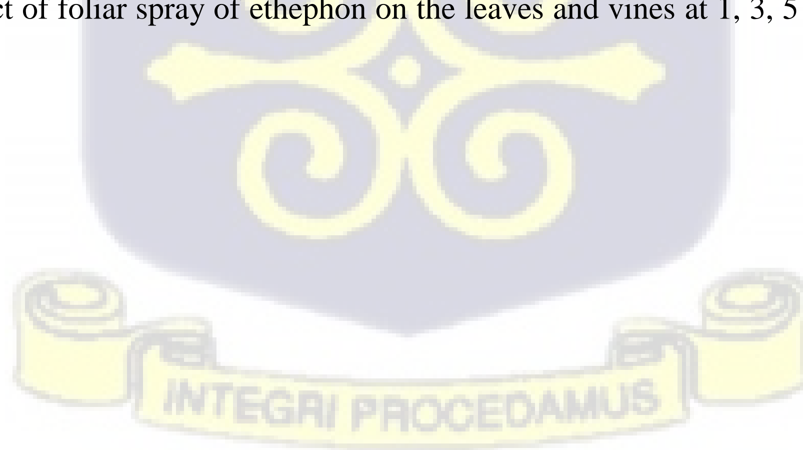
4.1.2 Effect of pre-harvest ethephon treatment application on the physiology of orange-fleshed sweetpotato

The ethephon applied had an effect on the leaves and vines of the orange-fleshed sweetpotatoes on the field. Rapid yellowing and browning of the leaves and vines days after the application was observed (**Figure 4.2**).

Figure 4.2 Effect of foliar spray of ethephon on the leaves and vines at 1, 3, 5 and 7 days before harvesting



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4.2 Effect of pre- and post-harvest calcium and ethephon treatment on the quality of orange-fleshed sweetpotato

4.2.1 Effect of pre-harvest calcium and ethephon treatment on the Percentage weight loss of orange-fleshed sweetpotato

At the end of the entire storage period (28 days), there was a steady rise in the percentage weight loss amongst all the treatments pre-applied to the storage roots. The percentage mean weight loss of the storage roots pre-treated with the combination of calcium chloride 2% and ethephon was significantly different ($p \leq 0.05$) from the rest of the treatments and the control. Storage roots treated with the combination of calcium 2% and ethephon suffered the highest mean weight loss of about 48.50%, followed by calcium 2%, calcium 4%, the combination of calcium 4% and ethephon, and ethephon which had mean weight losses of 39.04%, 38.17%, 32.78% and 22.75%, respectively (**Figure 4.3**).

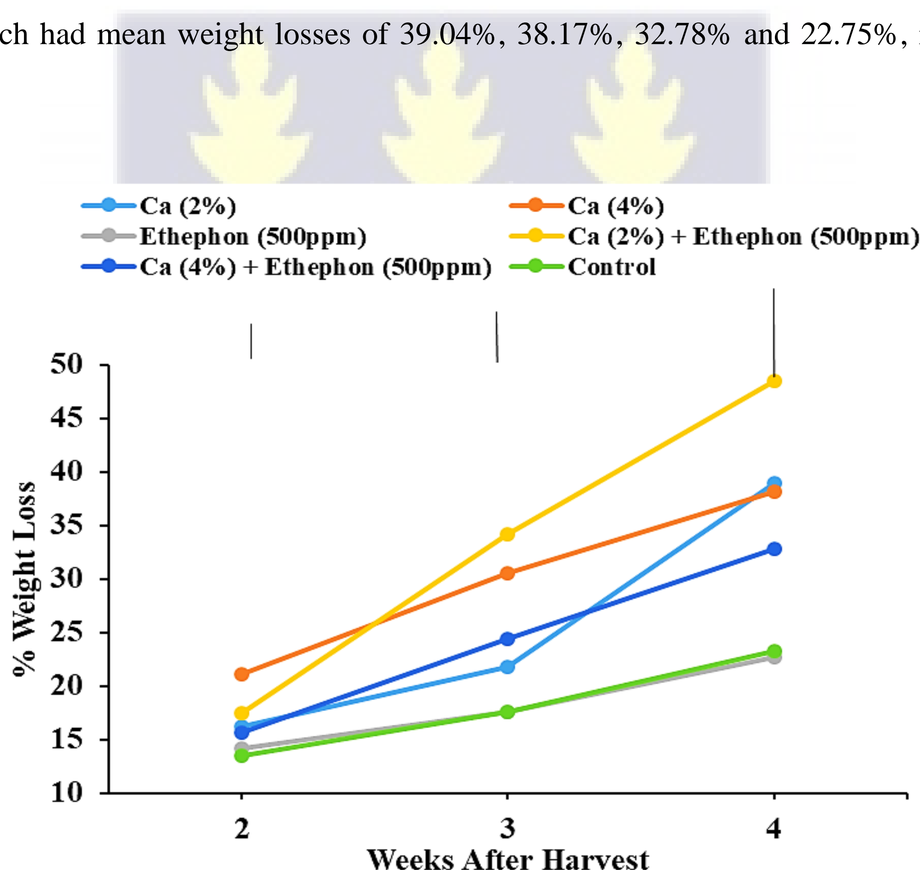

Figure 4.3: Percentage weight loss of *I. batatas* at **pre-harvest** application of Calcium and Ethephon

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Table 1: Percentage weight loss mean separation of *I. batatas* at **pre-harvest** application of calcium and ethephon after the storage period.

	Means
Ethephon (500ppm)	22.75 a
Control	23.27 a
Ca 4% + Ethephon (500ppm)	32.78 b
Ca 4%	38.17 b
Ca 2%	39.04 b
Ca 2% + Ethephon (500ppm)	48.48 c

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4.2.2 Effect of post-harvest calcium and ethephon treatment on the percentage weight loss orange-fleshed sweetpotato

For the post-harvest application of the treatments, there was a steady rise in the percentage weight loss of all the treatments applied to the storage roots. The percentage mean weight loss of the storage roots treated with the combination of calcium chloride 4% and ethephon and ethephon alone were significantly higher ($p \leq 0.05$) among the treatments. Roots treated with those two treatments suffered the highest mean weight losses of about 49.86% and 38.53% respectively. The rest of the treatments, the combination of calcium 2% and ethephon, calcium 2% and calcium 4% had mean weight losses of 32.05%, 28.12% and 27.08%, respectively (**Figure 4.4**).

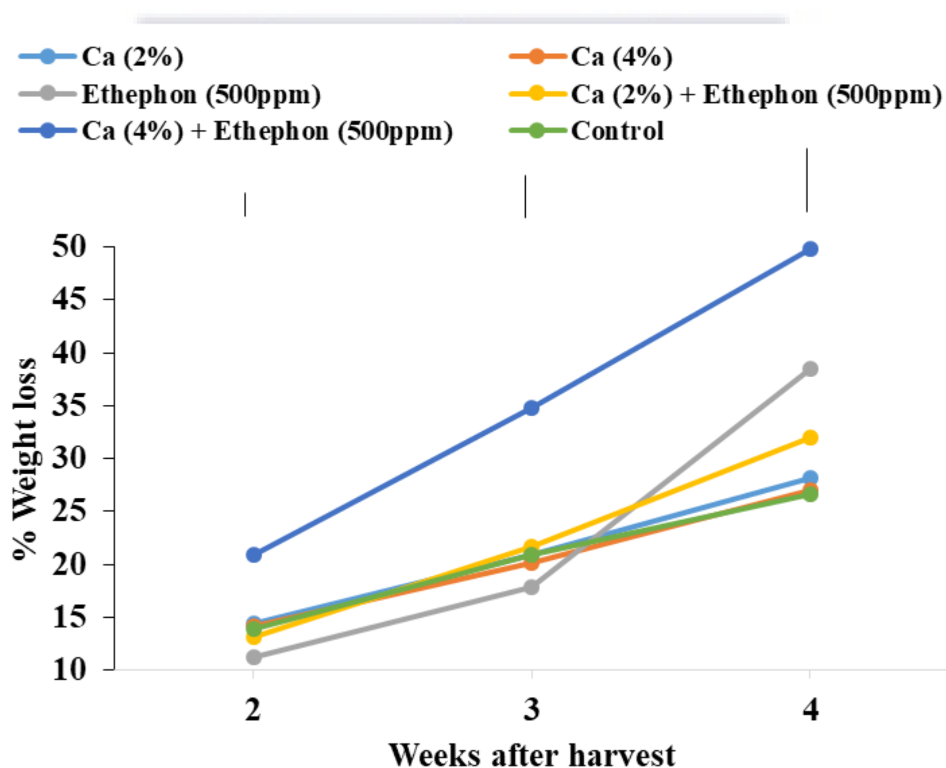

Figure 4.4: Percentage weight loss of *I. batatas* at **post-harvest** application of Calcium and Ethephon

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Table 2: Percentage weight loss mean separation of *I. batatas* at **post-harvest** application of calcium and ethephon after the storage period.

	Means
Control	26.60 a
Ca 4%	27.08 a
Ca 2%	28.12 a
Ca 2% + Ethephon (500ppm)	32.05 a
Ethephon (500ppm)	38.53 b
Ca 4% + Ethephon (500ppm)	49.86 c

4.2.3 Effect of pre-harvest calcium and ethephon treatment on the percentage shrinkage of orange-fleshed sweetpotato

At the end of the entire storage period, there was a steady rise in the percentage shrinkage of all the treatments applied to the roots. The percentage mean shrinkage of the roots treated with calcium chloride 2% and 4% were significantly different ($p \leq 0.05$) from the rest of the treatments and the control. Roots treated with calcium chloride 2% and 4% had significantly higher ($p \leq 0.05$) root shrinkage followed by the combination of calcium 4%, 2% and ethephon. There was no significant difference in the ethephon treated roots and the control. The mean percentage shrinkage at the end of the entire storage period was 22.18%, 19.40%, 15.77%, 13.82%, 10.55% and 7.94% for calcium 4%, calcium 2%, calcium 4% and ethephon, calcium 2% and ethephon, control and ethephon respectively (**figure 4.5**).

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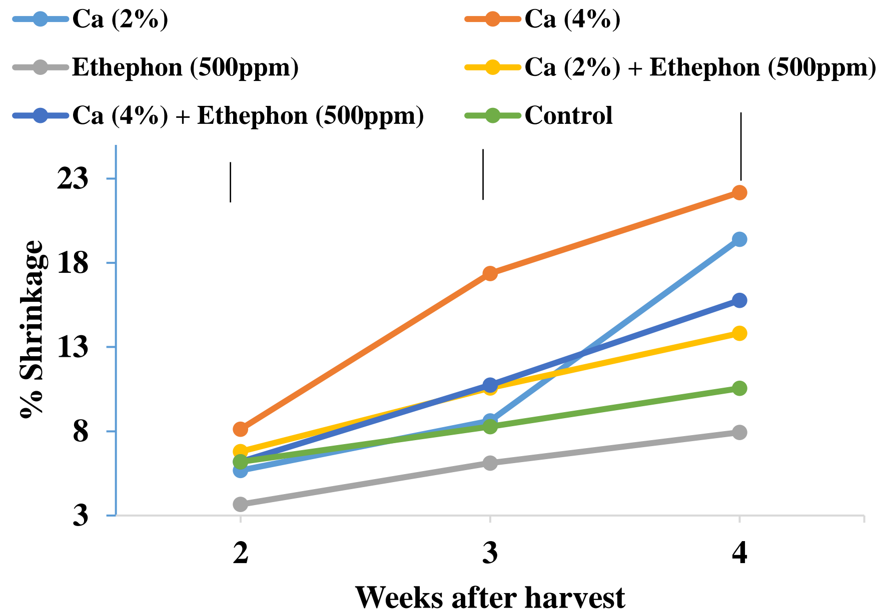

Figure 4.5: Percentage shrinkage of *I. batatas* at **pre-harvest** application of calcium and ethephon

Table 3: Percentage shrinkage mean separation of *I. batatas* at **pre-harvest** application of calcium and ethephon after the storage period.

	Means	
Ethephon (500ppm)	7.94	a
Control	10.55	ab
Ca 2% + Ethephon (500ppm)	13.82	bc
Ca 4% + Ethephon (500ppm)	15.77	bcd
Ca 2%	19.40	de
Ca 4%	22.18	e

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4.2.4 Effect of post-harvest calcium and ethephon treatment on the percentage shrinkage orange-fleshed sweetpotato

For the post-harvest application of the treatments, there was a steady rise in the percentage shrinkage of all the treatments applied to the storage roots. The percentage mean shrinkage of the storage roots treated with the combination of calcium chloride 4% and ethephon was significantly higher ($p \leq 0.05$) from the rest of the treatments and the control. There was no significant difference between the ethephon and calcium 2% treated roots and the control. The mean percentage shrinkage at the end of the entire storage period was 29.43%, 16.81%, 15.33%, 13.83%, 13.35% and 11.89% for calcium 4% and ethephon, calcium 4%, ethephon, calcium 2%, calcium 2% and ethephon, and control respectively (**Figure 4.6**).

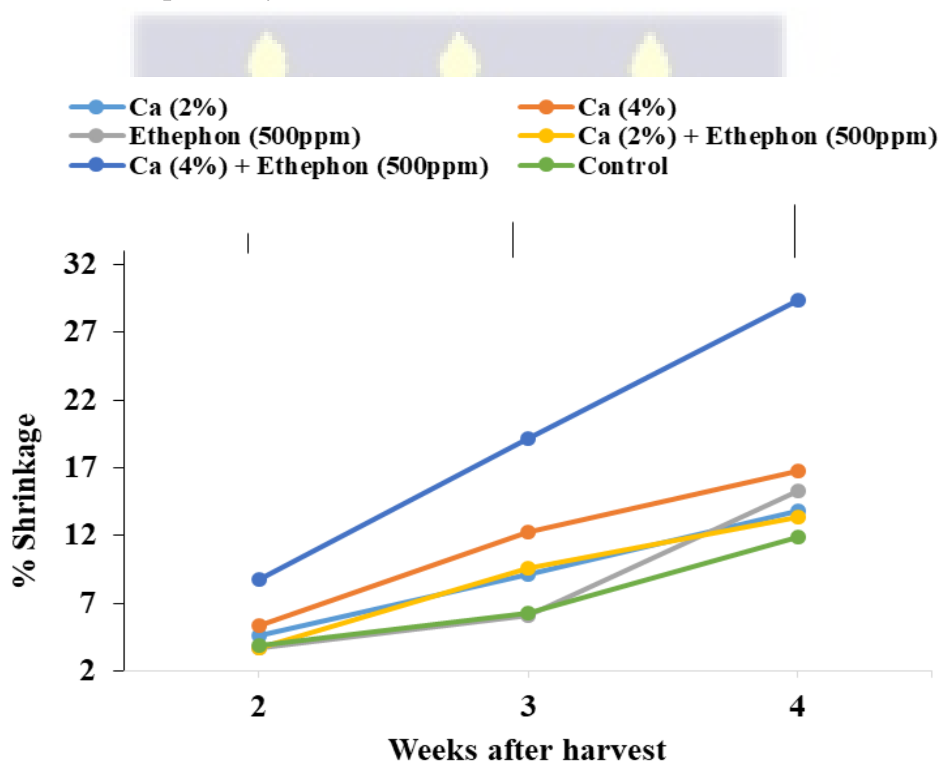

Figure 4.6: Percentage shrinkage of *I. batatas* at **post-harvest** application of calcium and ethephon

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Table 4: Percentage shrinkage mean separation of *I. batatas* at **post-harvest** application of calcium and ethephon after the storage period.

	means
Control	11.89 a
Ca 2% + Ethephon (500ppm)	13.35 ab
Ca 2%	13.83 ab
Ethephon (500ppm)	15.33 ab
Ca 4%	16.81 b
Ca 4% + Ethephon (500ppm)	29.43 c

4.2.5 Effect of pre- and post-harvest calcium and ethephon treatment on orange-fleshed sweetpotato sprouting

The treated and untreated (control) orange-fleshed sweetpotato storage roots showed no signs of sprouting after the storage period in both the pre-harvest and post-harvest application of the treatment.

4.3 Effect of pre- and post-harvest calcium and ethephon treatment on the shelf-life of orange-fleshed sweetpotato

4.3.1 Effect of pre-harvest calcium and ethephon treatment on orange-fleshed sweetpotato decay

Majority of the storage roots (93%) pre-treated with the combination of calcium 2% and ethephon got decayed. About 87%, 80%, 67% and 60% of the roots pre-treated with ethephon, calcium 2%, the combination of calcium 4% and ethephon, and control, respectively got decayed with calcium

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4% having the lowest percentage (53%) of decayed roots (**Table 5**).

Table 5: Decay percentage of *I. batatas* at **pre-harvest** application of calcium and ethephon

Treatment (pre-harvest)	Number of decayed roots	Decay percentage (%)
Calcium 2%	12	80
Calcium 4%	8	53
Ethephon 500 ppm	13	87
Calcium 2% + Ethephon 500 ppm	14	93
Calcium 4% + Ethephon 500 ppm	10	67
Control	9	60

The rate of decay was severe for the sweetpotato storage roots applied with the combination of calcium chloride 2% and ethephon at the end of the storage period, calcium chloride 2% treated roots showed a moderately severe decay rate as compared to the calcium 4%, ethephon, control and the combination of calcium chloride 4% and ethephon which showed a moderate decay rate (**table 6**).

Table 6: Severity of decay of *I. batatas* at **pre-harvest** application of treatments after storage.

Treatment (Pre harvest)	Decay score
Calcium Chloride 2%	Moderately severe
Calcium Chloride 4%	Moderate
Ethephon 500 ppm	Moderate
Calcium Chloride 2% + Ethephon 500 ppm	Severe
Calcium Chloride 4% + Ethephon 500 ppm	Moderate
Control	Moderate

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4.3.2 Effect of post-harvest calcium and ethephon treatment on orange-fleshed sweetpotato decay

Majority of the storage roots (87%) treated with the combination of calcium 2% and ethephon got decayed. About 80%, 73%, 67% and 47% of the storage roots treated with the combination of calcium 4% and ethephon, control, ethephon and calcium 2% respectively got decayed, with calcium 4% having the lowest percentage (20%) of decayed storage roots (**Table 7**).

Table 7: Decay percentage of *I. batatas* at **postharvest** application of calcium and ethephon

Treatment (postharvest)	Number of decayed roots	Decay percentage (%)
Calcium 2%	7	47
Calcium 4%	3	20
Ethephon 500 ppm	10	67
Calcium 2% + Ethephon 500 ppm	13	87
Calcium 4% + Ethephon 500 ppm	12	80
Control	11	73

The rate of decay was moderately severe in the sweetpotato storage roots treated with ethephon, combination of calcium chloride 2% and ethephon, and combination of calcium chloride 4% and ethephon. Calcium chloride 2% and calcium chloride 4% treated storage roots showed a slight decay rate as compared to the control which showed a moderate decay rate (**Table 8**).

Table 8: Severity of decay of *I. batatas* at **post-harvest** application of treatments after storage.

Treatment (Post-harvest)	Decay score
Calcium Chloride 2%	Slight
Calcium Chloride 4%	Slight
Ethephon 500 ppm	Moderately severe

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Treatment (Post-harvest)	Decay score
Calcium Chloride 2% + Ethephon 500 ppm	Moderately severe
Calcium Chloride 4% + Ethephon 500 ppm	Moderately severe
Control	Moderate

4.4 Effect of calcium chloride and ethephon on the biochemical properties of the orange-fleshed sweetpotato

The FTIR spectra of all the twelve (12) different samples are shown in Figure 4.7. The band at 3302 cm^{-1} is assigned to the stretching vibration of the bonded O-H group, and the band at 2934 cm^{-1} is assigned to C-H bond stretching associated with the ring methane hydrogen atoms. The band at 1642 cm^{-1} is assigned to the C-H bending vibrations, and the band at 1007 cm^{-1} is assigned to the stretching vibration of the C=O group. The application of the different treatments had no significant effect on the molecular structure of the sweetpotato. No new band losses were associated with the application of the treatments and no new bands were added.

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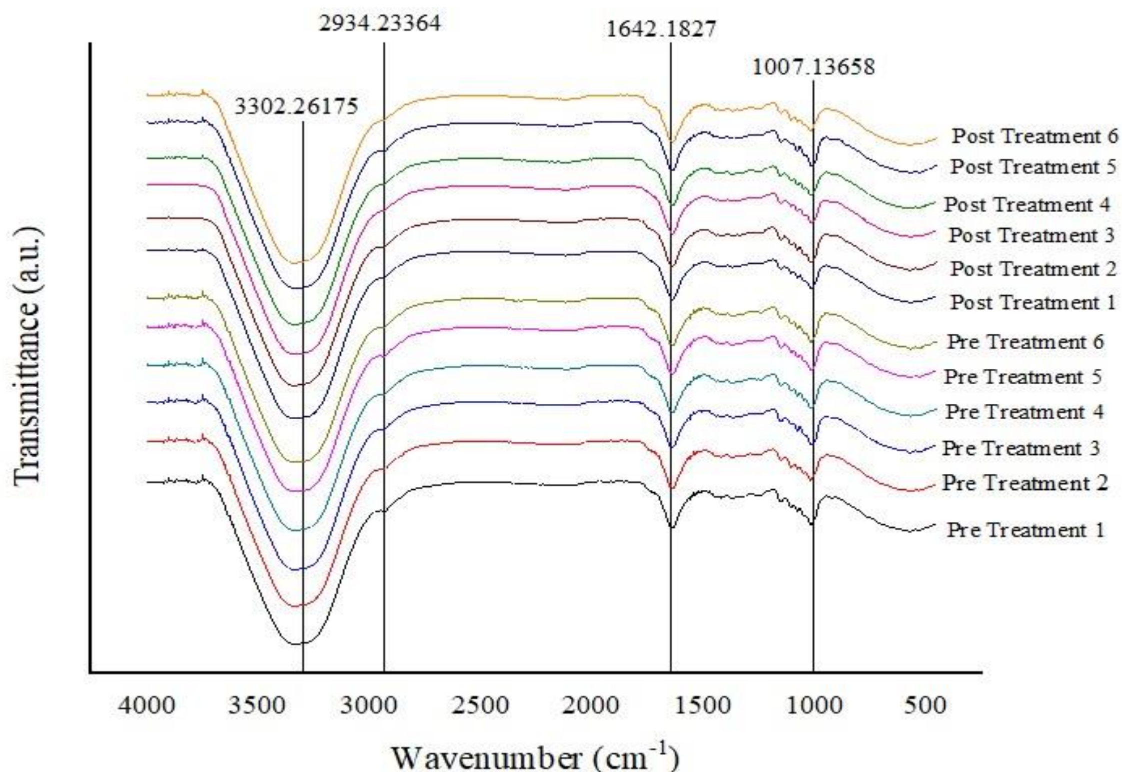

Figure 4.7: FTIR spectra of the six different treatments, pre and post applied. (Treatment 1: calcium 2%, treatment 2: calcium 4%, treatment 3: ethephon, treatment 4: calcium 2% + ethephon, treatment 5: calcium 4% + ethephon, treatment 6: control

4.5 Isolated and identified fungi from the sweetpotato storage roots.

At the end of the storage period, *Botryodiplodia theobromae* (Figure 4.8) and *Colletotrichum* spp. (Figure 4.11) were the two major fungi isolated from the sweetpotato storage roots. The mycelia of the fungi grew on the sweetpotato storage roots (Figures 4.8 and 4.11).

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Figure 4.8. Decayed orange-fleshed sweetpotato storage root after 28 days of storage with *Botryodiplodia theobromae* spores. The red arrow indicates acervulus

Subcultures of *Botryodiplodia theobromae* on PDA. An indication of the various growth stages of *Botryodiplodia theobromae* on PDA from the initial and intermediate stages to the final darkening stage (**figure 4.9**).

Figure 4.9: *Botryodiplodia theobromae* cultured on PDA. Initial stage (A) Intermediate stage (B) Fully grown stage(C)

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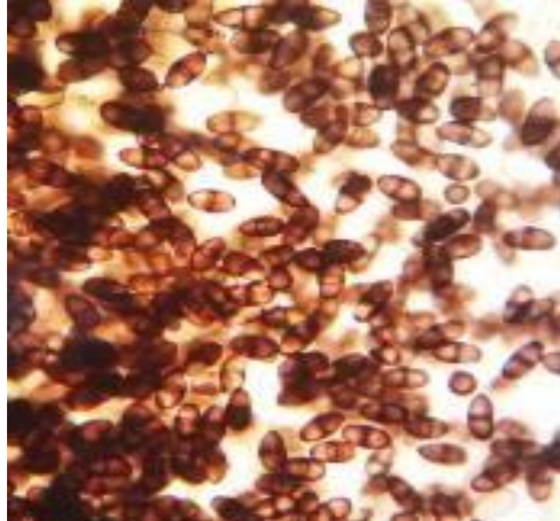

Figure 4.10: *Botryodiplodia theobromae* spores as observed under the compound microscope.

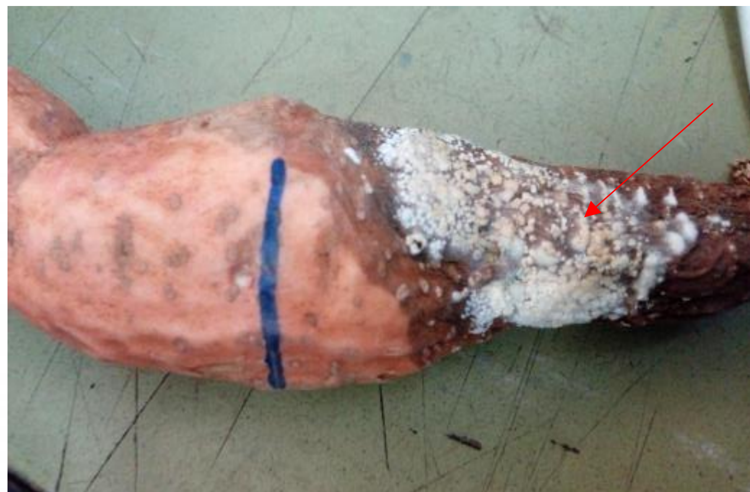

Figure 4.11. Decayed orange-fleshed sweetpotato storage root after 28 days of storage with *Colletotrichum* spp. spores. The red arrow indicates acervulus

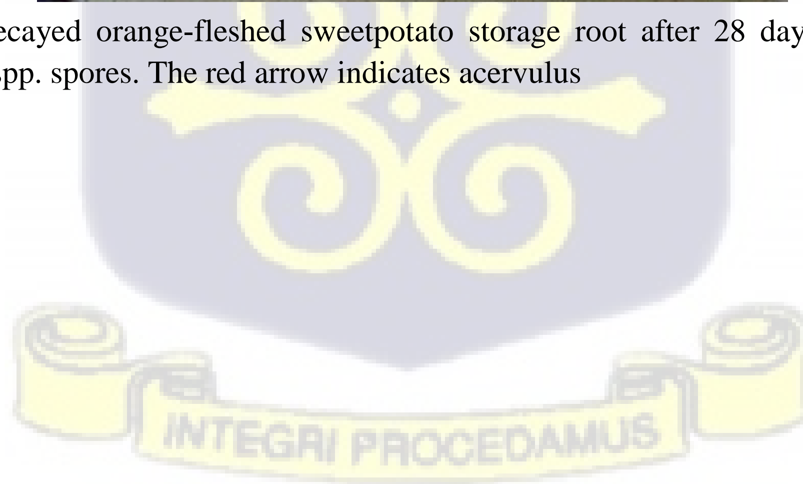

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Figure 4.12: 15 days old culture of *Colletotrichum* spp. on PDA.

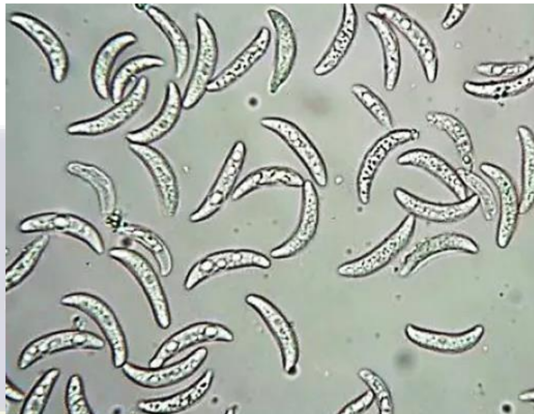

Figure 4.13: *Colletotrichum* spp. spores as observed under the compound microscope.

4.6 Pathogenicity test

After 12 days of inoculation, the same pathogen (*Colletotrichum* spp.) caused disease on healthy orange-fleshed sweetpotato storage roots. Similar symptoms of the pathogen on the previous infected storage roots were observed on the inoculated sweetpotato storage roots. And the same pathogen (*Colletotrichum* spp.) was seen under the compound microscope after isolation.

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4.7 Antifungal susceptibility test

There was growth of *Botryodiplodia theobromae* in the various treatment after the bioassay. A represents the standard fungicide, B and C represents the growth of *Botryodiplodia theobromae* in calcium chloride 4% and 2% respectively, D represents the control (**Figure 4.14**).

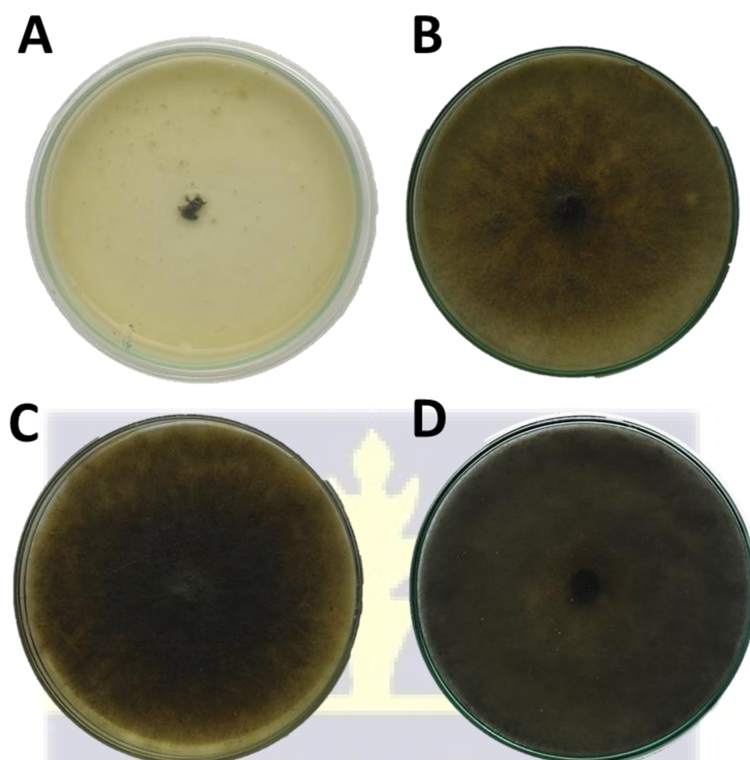

Figure 4.14: Growth of *Botryodiplodia theobromae* in the various treatments after the bioassay. Standard fungicide (A), Calcium Chloride 4% (B), Calcium Chloride 2% (C), Control (D).

The percentage inhibition of *Botryodiplodia theobromae* by calcium chloride 2% and 4% showed that the treatments were able to inhibit mycelia growth of *Botryodiplodia theobromae* by 0%, but the standard fungicide was able to inhibit it by 100% (**Table 9**).

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Table 9: Percentage inhibition of *Botryodiplodia theobromae* by the various treatments.

TREATMENT	INHIBITION PERCENTAGE (%)
Calcium Chloride 2%	0
Calcium Chloride 4%	0
Standard fungicide (Mancozeb)	100
Control	0

The growth of *Colletotrichum* spp. in the various treatment after the bioassay. A represents the standard fungicide, B and C represents the growth of *Colletotrichum* spp. in calcium chloride 4% and 2% respectively, D represents the control (**Figure 4.15**).

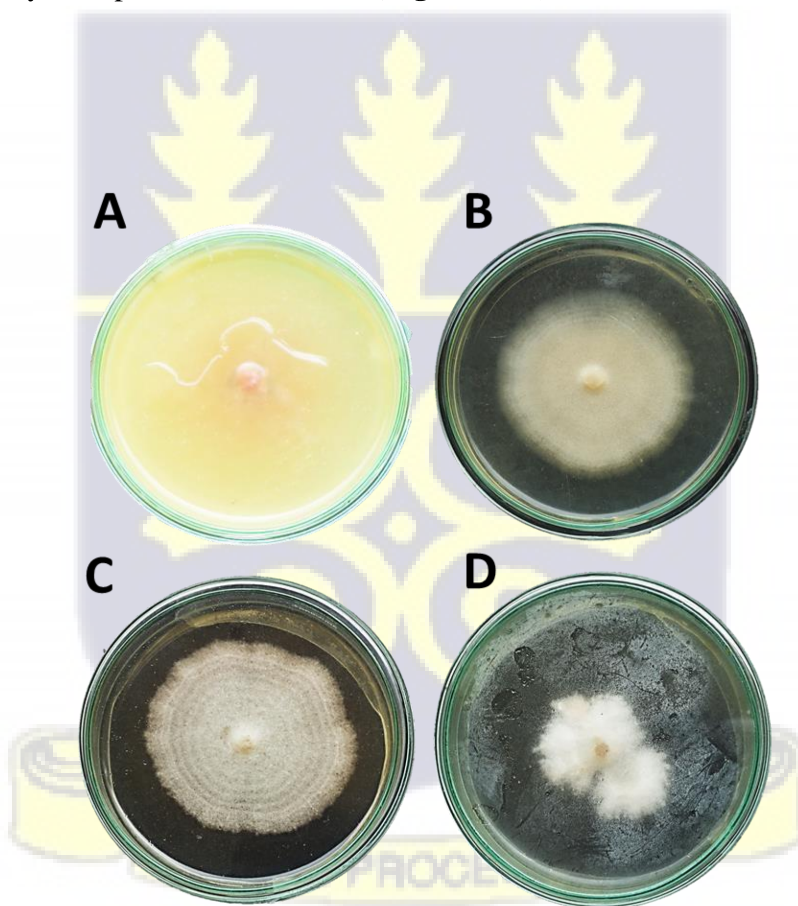

Figure 4.15: Growth of *Colletotrichum* spp. in the various treatments after the bioassay.

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Standard fungicide (A), Calcium Chloride 4% (B), Calcium Chloride 2% (C), Control (D).

The percentage inhibition of *Colletotrichum* spp. by calcium chloride 2% and 4% showed that the treatments were able to inhibit it by 28% and 33% respectively, but the standard fungicide was able to inhibit it by 100% (**Table 10**).

Table 10: Percentage inhibition of *Colletotrichum* spp. the various treatments.

TREATMENT	INHIBITION PERCENTAGE (%)
Calcium Chloride 2%	28
Calcium Chloride 4%	33
Standard fungicide (Mancozeb + Carbendazim)	100
Control	50

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CHAPTER FIVE

5.0 DISCUSSION

The use of chemical sprays on the field are to modify the ripening process and the time of maturity, and these are known to have an influence on the storage and the quality of produce on the market (Siddiqui *et al*., 2014). According to Diaz-Corona *et al*., (2020), firmness of crops can be maintained through the application of calcium salts which is achieved by stabilizing cell membranes and enhancing cell turgor. A study conducted by Bhalerao *et al*., (2010) showed that the pre-harvest application of 1% concentration calcium chloride reduced the percentage physiological loss in weight during storage and also improved the physicochemical qualities of sapota fruits. The combination of calcium chloride (2% concentration) and yeast antagonist candida spp. was also able to control gray and blue molds on apples and pears (McLaughlin *et al*., 1990; Wisniewski *et al*., 1995; Singh, 2005; Zhang *et al*., 2005). In contrast, the combination of calcium chloride and ethephon pre-applied and post-applied to the storage roots in this study had the highest physiological storage root weight loss and shrinkage. This could be due to water loss from the storage roots through respiration and transpiration during storage (Edmunds *et al*., 2008). The high ethephon concentration utilized could also be a factor for the high physiological weight loss of the storage roots. This is because, Siteinie (2016) observed higher dry matter content and lower weight loss when sweetpotato storage roots were treated with a lower ethephon (100 ppm) concentration. The study confirmed why the sweetpotato storage roots had a high physiological weight loss from our study with the 500 ppm concentration of ethephon used.

Ethylene is an important commercial hormone used by farmers or crop producers to preserve climacteric and non-climacteric produce by either extending or reducing the shelf-life of produce (Abeles, 1969). Amoah (2014) conducted a study and concluded that the exogenous application of

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ethylene on low ethylene producing crops has the ability to regulate dormancy, sprouting and senescence. Similar results were obtained from this study with all the sweetpotato storage roots showing no signs of sprouting. Also, the quality of sweetpotato storage roots and other agricultural produce can be maintained with ethylene application (Cheema *et al*., 2008). A study conducted by Wang *et al*. (2013) on sweetpotato showed that the pre-harvest application of ethephon to the leaves and vines reduced the skinning effect of storage roots. In this study, pre-harvest ethephon application had a yellowing effect on the leaves which was then followed by abscission of the leaves. Similar results to determine ethephon-induced effects on leaf senescence and chlorophyll content by Chen *et al*., (2010) was observed. The sweetpotato leaves detached and senesced in those treated with ethephon compared to the other treatments within six days after application.

According to da Silva & Clark (2012) and Stokes *et al*. (2012), sweetpotato pathogens include *Fusarium solani, Macrophomina phaseolina, Lasiodiplodia theobromae*, and *Diaporthe batatatis*. However, the most pathogenic fungi include *Fusarium oxysporum*, *Aspergillus niger*, soft rot (*Rhizopus stolonifer, Rhizopus oryzae*), Java black rot (*Botryodiplodia theobromae*), Black rot (*Ceratocystis fimbriata*), *Sclerotium* rot (*Sclerotium rolfsii*), charcoal rot (*Macrophomina phaseolina*), *Curvularia lunata* (*Cochliobulus lunutus*), *Rhizoctonia solani*, *Plenodomus destruens*, and *Penicillium* spp. (Ray & Nedunchezhiyan, 2012; Olaitan, 2012). The orange-fleshed sweetpotato storage roots from this study were infected with two different pathogens, *Colletotrichum* spp. and *Botryodiplodia theobromae*. Higher decay losses were realized in the sweetpotato storage roots pretreated with ethephon and the combination of calcium and ethephon a month after storage. This confirmed an experiment conducted by Arancibia *et al*. (2013) to determine the possible relationship between pre-harvest foliar ethephon application and root tip rot showed that after 1-2 months of root storage, tip rot occurred in the stored storage roots treated

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with the ethephon (Arancibia *et al*., 2013). This could be due to the ripening effect of the ethephon on the sweetpotato storage roots.

The application of the different treatments had no significant effect on the molecular structure of the sweetpotato. The treatment application neither led to the formation of new functional groups nor altered the short-range structures of the sweetpotato sample. The band at 3302 cm^{-1} represented the stretching vibration of the bonded O-H group of starch or polyphenols which signified the presence of starch or polyphenols in the sweetpotato roots (Han *et al*., 2020). The amide I band at 1007 cm^{-1} represented the stretching vibration of the bonded C=O group of the peptide linkages showing signs of proteins in the sweetpotato storage roots (Krekora *et al*., 2020). The obtained result is similar to a study conducted by Siteinie, which showed that the application of ethephon had no significant effect on the sucrose content of the sweetpotato storage roots (Siteinie, 2016). This observation was previously made by Cheema *et al*. (2013). But with the glucose and fructose content, ethephon application led to the decline in their concentrations (Amoah, 2014; Sitienei, 2016). The decline was due to the respiration induced by ethylene in the sweetpotato storage roots. In contrast, the current study showed no difference from the untreated roots in the glucose and fructose content of the sweetpotato storage roots after the treatment application.

Calcium chloride on mycelial growth of certain fungus has been assessed and has proven to be effective in inhibiting their growth, some reported cases are, *Fusarium oxysporum* f.sp. cepae (the causal agent of onion rot) (Turkkan, 2013), on the fungal growth of *Alternaria alternata, Alternaria solani, Aspergillus niger, Botrytis cinerea, Fusarium solani* isolated from guava fruits (Hassanein *et al*., 2018) and the growth of *Lasiodiplodia theobromae* isolates from mango (Nur Fatimma *et al*., 2018). A study by Eryani-Raqeeb *et al*. (2009) showed that spore germination of

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Colletotricum gloeosporiodes was effectively inhibited by the post-harvest application of calcium (calcium dip) on papaya and on red-flesh dragon fruit (*Hylocereus polyhizus*) (Awang *et al*., 2011). This finding was later shown in other studies and confirmed the reduction of anthracnose lesion diameters in infected papaya fruits (Madani *et al*., 2016; Ayon-Reyna *et al*., 2017). Likewise that of *Botryosphaeria dothidea* and *Botrytis cinerea* on apples (Biggs *et al*., 1997; El-Gali, 2008) and *Monilinia fructicola* on peaches (Biggs, 2004). Due to the inhibitory effect of calcium, calcium chloride concentrations of 2% and 4% was tested by agar diffusion plate method in this study to assess the growth of *Botryodiplodia theobromae* and *Colletotricum* isolated from the infected sweetpotato storage roots. The results showed that calcium chloride at both concentrations were not able to inhibit spore germination and hyphal growth of the two different pathogens. Contrary to this, another study conducted in 2014 showed that calcium chloride at 1.5% and 2% significantly inhibited spore germination and hyphal growth of *Colletotrichum acutatum* and *Colletotricum gloeosporioides* isolated from apple anthracnose lesions (Stosic *et al*., 2014). Moreover, a lower concentration of 1%, calcium chloride was also able to significantly decrease spore germination of *Colletotricum gloeosporioides* Penz (Madani *et* al., 2014). A recent study confirmed the inhibitory effect of calcium chloride (in vitro) on *Colletotricum gloeosporioides*, and the protection of pisang awak banana and chilli fruits from anthracnose damage by in vivo application (Le Thanh Toan *et al*., 2019). However in this current study, the application of 4% calcium chloride rather boosted the growth of *Colletotricum*. And this could be due to the increased concentration of calcium chloride used. Tian *et al*., (2002) showed that in vitro calcium chloride testing at 2% also significantly inhibited spore germination and growth of the *Rhizopus stolonifer* isolates from peach and nectarine fruits, which was later confirmed by Ismail *et al*., (2010). A similar result was reported even with a higher calcium chloride concentration of 4% on mycelia growth of *Rhizopus stolonifer* isolates from guava fruits (Hassanein *et al*., 2018).

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

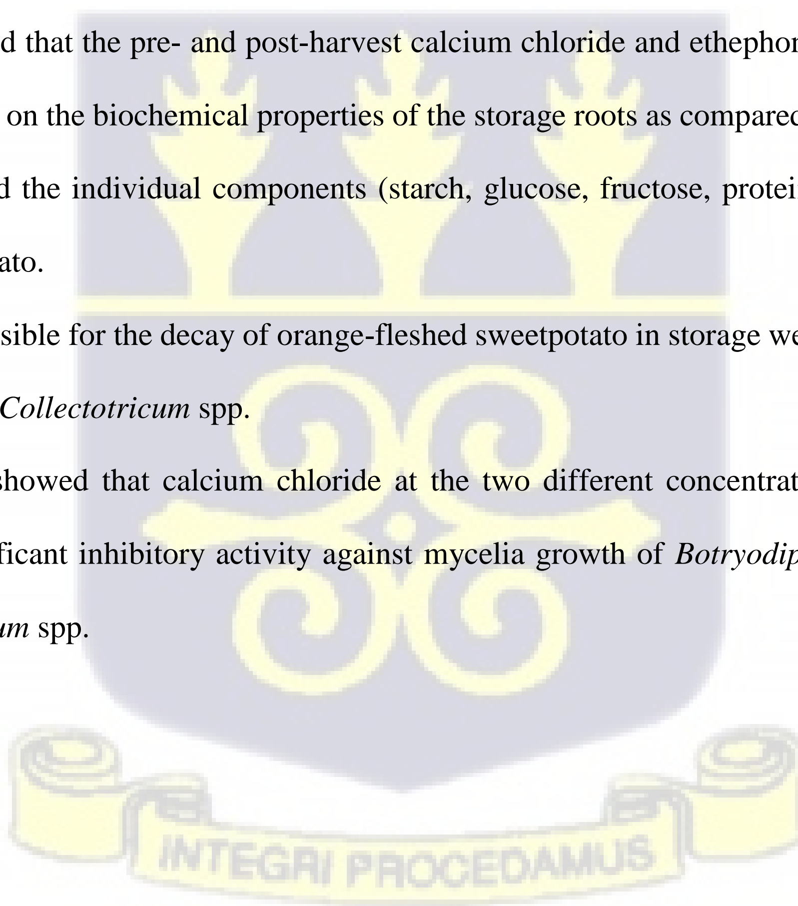
The overall quality and shelf-life of the orange-fleshed sweetpotatoes after applying calcium chloride both on the field and after harvest was not improved or prolonged. The storage roots after the storage period showed signs of decay, shrinkage and weight loss. The purpose of calcium chloride application on the storage roots to enhance firmness was not obtained.

The overall quality and shelf-life of the orange-fleshed sweetpotatoes after applying ethephon both on the field and after harvest was not improved or prolonged. The storage roots after the storage period showed signs of decay, shrinkage and weight loss.

The study showed that the pre- and post-harvest calcium chloride and ethephon treatment had no significant effect on the biochemical properties of the storage roots as compared to the control but rather maintained the individual components (starch, glucose, fructose, proteins) of the orange-fleshed sweetpotato.

The fungi responsible for the decay of orange-fleshed sweetpotato in storage were *Botryodiplodia theobromae* and *Collectotricum* spp.

The study also showed that calcium chloride at the two different concentration (2% and 4%) possess no significant inhibitory activity against mycelia growth of *Botryodiplodia theobromae* and *Collectotricum* spp.



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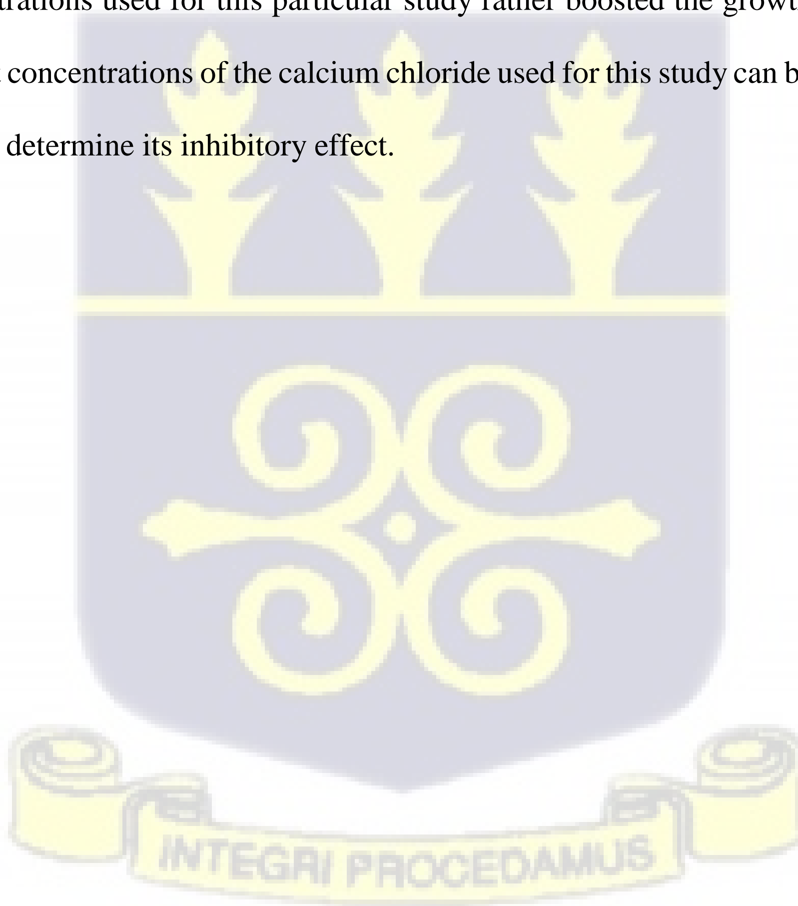
6.2 Recommendation

Extensive studies can be conducted at different calcium chloride application periods including the sixth and twelfth weeks on the orange-fleshed sweetpotato since the calcium chloride was only applied six weeks and twelve weeks after cultivation. The exact concentrations of calcium chloride and ethephon used in this study can be used on other sweetpotato varieties and other root crops to compare their effects on the shelf-life and quality. Also, different calcium chloride and ethephon mode of application or dipping times can be assessed to compare their effect on the quality and shelf-life of orange-fleshed sweetpotato. Higher concentrations of the calcium chloride can be used in the inhibition of the mycelia growth of *Botryodiplodia theobromae* and lesser concentrations of the calcium chloride can be used in the inhibition of the mycelia growth of *Collectotricum* spp. since the concentrations used for this particular study rather boosted the growth of the pathogen. Finally, the exact concentrations of the calcium chloride used for this study can be tested on diverse fungal species to determine its inhibitory effect.



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APPENDIX

ANALYSIS OF VARIANCE (ANOVA) FOR THE STUDY

Appendix 1: ANOVA for percentage weight loss at 2 weeks for the pre-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	31.215	15.608	6.59	
Rep.*Units* stratum					
Treatment	5	109.825	21.965	9.28	0.002
Residual	10	23.672	2.367		
Total	17	164.712			

Appendix 2: ANOVA for percentage weight loss at 3 weeks for the pre-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	49.447	24.724	4.58	
Rep.*Units* stratum					
Treatment	5	658.778	131.756	24.40	<.001
Residual	10	54.002	5.400		
Total	17	762.227			

Appendix 3: ANOVA for percentage weight loss at 4 weeks for the pre-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	32.82	16.41	0.86	
Rep.*Units* stratum					
Treatment	5	1486.57	297.31	15.55	<.001
Residual	10	191.22	19.12		
Total	17	1710.61			

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Appendix 4: ANOVA for percentage root shrinkage at 2 weeks for the pre-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum					
	2	14.478	7.239	5.13	
Rep.*Units* stratum					
Treatment	5	32.294	6.459	4.58	0.020
Residual	10	14.107	1.411		
Total	17	60.879			

Appendix 5: ANOVA for percentage root shrinkage at 3 weeks for the pre-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	23.124	11.562	4.64	
Rep.*Units* stratum					
Treatment	5	223.348	44.670	17.93	<.001
Residual	10	24.918	2.492		
Total	17	271.390			

Appendix 6: ANOVA for percentage root shrinkage at 4 weeks for the pre-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	36.413	18.207	3.64	
Rep.*Units* stratum					
Treatment	5	427.438	85.488	17.10	<.001
Residual	10	49.984	4.998		
Total	17	513.835			

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Appendix 7: ANOVA for percentage weight loss at 2 weeks for the post-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	165.028	33.006	12.50	<.001
Residual	12	31.689	2.641		
Total	17	196.717			

Appendix 8: ANOVA for percentage weight loss at 3 weeks for the post-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	551.371	110.274	18.72	<.001
Residual	12	70.670	5.889		
Total	17	622.041			

Appendix 9: ANOVA for percentage weight loss at 4 weeks for the post-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1237.90	247.58	23.74	<.001
Residual	12	125.17	10.43		
Total	17	1363.07			

Appendix 10: ANOVA for percentage root shrinkage at 2 weeks for the post-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	57.2690	11.4538	12.03	<.001
Residual	12	11.4262	0.9522		
Total	17	68.6952			

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Appendix 11: ANOVA for percentage root shrinkage at 3 weeks for the post-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	355.164	71.033	19.48	<.001
Residual	12	43.767	3.647		
Total	17	398.930			

Appendix 12: ANOVA for percentage root shrinkage at 4 weeks for the post-harvest application

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	619.652	123.930	20.72	<.001
Residual	12	71.778	5.982		
Total	17	691.431			

Appendix 13: Means of percentage weight loss at 2nd, 3rd and 4th week for the pre-harvest application

Treatment	2nd week	3rd week	4th week
Calcium 2%	16.26	21.75	39.00
Calcium 4%	21.07	30.49	28.20
Ethephon 500ppm	14.20	17.52	22.70
Calcium 2% + Ethephon 500ppm	17.44	34.17	48.50
Calcium 4% + Ethephon 500ppm	15.62	24.35	32.80
Control	13.53	17.52	23.30

Appendix 14: Means of percentage root shrinkage at 2nd, 3rd and 4th week for the pre-harvest application

Treatment	2nd week	3rd week	4th week
Calcium 2%	5.68	8.62	19.40
Calcium 4%	8.13	17.37	22.18
Ethephon 500ppm	3.66	6.11	7.94
Calcium 2% + Ethephon 500ppm	6.80	10.58	13.82
Calcium 4% + Ethephon 500ppm	6.19	10.75	15.77
Control	6.19	8.29	10.55

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Appendix 15: Means of percentage weight loss at 2nd, 3rd and 4th week for the post-harvest application

Treatment	2nd week	3rd week	4th week
Calcium 2%	14.44	20.90	28.12
Calcium 4%	14.18	20.16	27.08
Ethephon 500ppm	11.19	17.79	38.53
Calcium 2% + Ethephon 500ppm	13.13	21.68	32.05
Calcium 4% + Ethephon 500ppm	20.97	34.78	49.86
Control	13.89	20.97	26.60

Appendix 16: Means of percentage root shrinkage at 2nd, 3rd and 4th week for the post-harvest application

Treatment	2nd week	3rd week	4th week
Calcium 2%	4.62	9.13	13.83
Calcium 4%	5.30	12.21	16.81
Ethephon 500ppm	3.64	6.07	15.33
Calcium 2% + Ethephon 500ppm	3.73	9.61	13.35
Calcium 4% + Ethephon 500ppm	8.75	19.19	29.43
Control	3.84	6.30	11.89