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FUNGICIDAL POTENTIAL OF THREE PLANT EXTRACTS IN THE MANAGEMENT OF ROOT ROT DISEASE OF SWEET POTATO [*Ipomoea batatas* (L.) Lam] CAUSED BY FUNGAL PATHOGENS.

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BY

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OCTOBER, 2023

DECLARATION

I, Yakubu Fatawu Ware hereby declare that this is an original result of my work and no previous submission for a degree or any certificate has been done here or elsewhere. Except information relevant to this work from other authors has been cited and duly acknowledged by way of references.

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We hereby declare that the preparation and presentation of the thesis were supervised per the guidelines on supervision laid down by the University for Development Studies.

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ABSTRACT

This study sought to find eco-friendly alternative management of root rot disease of sweet potato in storage as often used synthetic pesticides have detrimental effects not only on the consumer but the environment. This study therefore investigated the use of three plant extracts with potential in managing sweet potato root tuber rots in Northern Ghana. Phytochemical analysis was conducted on both aqueous and ethanol extracts of leaves of bitter leaf (*Vernonia amygdalina*), lemon grass (*Cymbopogon citratus*) and holy basil (*Ocimum sanctum*). The antifungal activities of the aqueous and ethanol plant extracts were tested *in vitro* at 50 and 100% extract concentrations against the mycelia growth of isolated sweet potato root tuber rot fungal pathogens while 10% basic aqueous concentration of each of the plant extract was tested *in vivo* for weight loss of root tubers, severity of rot, lesion diameter and sprout count. The phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, and phytosterols but varied among the plant extracts tested. Seven fungal species namely; *Aspergillus niger*, *Fusarium oxysporum*, *Lasiodiplodia theobromae*, *Aspergillus flavus*, *Aspergillus clavatus*, *Trichoderma harzianum* and *Rhizopus stolonifer* were isolated from both white-flesh and orange-flesh sweet potato varieties. However, five isolates namely; *A, niger, F. oxysporum, L. theobromae*, *T. harzianum* and *R. stolonifer* were pathogenic on fresh and healthy root tubers after pathogenicity. *Aspergillus niger* had the highest frequency of occurrence and the highest necrotic lesion on both white-flesh and orange-flesh varieties. The mycelia growth was significantly ($P < 0.05$) reduced by each of the plant extracts at both 50% and 100% concentration levels in both aqueous and ethanol solvents. *Vernonia amygdalina* and *Cymbopogon citratus* were highly efficacious on *Fusarium oxysporum* than any of the isolated fungal pathogens. The plant extracts reduced the sweet potato root tuber weight loss significantly ($P < 0.05$) from week one to week six in storage. Also,

root tuber rot and sprout were suppressed by *Vernonia amygdalina* and *Cymbopogon citratus* respectively. *Vernonia amygdalina* managed sweet potato root tuber rots to the barest minimum followed by *Cymbopogon citratus* and then *Ocimum sanctum*. This study showed that aqueous and ethanol extracts of leaves of *Vernonia amygdalina*, *Cymbopogon citratus,* and *Ocimum sanctum* have fungicidal properties against sweet potato root rot fungal pathogens (*A. niger, F. oxysporum, L. theobromae*, *T. harzianum* and *R. stolonifer*); hence can be exploited in the management of sweet potato root tuber rot in storage.

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DEDICATION

To my mother Mrs Fatuma Yakubu for her support and prayers. Also, my brothers and sisters; Yakubu Salifu, Alhassan Yakubu, Banwuolaima and Aminatu and all your children for giving me the love and your support, Allah Almighty richly bless you.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Sweet potato (*Ipomoea batatas* L. Lam) is one of the world's most important root crops (Scruggs and Quesada-Ocampo, 2016) with projected production of 89.49 million tonnes (Balasubramanian, 2023) and ranked as the seventh most important cultivated root crop in the world (Sugri *et al.*, 2017; EdunA *et al.*, 2019). Asia is the largest producer with almost 66 % of projected world production and Africa being the second largest producer with 28.3 % (Cartabiano-Leite *et al.*, 2020). China, Tanzania, Nigeria, Uganda, and Indonesia are the world's top five producers of sweet potatoes, with China producing the most at about 75.6 million tonnes, followed by Tanzania and Nigeria at about 3.57 and 2.73 million tonnes, respectively (Sugri *et al.*, 2017). In developing countries, it is among the top five most significant crops including cassava, rice, maize and wheat (Elameen *et al.*, 2008). In Ghana, except for yam (*Dioscorea* spp.), cassava (*Manihot esculenta* Crantz), and taro (*Colocasia* spp.), sweet potato is the next enviable tuber crop on the menu of many Ghanaians concerning tuber crops in the country (Sugri *et al.*, 2017). The crop is most prominent among smallholder farmers who extensively grow the crop in the Northern, Upper East, Upper West, Central, and Volta Regions of Ghana (Bidzakin *et al.*, 2014). In Ghana, it is reported that the estimated area for production per annum gives 0.132 tonnes from an area of 9,622 ha (MoFA, 2013; Sugri *et al.*, 2017). Sweet potato serves as a source of rich carbohydrates, vitamin A, which is lacking in many children under six years in Sub-Saharan African countries, particularly in Ghana (Ayensu *et al.*, 2019). It is a prominent food security crop due to its drought-tolerant ability as well as income-earning root tuber crop that does well in the Guinea savanna region of Ghana. However, in a tropical country like Ghana, sweet potato root tubers are subjected to

microbial attacks especially fungi after harvest which constitute losses to the farmer. Sweet potato root tubers are vulnerable to attack when wounds and cuts are inflicted on the skin at harvest or during transportation. Several fungi have been reported to sufficiently induced rots on sweet potato root tubers either on the field or in storage. The most notably fungal pathogens causing sweet potato rots in the tropics include; *Aspergillus* spp., *Botryodiplodia theobromae*, *Fusarium* spp., *Ceratocystis fimbriata*, *Macrophomina phaseolina* and *Rhizopus stolonifer* (Beckley and Awoyemi, 2021). Interestingly, the use of botanicals in the management of fungal pathogens is gaining momentum in Ghana due to the shift from synthetic chemicals in the treatment of fungal pathogens causing sweet potato root tubers rots which causes health implications to the consumer. With this in perspective, many researchers have adopted plant-driven extracts as the safer alternatives in the control of root tuber rots (Fokunang *et al*., 2000; Anukwuorji *et al*., 2013; Tartoura *et al*., 2015; Giri *et al*., 2020).

1.2 Problem statement

The crop is grown in the Volta, Middle belts (Bono, Bono East, and Ahafo regions) and Upper regions of Ghana. The crop is a reserve food source and helps prevent food shortages until the next harvest of other staple crops. Due to its ability to generate significant yields in a short growing season even with little rainfall, it is especially used as a security crop in Northern Ghana. However, when sweet potato roots are detached from the main plant it becomes perishable and the shelf-life is shortened to a few weeks of storage as compared with other staple crops (Ray and Tomlins, 2010). Ray and Tomlins (2010) further stated that fresh roots are weak in mechanical strength due to their high moisture content ranging between 50 % to 70 % coupled with a high respiration rate which generates heat and damages the skin by causing it to become softer. Inappropriate handling of root tubers that causes mechanically injuries including abrasion, cuts and

bruises serve as a site for entry to microorganisms. Opara and Onuoha, (2014) reported that the ability of the fresh roots to succumb to microorganisms attack leads to post-harvest losses as a result of physical, physiological, or pathological reasons, or a combination of these factors. Post-harvest loss is a major hindrance to long shelf-life of the root tubers in storage (EdunA *et al.*, 2019). Singh *et al.* (2016) reported that weight loss, sprouting, weevil damage and microbial attack are the factors that facilitates postharvest quality deterioration and rots (Olaitan, 2012; Clark *et al.*, 2013). Many different microbes, primarily moulds, have been associated to tuber rotting, but only a small number are the main pathogens (Clark *et al.*, 2013). *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer, Botryodiplodia theobroma*, and *Penicillium* sp. are among the fungi linked to sweet potato rots in the tropics (Olaitan, 2012).

1.3 Justification

In developing countries, food shortage occurs annually and it is estimated that one billion people suffer from extreme hunger with 10 % of them possibly passing away due to this food shortage (Alum, 2021). Many blame this problem on the upsurge in the human population creating pressure on the food supply (Urom *et al.*, 2022). The remedy to this problem is to tackle food insecurity for the ever-increasing human population while putting in measures that will ensure long-term sustainable development in these developing countries (Alum, 2021). By 2050, it is estimated that food production must be up by 70 % to be able to cater for the world's 9.3 billion people (Alum, 2021). However, food output is still very low worldwide with large quantities of food and the potential food security crop like sweet potato are lost owing to infestations and spoilage by microorganisms and lack of inadequate agricultural storage (Kana *et al.*, 2012; Alum, 2021). To overcome these losses, farmers resorted to fungicides application and 70-99% of them never successfully reach their targets (Pang *et al.*, 2021). It has been demonstrated

that the presence of these fungicides negatively impacts soil and surface water quality, as well as terrestrial organisms (Sartori *et al.*, 2020; Pang *et al.*, 2021). The excessive reliance on synthetic fungicides for eradicating post-harvest infections has recently been reduced through extensive research for alternative methods (Zhang *et al.*, 2018). However, to address this growing issue, safe and environmental solutions must be found for perishable crops, such as sweet potatoes (Pang *et al.*, 2021).

Reduction of sweet potato losses at the postharvest stage requires research that will provide preliminary data on appropriate handling, treatment, curing, and storage. Currently, the serious threat to prolonged shelf-life of sweet potatoes into the lean- season in Ghana is storage diseases (Linus, 2014b). Reducing the amount of sweet potato that deteriorates after harvest with botanicals has enormous advantages including a reduction in food shortage while being environmentally friendly, climatic-wise, and safer water bodies in developing countries.

1.4 Main objective

To investigate the use of three plant extracts with potential in managing sweet potato root tuber rot diseases caused fungal pathogens in northern Ghana.

1.4.1 The specific objectives are;

- To determine the phytochemical properties of aqueous and ethanol extracts of bitter leaf, holy basil, and lemon grass.
- To assess the fungitoxicity of aqueous and ethanol extracts of bitter leaf, holy basil and lemon grass on the mycelia growth of pathogenic fungi isolated from sweet potato root rot.
- To evaluate the efficacy of aqueous extracts of bitter leaf, holy basil, and lemongrass in managing sweet potato root rot.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and taxonomy of sweet potato

Sweet potato is native to tropical areas of Central and South America. It is believed to be domesticated in Central America 5,000 years ago (Gichuhi *et al.*, 2014; Mu and Li, 2019). It was disseminated to all now sweet potato growing regions of the world particularly in the tropical and sub-tropical regions (Gichuhi *et al.*, 2014; Mu and Li, 2019). The discovery of historic remnants of sweet potatoes was made in Polynesia by archaeologists through the use of radiocarbon dating in the early years between CE 1000 – 1100 (Mu and Li, 2019). Ray and Tomlins, (2010) also reported that dried roots of sweet potatoes were discovered through radiocarbon dating in the caves of Chilca, Peru between 8000 – 10000 years ago. Researchers analysed the DNA of 1245 sweet potato samples from different varieties coming from Asia and the Americas and it was established that sweet potatoes were found 400 years earlier in Polynesia before the voyager of Christopher Columbus and his counterparts in 1942 (Roullier *et al.*, 2013; Mu and Li, 2019). Genetic research points to the possibility that sweet potatoes were once transported by locals from island to island in the Pacific, where they progressively expanded from the Americas to growing regions (Alum, 2021).

Sweet potato is a dicotyledonous plant and a member of the *Convolvulaceae* family, genus *Ipomoea*, along with closely related wild species. The plant is a herbaceous perennial vine with an alternating heart-shaped or palmately lobed leaves (Nanbol and Namo, 2019). The long, tapering edible with smooth skin differs in colour from yellow to orange, red, brown, purple and beige. The root tubers are varied in numerous colours white, beige, red, pink, yellow, orange, or purple are all possible colours for its flesh. White or light yellow-fleshed sweet potato varieties are less in sugar and moist content

than orange-flesh, red or pink hence less sweet taste (Loebenstein, 2009). *Ipomoea* is a genus with approximately 600 species worldwide, 13 of which are found in the species batatas. All 13 of these species are indigenous to the Americas; the only one that is farmed and hexaploidy (6x = 90) is the sweet potato. Due to the variations in their ploidy (chromosome level) and sophisticated compatibility and sterility mechanisms, these wild species do not spontaneously cross-pollinate with sweet potatoes.

2.2 Production of sweet potato

Besides wheat, rice, maize, potato, barley, and cassava, sweet potato ranks seventh in terms of consumption (Stathers *et al.*, 2018). Globally, sweet potato farms occupied approximately 8 million hectares in 2018 and root tubers were produced 91 million tons giving an average of 11 tons per hectare (Cartabiano-Leite *et al.*, 2020). Approximately 95 % of the world's sweet potatoes are produced in developing countries (Gobena *et al.*, 2022). This led to the separation of China's production estimates to get the real figures for Africa. Sweet potato is notably important in East and Central African countries that border the Great Lakes prominent among them are Malawi, Madagascar in Southern Africa; and Nigeria in West Africa (Stathers *et al.*, 2018). Scott, (2021) suggested that sweet potato in developing countries will be the major resorted to root tuber crop with respect to nutritional needs and food security. The steady expansion in sweet potato acreage and output in emerging countries demonstrated its value in the livelihoods of people in those countries particularly in Asia, Africa, and Latin America (Devaux *et al.*, 2020). Sweet potato is reported to be produced on 19.2 million hectares of land worldwide, with an annual production of 376.8 MT (Tiwari *et al.*, 2021a; Riaz *et al.*, 2022) which is doubled in both land for cultivation and output for what was recorded a decade ago. China and India, the two top sweet potato producers, produce 90.3 and 52.5

MT of sweet potatoes, respectively, accounting for 38 % of world potato production (Tiwari *et al.*, 2021b).

2.3 Importance of sweet potato in Sub-Saharan Africa

The crop is one of the most widely produced root crops in Africa, with yield production of 26.80 metric tonnes of roots in 2020 from a production area of 4.214 million hectares (Balasubramanian, 2023). It is primarily farmed on small plots for consumption, earning it the term "poor man's food" (Woolfe, 1992). It is known that women produce the majority of sweet potatoes in Sub-Saharan Africa which gave it another name ‘women’s crop’. The crop is especially important in Eastern and Central African countries surrounding the Great Lakes (Stathers *et al.*, 2018). It grows faster compared to any other important root tuber crop in Africa and it is usually cultivated at 2300 m above sea level (Gobena *et al.*, 2022). Compared to other important staples, sweet potatoes generate more food per unit area and per unit of time (Snowdon, 1990). In contrast to other crops, a significant and increasing pandemic of the cassava brown streak virus, it tolerates uncommon dry spells and produces even on less fertile soils. Sweet potato and cassava are often grown on the same farm or in the same region as backup crops. It is estimated that in 2019, cassava covers 22 million hectares in Sub-Saharan Africa (Adebayo, 2022) and when it fails, farmers frequently turn to sweet potato to replace the energy provided by cassava.

Many nations have seen shrinking cultivable land due to population surge which is affecting cropping patterns. This has caused farmers to seek out more efficient ways to produce food, such as using genetically modified crops that require fewer resources. Additionally, rising global temperatures have had an impact on crop production, leading to stressed soils and water shortages However, the reluctance of Africa farmers to use

GMO's, sweet potatoes are a popular choice of root crops because they produce higher yields per unit area than grain crops.

Reducing support for maize production is a second important factor in changing cropping patterns. In the post-colonial era, Sub-Saharan African governments' pursuit of food self-sufficiency resulted in a wide range of policies that fostered an excessive reliance on maize. Over the recent decade, governments, particularly in Southern Africa, have acknowledged the enormous cost of the programs and begun to withdraw some of the support. Decreased irrigation, fertilizer, seed, and marketing cost subsidies resulted in a decrease in maize area. Farmers frequently incorporate sturdy, low-cost sweet potato in their search for alternatives. Rising grain prices as a result of the current food crisis have diverted farmers attention to the significance of root and tuber crops in food security in Africa. As agriculture becomes more market-oriented, one of the many crops that farmers may cultivate to offer monetary remuneration along with household food security is sweet potato. As a result, supply and demand factors are becoming more and more crucial in determining the role of sweet potato in a smallholder farming sector that is more focused on the market. Fresh roots and vines are available in some countries (like Uganda), but the market size is often small. In response to factors such as price, convenience, and status, sweet potato consumption declines with urbanization. Through enhanced marketing techniques, the sweet potato's untapped potential can be explored (Tomlins *et al.*, 2000) and increasing demand for fresh roots and value-added sweet potato-based goods would improve rural farmers' productivity and well-being, especially for women, and help fulfil the rising need for food in cities.(Hossain *et al.*, 2021).

2.4 Nutritional composition of sweet potato

Frequent intake of vegetables as well as fruits cut down the risk of cardiovascular diseases (Zubrzycki *et al.*, 2013). Sweet potato can reduce the risk of these cardiovascular diseases

as it has antioxidant properties such as polyphenols which are the main constituents to preventing these diseases (Johnson and Pace, 2010). Sweet potatoes are a healthy root crop that includes considerable levels of beta carotene, vitamin C, and fibre, especially in cultivars with vibrantly coloured roots (Brandenberger *et al.*, 2014). According to Alum, (2021), 100 g of sweet potato contains 360 kj, 3.0 g, 20.1 g, 0.1 g, 1.6 g, 30 mg, 25.0 mg, 337 g, 0.6 g, 0.1 mg, 47 mg, 0.1 mg, 55 mg, 0.3 mg, 11 Ng, 0.61 mg, 79.8 %, 0.8 mg, 0.25 mg of energy, fibre, carbohydrates, fat, protein, calcium, magnesium, potassium, iron, thiamine, phosphorus, riboflavin, sodium, zinc, folate, Niacin (B3), water, pantothenic Acid, vitamin (B6) and about 2.4 mg for vitamin C respectively. Additionally, it is said that a number of minerals and xenobiotic phytochemicals, such as antioxidant, anticoagulant, and anti-diabetic properties, may be found in sweet potatoes (Escobar-Puentes *et al.*, 2022). Previously, Johnson and Pace (2010) found that sweet potato leaves contain high concentrations of vitamins, minerals, antioxidants, dietary fibre, and essential fatty acids, all of which are crucial for promoting health. Scientists in the United States have created genetically modified sweet potato plants that contain edible vaccinations against hepatitis B and the Norwalk virus (Saxena and Rawat, 2014). New edible vaccines like these could provide low-cost health protection in the future. According to Paul *et al.* (2021) and Escobar-Puentes *et al.* (2022), sweet potato tubers or leaves have significant therapeutic implications for hyperglycemia in type 2 diabetes mellitus, cardiovascular health, and inflammation. This suggests that sweet potato foods may one day be used to treat the developed world's rising obesity rates.

2.5 Sweet potato production constraints

Several obstacles must be overcome by sweet potato farmers in Africa to produce food for the household as well as generate income from crops. Among these constraints that needed immediate attention include; poor soils, ineffective farming methods, use of

regional sweet potato cultivars, land ownership, and damage from diseases and pests are a few of these limitations. Poor agricultural techniques, lack of suitable markets, subpar farming methods, and low resistance to sweet potato viral diseases are not exceptions to the woes of the African farmer (Echodu *et al*., 2019). The biggest of these restrictions is post-harvest crop deterioration as sweet potato farmers are devastated in attempts of recouping what they have invested into the production.

2.5.1 Post-harvest losses of sweet potato

During post-harvest storage and in the field, pests, diseases, and weeds can cause yield losses. Chaturvedi *et al.* (2011) estimated that yield losses ranged from 10 to 30% of crop production. It is reported that plant diseases result in direct and indirect losses, including reduced crop quality and quantity, increasing production costs, endangering animals, environmental health, a restriction on the crops/varieties that may be grown, and the loss of natural resources (Kumar *et al.*, 2009). To avoid food losses, thus, it is crucial to prevent and control diseases. A study by Kitinoja *et al.* (2018) indicates that about 20% of all grains are lost between harvest and consumption, and 44% of roots and tubers are lost. The post-harvest losses indicate that more attention must be paid to reducing these losses to resolve the world's food security issues. Worldwide, one-third of the food produced is lost or wasted, amounting to 1.3 billion tonnes per year (Valariño *et al.*, 2017). This is a huge amount of food that is not available for people who need it. Moreover, the production of such food requires a large amount of energy and resources, which could be saved if the food is not wasted. This is why reducing food waste is essential in making the world more sustainable.

2.5.2 Post-harvest deterioration of sweet potato tubers

Failure to follow proper curing and packing, as well as poor storage conditions, can result in tuber damage, peeling, water loss, and microbial assaults, may all outcome

considerable losses after harvest (Balasubramanian, 2023). In the field, during harvest and after harvest handling, storage, and distribution, spoilage-causing microorganisms can be introduced to the crop at any time (Sugri *et al.*, 2017). Fungi are typically the most frequently involved causative factors in root crop deterioration (Sugri, *et al.*, 2017). Sweet potatoes are susceptible to several fungi-related diseases, including surface rots, scurf disease root rot, soft rot, black rot, Java black rot etc. A lot of the diseases that afflict the root tubers in storage initially developed on planting materials like scurf or in the field. The breakdown of host tissue by microbes, which frequently occurs quickly and extensively, leads to pathological degeneration. Initial infection typically is visible at the locations of physical damage or at the point where the root attaches to the plant. A single or small number of pathogenic bacteria often start the attack pattern, which is then trailed by numerous saprophytes that may considerably amplify the initial damage. The disease development is influenced by the associated microorganism's capacity for growth and enzyme synthesis as well as the physiological state of the infected tissues. These microorganisms break down sweet potatoes by producing a wide range of hydrolysing enzymes such as cellulases, pectinases, xylanases, and proteases which promote tissue maceration and cell death, allowing microorganisms to take the nutrients from the dead plant tissues. This subsequently changes the quality of the root tuber flour, making it unsuitable for consumption or causing a major drop in market value.

2.6 Major post-harvest diseases of sweet potato

Several infections may severely impact potato production, either directly or indirectly. According to Clark *et al.* (2013), diseases affecting plants brought on by biological and/or non-biological causes are a significant factor in restricting the production of high-end categories of sweet potato tubers. Tiwari *et al.* (2021a) reported that, in the potato production chain, fungal infections are a key limiting factor that can lead to financial

losses both in the field and during transport and storage. When being harvested, transported, or stored in cold storage, newly harvested potato tubers, which contain around 70 % water, are susceptible to rots, galls, and blemish diseases. These factors facilitate the postharvest losses of sweet potato. Many phytopathogenic *Fusarium* species threaten sweet potato production, which may cause wilt of potatoes and dry rot of tubers during storage (Bojanowski *et al.*, 2013; Tiwari *et al.*, 2021b).

2.6.1 **Fusarium rots**

In all regions where sweet potato is grown, *Fusarium* species cause the Fusarium dry rot disease. The distribution of these species varies depending on the season, location, and accessibility of certain potato cultivars (Tiwari *et al.*, 2020b). According to Cullen *et al.*, (2005), more than 13 different *Fusarium* spp. have been identified as the root cause of Fusarium dry rot. According to Du *et al.*, (2012), losses from dry rots might range from 25 to 60% during storage. The annual financial damage caused by this disease is between $100 and $250 million in the United States alone (https://www.ars.usda.gov). According to studies, 88% of all post-harvest losses in the Chinese province of Gansu were attributable to dry rot disease (Du *et al.*, 2012). In the Michigan state of the United States, almost 50% of sweet potato seed tubers had *Fusarium* species infections (Gachango *et al.*, 2012). Similar to this, *F. sambucinum* (FS) is described as the most aggressive fungus species causing dry rot in tubers in Europe, China, and North America (Du *et al.*, 2012). According to Gildemacher *et al.* (2009), *F. coeruleum* (Libert) is the most common fungus found in British cold storage facilities. The most frequent fungi causing dry rot in North Dakota and Michigan, respectively, are *F. graminearum* (Schwabe) and *F. oxysporum* (FO). *F. oxysporum, F. solani*, and *F. sambucinum* are often found in cold storage in tropical and subtropical areas of India (Sagar *et al.*, 2011; Tiwari *et al.*, 2021b). Necrotic wrinkled, brown to black, depressed tuber patches that diminish the dry matter

and cause shrivelled flesh are signs of tuber dry rot. When stored at a temperature between 5 and 30 ℃, these wrinkled patches and necrotic lesions develop mycelial growth that is creamy white, pink, or orange in colour (Bojanowski *et al.*, 2013; Elsherbiny *et al.*, 2016).

2.6.2 **Java black rot**

Botryodiplodia theobromae is the causative organism for Black Java rot of sweet potato and the most common storage disease in tropical and subtropical areas, including West Africa, Asia, and the subtropical zone of the United States (Sowley and Oduro, 2002; Ray and Edison, 2005; Ray and Tomlins, 2010). The proximal end of the root or other incision locations are typically where the rot starts to spread. The diseased tissues start out looking yellowish-brown before turning black. After six to eight weeks of storage, the afflicted roots exhibit dark patches on the outside that house numerous pycnidia while the tissues within turn yellow and eventually turn black. Roots that have deteriorated become withered, brittle, and mummified. The primary risk factor for Botryodiplodia infection is wounding. For *B. theobromae* to flourish, the ideal temperature and relative humidity are 25 – 35 ℃ and 85 – 90%, respectively.

2.6.3 **Black rot**

Anywhere sweet potatoes are cultivated intensively, black rot, which is caused by *Ceratocystis fimbriata* (Mohsin *et al.*, 2021), has been a concern. The pathogen causes numerous damages to sweet potato in transplanted beds, fields, and in storage. Aside from the quality loss and tuber deterioration caused by *Ceratocystis fimbriata* in storage but also gives a distinct bitter taste. The initial symptoms are usually small, circular, slightly sunken, and dark-brown spots. In the field, healthy sweet potato can be penetrated by *C. fimbriata* through the skin but preferable lateral roots, lenticels, and wounds are used (Stahr and Quesada-Ocampo, 2019). Despite efforts to eradicate the disease using fungicides such as thiabendazole on seed roots and removing transplants above the soil

line, the disease still occurs mainly in the United States, New Zealand, and Japan (Stahr, 2021). Other tropical and subtropical areas including Papua New Guinea, Haiti, Peru, and Vietnam continue to view it as a significant post-harvest disease. However, sweet potato-growing Asian nations including the largest producer of sweet potato China, and Pakistan, Nepal, India have not yet detected the rot (Ray and Edison, 2005).

2.6.4 Bacterial soft rot

Bacterial soft rot of potato is an important disease due to its losses during crop development and storage (Bdliya and Dahiru, 2006). It is caused by *Erwinia carotovora*, which is commonly associated with soft rot tubers of potatoes and other vegetables (Prajapat *et al.*, 2013). It is destructive, especially in transit and storage (Ikechi-Nwogu and Nworuka, 2023).

2.6.5 Rhizopus soft rot

Sweet potato storage soft root rot is caused by *Rhizopus* spp. in many sweet potato growing regions (Penyimpanan *et al.*, 2016). The sweet potato is prone to a variety of pathogens in the field as well as in storage though Rhizopus soft rot is the most destructive pathogen accounting for about 2 % in storage rot before reaching the market place (Clark and Moyer, 1988; Scruggs and Quesada-Ocampo, 2016). According to Scruggs and Quesada-Ocampo (2016), *Rhizopus* sp. takes advantage of natural openings and wounds that resulted from mechanical tools and pests during farming operations. It is further reported that, the susceptibility of sweet potato roots to *Rhizopus* spp. is determinant on the type of wound and the storage time (Scruggs and Quesada-Ocampo, 2016) while Holmes and Stange (2002), reported in their study that, the disease development is conducive with bruising as compared to other type of injuries such as breaking, scraping and puncturing. It is reported that Rhizopus soft rot symptoms are visible between 3 to 5 months after harvest due to decreasing susceptibility within this period (Holmes and

Stange, 2002). The infection of the disease on sweet potato can be reduced by curing the tubers at higher temperature and humidity as it creates suberization on the injured portions thereby serving as a barrier to the entry of the pathogen. The spores of *Rhizopus* spp. are air-borne which make them easier to over winter on crop debris, fruits and vegetables as well as tools and equipment (Scruggs and Quesada-Ocampo, 2016). The distinctive characteristics of Rhizopus soft rot are the soft, watery and stringy sweet potato which usually occurs around the wounded region of the infection however, (Clark, 1980) reported that 'whiskers' is the most distinguishing feature which usually arise from the periderm around the root and occur once awhile.

2.6.6 Charcoal rot

The fungus *Macrophomina phaseolina* is responsible for the charcoal rot on sweet potato, and it exclusively affects fleshy roots during storage (Akinbo *et al.*, 2016). Other plant components are not attacked by the fungus. Starting on the root's surface, the infection spreads via the vascular ring and toward the pith. The fungus is widespread around the world and affects a variety of plant types. It is soil-borne and can exist autonomously as sclerotia or saprophytically on plant detritus. No control measures are known.

2.7 Causes of sweet potato rots

Sweet potato rots are caused by biological and environmental factors both in the field and in storage. However, the most prominent among them are as follows:

2.7.1 Mechanical injuries

According to reports, sweet potatoes have delicate, thin skin that is readily damaged by cuts and abrasions when being harvested, transported, or distributed. The epidermis of the roots is damaged when harvesting equipment strikes them or when they are dropped into containers (Gambari and Okinedo, 2020). If the sweet potatoes are handled harshly

or stored in containers with sharp edges, they may be damaged or bruised, which might lead to microbial infection (Gambari and Okinedo, 2020). The sweet potato root's undamaged skin serves as a defence against infection penetration and moisture loss. Infections can be facilitated by mechanical injuries that serve as entrance routes for microbial pathogens. Therefore, it is important to conduct careful harvest and post-harvest management to prevent damage and promote root quality, particularly during extended storage.

2.7.2 Temperature

In the last decade, global temperature has risen due to the increased levels of CO_2 and other greenhouse gases (Chen and Setter, 2021). It is estimated that these trends could impact crop production and food security (Jarvis *et al.*, 2012; Chen and Setter, 2021). It is reported that temperature plays a major role in lengthening the natural dormancy of potato (*Solanum tuberosum* L.) as long as the storage temperatures are low *(*Murigi *et al.*, 2021) by managing sprout development, tuber weight loss, and tuber quality. Murigi *et al.* (2021) and Paul *et al.* (2016) reported that long-term storage can be achieved by storing potato at low temperatures (2 – 4 ℃) as it will inhibit sprout development. However, reducing sugars (fructose and glucose) are accumulated at 2 – 4 ℃ thereby giving a sweet taste to potato tubers. It is reported that tuber maturation, cooling down, and long-term storage are the three stages of long-term storage concerning recommended temperature and humidity (Krochmal-Marczak *et al.*, 2020; Vithu *et al.*, 2020). At high temperatures though, sweet potato tubers are vulnerable to rots due to microbial attacks and become worst when tubers suffered from injuries such as cuts, bruises, insects and pests etc.

2.7.3 Pests and diseases

Though the benefits of sweet potato are huge in Sub-Saharan Africa and the world at large, unfortunately, its production is heavily plagued with insect pests and diseases (Musa *et al.*, 2022), and microbial deterioration (Nwaneri *et al.*, 2020) in both fields and storage. Many researchers have reported that the most devastating major insect pests preventing sweet potato production in Sub-Saharan Africa are the sweet potato weevils (*Cylas puncticollis* Boheman, Coleoptera; Curculionidae) and the flea beetles (*P. cruciferae* Goeze, Coleoptera: Chrysomelidae) drastically reducing sweet potato yield (Hue and Low, 2015; Okpara *et al.*, 2021; Musa *et al.*, 2022). The major and minor insect pest infestation has been reported to reduce sweet potato yield by 20 – 75 % (Alehegne, 2007). It is reported that the adult *C. formicarius* is a notorious pest as it can destroy the petioles, vines, and crowns as well as the tubers in storage while the adult female lay their eggs underneath the vine epidermis and storage roots as well (Dongzhen *et al.*, 2020). The larvae however dig tunnels into the vines and storage roots where they deposit their faeces (Reddy *et al.*, 2014). Feeding activities of these insect pests cause the production of unwanted substances including terpenoids and phenolic compounds which give a bitter taste, inedibility, and unpleasant smell making the storage roots unmarketable (Dongzhen *et al.*, 2020).

2.8 Sweet potato disease management

A variety of fungal diseases can affect sweet potatoes, and more than 40 pathogens are associated to cause disease infections both in the field as well as in storage in sweet potato-growing regions around the world (Hedge *et al.*, 2012). Postharvest spoilage of sweet potato root tubers is a major constraint in Ghana and extensive research has been conducted to find effective control measures for recommendation for sweet potato farmers. Physical and chemical methods have been named the two major control methods

in Ghana (Sowley, 1999). However, recent research on the management of postharvest sweet potato losses has been focused on the use of botanicals that are health-wise safe and eco-friendly for all actors in the production of sweet potatoes in the tropics (Linus, 2014b; Kwodaga *et al*., 2019; Giri *et al*., 2020). Below are some of the improved methods for managing sweet potato diseases.

2.8.1 Good agricultural practices

According to Karavidas *et al.* (2022), with the ever-increasing human population estimated to be around 10.4 billion people by 2067 with Africa and Asia to contribute to three-quarters of this population growth (Chojnacka *et al.*, 2020). Therefore, safe agronomic practices with integrated disease management could be the way forward in the 21st century for sustainable agriculture in Africa. The practice of slash and burn in most countries in West Africa in land preparation for the new farming season depletes the soil major and micronutrients depriving plants of the needed support for growth. However, the demand for synthetic chemical fertilizers, chemical pesticides, and freshwater quality could be drastically reduced if developed and efficient methods including; irrigation, protection, and crop fertilization are used without sacrificing yield and quality (Young *et al.*, 2022). On the United Nations Sustainable Development Goals (UN-SDGs), biological-based practices for crop production which creates an eco-friendly environment as well as long-term profitability are the priorities for irradicating malnutrition and food security (Karavidas *et al.*, 2022). For sustainable agriculture, intercropping, little or no-tillage management, and organic farming are some of the techniques that one can use to promote soil biodiversity as well as enhance the soil profile and health (Morugán-Coronado *et al.*, 2020). Reduced tillage also necessitates drastically reducing the size, power, and usage of agricultural equipment, which lowers management costs and greenhouse gas emissions (Ghimire *et al.*, 2017). High-yielding cultivars that are also

resistant to biotic and abiotic stresses to sweet potatoes in the field which gives yield quality without disturbing the ecological system as well as improves nutrient uptake and water absorption from the soil could be considered as good agronomic practice. Additionally, because of its potential to increase yield and product quality as well as owing to legislative limits on soil fumigants and pesticides to battle soil-borne diseases, the use of hydroponics is becoming more and more significant in sustainable agricultural systems (Karavidas *et al.*, 2022).

2.8.2 Curing

To reduce storage losses, seed or marketable stocks need to be cured as soon as they are harvested. Curing entails regulating the relative humidity and temperature while supplying good aeration for a number of days depending on the location. For a wound to heal as quickly as possible, the curing process needs a temperature range of 27 ℃ - 32 ℃, relative humidity of 85 – 90 %, and good ventilation to eliminate carbon dioxide from the curing region. A protective cork covering will form over the entire root surface as cuts and bruises heal. Suberin, a waxy substance, is also deposited. To prevent moisture loss and decay-causing organisms, the cork layer and suberin serve as a barrier. Tortoe *et al.* (2014) reported on tuber crops that curing enables damaged roots and root vegetables to heal and inhibit microorganisms from attack. Substantial shrinking was decreased by approximately 1% in fresh weight of tubers after 3 days between 35 °C - 40 °C and 80% - 90% at temperature and relative humidity respectively (Demeaux and Vivier, 1984). It has been suggested that curing sweet potato roots may enhance their preservation (Tortoe *et al.*, 2014). According to reports, higher temperatures and humidity cause yam tubers to produce cork cells, which completely seal the lesions (Bautista, 1990). Cork cells are subsequently created in the cork cambium and transported into the wound sites, where they seal the wound with several layers of periderm. This layer significantly slows down

the desiccation process and guards against bacterial and fungal growth (Tortoe *et al.,* 2014). During periderm production, the metabolic reactions produce water, carbon dioxide, and heat that are released into the atmosphere as a result of starch expiration.

2.8.3 Refrigeration

A refrigerator slows the metabolism of pathogens, which often prevents the development of putrefaction. When the product reaches ambient temperature, putrefaction will begin again since the putrefaction organisms are rarely killed. However, this method is expensive to be carried out by peasant sweet potato farmers (Tartoura *et al.*, 2015), particularly in Ghana. Otherwise storing root tubers at 4 – 5 ℃ and 7 – 10 ℃ are ideal temperatures for seed and fresh sweet potato for market respectively (Giri *et al.*, 2020) though undesirable sweetening in the tubers is produced due to the convection of starch into sugars caused by low temperature (Giri *et al.*, 2020).

2.8.4 Handling methods

Proper root tuber handling and harvesting methods are the only way to reduce mechanical damage. Tropical regions generally lack well-developed food handling practices, and it is all too common to treat fresh vegetables like inert objects. Proper packing must be prioritized, especially if the fruit will be consumed distant from the manufacturing location. For processing and transporting root crops in the tropics, boxes or cartons are generally regarded as being significantly more suited than huge sacks (Sowley and Oduro, 2002).

2.9 Sweet potato decay control treatment

2.9.1 Chemical treatment strategies

Chemicals are still the major choice for sweet potato farmers in Ghana for managing sweet potato root tuber diseases despite their numerous dangers. The ingestion of crop

products treated with synthetic chemicals has been linked to negative health impacts, including teratogenicity, allergies, and the mortality of animals, according to recent research, which has raised concerns (Nwaneri *et al.*, 2020). Typically, the tactics employed for the application of these chemicals include; fumigation, dip or drench treatments, and pre-and/or postharvest sprays. However, some of these methods are affected by the time of application on crop produce. For instance, vegetables get contaminated by viruses at the pre-harvest stage after spraying. It is often recommended for fungicides to be applied on sweet potato plants on the field (Coates and Johnson, 1997). Preharvest sprays often reduce the amount of surface inoculum and avoid contamination and infection during harvest and postharvest. To prevent infections from spreading throughout the postharvest handling chain, including storage, fungicides used throughout the postharvest process must suppress latent infections. Postharvest fungicides can be applied via waxes, coatings, fumigants, treated wraps, box liners, sprays, dips, and fumigants. Frequently employed dips and sprays might be aqueous solutions, suspensions, or emulsions, depending on the substance. Fungicides such as benzimidazoles (e.g. benomyl and thiabendazole) and demethylation inhibitor fungicides (e.g. prochloraz and immazalil) are frequently used as dips or sprays. Ammonia, ozone, and carbon dioxide are among other fumigants that are employed in developed countries. A commonly used fungicide, mancozeb is categorized as a contact fungicide with preventative action. By producing a molecule containing metal-containing enzymes, especially ATP-producing enzymes, it inhibits the action of fungal enzymes. This fungicide protects fruits, vegetables, nuts, and field crops from fungal diseases, including rusted roses, apple scabs, pear scabs, leaf spots, and potato blight. Furthermore, mancozeb can also be used to treat cotton, potato, maize, safflower, sorghum, peanuts, tomatoes, flax, and cereal grains. Junaid *et al.* (2013) observed that mancozeb was the most

successful in inhibiting the development of *F. solani* and *F. oxysporum*, and that zineb was also efficient at controlling *F. solani*. At the least advised dose of 500 ppm, the combination of cymoxanil + mancozeb, carbendazim + mancozeb, and tricyclazole + mancozeb was shown to be the most efficient. Despite being effective against postharvest fungal infections, fungicides primarily used to manage postharvest diseases have been extensively studied for carcinogenic and other serious health hazards (Daniel, 2014) as they can induce acute toxicity, and some can also cause chronic diseases. Ray and Ravi, (2005) reported that several environmental and health problems have been connected to the use of chemical pesticides. Also, most sweet potato packing facilities employ the fungicide dicloran (Botran) as a spray or dip treatment on the packing line to reduce losses from Rhizopus soft rot, a post-harvest disease caused by one of the principal sweet potato pathogens.

2.9.2 Antagonistic microorganisms

The use of microorganisms as biological control agents as a component of an integrated disease management or used separately (Stathers *et al.*, 2018). A genetically stable organism that can be effective at low concentrations and operates against a wide spectrum of infections on diverse food commodities has been characterized as a suitable antagonist. The antagonist should have minimal nutritional needs, be able to survive under adverse environmental conditions, and be able to establish itself in fermenters on inexpensive substrates. Furthermore, an ideal antagonist should not be pathogenic to the host crop and should not create metabolites that are hazardous to human beings as well as plants. Aside from that, it is also necessary that it is able to withstand common pesticides and complement other chemical and physical therapies effectively. The aforementioned traits enable useful microbes to combat pathogenic organisms by producing antibiotics, through competition, parasitism, direct contact, or by developing resistance. "Biosave"

(*Pseudomonas syringae* Van Hall) and Shemer" (*Metschnikowia fructicola*), are registered in both the United States and Israel to control sweet potato, potato, and carrot diseases, are a couple of examples of commercially available antagonistic products (Eshel *et al.*, 2009).

2.9.3 Secondary compounds of plants

Plant bioactive compounds have received attention recently as a potential new postharvest disease control strategy. The secondary metabolites that plants create are diverse and, in many cases, physiologically active. These compounds are also having antioxidant, antibacterial, bioregulatory, and allelopathic capabilities (Moomin *et al.*, 2023). This collection of substances includes; phenols, flavones, phenolic acids, quinones, flavonoids, coumarins, tannins, and flavanols as significant subclasses. These chemical classes have antibacterial properties and act as defence mechanisms for plants over harmful microbes. The site(s) and quantity of hydroxyl groups that are present in the phenolic compound are what cause it to be hazardous to microorganisms. Plants produce flavones, flavonoids, and flavanols, which are phenolic compounds containing a single carbonyl group. These compounds are frequently reported to be effective *in vitro* as antimicrobials against a variety of pathogens. Environmental factors, the time the plant part was gathered, how it was dried, storage conditions, and isolation techniques, among others, all affect the yielding ability of the biological properties at a given moment.

2.9.4 Botanical treatment

Plant extracts are generally preferred over synthetic chemicals when managing diseases due to the unsafe health conditions they pose (Opiyo *et al*., 2011). Chemicals of plant origin have lately received significant interest worldwide (Endersby and Morgan, 1991; Ware and Whitacre, 2000) due to their antifungal, antibacterial properties and environmentally friendly exposure. Botanical pesticides are significant class of naturally

occurring, frequently weak crop protectants that, relative to traditional pesticides, typically do less harm to people and the environment and have fewer long-term side effects (Pavela, 2009). According to Ivbijaro, (2012), using botanicals to protect plants from pathogens offers a number of clear benefits. The use of botanicals as treatment of pathogens has long been considered an attractive alternative to synthetic fungicides, as they have little or no impact on the overall ecosystem and are also much safer for humans to use (Isman, 2006; Okpara *et al.*, 2021). Many researchers have expressed interest in exploring plant derivatives as a possible substitute to synthetic pesticides to avoid harmful or unfavourable side effects (Yang *et al.*, 2010). Due to the limitations associated with conventional chemical-based control methods, the focus has recently shifted toward the use of plant extracts as innovative fungicides (Okigbo and Ogbonnaya, 2006). The extraction of important bioactive plant tissue fractions from plants using select appropriate technologies has been a key focus of research into biopesticide extraction methods. When extracting compounds from plant tissue, it's important to consider the polarity of the solvent being used. The effectiveness of the plant extract will depend on a variety of factors, such as the type of plant material being used, the solvent of choice, and the extraction method. It's also worth noting that the type of solvent used can greatly impact the analysis of biologically active compounds in the plant material. With this in perspective, Alam *et al.* (2016) reported that low toxicity, preservation action, ability to trigger the resulting compound to dissociate and ease of evaporation are the appropriate properties of an ideal solvent extraction from plants. The extraction solvent should not be harmful and should not affect the bioassay because the finished product will still include residues of the extraction solvent. The chemicals that need to be eliminated will also have an impact on the choice. For the first examination of plants for possible antimicrobial activities, crude or alcohol extractions are typically performed, and different organic

solvent extraction procedures can be used as a follow-up. Many plant species have been exploited in the search for distinct properties which could act against microbes. Nwaneri *et al.* (2020) reported that *R. stolonifer* causing sweet potato soft rot in Northern Nigeria can be greatly managed by the use of *Azadirachta indica* and *Moringa oleifera* extracts. Similar studies were conducted in Southern Nigeria also, by Amienyo and Ataga, (2007) by using three extracts namely; *Zingiber officinalis*, *Alchomia cordifolia,* and *Garcinia kola* to control rot on tubers caused by *Botryodiplodia theobroma*. Again, Linus, (2014a), used neem, ginger, and onion to inhibit the growth of *R. stolonifer* and *Aspergillus flavus* causing rots on three different sweet potato varieties in Ghana. The neem extracts inhibited *R. stolonifer* and *A. flavus* at 62.5 % and 56.2 % respectively while ginger and onion inhibited *A. flavus* at 42.7 % and 35.3 % respectively. Linus, (2014b) reported that a combination of three or four related chemicals and more than 20 minor compounds helps neem (*Azadirachta indica*) defend itself against pathogens and pests.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted in the Microbiology Laboratory of the Spanish Laboratory at Nyankpala Campus of the University for Development Studies. It is located at Longitude 0˚ 58″ 42′ W and latitude 9˚ 25″ 41′ N situated at 1057 mm above sea level. The area has unimodal rainfall of about 1500 mm per annum with a relative humidity of 60% in the Guineas Savanna Ecological Zone of Ghana.

3.2 Source of experimental materials

Petri dishes (glass and plastic), Potato Dextrose Agar (PDA) (OxoideTH), hand gloves, 75% alcohol, cotton, and protective wear were procured from LabChem Limited in Kumasi. Relative humidity chambers were built using a 150 cm × 50 cm × 30 cm binding wire frame which was inserted into a white polyethylene bag.

3.2.1 Source of sweet potato tubers

Sweet potato varieties both white flesh and orange flesh were procured at the farm gate from sweet potato farmers in Nyankpala and the neighbouring communities and were sorted into uniform sizes 30 g – 80 g before being transported to the Spanish Laboratory. They were cured in the open sun for one week before being kept on raised platforms in an airy open space in the Spanish Laboratory till the commencement of the experiment.

3.2.2 Sources of botanicals

Holy basil (*Ocimum sanctum*) was collected in the Nyankpala township and its environs while lemon grass (*Cymbopogon citratus)* was obtained at the Nyankpala campus of the University for Development Studies. Bitter leaf (*Vernonia amaygdalina*) was, however,

obtained at Damango (125 km from Nyankpala) in the Savanna Region due to insufficient availability of it in Nyankpala and its environs.

3.3 *In vitro* studies

3.3.1 Preparation of plant extracts

Each botanical was washed under running tap water after harvesting and then air dried on standing tables under a shaded environment till it was completely air dried. The dried samples were sent to the Savanna Agricultural Research Institute (SARI) of the Council for Scientific and Industrial Research (CSIR) food laboratory in Nyankpala for grinding. They were grounded into powder using a grinding mill fitted with a 2 mm sieve. One hundred and eighty (180) g of each extract powder was weighed and kept in aseptic bottles and dissolves in 1.8 litres of distilled water to make 10 % basic concentrated solutions. The extracts were agitated vigorously and left to stand for 24 hours. They were filtered using sterilized Whatman filter paper No. 4 and the filtrate was used as the extracts for the *in vivo* studies.

3.3.2 Phytochemical analysis of plant extracts

The qualitative phytochemical analysis of the three plant extracts were conducted in aqueous and ethanol extractions methods. Preliminary screening for the qualitative analysis of the crude powder of the plant samples was carried out in each of the extraction methods according to the method of Nandagoapalan *et al*. (2018) with some modifications.

3.3.2.1 Detection of Alkaloids

(a) Individual extracts were diluted in dilute hydrochloric acid and filtered

Wagner's Test: Wagner's reagent (Iodine in Potassium Iodide) was applied to the filtrates. The presence of alkaloids is indicated by the formation of brown/reddish precipitate (De *et al*., 2010).

(b) Hager's Test: Hager's reagent (saturated picric acid solution) was applied to the extracts. The production of yellow-coloured precipitate confirmed the presence of alkaloids (De *et al*., 2010).

3.3.2.2 Detection of saponins

Froth Test: Extracts were diluted to 20 ml with distilled water and shaken in a graduated cylinder for 15 minutes. The presence of saponins was indicated by the creation of a 1cm layer of foam (Tyagi and Agarwal, 2017).

3.3.2.3 Detection of phytosterols

Salkowski's Test: Extracts were chloroform-treated and filtered. The filtrates were agitated and left to stand after being treated with a few drops of concentrated sulphuric acid. The presence of triterpenes is indicated by the emergence of golden yellow tint.

3.3.2.4 Detection of phenols

Ferric Chloride Test: Three to four drops of ferric chloride solution were applied to the extracts. The presence of phenols is indicated by the creation of a bluish-black colouration(Pandey and Tripathi, 2014).

3.3.2.5 Detection of flavonoids.

Lead Acetate Test: A few drops of lead acetate solution were applied to the extracts. The presence of Flavonoids was shown by the production of a yellow precipitate (Bhandary *et al*., 2012).

3.3.3 Preparation of sweet potato root tubers

Matured and wholesome sweet potato varieties of white-flesh (Plate 3:1 (A)) and orange-flesh (Plate 3:1 (B)) were selected and weight to give a range of 30 – 80 g of tuber size for the experiment. The selected root tubers were washed under a tap running water to remove soils and unwanted impurities since they were collected at the farm gate. They were air-dried on a sterilized workbench at a room temperature.

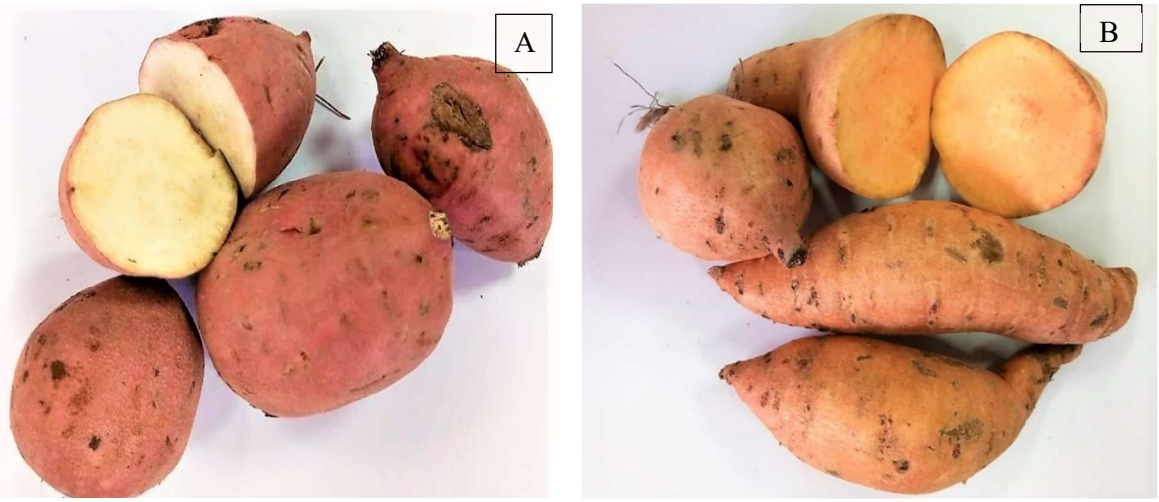

Plate 3.1: Healthy sweet potato varieties white-flesh sweet potato (WFSP) (A) and orange-flesh sweet potato (OFSP) (B)

3.3.4 Isolation of pathogens causing sweet potato rots

3.3.4.1 Preparation of culture media

At the manufacturer's specification, thirty-nine (39) g of PDA was weighed using a weighing balance (Aczet Thailand Co. Ltd.; CY 324C; 320 g) and kept in a 1 litre media bottle. One tablet of amoxicillin (500 mg (September 2022); Zylomox, Zydus Cadila, India) was added to inhibit the growth of bacteria. One litre of distilled water was added and then kept in a heated water bath for the dissolution of the PDA. The dissolved PDA was autoclaved at 121 ℃ for 15 minutes. After autoclaving, the PDA was allowed to cool

in a sterilized laminar air flow chamber before pouring it into Petri plates (90.8 mm $\times$ 87.5 mm for solidification.

3.3.4.2 Isolation and Identification of pathogens from rotted sweet potato tubers.

Sweet potato tubers with rot symptoms (Plates 3. 2; A and B) were washed under running tap water and allowed to dry under room temperature. Small tissues were excised containing the periphery of both healthy and diseased parts of the rotten tubers and then soaked in 75 % alcohol for 5 seconds before removal and placed on sterilized clean tissue papers in a Laminar air flow chamber to dry under room temperature of 30 ℃. The dried diseased tissues were plated separately on the solidified PDA according to the variety and their Labelling. The plates were wrapped with cling film and placed on a sterilized workbench at a temperature of 27 ± 2 ℃ and were left for six days of incubation. Upon the growth of mycelia on the incubated plates, subculturing was done on a freshly prepared PDA as discussed (section 3.3.2.1: Preparation of culture media) above and repeated subculturing was carried out till pure culture of a single species was obtained.

After obtaining the pure culture, the isolated pathogens were identified using light microscopy, and the colour and colony features of the isolates were studied and described using microscope. Microscopic slides of the organisms were prepared, fixed, mounted, and inspected under the microscope to determine the pathogen characteristics using Campbell and Johnson (2013) and Barnett and Hunter (1998).

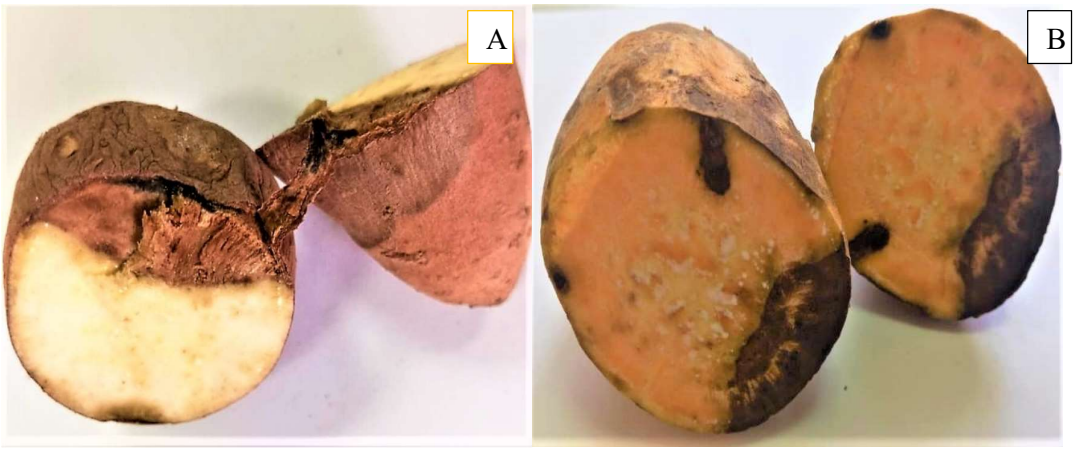

Plate 3.2: Rotten sweet potato tubers white-flesh variety (A) and orange-flesh variety (B)

3.4 Pathogenicity studies

This was conducted according to the method of Beckley and Awoyemi, (2021) with some modifications. Fresh and healthy sweet potato tubers were washed under running tap water and allowed to dry before surface sterilizing with 75 % of alcohol. A 5 mm cork borer was used to create a cylindrical hole on each tuber with the removal of the tissue core. A six-day-old of 3 mm mycelia plug of each of the isolated pure fungus culture was placed into each hole at a depth of 5 mm created on the tubers and then covered up with the cylindrical tissue core before using Vaseline to completely sealed it off to prevent drying of the mycelia plug. The inoculated tubers were placed on cotton wool wet with 20 ml of distilled water in relative humidity chambers made by inserting a binding wire frame of 100 cm × 50 cm × 30 cm to give an area of 150,000 cm^3 into a white polyethylene bag of doubled-layered. The relative humidity chambers were sealed off and left for 14 days at a room temperature of 27 ± 2 ℃. Controls were carried out with uninoculated PDA placed in the holes created on the tubers. After, 14 days of incubation tubers were

brought out and assessed for disease development before re-isolation of the pathogens on fresh prepared PDA for confirmation of Koch's postulates.

3.5 Experimental design

The experiment was a 2 × 5 factor experiments laid out in a Completely Randomized Design (CRD) with three replications. The treatment consisted of two sweet potato varieties; orange-flesh and white-flesh and three plant extracts; bitter leaf, lemon grass, and holy basil with fungicide and water being positive and negative controls respectively. Each sweet potato variety was treated separately with the plant extracts to give a 1 × 5 single factor experiment.

3.5.1 Treatment combinations for sweet potato variety against extracts

Table 3.1: Treatment combination of plant extracts

Variety	**Extracts**				
	Lemon grass (Lg)	Holy basil (Hb)	Bitter leaf (Bl)	Negative control (H_2O)	Positive control (fungicide)
OFSP	Lg + OFSP	Hb + OFSP	Bl + OFSP	H_2O + OFSP	Fungicide + OFSP
WFSP	Lg + WFSP	Hb + WFSP	Bl + WFSP	H_2O + WFSP	Fungicide + WFSP

3.6 Parameters studied

3.6.1 *In vitro* studies

3.6.1.1 Percentage of disease occurrence (%)

Four cylindrical mycelium plugs of six days old cultured isolated fungal pathogens were placed equidistantly to each other on the solidified PDA media in each petri dish. The growth of each mycelium plugs on the PDA represents 25 % of occurrence hence all four mycelia growth on the PDA represents 100 % occurrence. Records of the number of times each type of organism was observed on the incubated sweet potato petri dish were used to determine the percentage occurrence of the micro-flora using the formula adopted by Maranzu, (2019).

$$Percentage\ of\ occurrence\ (\%) = \frac{No.of\ observation\ of\ mycelia\ growth\ of\ the\ pathogen\ in\ the\ petri\ dish}{Total\ number\ of\ observation\ in\ the\ petri\ dish} \times 100$$

3.6.1.2 Weight loss

Three healthy root tubers from each sweet potato variety with uniform weight range of 30 – 80 g were selected, surface sterilized with 75 % ethanol, and then weighed using a weighing balance (Aczet Thailand Co., Ltd.; CY 324C; 320 g). The tubers were dipped to completely submerged in each of the aqueous solutions of the extract prepared for 10 minutes (Linus, 2014a) before removal and air-dry on sterilized tissue papers. The treated root tubers were then stored at room temperature of 27 $\pm$ 2 ℃. The weight of the treated tubers was taken at 7 days intervals. Each treatment was replicated three times. Percentage (%) weight loss was calculated on a weekly basis by the formulae used by (Kuyu *et al.*, 2019);

$$Weight\ loss(\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

3.6.1.3 Sprout

Tuber sprout was observed each week and those that sprouted each week were counted and then severed. At the end of the storage period, all the sprout count for each treatment was summed up and then averaged to get the sprout count for each treatment.

3.6.1.4 Antifungal activity of plant extracts against isolated pathogens

The five test fungi isolated; *Trichoderma harzianum*, *Fusarium oxysporium*, *Rhizopus stolonifer*, *Aspergillus niger*, and *Lasiodiplodia theobromae* from rotted sweet potato tubers were used for this set-up. The food poisoned method of Potato Dextrose Agar (PDA) medium with crude plant extracts was used to test the efficacy of the plant extracts against the isolated fungi pathogens according to the method used by Balouiri *et al*. (2016). The plant extracts were in two levels 100 % and 50 % concentrations. Five millilitres of each extract were dispensed into the Petri plate using a sterilized disposable syringe before pouring the prepared PDA into it and calmly swelled to get a uniform mixture for the 100 % concentration level while 2.5 millilitres each of both the extract and distilled water were dispensed into each of the Petri plates for 50 % concentration level and swelled for uniform mixture before dispensing the prepared PDA. The extracts with the PDA were allowed to solidify before centrally inoculating 5 mm mycelia plugs of six days old cultures of each of the five test fungi on the Petri plates according to their Labelling. Each treatment was replicated three times with Negative and Positive controls of water and fungicides.

Initially, two perpendicular lines were drawn at the reversed side of the Petri plates and the intersection becomes the centre of the plate. A 5 mm mycelial plug of six days old

culture of each test fungi was placed in the intersected point where the two perpendicular lines meet. Both negative and positive control experiments were set up without the extract where water was used for the negative control. Mancozeb (a synthetic chemical) was prepared per the method used by Gwa and Richard, (2018) by weighing 4 g of the chemical and dissolving it in 1 L of distilled water and stirring to make a concentration of 4 g/L for the positive control. Extract toxicity was determined with respect to percentage colony inhibition and calculated by the formulae used by Amadioha, (2003) with some modifications.

$$Growth\ inhibition\ (\%) = \frac{RC - RT}{RC} \times 100$$

Where;

RC = Average radius of control

RT = Average radius of a fungal colony with treatment.

3.6.2 *In vivo* experiment

3.6.2.1 Virulence of test fungi

Fresh and healthy tubers were washed under running tap water and allowed to dry before surface sterilizing with 75 % of alcohol. A 5 mm cork borer was used to create a cylindrical hole on each tuber with the removal of the tissue core. A six-day-old isolated fungi culture of the test pathogens isolated viz. *Fusarium oxysporum*, *Lasiodiplodia theobromae, Trichoderma harzianum, Rhizopus stolonifer,* and *Aspergillus niger* was each placed separately into the hole created on the tubers and then covered up with the cylindrical tissue core before using Vaseline to completely sealed it off to prevent drying of the mycelia plug. The inoculated tubers were placed on cotton wool wet with 20 ml of distilled water in relative humidity chambers made by inserting a binding wire frame into

a 150 cm white polyethylene. The relative humidity chambers were sealed off and left for 14 days at a room temperature of 27 ± 2 ℃. Controls were carried out with uninoculated PDA placed in the holes created on the tubers. After, 10 days tubers were removed and examined for disease incidence (Scruggs and Quesada-Ocampo, 2016) and were cut diametrically across the point of inoculated mycelia plugs for measurement.

3.6.2.2 Rot severity (%)

This was conducted based on the protocols adopted by Amadioha, (2004) and Chukwuebuka *et al.*, (2016). Where one root tuber from each treatment was inoculated with a 5 mm diameter disc of isolated pathogens and replicated three times and incubated in relative humidity chambers for 11 days (Scruggs and Quesada-Ocampo, 2016). Rot initiation and development in the root tubers were assessed as a percentage of the final weight of inoculated and treated tubers by the rot pathogens using the formula by Chukwuebuka *et al.*, (2016);

$$Rot\ severity\ (\%) = \frac{W - w}{W} \times 100$$

Where;

W = Final weight of inoculated and rotted tubers at the end of storage

w = weight of rotted part of sweet potato

3.6.2.3 Growth inhibition (%) of test fungi in tuber sample

The antifungal effect of the extracts on the pathogens on the tuber samples in *in-vivo* was determined by transversely cutting across the point of inoculation with a sterilized knife and measuring the diameter of the rot lesion. The percentage growth inhibition was calculated by the formulae adopted by Amadioha, (2003);

$$Growth\ inhibition\ (\%) = \frac{dc - dt}{dc} \times 100$$

Where;

dc = Average diameter of the rotted fungal lesion in the control experiment

dt = Average diameter of rotted fungal lesion of treatment

3.6.2.4 Depth of lesion of rot

The depth of the rot lesion was measured after diametrically cutting across the point of inoculation of the mycelia plugs.

3.7 Data analysis

Data collected were subjected to general Analysis of Variance (ANOVA) of the Genstat Statistical Software Package edition 12. Square root transformation $\sqrt{(x+1)}$ was performed on fungal percentage occurrence, sprout percentage, growth inhibition, and rot severity. Means were separated using the Least Significant Difference at a 5 % probability level and the results obtained were presented in tables and graphs.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical analysis of plant extracts

4.1.1 Aqueous

The screen of the three plant extracts for phytochemical properties in aqueous solvent detected that, flavonoids, saponins, and phytosterols were present in bitter leaves (*Vernonia amygdalina*). Alkaloids and flavonoids were detected in the extract of holy basil (*Ocimum sanctum*) while lemon grass (*Cymbopogon citratus*) contained flavonoids only in the aqueous solvent (Table 4.1).

Table 4.1: Phytochemical analysis of plant extracts in aqueous solvent

Plant extracts	Phytochemicals				
	Alkaloids	Flavonoids	Saponins	Phenolic	Phytosterols (Triterpenes)
Vernonia amygdalina	-	+	+	-	+
Cymbopogon citratus	-	+	-	-	-
Ocimum sanctum	+	+	-	-	-

The plus sign (+) indicates the presence of the chemical property while the minus sign (-) indicates the absence of that chemical property.

4.1.2 Ethanol

Similarly, to aqueous solvent, ethanol solvent detected flavonoids, saponins, and phytosterols being present in bitter leaf extracts followed by alkaloids and flavonoids contained in the extract of holy basil while lemon grass containing only flavonoids among all the phytochemical constituents screened for as shown in Table 4.2.

Table 4.2: Phytochemical analysis of plant extracts in ethanol solvent

Plant extracts	Phytochemicals				
	Alkaloids	Flavonoids	Saponins	Phenolic	Phytosterols (Triterpenes)
Vernonia amygdalina	-	+	+	-	+
Cymbopogon citratus	-	+	-	-	-
Ocimum sanctum	+	+	-	-	-

+ indicates the presence of the chemical property while the - indicate the absence of that chemical property.

4.2 Isolation and identification of pathogens causing sweet potato root tuber rots

Seven fungal isolates were isolated from both diseased white flesh and orange flesh sweet potato root tuber varieties. The fungal isolates were *Aspergillus clavatus, Aspergillus flavus, Lasiodiplodia theobromae, Aspergillus niger, Fusarium oxysporum* Schlectend, *Rhizopus stolonifer* (Ehrenb: Fr) Vuillemin and *Trichoderma harzianum*. However, two isolates namely; *Aspergillus clavatus,* and *Aspergillus flavus* were found not to have symptoms of disease development after fourteen days of incubation.

Fusarium oxysporum Schlectend

Mycelia growth of the fungus was observed visibly on the PDA plate after two days of incubation. The mycelium was a compact whitish cottony with a dark-purple under-surface (Plate 4.1). Spores ranged from ellipsoid to kidney shape or tapering to septate with chains being formed by chlamydospores.

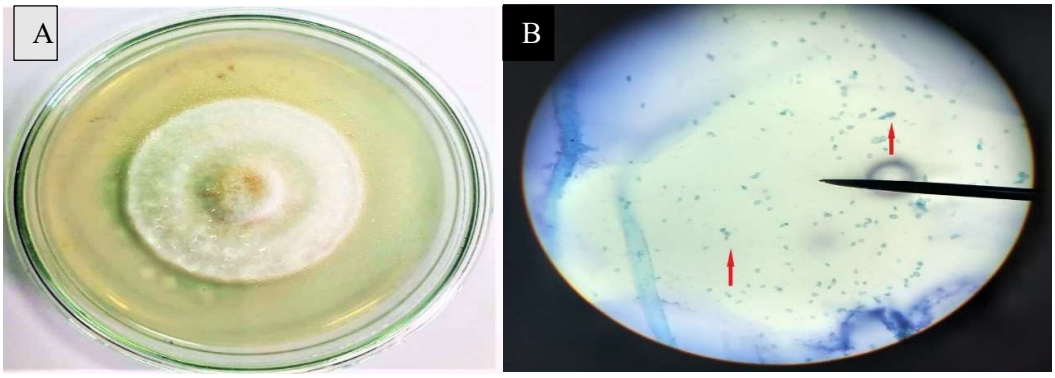

Plate 4.1: Cultured *Fusarium oxysporum* in Petri dish after 5 days (A) and Microscopic view (red arrows) (× 400 LPCB) (B)

Rhizopus stolonifer, (Ehrenberg: Fr) Vuillemin

The fungus appears cottons-fluffy mass on the PDA plate after 24 hours of inoculation under the ambient temperature of 27 ± 2 °C. The mycelium becomes dark-cottonish with age. Mycelium formed a polygon or angular in shape with sporangiophores measuring 5 cm in 5 days of incubation (Plate 4.2).

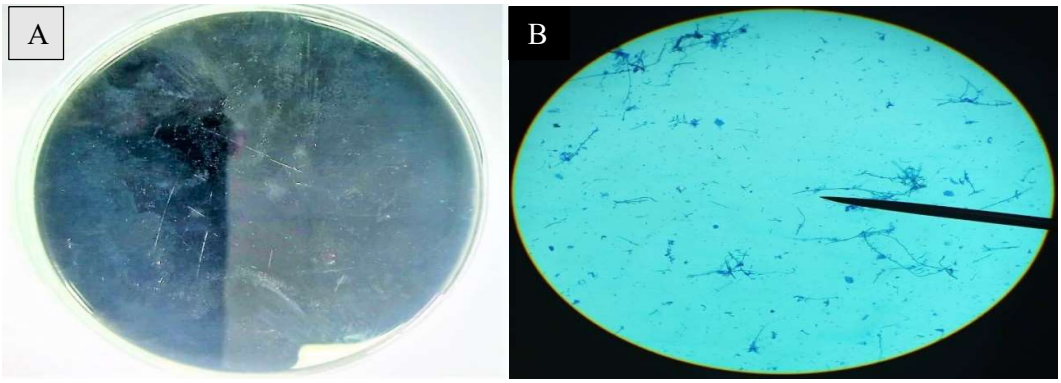

Plate 4.2: *Rhizopus stolonifer* after 5 days of culture (A) and Microscopic view (× 400 LPCB) (B)

Lasiodiplodia theobromae Pat. Griffon and Maubl.

The fungus produced a rapidly growing fluffy mycelia mass on PDA after 24 hours of inoculation. White to pale greenish-grey colony was observed after 5 days of incubation and gave dark-gray colour with old age. Mycelium can be described as shades of gray and black pigmentation. The conidia were found to have ellipsoidal ovoid to tapering truncated base (Plate 4.3).

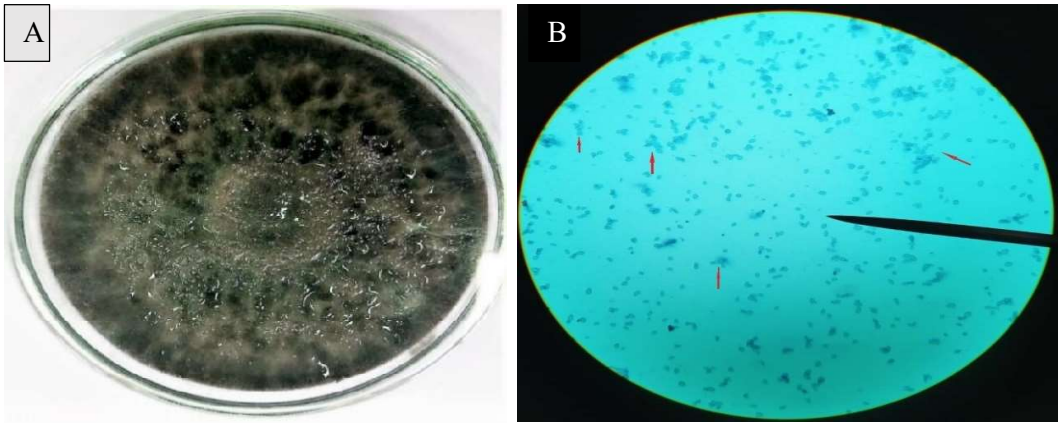

Plate 4.3: *Lasiodiplodia theobrom*ae after 5 days of culture (A) and Microscopic view (× 400 LPCB) indicated by red arrows (B)

Trichoderma harzianum, Pearson ex Gray

It was observed that the fungus grows rapidly under an ambient temperature of 27 ± 2 ℃. The colony becomes matured after 5 days of incubation to give yellow-green patches with concentric rings progressing on fresh PDA in the Petri plate. Conidiophores are attached at an angle to give the form a pyramidal configuration (Plate 4.4).

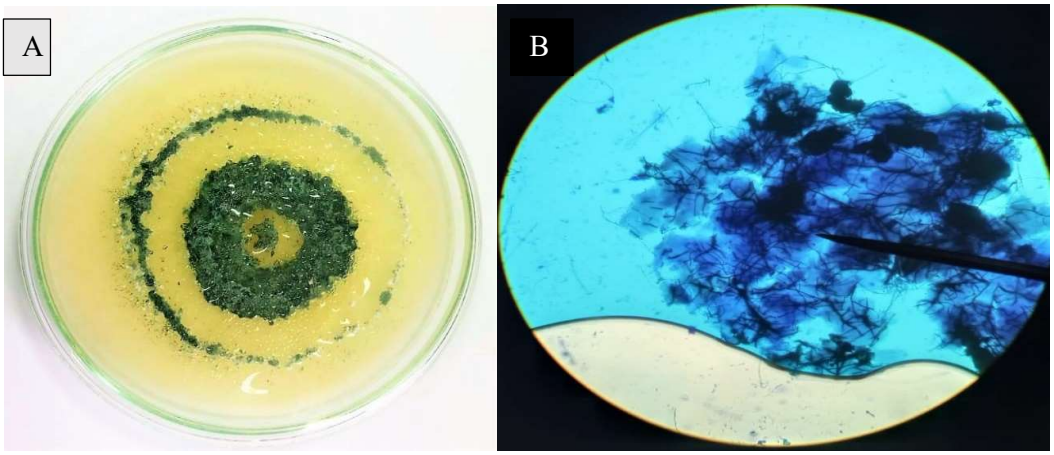

Plate 4.4: Cultured *Trichoderma harzianum* on PDA in Petri dish after 3 days (A) and Microscopic view (× 400 LPCB) (B)

Aspergillus niger, Currie

The fungus initially gives cottony whitish growth within 24 hours of inoculation before turning black with age. Black colour is observed from 24 to 48 hours of inoculation which is initiated by the production of conidial spores. The conidial spores are aerial and usually give oval shape growth on PDA. It quickly matures within 4 to 5 days of incubation and produces radial fissures on the PDA (Plate 4.5).

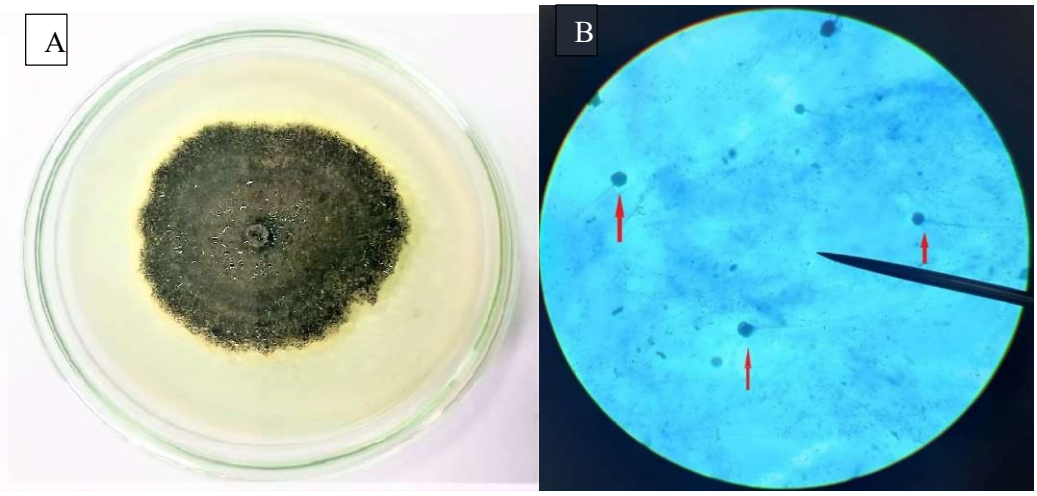

Plate 4.5: *Aspergillus niger* after 3 days of culture on Petri dish (A) and Microscopic view (B)

4.3 Percentage (%) occurrence of fungi causing sweet potato tuber rot.

Figure 1 below indicates the seven fungi that were isolated from the diseased sweet potato root tubers sampled from the farm gate in Nyankpala and its neighbouring communities, *Aspergillus niger* was the most frequently obtained fungal pathogen causing sweet potato root rot in both white-flesh (92 %) and orange-flesh (72 %) sweet potato varieties. Generally, *A. niger* was significantly ($P \leq 0.05$) higher than any of the other fungal isolates in the frequency of occurrence. However, *A. niger* recorded no significant difference ($P > 0.05$) between the two sweet potato varieties while *Lasiodiplodea theobromae* had the least frequency of occurrence with 3.3 % followed by *T. harzianum* (5.6 %) in increasing order in orange-flesh sweet potato variety. For the white-flesh sweet potato, *R. stolonifer (*27 %) *A. flavus* (25 %), *F. oxysporum* (10.6 %), and *T. harzianum* (10 %) recorded a decrease pattern in frequency of occurrence whiles *A. clavatus* and *L. theobromae* were not encountered on white-flesh variety.

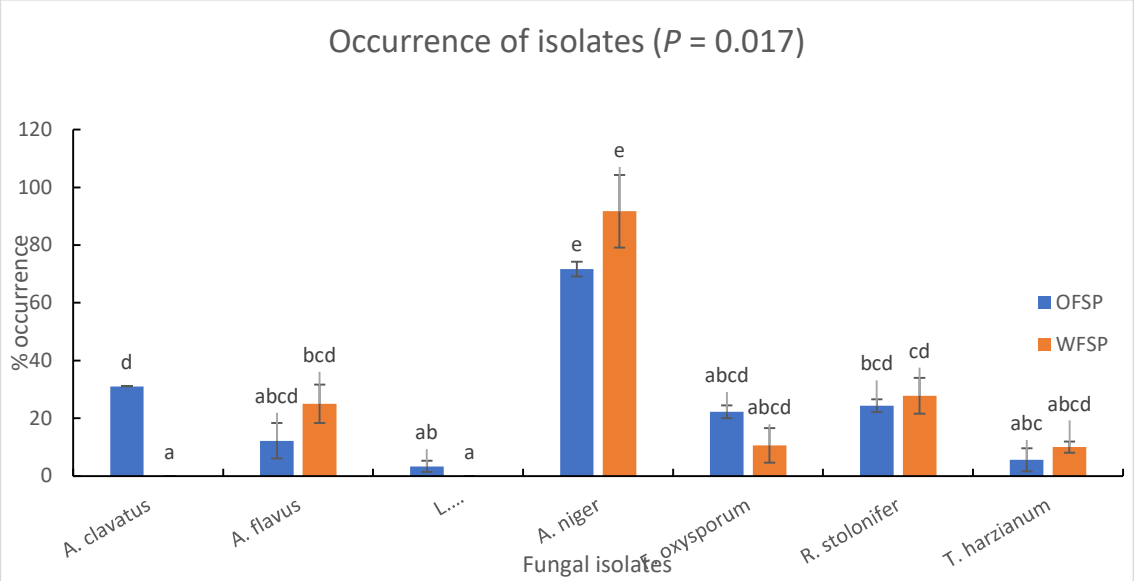

Figure 1: Percentage of occurrence of fungal isolates from the two sweet potato varieties.

Bars with the same letters are not significantly (P > 0.05) different whiles bars with different letters indicate significant (P < 0.05) differences between the sweet potato varieties.

4.4 Pathogenicity of fungal isolates

Five fungal species out of the seven isolated from the diseased sweet potato root tubers were found to be pathogenic on healthy root sweet potato tubers of both the white-flesh and orange-flesh varieties. Of the seven fungal isolates, *A. niger, F. oxysporum, L. theobromae, R. stolonifer,* and *T. harzianum* were pathogenic whiles *A. flavus* and *A. clavatus* were found not to be pathogenic after subjecting them to Koch's postulate.

4.5 Virulence of isolated fungal pathogens

After fourteen days of incubation, root tubers were transversely dissected to assess the severity of rots, and *A. niger* was found to be more severe on orange-flesh sweet potato while both *R. stolonifer* and *L. theobromae* recorded the same necrotic areas on white-flesh sweet potato. However, *Lasiodiplodia theobromae* (1.08) recorded less extensive rot in orange-flesh sweet potato compared to its counterparts except for the Control (0.0). *Trichoderma harzianum* also recorded the least necrotic area with 1.09 on white-flesh sweet potato among the fungal isolates although a significant ($P > 0.05$) difference was not recorded between them (Table 4.3).

Table 4.3: Necrotic area caused by fungal isolates on sweet potato varieties

Name of fungal isolate used in inoculation	Type of sweet potato and Lesion size (cm^2)	
	Orange Flesh	White Flesh
Control	0.00 (0.00) [a]	0.00 (0.00) [a]
Fusarium oxysporum	1.15 (0.01) [b]	1.1 (0.01) [b]
Lasiodiplodia theobramae	1.08(0.02) [b]	1.19(0.01) [d]
Trichoderma harzianum	1.13(0.03) [b]	1.09(0.02) [b]
Rhizopus stolonifera	1.11(0.04) [b]	1.19(0.02) [d]
Aspergillus niger	1.33(0.018) [c]	1.14(0.01) [c]
P value ($P<0.05$)	<.001	<.001
LSD	0.081	0.028
CV %	1.600	1.800

Means with ± Standard Error (in brackets) of necrotic lesion. Means with the same superscripts are not significantly ($P > 0.05$) different whiles means with different superscripts indicate significant ($P \leq 0.05$) differences among the pathogens.

4.6 Antifungal effect of plant extracts on fungal isolates *in vitro*

4.6.1 Aqueous

The inhibition of mycelia growth of the fungal isolates was greatly impacted among the various aqueous plant extract treatments as shown in Table 4.4. There were significant ($P \leq 0.05$) differences in the mycelia growth among the fungal isolates at both 100 and 50 % concentration levels. Generally, mycelia growth ranged from 2.33 ± 0.08 to 11 ± 0.38 mm for Lemon grass *F. oxysporum* (FLG) and Lemon grass *R. stolonifer* (RLG) as the lowest and highest respectively among the aqueous plant extracts at 100 % concentration level. Similarly, a range of 5.67 ± 0.22 to 13.67 ± 0.17 mm was recorded for holy basil *F. oxysporum* (FOS) and Lemon grass *R. stolonifer* (RLG) as the lowest and the highest at the 50 % concentration level. However, all the negative control treatments were significantly ($P \leq 0.05$) higher than any of the aqueous plant extract treatments at both 100 and 50 % concentration levels with *R. stolonifer* recording the highest mycelia growth and whiles that of the negative control for *F. oxysporum* recording 8 ± 14 mm as the least at both 100 and 50 % concentration levels (Table 4.4). The positive control treatments completely inhibited mycelia growth among all the fungal isolates.

4.6.2 Ethanol

Table 4.4 shows the significant differences ($P \leq 0.05$) among the various ethanol plant extract treatments at both 100 and 50 % concentration levels. The fungal isolate *R. stolonifer* recorded 12.83 ± 0.22 and 14.83 ± 0.22 mm as the highest mycelia growth inhibition at both 100 % and 50 % concentration levels. Similarly, *F. oxysporum* recorded

2.33 ± 0.08 mm as the least mycelia growth inhibition for the ethanol bitter leaf (BL) at 100 % concentration level. At 50 % concentration level, *F. oxysporum* recorded 7.08 ± 0.17 mm mycelia growth inhibition for both ethanol extracts of lemon grass (LG) and holy basil (HB). The negative control of the mycelia growth inhibition of the fungal isolates were significantly ($P \leq 0.05$) higher than any of the ethanol plant extracts except for the negative controls for *F. oxysporum* and *L. theobromae* at both 100 and 50 % concentration levels. However, the positive control of the ethanol extracts recorded complete mycelia growth inhibition at both 100 and 50 % concentration levels. It was observed that both aqueous and ethanol plant extracts recorded higher mycelial growth inhibition of the fungal isolates with increasing concentration levels of the plant extract treatments.

Table 4.4: Plant extracts antifungal activity against fungal isolates at 100 and 50 %concentration levels

Treatment	Mycelia growth (mm)			
	Aqueous		Ethanol	
	100%	50%	100%	50%
F –VE	8.00 (0.14) ^{hi}	8.00 (0.14) ^{cd}	8.00 (0.14) f	8.00(0.14) ^{bc}
L –VE	12.67(0.33) ^{l}	12.67 (0.33) ^{i}	12.67(0.33) ^{g}	12.67(0.33) ^{j}
T –VE	14.08 (0.22) ^{m}	14.08(0.22) ^{k}	14.08 (0.22) ^{h}	14.08(0.22) ^{j}
R –VE	19.33 (0.36) ^{n}	19.33(0.36) ^{l}	19.33(0.36) ^{i}	19.33(0.36) ^{l}
A –VE	12.83(0.08) ^{lm}	12.83(0.08) ij	12.83(0.08) ^{gh}	12.83(0.08) ^{j}
FBL	3.55(0.05) ^{bc}	7.67(0.17) ^{c}	2.33(0.08) ^{b}	7.08(0.17) ^{b}
FLG	2.33(0.08) ^{b}	6.33(0.08) ^{b}	4.58(0.08) ^{c}	8.83(0.3) ^{cd}
FHB	4.083(0.17) ^{cd}	5.67(0.22) ^{b}	5.00(0.14) ^{cd}	7.08(0.08) ^{b}
LBL	4.67(0.08) ^{cde}	8.67(0.17) ^{de}	5.58(0.08) ^{cde}	8.67(0.08) ^{c}
LLG	4.67(0.08) ^{cde}	10.42(0.17) ^{g}	6.42 (0.22) ^{e}	9.67(0.3) ^{de}
LHB	5.08(0.17) ^{def}	9.67(0.08) ^{fg}	8.82 (0.17) ^{f}	10.33(0.17) ^{ef}
TBL	5.92(0.44) ^{ef}	9.17(0.22) ^{ef}	8.17(0.22) ^{f}	8.58(0.08) ^{c}
TLG	9.25(0.25) ^{ij}	9.18(0.12) ^{ef}	6.08d(0.17) ^{e}	11(0.25) ^{fgh}
THB	6.08(0.08) ^{f}	10.25(0.14) ^{g}	9.25(0.5) ^{f}	11.08(0.08) ^{fgh}
RBL	9.00(0.25) ^{ij}	12.00(0.14) ^{hi}	8.17(0.46) ^{f}	12.58(0.08) ^{j}
RLG	11(0.38) ^{k}	13.67(0.17) ^{jk}	9.17(0.68) ^{f}	11.5(0.25) ^{ghi}
RHB	10.17(0.36) ^{jk}	12.75(0.14) ^{i}	12.83(0.22) ^{gh}	14.83(0.22) ^{k}
ABL	7.42(0.58) ^{gh}	11.58(0.08) ^{h}	8.83 (0.08) ^{f}	12.33(0.08) ^{ij}
ALG	8.25(0.29) ^{hi}	11.58(0.08) ^{h}	9.33 (0.08) ^{f}	10.58(0.08) ^{efg}
AHB	6.17((0.08) ^{fg}	10.25(0.14) ^{g}	8.25 (0.27) ^{f}	11.58(0.17) ^{hi}
F +VE	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}
L +VE	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}
T +VE	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}
R +VE	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}
A +VE	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}	0.00(0.00) ^{a}
P<0.05	<.001	<.001	<.001	<.001
LSD	0.688	0.45	0.731	0.51
CV %	0.900	0.800	0.300	0.700

Means with ± Standard Errors (brackets) of inhibition rates of fungal isolates against plant extracts *in vitro*. Fungal isolates *Fusarium oxysporum* (F), *Lasiodiplodia theobromae* (L), *Trichoderma harzianum* (T), *Rhizopus stolonifer* (R), and *Aspergillus niger* (A) treated with plant extracts; *Fusarium* Bitter leaf (FBL), *Fusarium* Lemon grass (FLG), *Lasiodiplodia* Bitter leaf (LBL), *Lasiodiplodia* Lemon grass (LLG), *Lasiodiplodia* Holy

basil (LHB), *Trichoderma* Bitter leaf (TBL), *Trichoderma* Lemon grass (TLG), *Trichoderma* Holy basil (THB), *Rhizopus* Bitter leaf (RBL), *Rhizopus* Lemon grass (RLG), *Rhizopus* Holy basil (RHB), *A. niger* Bitter leaf (ABL), *A. niger* Lemon grass (ALG), *A. niger* Holy basil (AHB) and *Fusarium* Holy basil (FHB) at 100% and 50% concentration levels respectively. Means with the same letters are not significantly ($P > 0.05$) different whiles means with different letters indicate significant ($P \leq 0.05$) differences among the treatments.

4.7 Effect of plant extracts on weight loss of sweet potato varieties

4.7.1 Effect of plant extracts on weight loss of orange-flesh sweet potato

There was a weekly interactive effect of plant extracts on weight loss of stored root tubers of orange-flesh sweet potato variety. Orange-flesh root tuber without treatment on the first week of storage recorded the highest weight loss of 20.8 % which is significantly ($P \leq 0.05$) higher than all of the weight loss in the storage period while week 6 (0.73 %) recorded the least weight loss. Across the Weeks, week 6 recorded both the highest and lowest weight loss at 5.62 % and 0.73 % respectively for holy basil and lemon grass. However, in week 3, lemon grass recorded the second highest weight loss with 5.43 % which was not significantly different ($P \leq 0.05$) in comparison with that of holy basil in week 6. The positive control recorded 3.57 %, 3.27 %, 3.1 % etc. in weeks 2, 4, and 1 respectively in weight loss (Table 4.5).

Table 4.5: Effect of plant extracts on weight loss of orange-flesh sweet potato

Treatment	Percentage weight loss						
	Init. Weight	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Negative	83.3(8.04) [b]	20.8(5.52) [b]	12.22(1.89) [b]	5.63(1.09) [b]	8.18(1.029) [b]	12.76(0.76) [c]	6.59(0.05) [c]
Bitter leaf	31.1(4.9) [a]	2.8(0.07) [a]	2.40(0.16) [a]	2.79(0.29) [a]	3.89(0.31) [a]	1.12(0.10) [a]	3.34(1.43) [ab]
Lemon grass	43.2(6.97) [a]	3.2(0.014) [a]	2.75(0.33) [a]	3.18(0.11) [ab]	5.43(0.07) [a]	2.45(0.11) [ab]	0.73(0.22) [a]
Holy basil	47.1(8.16) [a]	3.3(0.08) [a]	2.01(0.22) [a]	4.369(0.20) [ab]	4.61(0.18) [a]	2.71(0.10) [b]	5.62(0.23) [bc]
Positive con.	29.5(1.48) [a]	3.1(0.05) [a]	3.56(0.035) [a]	2.01(0.023) [a]	3.27(0.19) [a]	0.90(0.14) [a]	2.61(0.15) [ab]
Mean	46.8	6.7	4.59	3.59	5.08	3.99	3.78
(P<0.05)	0.001	0.003	<.001	0.008	<.001	<.001	0.001
LSD	19.65	8.07	2.78	1.70	1.62	1.04	2.08
CV %	12.90	28.60	15.6	10.70	7.50	9.30	15.20

*Means with ± Standard Errors in brackets. Different superscript letters indicate significant differences ($P \leq 0.05$) among the extracts. Values with the same letters are not significantly different ($P > 0.05$)

4.7.2 Effect of plant extracts on weight loss of white-flesh sweet potato (WFSP)

Similarly, with the results in section 4.5.1, Table 4.6 shows the interactive effect of plant extracts on weight loss of white-flesh sweet potato variety across weeks of storage. There were significant differences ($P \leq 0.05$) in weight loss in all the weeks of storage except in weeks 5 and 6. It was observed that the white-flesh sweet potato root tuber without treatment recorded the highest weight loss in week 4 (10.35%) which is significantly higher than all of the weight loss across the weeks in storage except for week 5 recorded 13.8 % and 11.5 % for the extracts of bitter leaf and holy basil respectively. Similarly, week 6 also recorded 16.1 %, 13.1 %, and 10.8 % weight loss for bitter leaf, lemon grass, and holy basil respectively in comparison from week 1 to week 4. Among the plant extracts, bitter leaf recorded the highest and as well as lowest weight loss with 16.1 % and 3.47 % in weeks 6 and 2 respectively. The second highest weight loss was recorded by lemon grass (13.8 %) in week 5 and then followed by holy basil with 13.2 % in week 6. The Negative control lost more weight than the Positive control in all the weeks of storage with the exception of week 6 with the Positive control recording 10.8 % weight loss in comparison to 8.7 % for the Negative control in the same week 6.

Table 4.6: Effect of plant extracts on weight loss of white-flesh sweet potato (WFSP)

Treatment	Percentage weight loss						
	Int. Weight	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Negative control	68.60 ^{ab}	9.63(0.42) ^{c}	8.12(.55) ^{b}	8.01(0.42) ^{c}	10.35(0.35) ^{d}	8.30(1.71) ^{a}	8.70(3.65) ^{a}
Vernonia amygdalina	81.50 ^{ab}	6.21(0.20) ^{b}	3.47(0.26) ^{a}	5.80(0.63) ^{b}	3.60(0.03) ^{a}	13.80(0.82) ^{a}	16.10(5.15) ^{a}
Cymbopogon citratus	105.10 ^{ab}	5.98(0.08) ^{b}	3.71(0.12) ^{a}	4.33(0.28) ^{ab}	7.97(0.38) ^{c}	6.90(0.39) ^{a}	13.10(1.53) ^{a}
Ocimum sanctum	74.60 ^{ab}	5.78(0.05) ^{b}	5.06(0.19) ^{a}	5.36(0.10) ^{ab}	4.20(0.15) ^{a}	11.5(2.98) ^{a}	13.20(1.36) ^{a}
Positive control	61.20 ^{a}	4.22(0.26) ^{a}	4.04(0.32) ^{a}	3.88(0.26) ^{a}	5.95(0.25) ^{b}	6.90(2.77) ^{a}	10.80(6.09) ^{a}
Mean	78.20	6.36	4.88	5.48	6.41	9.50	12.40
P <0.05	0.052	<.001	<.001	<.001	<.001	0.215	0.783
LSD	28.25	0.88	1.18	1.09	0.92	7.32	13.89
CV%	5.90	0.40	1.50	7.60	2.30	3.30	18.20

*Means with ± Standard Error (brackets). Different superscript letters indicate significant ($P \leq 0.05$) differences among the extracts. Values with the same letters are not significantly different ($P > 0.05$).

4.8 Effect of plant extracts on the depth of lesion of sweet potato varieties in *in vivo.*

4.8.1 White-flesh sweet potato (WFSP) Variety

Table 4.7 shows the impact of three plant extracts on the lesion diameter of white-flesh sweet potato root tuber *in vivo*. Generally, there were significant differences ($P \leq 0.05$) in the effectiveness of the plant extracts in the control of rot on inoculated root tubers on white-flesh sweet potato. Root tubers inoculated without treatment recorded the highest lesion diameter with 23 ± 0.58 mm for *R. stolonifer* and was not significantly different ($P < 0.05$) from the inoculated root tubers without treatment for *A. niger* (22.67 ± 0.33 mm) while the least lesion diameter was recorded by *T. harzianum* (16.67 ± 2.7 mm) without treatment. Among the plant extracts, *R. srolonifer* recorded a greater lesion diameter with 21.67 ± 1.86 mm for bitter leaf extract, followed by the extract of hoy basil recording 20.33 ± 5.78 mm as the second highest for *A. niger* and the least impact in terms of lesion diameter was recorded by lemon grass (6.67 ± 0.33 mm) for *T. harzianum*. The positive controls recorded the lesion diameter in a range between 9.17 ±1.9 to 14 ± 0.58 mm which was not significantly different from the extract treatments.

Table 4.7: Effect of plant extracts on white-flesh sweet potato root tuber rot lesion caused by fungal pathogens

Pathogen	Lesion diameter (mm)				
	Negative control	Bitter leaf	Lemon grass	Holy basil	Positive control
F. oxysporum	17.33(2.85) ^{abcde}	8.33(0.33) ^{ab}	7.33(0.33) ^{a}	6.87(0.59) ^{a}	10.33(1.20) ^{abcde}
L. theobromae	21.33(3.18) ^{cde}	17.00(3.06) ^{abcde}	16.50(0.29) ^{abcde}	8.00(0.58) ^{ab}	9.17(1.91) ^{abc}
T. harzianum	16.67(2.73) ^{abcde}	15.30(3.84) ^{abcde}	6.67(0.33) ^{a}	9.83(2.35) ^{abcd}	14.0(0.58) ^{abcde}
R. stolonifer	23.00(0.58) ^{e}	21.67(1.86) ^{cde}	15.50(0.29) ^{abcde}	19.00(4) ^{abcde}	13.83(0.73) ^{abcde}
A. niger	22.67(0.33) ^{de}	15.00(4.04) ^{abcde}	17.67(0.33) ^{abcde}	20.33(5.78) ^{bcde}	12.67(0.33) ^{abcde}
Mean = 14.64	Sig. Level ($P < 0.05$) = <.001		CV % = 6.68		

*Means of depth lesion with Standard Error of means (± SE). Bitter leaf (*Vernonia amygdalina*), Lemon grass (*Cymbopogon citratus*) and Holy basil (*Ocimum sanctum*). Different superscript letters indicate significant differences ($P \leq 0.05$) among the extracts. Values with the same letters are not significantly different ($P > 0.05$).

4.8.2 Orange-flesh sweet potato (OFSP) variety.

Table 4.8 shows the results of rot lesion on orange-flesh root tubers inoculated with fungal isolates as indicated in Plate 4.6 with red arrows. The results revealed significant differences ($P \leq 0.05$) in the lesion diameter. Root tubers inoculated without treatment for *F. oxysporum* (28.7 ± 0.17) recorded greater lesion diameter although there were no statistical differences between other fungal isolates except for *R. stolonifer* (22.6 ± 0.33). With the plant extracts, lemon grass had the highest lesion diameter for *A. niger* with 26.83 ± 1.01 and was however not significantly different ($P \geq 0.05$) in comparison with holy basil for *F. oxysporum* (26.67 ± 1.45) and *L. theobromae* (24.33 ± 2.31) whiles bitter leaf extract recording the least lesion diameter for *F. oxysporum* (8 ± 0.29). Interestingly, there were no significant differences ($P \geq 0.05$) between the positive control and that of the extract treatments on the lesion diameter.

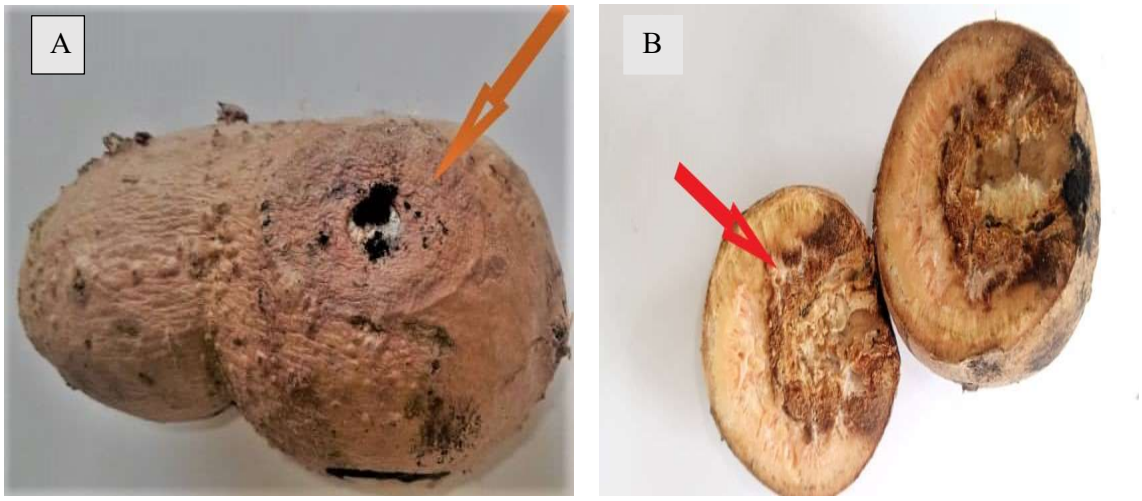

Plate 4.6: Rot lesion on whole tuber (A) and on dissected tuber (B) indicated with red arrows.

Table 4.8: Effect of plant extracts on Orange-flesh sweet potato root tuber rot lesion caused by fungal pathogens

Pathogen	Lesion diameter (mm)				
	Negative control	Bitter leaf	Lemon grass	Holy basil	Positive control
F. oxysporum	28.70(0.17) j	8.00(0.29) ^{a}	9.33(0.33) ^{ab}	26.67(1.45) ^{hij}	20.00(1.15) ^{defghij}
L. theobramae	27.00(4.38) ^{hij}	10.17(2.02) ^{abc}	14.00(4.38) ^{abcdef}	24.33(2.31) ^{ghij}	19.00(3.46) ^{cdefghi}
T. harzianum	27.67(2.96) ^{ij}	11.50(0.29) ^{abcd}	13.67(0.33) ^{abcdef}	13.33(0.88) ^{abcde}	18.33(0.67) ^{bcdefgh}
R. stolonifer	22.67(0.33) ^{fghij}	20.00(0.29) ^{defgghij}	14.50(0.87) ^{abcdef}	18.50(2.57) ^{cdefgh}	16.17(0.17) ^{abcdefg}
A. niger	26.33(3.84) ^{hij}	15.17(0.6) ^{abcdef}	26.83(1.01) ^{hij}	22.00(2.08) ^{efghij}	21.33(4.37) ^{efghij}
Grand mean = 18.99	Sig. level ($P < 0.05$) = <.001		CV % = 2.60		

*Means of depth lesion with Standard Error of means (± SE). Bitter leaf (*Vernonia amygdalina*), Lemon grass (*Cymbopogon citratus*) and Holy basil (*Ocimum sanctum*). Different superscript letters indicate significant differences ($P \leq 0.05$) among the extracts. Values with the same letters are not significantly different ($P > 0.05$).

4.9 Antifungal activity of plant extracts on rot severity

4.9.1 White-flesh sweet potato (WFSP)

The results show that, the plant extracts sufficiently ($P \leq 0.05$) reduced rot severity in inoculated root tubers with the isolated pathogens after fourteen days of incubation in humid chambers (Table 4.9).Generally, it was observed that 74 % (26 % rotten) and 71 % (29 % rotten) were the highest mean percentage diameter recorded by bitter leaf extract as the first and second best in reducing rot severity on white-flesh sweet potato root tubers, followed by lemongrass with 67 % (33 % rotten) whereas holy basil produced the most rot among the plant extracts with 58 % (42 % unrotten). The standard check (Negative control), root tubers without treatment inoculated with *Trichoderma harzianum* recorded the greatest rot with 78 % (22 % unrotten), followed by root tubers inoculated with *R. stolonifer* with 76 % (24 % unrotten), and *F. oxysporum* inoculated root tubers recording 63 % (37 % unrotten) as the best performance among the untreated root tubers used for negative control while *R. stolonifer* recording less rot among the root tubers with fungicide.

4.9.2 Orange-flesh sweet potato (OFSP)

The effect of the plant extracts on the percentage rot severity during the incubation period showed significant differences ($P \leq 0.05$) on the orange-flesh sweet potato root tuber (Table 4.10). Generally, across the extracts, holy basil inoculated *F. oxysporum* outperformed both bitter leaf and lemon grass extracts with rot reduction of 65 % (35 % rotten) although it was closely followed by lemon grass inoculated *F. oxysporum* with 61 % reduction (39 % rotten) while bitter leaf inoculated with *A. niger* significantly ($P \leq 0.05$) recorded the highest rot severity among the extracts with 74 % (26 % unrotten). Positive control inoculated *T. harzianum* recorded the least rot development with 73 % of the root tuber recording no rot which was not significantly different ($P \leq 0.05$) from root tubers inoculated with other isolated pathogens. The negative control inoculated with *A. niger* recorded the highest rot with 13 % of the root tuber being wholesome.

Table 4.9: Antifungal activity of plant extracts on rot severity of White-flesh sweet potato

	Plant Extracts				
Pathogen	Negative Control	Bitter leaf	Lemon grass	Holy basil	Positive control
F. oxysporum	37.22(1.56) ^{bc}	73.90(1.11) ^{j}	67.31(1.55) ^{ghij}	64.37(1.02) ^{efghij}	60.85(7.67) ^{efghi}
L. theobromae	25.65(1.05) ^{ab}	66.59(0.56) ^{ghij}	61.29(1.41) ^{efghij}	56.08(2.38) ^{efg}	64.63(1.34) ^{efghij}
T. harzianum	21.91(0.19) ^{a}	71.21(0.31) ^{ij}	61.36(0.95) ^{efghij}	52.45(0.27) ^{de}	68.73(2.76) ^{ghij}
R. stolonifer	23.67(1.37) ^{a}	66.23(1.74) ^{ghij}	64.67(1.63) ^{efghij}	56.24(2.69) ^{efgh}	68.88(1.78) ^{hij}
A. niger	26.80(3.45) ^{ab}	58.95(0.82) ^{efghi}	53.43(1.45) ^{def}	41.52(4.42) ^{cd}	65.68 (0.06) ^{fghij}
Grand mean = 55.18	Sig. level ($P \leq 0.05$) = <.001		LSD = 6.61	CV% =1.8	

Means of rot severity with Standard Error of means (± SE). Bitter leaf (*Vernonia amygdalina*), Lemon grass (*Cymbopogon citratus*) and Holy basil (*Ocimum sanctum*). Different superscript letters indicate significant differences ($P \leq 0.05$) among the extracts. Values with the same letters are not significantly different ($P > 0.05$).

Table 4.10: Antifungal activity of plant extracts on rot severity (%) of orange-flesh sweet potato

	Plant Extracts				
Pathogen	Negative control	Bitter leaf	Lemon grass	Holy basil	Positive control
F. oxysporum	32.86(2.38) ^{bcde}	59.93(2.47) ^{ijklm}	60.61(2.28) ^{ijklm}	65.03(0.49) ^{jklm}	65.29(3.0) ^{klm}
L. theobromae	32.86(3.99) ^{bcde}	47.08(3.8) ^{efghi}	53.00(3.68) ^{fghijk}	53.76(2.43) ^{ghijk}	71.17(1.35) ^{lm}
T. harzianum	40.15(3.79) ^{cdefg}	52.13(0.68) ^{fghijk}	31.19(2.11) ^{bcd}	56.55(1.64) ^{hijkl}	73.22(1.20)m
R. stolonifer	22.06(5.13) ^{ab}	31.73(2.17) ^{bcd}	50.02(1.85) ^{fghij}	43.50(1.24) ^{defgh}	64.51(1.26) ^{jklm}
A. niger	13.42(0.97) ^{a}	25.88(2.01) ^{abc}	34.72(4.93) ^{bcde}	38.60(3.72) ^{cdef}	61.36(2.30) ^{ijklm}
Grand mean = 47.23	Sig. level ($P < 0.05$) =<.001	LSD = 7.82	CV% = 1.7		

* Means of rot severity with Standard Error of means (± SE). Bitter leaf (*Vernonia amygdalina*), Lemon grass (*Cymbopogon citratus*) and Holy basil (*Ocimum sanctum*). Different superscript letters indicate significant differences ($P \leq 0.05$) among the extracts. Values with the same letters are not significantly different ($P > 0.05$

4.10 Effect of plant extracts on sprout development in sweet potato varieties

There was a significant interaction between variety and plant extracts on sprout percentage in the sweet potato varieties (Table 4.11). Sweet potato root tubers without treatment for both white-flesh (WFSP) and orange-flesh (OFSP) varieties recorded the highest sprout percentage of 37.3 % and 30.6 % respectively. Contrary to the negative control for both varieties, the positive control recorded no development of sprout in WFSP that to the positive control for OFSP recording 6 %. Across the plant extracts, lemon grass produced the highest as well as the lowest sprout count in both OFSP and WFSP with 29.47 % and 8.33 % respectively, followed by holy basil recording 21.87 % and 18.20 % for both OFSP and WFSP while bitter leaf recording the least percentage sprout count of 12.54 % and 13.91 % in decreasing manner for both OFSP and WFSP. Generally, it was observed that OFSP-treated extracts produced more sprout count than WFSP-treated extract root tubers except for treated bitter leaf white-flesh sweet potato.

Table 4.11: Effect of plant extracts on sprout count of sweet potato varieties

	Sprout count (%)	
Treatment	OFSP	WFSP
Negative control	30.60 (0.87) ^{d}	37.30 (3.24) ^{e}
Bitter leaf	12.54 (0.42) ^{b}	13.91 (0.73) ^{c}
Lemon grass	29.47 (0.32) ^{d}	8.33 (0.71) ^{b}
Holy basil	21.87 (0.69) ^{c}	18.20 (1.10) ^{d}
Positive control	6.00 (0.50) ^{a}	0.00 (0.00) ^{a}
$P < 0.01$	<.001	<.001
LSD	2.09	2.814
CV %	1.4	5.3

*Means with Standard Errors (in brackets). The same superscript letters are not significant ($P > 0.05$) while superscripts with different letters have significant ($P \leq 0.05$) differences between the means.

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CHAPTER FIVE

5.0 DISCUSSION

5.1 Phytochemical properties of plant extracts

The qualitative phytochemical analysis of the aqueous and ethanol extracts of bitter leaf, holy basil, and lemon grass revealed the presence of alkaloids, flavonoids, saponins, and phytosterols respectively. This agrees with the work of many researchers (Udochukwu *et al*., 2015; Borah and Biswas, 2018; Gupta *et al*., 2019; Panchal and Parvez, 2019; Bhardwaj, 2020; Eraga and Ijeh, 2021) who also determined the presence of these phytochemical components in bitter leaf, lemongrass, and holy basil and has antimicrobial activity against fungal pathogens. The presence of these phytochemical constituents in the plant extracts might have been responsible for the antifungal activity against test fungal pathogens (*A. niger, F. oxysporum, T. harzianum*, *L. theobromae* and *R. stolonifer*). Each of these phytochemical components has its function for the inhibition of the isolated fungal pathogen. For example, saponin has anti-inflammatory as well as detergent properties (Unegbu *et al.*, 2020). Alkaloids have a heterocyclic ring with a complicated structure and tend to be poisonous for the protection of plant species (Unegbu *et al.*, 2020). They are usually produced as metabolic waste products and have been implicated in the antibacterial action of many substances (Erasto *et al.*, 2007). Among the plant extracts, the bitter leaf was found to have more phytochemical components in both solvent extractions. As a result, this might have explained its highest antimicrobial activity on the isolated fungal pathogens than those of the holy basil and lemon grass. However, the phytochemical constituents of the aqueous and ethanol extracts of the bitter leaf, holy basil, and lemon grass used in this study were fewer compared to those detected in other studies (Borah and Biswas, 2018; Bhardwaj, 2020; Eraga and Ijeh, 2021). This could be attributed to many factors including; solvent extraction, extraction method, age

of the plant, time of the harvest, and part of the plant harvested (Li *et al.*, 2012; Ramasar *et al.*, 2022; Moomin *et al.*, 2023). It is reported by Moomin *et al.* (2023) and Kumar *et al.* (2017) that seasons, temperature, rain, and wind patterns also affect the phytochemical components of plants. Furthermore, in India, *Aloe vera* sampled from various altitudes, temperatures, and different rainfall patterns was observed to contain different amounts of alkaloids, glycosides, flavonoids, phenolic compounds, and saponins (Kumar *et al.*, 2017). These factors might have affected the availability of the phytochemical components of the plant extracts as the plants used were harvested at different locations in both the Northern and Savanna regions in Ghana. Furthermore, the temperature at which these plant leaves were stored before analysis might have also played a role in the amounts of secondary metabolites in these plant extracts. Javadi *et al.* (2015) reported that the phytochemical components of *Cosmos caudatus* such as lycopene, benzoic acid, catechin and α-tocopherol evaporated at room temperature after 24 hours in storage. Interestingly, in this study plant leaves were stored at 27 ± 2 ℃ before processing into grounded powder for the phytochemical analysis which would have affected the phytochemical yielding ability of the plant extracts. Amagloh *et al.* (2021) reported that different processing methods affect the retention of phytochemical properties. Li *et al.* (2012) reported higher antioxidant activities at 4 ℃ temperature were maintained in fruits than stored at 25 ℃. Park *et al.* (2011) reported that at 15 ℃ broccoli florets lose more in chlorophyll, ascorbic acid, beta-carotene, and total antioxidant contents than been stored at 4 ℃. This suggests that at lower temperatures phytochemical components of plants can be preserved than in higher temperatures.

5.2 Isolation and Identification of pathogens causing sweet potato root rot

Seven fungal species were isolated from the rotten sweet potato root tubers. These were; *Aspergillus niger*, *Aspergillus clavatus, Aspergillus flavus, Lasiodiplodia theobromae,*

Trichoderma harzianum, Fusarium oxysporum and *Rhizopus stolonifer*. However, five fungal isolates *A. niger, F. oxysporum, T. harzianum*, *L. theobromae* and *R. stolonifer* have been shown to cause sweet potato root rot after they were inoculated on fresh healthy root tubers while *A. flavus* and *A. clavatus* inoculated tubers were not found to exhibit rot symptoms. Based on the pathogenicity conducted, the five fungal isolates as confirmed by Koch's postulate are all implicated by many researchers as the cause of root rots in tubers in storage in all sweet potato growing regions in the tropics (Opiyo *et al.*, 2011; Scruggs and Quesada-Ocampo, 2016; Beckley and Awoyemi, 2021; Gyasi *et al.*, 2022). The study finding of *A. flavus* not exhibiting root rot symptoms after pathogenicity is contrary to that of Gyasi *et al.* (2022) while *A. clavatus* which was not found to cause sweet potato root rot in this study confirms the work of Sugri *et al.* (2020) who listed the most common fungal pathogens causing root tuber rots in sweet potato in Ghana which did not include *A. clavatus*. This could be attributed to unfavourable temperature for the development of *A. flavus* as root tubers were stored at a temperature of 27 $\pm$ 2 ℃ under room conditions. Baazeem *et al*. (2021) reported in his research work that *A. flavus* can effectively grow at a temperature range of 25 – 35 ℃.

Among the fungal isolates, *A. niger* was the most commonly occurred root rot fungal pathogen in both white-flesh and orange-flesh sweet potato varieties, as well as the most virulent fungal pathogen in orange-flesh sweet potato but however, produced moderate necrotic lesion in white-flesh variety during pathogenicity test. This conforms with that of Gyasi *et al.* (2022) who reported *A. niger* being the frequently obtained root rot pathogen on sweet potato root tubers in the Eastern region of Ghana but in contrast to the same report it produced the least lesion diameter of 12. 7 mm. This could be the reason that its spores are easily suspended and dispersed by air currents. Furthermore, the relative humidity in the growing chambers might have played a major role in the pathogens to

produced root rot symptoms. This supports the assertion by Scruggs and Ocampo-Quesada (2016) and Beckley and Awoyemi (2021) that the rate of fungi infection is relative to relative humidity.

Necrotic-wise *R. stolonifer* produced a greater area in white-flesh variety in comparison to *A. niger*. *R. stolonifer* has been reported as a sweet potato tuber root rot microorganism causing soft rot in root tubers in the tropics (Scruggs and Ocampo-Quesada 2016). They complimented that *R. stolonifer* is best controlled at the temperature of 13 ℃ while complete decay of root tubers results at 29 ℃. This could explain why it produced the highest necrotic area with *L. theobromae* in the white-flesh sweet potato variety as root tubers were stored at a temperature of 27 ± 2 ℃. However, this is in contrast to the report by Clark *et al.* (2013) that a greater necrotic lesion is produced at a temperature range between 6 ℃ to 22 ℃.

Similarly, *F. oxysporum* has been reported to cause moderate root tuber rot on sweet potatoes (Beckley and Awoyemi, 2021) which agrees with this study for the necrotic area produced and frequency of occurrence on white-flesh variety however, this was contrary to the part of orange-flesh as it produced the greater necrotic area after *A. niger* which correspond to the findings of Paul *et al.* (2021) who stated that the disease caused by *F. oxysporum* is the most damaging disease than any other disease in storage in all sweet potato growing regions. Scruggs and Quesada-Ocampo (2016) emphasized that the rate of disease infection and progression of *Fusarium* spp., on sweet potato tubers depend on the method of inoculation which could have been the case in this study and potentially affects the outcome.

L. theobromae has been reported to cause Java black rots on sweet potato root tubers in Ghana. The fungus was isolated from root tubers of sweet potatoes with the largest rot lesion diameter of 27.1 mm (Gyasi *et al.*, 2022) and this study was no exception as it

produced a 1.19 cm^2 diameter in white-flesh sweet potato. In the studies of Okigbo and Emeka, (2010) and Gyasi *et al.* (2022), they discovered that *L. theobromae* is one of the most virulent pathogens causing root rots in sweet potato tubers in storage.

Trichoderma spp. have been reported to be a pathogen causing green mould disease in sweet potato (Clark *et al.*, 2013; Yang *et al.*, 2021) though, there was no report on *T. harzianum* causing sweet potato root rot in Ghana at the time of this study. Regardless, Yang *et al.* (2021) reported its presence in Guangdong Province, China as the cause of green mould disease, *Trichoderma* spp. is well known for biological control of pathogenic fungal species (Mazrou *et al.*, 2020).

5.3 Antifungal effect of plant extracts on the mycelia growth of isolated pathogens

All the three plant extracts (Bitter leaf, Lemon grass, and holy basil) in both aqueous and ethanol extraction methods tested *in vitro* against the five isolated fungi, *F. oxysporum, L. theobromae, T. harzianum, R. stolonifera,* and *A. niger* were found to have a fungitoxicity effect on the radial mycelia growth of the test organisms. This could be a result of the antifungal activity of the three plant extracts. This conforms with earlier reports by other researchers that the antifungal activities of plant extracts on fungi are due to secondary metabolites possessed by plants (Anukwuorji *et al.*, 2013; Kwodaga *et al.*, 2019). However, the antifungal activity on the tested fungi varied with the concentration levels as well as the extraction method (aqueous or ethanol) on the radial growth inhibition of the pathogens in this study.

The plant extracts gave varying performances on the test fungi. Generally, lemon grass was highly efficacious on *F. oxysporum* in both aqueous and ethanol solvents. This might have resulted due to the highly therapeutic ability of lemon grass with the possession of

flavonoids on *F. oxysporum* as seen in the phytochemical analysis. This assertion was earlier proved by Dong and Thuy (2021), Mukarram *et al.* (2021), and Oniha *et al.* (2023) that the fungitoxicity effect on several fungal strains is due to the presence of flavones, flavonoids, and phenols in lemon grass oil extracts. In their studies, Helal *et al.* (2007) and Mukarram *et al.* (2021) reported that lemon grass extracts can rupture the plasma membrane and disorganize the cell mitochondrion leading to leakage of ions. As a result, signal transduction and fungal germination can be adversely affected (Alviano *et al.*, 2005). Bitter leaf extract performed moderately on the test fungi with greater inhibition occurring on *F. oxysporum* at both 100% and 50 % concentration levels in both solvent extractions although it contains more phytochemical components than that of lemon grass in both aqueous and ethanol solvents. The reason could be due to highly therapeutic potential against *F. oxysporum* which is indicative of the active secondary metabolites it contains which can inhibit the growth of fungi causing sweet potato root rots. This finding agrees with that of John *et al.* (2016) who found that the mycelial growth of *F. oxysporum* could be sufficiently reduced by 32.26 % with the application of bitter leaf extract. A similar observation was also made on *R. stolonifer* with a reduction of 22.58 % with the application of bitter leaf extract (John *et al.*, 2016), which agrees with this study's findings on the phytochemical properties exhibited by bitter leaf on the inhibition of the mycelia growth of the test fungi.

Among the plant extracts holy basil gave the least efficacy performance on the test fungi although it significantly possessed therapeutic potential for the inhibition of the mycelial growth of the test fungi. However, the lower performance could be attributed to many factors such as the part of the plant, extraction method, and age of the plant used. This corroborates with the findings of Anukwuorji *et al.* (2013) that the phytochemical content of plants can be altered for the plant age, method of extraction as well as harvesting time

of the plant. Regardless, holy basil has been accorded the status of antifungal, antibacterial, antiviral as well as antiprotozoal properties to its numerous species (Dixit *et al.*, 2021).

In this study, the solvent extraction method used might have played a major role in the antifungal activity of the plant extracts on the test organisms. In general, the aqueous extraction method gave more encouraging performance than the ethanol extraction method on the tested fungi although a significant difference did not occur between them which is contrary to the findings by Balakumar *et al.* (2011); Nagappan, (2012) and Anukwuorji *et al.* (2013) on ethanol solvent giving better performance on fungi than aqueous solvent in their various studies. This could be attributed to the fact that the ethanol extraction method might have led to the volatility of some of the phytochemical constituents during the preparation process which was otherwise in the aqueous preparation process, hence; adversely affecting the full inhibition potential of the ethanolic extracts of the plants. On the contrary note, Anukwuorji *et al.* (2013), reported that water is an inorganic solvent and hence gave a lower performance on mycelia growth *in vitro* against fungal pathogens causing sweet potato root rots which resulted from the inability of the aqueous plant extracts to completely dissolve the biologically active properties responsible for antimicrobial activities in these plants. At the concentration levels, there differ antifungal activity in the plant extracts with higher fungitoxicity at 100 % concentration level in both the aqueous and ethanolic extracts. In their studies, Clark *et al.* (2013) and Ijato *et al.* (2010) reported that the higher the concentration of plant extract, the better the inhibitory performance against pathogens.

Comparatively, there may be some correlation between the antifungal activities of the plant extracts and their biologically active constituents. The findings concur with those of Kwodaga *et al.* (2019) and Fokunang *et al.* (2000), who asserted that the antifungal

activity of any plant extract depends on its active phytochemicals. This suggest that the higher the phytochemical properties of a plant extract, the higher the antifungal activity of the extract against the test organisms. There may be a direct correlation between a plant extract's concentration and the quantity of secondary metabolites it contains. As a result, raising the concentration of any extract can boost its antifungal effectiveness.

5.4 Effect of plant extracts on weight loss of sweet potato varieties

Weight loss is one of the principal components of root tubers in storage (Castillo-Matamoros *et al.*, 2014; Maalekuu *et al.*, 2015; Zhang *et al.*, 2018). The results obtained in this study suggest that sweet potato root tubers in weight loss were affected by the root tuber size, plant extracts, and the sweet potato variety. The rapid deterioration encountered in weight loss during the storage period decreases from the first week of storage to the last week of storage. Bigger root tubers in both varieties were observed to have greater weight loss in comparison to medium size root tubers. It is reported that the bigger the surface area of the tuber, the greater the transfer of moisture into the environment and vice versa (Dramani, 2013b). This natural phenomenon could be attributed to the high moisture content of the root tubers in the initial stage of storage. This corroborates with the findings of Kou *et al.* (2023) that weight loss is experienced due to the dissipation of high moisture content (50 % - 80 %) from the surface of the sweet potato root tubers in storage. This results in delicate skin as well as tuber shrivelling at the tuber tips.

In comparison, the rate of weight loss was affected by variety. Orange-flesh variety experienced high weight loss than the white-flesh sweet potato variety. Similar studies conducted by Picha (1985), suggested that the white-flesh variety was stored longer due to its low weight loss and pithiness. This might be due to the physicochemical variation

between the two sweet potato varieties. This assertion is in support of Dramani (2013a) who reported that hemicellulose and cellulose except for lignin were altered with weight loss in yam tuber varieties after storage. On the contrary, Afoakwa and Sefa-Dedeh, (2001) deduced that fibre content increases in tubers after long storage but varies with tuber variety. Furthermore, the higher weight loss by the orange-flesh variety may be due to the factors such as high dehydration as well as respiration and thin skin occurring in the orange-flesh variety. This view conforms with that of Kou *et al.* (2023) who stated that sweet potato varieties respire differently after treatment with ethylene. A higher respiration rate and undesirable environmental impacts might have affected the storage process and hence exacerbated excessive weight loss, which was associated with moisture loss of the orange-flesh variety.

The performance on weight loss for the plant extracts varied between the two varieties. However, bitter leaf gave average best performance though much differences were not observed between bitter leaf and the other two plant extracts. This could be attributed to the phytochemical constituents of the bitter leaf containing flavonoids, saponins, and triterpenes which is correlated to the antioxidant activity on the stored root tubers.

5.5 Effect of plant extracts on rot depth lesion and severity

The plant extracts defence mechanism was tested on fresh and healthy root tubers inoculated with each test pathogen in *in vivo*. Necrotic lesions on the stored root tubers were more necrotic at the proximal point of inoculation on all the inoculated tubers. Averagely, the orange-flesh variety had a greater depth lesion as well as rot severity than that of the white-flesh variety. This could be attributed to the varietal differences in moisture content between the varieties. As stated in section (5.3) the high moisture content of the orange-flesh (50 – 80%) variety might have resulted in soft skin for easy colonization of the tuber. This corroborates with that of Gwa and Richard (2018) that the

variation in moisture content between the regions in the tuber is a major contributor to tuber rot severity by pathogens. The low rot severity in that of the white-flesh variety could be due to the phytochemical constituents possessed by the plant extracts in conjunction with the production of enzymes in the white-flesh variety that inhibited the pathogens in *in-vivo.* The chemical composition differs between varieties and the differences in sweet potato varieties' susceptibility to rot severity can be explained by the composition of the skin and tubers, in particular anti-fungal phenolic compounds. This supports the idea by Gwa and Richard (2018) that, 'ogaja' tubers were able to resist the attack of pathogens due to the production of phytoalexins in the yam tuber. Furthermore, some fungi can produce a considerable amount of pectolytic enzymes such as pectinase, amylase, and cellulase which assist in liquefying the tuber flesh for easy colonization (Beckley and Awoyemi, 2021). Interestingly, this action is facilitated by humid conditions coupled with higher temperatures occurring in the tropics as reported by Beckley and Awoyemi (2021). According to Sowley (1999), a fungus must penetrate and spread through a tissue firstly being receptive to fungal growth, then it must create the enzymes and metabolites that kill and soften the tissue.

Regardless, the three plant extracts used performed to the expectation of reducing the rot severity in both sweet potato varieties. This suggests that the plants have antifungal properties against the test organisms in *in vivo*.

This is in accordance with the reports of Gwa and Richard (2018) who used *Zingiber officinale*, *Azadirachta indica,* and *Piper guineense* in the control of rots causing yam tubers rots in storage. Similar results were obtained by Opiyo *et al*. (2011), who reported a sufficient reduction in rot severity with the use of *Terminalia brownii* on pathogens causing sweet potato tubers to rot. However, the effectiveness of the plant extracts on the tubers in *in vivo* differs with bitter leaf which happened to give a little better performance

above lemon grass while holy basil was less effective on *A. niger*. The less effectiveness of holy basil on *A. niger* in this study could be attributed to the concentration level of the plant extract as well as the solvent extraction method. This supports the report by Sisodia and Rathore (2023) where they used holy basil with different solvent methods of extraction to inhibit the growth of *A. brasiliensis* and reported that methanol, acetone, and chloroform extraction methods gave a zone of inhibition with increasing concentration levels while the aqueous did not show any inhibition.

5.6 Effect of plant extracts on percentage sprout count

The sprout percentage of the three plant extracts was only tested in the aqueous extraction method instead of both aqueous and ethanol solvent forms on both sweet potato varieties. This is because this study seeks to find easy and accessible methods of managing sweet potato root rots as well as reducing sprouting with locally available plant species for smallholder sweet potato farmers in Ghana. Orange-flesh variety had the highest sprout percentage which was manifested in the weight loss thus losing more weight than white-flesh variety in storage. In this study, the plant extracts are suggested to have the ability to inhibit the sprout of sweet potato root tubers in storage than root tubers without treatment. This report is in agreement with that of Belay *et al.* (2022) who reported the inhibition of sprouting on the *Jalene* sweet potato variety in Ethiopia after applying garlic and rosemary essential oils to sweet potato tubers. This indicates that the plant extracts possess some chemical constituents or inhibitors that can sufficiently reduce the sprouting of sweet potato root tubers in storage. It is assumed that essential oils prevent sprouting because their primary constituents can limit meristematic cell division activities in comparison to the actions of auxins and cytokinins (Belay *et al.*, 2022). The least sprouting percentage produced by WFSP (8.33 %) treated with lemon grass extract is in conjunction with the findings of Giri *et al.* (2020) where 8.25 % was reported to be the

least percent sprout when lemon grass extracts were used in their experiment on sweet potato root tubers. They further reported that lemon grass oil significantly reduced sweet potato sprout to 13 % after 90 days of storage. The effectiveness of lemon grass in suppressing sweet potato sprouts could be attributed to it containing citral which is an effective sprout suppressant (Giri *et al.*, 2020). However, the highest percent sprout as produced by the OFSP variety treated with lemon grass extract is in contrast to what was observed on WFSP lemon grass treated could be due to their genetic variation. This assertion is supported by the works of Belay *et al.* (2022) and Frazier *et al.* (2004) who reported that due to genetic variation sprouting of sweet potato tubers varies. Contrary to their argument, Belay *et al.* (2022) reported that essential oils of plants could either inhibit or induce sprouting on sweet potato root tubers depending on the variety.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The findings of this study revealed that some plants possessed secondary metabolites for the protection of plants against diseases. The results obtained from the qualitative phytochemical analysis showed that alkaloids, flavonoids, saponins, and phytosterols were present in the plant extracts used in this study. This confirms the antifungal activity of the plant extracts against isolated fungal pathogens. The plant extracts in both aqueous and ethanol solvents contained the same amounts of phytochemical properties although bitter leaf contained flavonoids, saponins, and phytosterols while holy basil showed the presence of alkaloids and flavonoids, and lemon grass lastly containing flavonoids only in both aqueous and ethanol solvents.

Seven fungal species namely; *A. niger, L. theobromae, F. oxysporum, R. stolonifera, T. harzianum A. flavus* and *A. clavatu*s were isolated from sweet potato rotten root tubers. Five out of the seven fungi isolated (*A. niger, L. theobromae, F. oxysporum, T. harzianum* and *R. stolonifer*) were found to the pathogenic on fresh and healthy sweet potato root tubers through Koch's postulate. The *in vitro* treatments with the aqueous and ethanol plant extract significantly reduced ($P < 0.05$) the mycelia growth of the pathogens (*A. niger, L. theobromae, F. oxysporum, T. harzianum* and *R. stolonifer*) in comparison to their respective negative controls. At 100 % concentration level mycelia growth inhibition was greater as compared to the 50 % concentration levels of the various plant extracts in both aqueous and ethanol solvents. Sweet potato root tubers treated with bitter leaf extracts in *in vivo* had a promising performance on reduced weight loss, prevention of rot severity as well as percentage sprout of root tubers.

Comparably, among the three plant extracts with decreasing efficacy against isolated fungal pathogens, bitter leaf followed by lemon grass and holy basil gave promising botanical management of sweet potato root rots at the postharvest stage of sweet potato production.

6.2 Recommendations

1. Sweet potato farmers should adopt the usage of plant extracts instead of the use of synthetic pesticides in the management of root rot of sweet potato root tubers at the postharvest production stage.
2. Further research should be done to consider mixing these plant extracts in combination for the management of sweet potato root rots. Since this study showed that each plant extract has different phytochemical components hence in combination it will give a broad spectrum of antimicrobial activity against sweet potato root rot pathogens.
3. Research should be carried out to find out the synergic action of these plant extracts against other pathogens causing other sweet potato diseases such as bacterial diseases.

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APPENDICES

Appendix 1: Analysis of variance for antifungal activity of aqueous at 100 %

Variate: Aqueous

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	0.1586	0.0793	0.45	
Replication.*Units* stratum					

Treatment	24	1819.0199	75.7925	431.06	<.001
Residual	48	8.4397	0.1758		
Total	74	1827.6182			

Grand mean 6.582
CV % 0.9

Appendix 2: Analysis of variance for antifungal activity of ethanol at 100 %

Variate: Ethanol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	0.0217	0.0108	0.05	
Replication.*Units* stratum					
Treatment	24	1822.0800	75.9200	382.79	<.001
Residual	48	9.5200	0.1983		
Total		74	1831.6217		

Grand mean 7.173
CV % 0.3

Appendix 3: Analysis of variance for orange-flesh sweet potato rot severity

Variate: OFSP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	34.13	17.07	0.75	
Rep.*Units* stratum					
Treatment	24	19086.68	795.28	35.05	<.001
Residual	48	1089.24	22.69		
Total	74	20210.05			

Grand mean 47.23
CV % 1.7

Appendix 4: Analysis of variance for antifungal activity of aqueous at 50 %

Variate: Aqueous

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Replication stratum	2	0.23927	0.11963	1.56	
Replication.*Units* stratum					
Treatment	24	1941.84947	80.91039	1056.58	<.001
Residual	48	3.67573	0.07658		
Total	74	1945.76447			
Grand mean	8.631				
CV %	0.8				

Appendix 5: Analysis of variance for antifungal activity of ethanol at 50 %

Variate: Ethanol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	0.18667	0.09333	0.96	
Replication.*Units* stratum					
Treatment	24	1974.51333	82.27139	849.86	<.001
Residual	48	4.64667	0.09681		
Total	74	1979.34667			
Grand mean	8.907				
CV %	0.7				

Appendix 6: Analysis of variance for white-flesh sweet potato rot severity

Variate: WFSP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	48.70	24.35	1.50	
Rep.*Units* stratum					
Treatment	24	18519.66	771.65	47.60	<.001
Residual	48	778.16	16.21		
Total	74	19346.53			
Grand mean	55.18				
CV %	7.3				

Appendix 7: Analysis of variance for orange-flesh sweet potato sprout

Variate: OFSP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep_1 stratum		2	0.801	0.401	0.33	
Rep_1.*Units* stratum						
Treatment		4	1371.207	342.802	278.23	<.001
Residual		8	9.856	1.232		
Total		14	1381.865			
Grand mean	20.09					
CV %	1.4					

Appendix 8: Analysis of variance for white-flesh sweet potato sprout

Variate: WFSP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_1 stratum	2	6.776	3.388	1.52	
Rep_1.*Units* stratum					
Treatment	4	2329.886	582.472	260.79	<.001
Residual	8	17.868	2.234		
Total	14	2354.530			

Grand mean 15.55 CV % 5.3