CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Sweet potato (*Ipomoea batatas*) is a tuberous rooted perennial crop that is usually grown as annual crop. It originated from Central America (Ecocrop, 2010) and is widely grown as an important staple food in most parts of the world. There are over 403 varieties of sweet potato of which the flesh can be white, yellow, red, purple, pink, violet and orange or brown while the skin colour varies among yellow, red, orange and brown (Ecocrop, 2010). The crop has great food and health values. Many parts of the plant including the leaves, root and vines are edible. The roots are widely used as carbohydrate food; the tender leaves commonly eaten by man while the leafy stems are fed to livestock (Woolfer, 1992). Beside these, the crop has been noted to provide surprising health benefits including fighting cancer, diabetes, vitamin A deficiency, and inflammation; preventing arteriosclerosis, heart disease, depression, emphysema, arthritis, stroke, muscle cramp and stomach ulcers; reducing arthritis and inflammation and curing bronchitis and stomach ulcers (Alum *et al.*, 2013).

The population of Nigeria relies on sweet potato as a food security crop (Adeyonu *et al*., 2016). Unfortunately, sweet potato roots are susceptible to many microbial infections at different stages including field, harvest and storage (if they are not properly harvested and stored) and marketing stages. This type of spoilage commonly associated with sweet potatoes is a major constraint to the potential of sweet potato as food and health security crop (Echerenwa and Umechuruba, 2004). This result in many detrimental effects including deterioration in food quality characteristics, great loss in storage roots, unavailability of food produce during off-season and a

waste of farm inputs and scarce resources such as water. It also saps human effort and investments and adversely affects the people's economic access to crop produce. Microbial spoilage also compromises food (sweet potato) safety; posing a serious health concern (Walsh *et al*., 2004; Jain *et al*., 2011; Esnakula *et al*., 2013; Esnakula *et al*., 2013 and Georgiadou *et al.*, 2014) .

Due to the negative economic importance of fungal pathogens, control strategies are needed. Several postharvest pathogen control methods used include fungicide treatment, gamma irradiation and hydro-warming. These methods, though reported to have intermediate impacts in controlling spoilage and enhancing shelf life of sweet potato tubers (Ray and Ravi, 2005), have some drawbacks including unavailability to Nigerian farmers, unfriendly to environmental sustainability, phytotoxic to man and a great propensity to trigger resistance in the targeted pathogens (Okigbo and Nmeka, 2005). Given these drawbacks associated with the orthodox fungi and rot control approaches, focus hasin recent times, shifted toward exploitation of plant extracts as novel fungicides in plant protection (Okigbo and Nmeka, 2005; Okigbo and Omodamiro, 2006). Many botanicals have been extensively researched on and proved to possess antimicrobial properties; hence myriads of reports have been documented stating the uses of plant extracts to control plant diseases. Some plants tested for antimicrobial properties include *Chromolena odorata* (Siam weed), *Ocimum gratissimum* (wild basil), *Moringa oleifera* (moringa) and *Zingiber officinale* (Ginger) (Okigbo and Nmeka, 2005; Okigbo *et al.,* 2009a).

1.2 Statement of the Problem

Microbial pathogens affecting crops tend to vary in occurrence and distribution depending on the environment, crop physiology, harvesting and storage, thus the incidence of postharvest rot disease, the frequency of occurrence of different pathogens and their importance as primary pathogens of decay may change with reference to location. Therefore, to develop an effective disease

control programme for the sweet potato sector, it is important to know the fungal pathogens responsible for the disease within a location. Moreover, the development of potent and drawback-free decay control measures is critical to prevention of microbial spoilage and reduction of food losses to microbial attack. In spite of these recognitions, the occurrence and control of fungi associated with sweet potato spoilage in Ebonyi State notable for sweet potato production in South-Eastern Nigeria seem not to have received attention; yet there is apparently a steady increase in post-harvest microbial spoilage of sweet potato observed and reported by some farmers in the area.

Attention to postharvest rot control has been focused on the use of single plant extracts with antimicrobial activities that were always far less potent than those of synthetic chemicals employed as treatment checks in several investigations. Moreover, the antimicrobial activity of plant extracts that is observed in *in-vitro* conditions is quite different from its effect in complex food systems. In most cases antimicrobial activity is decreased due to interactions with food components. This could be a challenge in utilizing plant antimicrobials, as a higher concentration could result in unfavorable changes to the taste and aroma of food (Havelaar *et al.,* 2010). Combinations of extracts can lead to additive or synergistic effects on postharvest pathogens. Despite these recognitions, literature in Nigeria still lacks sufficient data on the potency of combined plant extracts against microbial spoilage pathogens for use in sweet potato preservation.

1.3 Justification for the Study

The developing nations of the world have always been in short supply of food. Around 1 billion people are being faced by severe hunger in these nations of which 10% actually die from hunger-related complications. This problem is further compounded by the accelerated increase in human population, which creates pressure on every form of food supply (Urom, 2014). Today, one of the main global challenges is how to ensure food security for a world growing population whilst

ensuring long-term sustainable development. According to the Food and Agricultural Organization, food production will need to grow by 70% to feed world population which will reach 9.3 billion by 2050. Worse still, in the meantime, while the number of food insecure population remains unacceptably high (FAO, 2010), each year and worldwide, massive quantities of food including sweet potatoes are lost due to spoilage and infestations from farm to folk. This problem arises due to inadequate agricultural storage and microbe-induced spoilages (Kana *et al.,* 2012).

Studies that will generate baseline information on the occurrence of postharvest spoilage fungi of sweet potato in Ebonyi state and a potent drawback-free strategy for controlling the rot pathogens are critical to reducing sweet potato yield losses at postharvest. The potential benefits of reducing postharvest losses of sweet potato to mycodeterioration are large. It is critical to alleviation of poverty while reducing pressure on ecosystems, climate and water. It is also a strategy for contributing to food security enhancement and closing the food gap between food available today and food needed in 2050 to adequately feed the planet's projected 9.3 billion people.

1.4. Aim of the Study:

The study was aimed at investigating the occurrence and biocontrol of the postharvest fungi responsible for sweet potato spoilage in Ebonyi State

1.5 Objectives

The objectives of the study were to:

1. isolate and characterize the fungi associated with sweet potato rots from Ebonyi State,
2. determine the seasonal percentage occurrence and pathogenicity of the isolated fungi,
3. determine the severity of the pathogenic fungi and susceptibility of Ebonyi farmers' most commonly grown sweet potato cultivars to rot,

4. evaluate the effect of fungi infection on the nutritional quality of sweet potato cultivars most commonly grown by Ebonyi farmers,
5. determine the *in vitro* antimicrobial activity of single and combined extracts of *Zingiber officinale* (ginger), *Garcinia kola* (bitter kola), *Allium sativum* (garlic) and *Moringa oleifera* (moringa) on the fungal pathogens
6. determine the *in vivo* antimicrobial activity of selected plant extract combinations using sweet potatoes as a food model

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Taxonomy of Sweet potato

Sweet potato originated in South Mexico and Central America. The crop has been cultivated for more than five thousand years and fossilized remains found in the Andes have been dated at about 8,000 years old. Genetic studies suggest the likelihood that in early times sweet potatoes were carried by the local people from island to island, spreading gradually across the Pacific from Central and South America to eastern Indonesia, New Guinea, Polynesia, and New Zealand. The rest of Asia accounts for 6 percent, Africa 5 percent, Latin America 1.5 percent, and the United States 0.45 percent

Taxonomically, the sweet potato (a dicotyledonous root crop) and the wild species closely related to it are classified in the family Convolvulaceae, genus *Ipomoea*.

2.2 Description of the Sweet Potato Plant

Sweet potato (Plate 2.1) is a member of the morning glory family. The crop is a perennial that is usually grown as an annual crop. It grows from underground tuberous roots with trailing, twisting stems that can be as long as twenty feet (Six meters).

Plate 2.1: *Ipomoea batatas* (Sweet Potato) Storage Roots
Source: Brooke *et al.* (2003).

Leaves are variable in shape, size and colour but are generally more or less heart-shaped and green with purple markings and are spirally arranged on the stem in a pattern known as 2/5 phyllotaxis. This means that there are five leaves spirally arranged in two circles around the stem for any two leaves are located in the same vertical plane on the stem. In relation to cultivar type, the edge of the leave may be entire, toothed or lobed. The single flowers are funnel shaped and white or pale purple but are rarely seen in temperate regions. Roots grow where stem nodes touch the ground, and most develop into the edible storage roots, usually four to ten storage roots per plant.

2.3 Importance of Sweet Potato in Sub-Saharan Africa

Sweet potato is an important staple crop in 130 countries worldwide, ranking fourth in production after rice, maize, and wheat (Ukpabi, 2009) with Nigeria being the fourth biggest producer in Sub Saharan Africa as at 2008 (FAO, 2008). In Africa, the bulk of production is consumed or locally traded, playing a crucial role in providing food security. The crop is particularly important crop for subsistence farmers in Africa and other developing countries. Sweet potato is a very important food security crop due to its relatively short growing period, tolerance to drought and high yield from poor soils. It is used as a famine reserve for many of these households (Ofor, 2011).

Sweet potato is grown generally for its storage roots, which are eaten fresh, steamed, or boiled. Sometimes the leaves are eaten as vegetables, the vines are fed to livestock, and the storage roots are processed into flour for use during the lean season. The crop tubers have been consumed in various forms in developing countries of Africa, including eating in cooked form, incorporation with yam during pounded yam preparation, processing into flours along with yams for edible dough (such as amala in Nigeria). Sweet potatoes can be substituted for potatoes, apples, or squash in almost any recipe. Cooked, mashed sweet potatoes are also used to replace some of the wheat flour in breads, cakes, muffins, and cookie recipes, as is sweet potato flour. In third world countries sweet potatoes are processed into starch, noodles, candy, desserts, and flour. This allows the farm household to extend the availability of the crop. In China, for example, sweet potato starch production has become an important cottage industry, while in Uganda sweet potatoes are sliced and dried, which allows them to be kept for about five months. Although usually the roots are eaten, young leaves and the tips of vines can be harvested, washed, and boiled as a green vegetable or added to stir-fries. All parts of the sweet potato are used as stock feed, although the roots are often cooked first

Sweet potatoes possess many positive health benefits including sources of anthocyanins, phenolic compounds and other bioactive compounds (Alum *et al.*, 2013). The vegetable crop also possesses antioxidant activities. Different species of sweet potatoes have gained popularity in many countries as a result of their health benefits (Leksrisompong *et al.*, 2012). In eastern and southern Africa some 3 million children under the age of five suffer from xerophthalmia or dry eye, which causes blindness. Dry eye is caused by a lack of vitamin A in the diet, and many of the affected children die within a few months of becoming blind. The yellow-and orange-fleshed varieties of sweet potatoes are high in beta-carotene, which can be converted into vitamin A in the intestines and liver. It has been shown that even small amounts of these sweet potatoes as a regular part of the diet will eliminate vitamin A deficiency in adults and children.

2.4 Nutritional Composition of Sweet Potatoes

The crop is highly rich in nutrient elements. A 100g edible portion of sweet potatoes yield 360kj energy, 20.1g carbohydrate, 3.0g dietary fibre, 0.1g fat, 1.6g protein, 30.0mg calcium, 0.6mg iron, 25.0mg magnesium, potassium 337mg, thiamine 0.1mg, riboflavin 0.1mg, phosphorus 47.0mg, sodium 55mg, zinc 0.3mg, folate 11Ng, pantothenic Acid 0.8mg, Niacin (B3) 0.61mg, 79.8% Water, about 2.4mg vitamin C is reported in sweet potato and 0.25mg Vitamin B_6. In spite of these, the nutrient compositions as documented by several authors have been revealed to vary from cultivar to cultivar. Eleazu and Ironua (2014) after evaluation of the composition of an unnamed sweet potato cultivar reported that, "the flour was observed to have good functional properties with a pH of 5.32±0.01, high percentage moisture content, indicative of poor shelf life characteristics and high chances of being attacked by microbes, low percentage dry matter, lipid, crude fibre and ash contents but a promising source of starch (20.78±0.02%), carotene (5.0±0.04 μg/g), protein (2.67±0.59%), carbohydrate (40.77±3.05%), energy (179.61±20.97 kcal/100 g), polyphenols, in addition to containing significant quantities of reducing sugar (1.58±0.53%)". In a study by Gebreegziabher *et al.* (2014) titled "Chemical Composition and *In Vitro* Dry Matter Digestibility of Vines and Roots of Four Sweet potato (*Ipomoea batatas*) Varieties Grown in Southern Ethiopia", root and vine proximate compositions were shown to vary with sweet potato cultivars.

2.5 Production of Sweet Potatoes

Sweet potato is adaptable and can grow under many different ecological conditions. It has a shorter growth period than most other root crops (3 to 5 months) and shows no marked seasonality: under suitable weather conditions it can be grown all year round. Adverse weather conditions rarely cause a complete crop loss. Sweet potatoes have been cultivated since about 3 000 B.C. Presently, Nigeria is the number one producer of sweet potato in Africa with annual output of 3.46 million

metric tons and globally the second largest producer after China. Being an important food security crop in Nigeria, it is widely grown in different agro-ecological zones largely by small scale farmers for home consumption and the surplus sold in local markets.

Sweet potato is propagated asexually from vine cuttings or sexually from seed. Propagation of sweet potato is done by vegetative propagation using one of the following methods: sprouting of whole storage roots (sprouts are then used as planting materials), and use of stem or vine cuttings from plants used for production or from multiplication plots. In the latter method green vines of approximately 30 cm length with at least three leaf nodes are planted into the soil. Sweet potato is most commonly grown on mounds or ridges, and occasionally on raised beds, or on the flat. Deep cultivation enhances root growth and bulking of the sweet potato roots. Mounds and ridges promote adequate drainage and ease of harvesting. Roots are harvested as the leaves begin to yellow in the fall. They are then brushed clean and left to cure. Traditionally, curing involved stacking the potatoes in the field or garden, covering them with sand, and leaving them for several weeks. Sweet potatoes in commercial production are cured in rooms with humidity between 75 percent and 80 percent and temperatures between 80°F and 86°F (27°C and 30°C). Curing heals cuts and reduces decay and shrinkage during storage, and it converts some starches to sugars, improving the flavour. Once cured, sweet potatoes can be stored for several months, and white-fleshed varieties last as long as ten months.

2.6 Factors Affecting Sweet Potato Production

African farmers are faced with several constraints in the production of food and cash crops. Some of these constraints are poor soils, poor farm practices, use of local varieties, land tenure and damages by diseases and pests. Other factors include low resistance to sweet potato virus disease, the lack of timely access to virus and pest-free planting material, insufficient knowledge and use of better

agronomic practices, lack of markets, poor soils and poor farm practices. The greatest of these constraints is that of post-harvest spoilage of farm produce.

2.7 Postharvest Microbial Deterioration of Sweet Potato

When sweet potato roots are not properly cured and well packed or when the storage conditions are inadequate, it may result in tuber injury, skinning, dehydration, and microbial attack, all factors that can lead to substantial post-harvest losses. Spoilage microorganisms can be introduced to the crop during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution. In root crop spoilage, fungi are often the most frequently involved causal agents. Fungal diseases which can afflict sweet potatoes include: surface rot, root rot, soft rot, Java black rot, scurf disease and black rot. Many of the diseases that affect sweet potatoes in storage are first established in the field or on planting material such as scurf. Other postharvest disease organisms are wind- or soil-borne as spores and are essentially ubiquitous (such as Rhizopus soft rot). Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices will provide dramatic reductions in yield loss due to spoilage at all subsequent steps.

Pathological deterioration results from frequently rapid and extensive breakdown of host tissue by microorganisms with initial infection usually occuring at the sites of physical damage or at the point of root attachment to the plant. The pattern of attack is usually initiated by a single or a few pathogenic microorganisms followed by several saprophytes that may greatly magnify the initial damage. The progress of any plant infection depends upon the growth potential and enzymatic production capabilities of the associated microorganism, in addition to the physiological status of the tissues that are infected. These microbes spoil sweet potato by producing a wide range of hydrolytic enzymes such as cellulases, pectinases, xylanases, and proteases. These enzymes are responsible for

tissue maceration and cell death, following which the microorganisms have access to the nutritional resources of the dead plant tissues. The rot changes the consistency of flour from the roots making them no longer suitable for consumption or causing a considerable loss in market value.

2.8 Factors Affecting Postharvest Microbial Deterioration of Sweet Potato

Infection of sweet potato roots may occur in the field, during harvesting**,** handling or in storage. The occurrence of postharvest diseases tends to vary from year to year. Outbreaks occur when pathogens are given an opportunity to proliferate. Many factors favour the proliferation of and damage of roots by microorganisms and include but are not limited to:

2.8.1 Environmental or Cultural Stress During Root Development that Influences the Physiology of the Root and Susceptibility to Infection.

2.8.2 Time of Vine Removal

While the roots of sweet potatoes are widely used as carbohydrate food, the young leaves are commonly eaten by man while the vines are fed to livestock. The leaves and vines are usually removed during the growing period of the sweet potato plant as green vegetables, planting materials or livestock feed. Kihurani (2004) in her studies, documented the effect of pre-harvest vine removal on root susceptibility to microbial infection); where vine removal before harvesting, and especially at two week was reported to significantly ($p<0.05$) enhance postharvest pathological deterioration of sweet potato roots.

2.8.3 The Type of Sweet Potato Cultivar

Sweet potato genotypes are known to vary widely in their susceptibility to postharvest diseases. The use of host resistance for disease management is appropriate because it ensures customer safety and minimum cost and labour to the producer. Fungi are the most

common and destructive postharvest pathogen of sweet potatoes. Sweet potato genotypes are known to vary widely in resistance to Rhizopus soft rot and other postharvest pathogens. A preliminary evaluation of local Kenyan and introduced germplasm in Tanzania and germplasm evaluation conducted in the United States of Americahas proved relativism in susceptibility of sweet potato cultivars to postharvest fungi. Variation in susceptibility to pathogenic infections is a function of the host genetic structure according to May and Anderson reported by Arash *et al.* (2013). Besides, the duration of the storage period and the type of wound that provides pathogen entry can influence the disease response (Holmes and Stange, 2002). Constant genotype-by-environment interactions can influence the degree of disease expression in host plants. Environmental variables including, but not limited to, ambient incubation temperature, host nutrient status and host age can also affect host susceptibility (Arash *et al.*, 2013). Understanding the interactions between pathogen and crop are necessary for the development of disease control practices. For instance, resistant plant genotypes can help constrain disease symptoms due to infections and limit pathogen spread. On the other hand, genotypes susceptible to infection may increase pathogen spread owing to their greater pathogen quantity, regardless of their symptom status.

2.8.4 Handling/Mechanical Injuries Factor

The intact skin of the sweet potato root acts as a barrier against pathogen entry and moisture loss. Mechanical injuries that become entry points for the infecting microorganisms can facilitate infections**.** Therefore, there is need to practice good harvest and postharvest handling to ensure that injuries are minimized, thereby promoting keeping quality of roots, especially during prolonged storage.

2.8.5 Curing Factor

Stored cured roots may last several months (over 10-12 months with only minimal deterioration of quality). Wounds inflicted on sweet potato roots during harvesting undergo a curing or healing process when the roots are promptly exposed to elevated temperature of 27-33°C and high relative humidity at 85 - 95% for four to seven days. Conventional curing is expensive and not easily affordable by many small sweet potato producers. “In-ground” storage is also practiced where mature roots are not harvested until they are needed for consumption or sale. In recent years, however, commercial sweet potato production has become popular and roots are increasingly harvested in bulk and transported to distant markets. During this brief storage period between harvesting and arrival at the market, the roots are inevitably exposed to high temperature conditions that favour microbial activity and high losses may occur. This need has necessitated development of simple and inexpensive curing structures and procedures which can be used effectively in different tropical environments.

2.8.6 Storage Temperature/Relative Humidity Factor

The length of time sweet potatoes can be held in storage without sacrificing quality will depend on the environment they are stored in. Sweet potatoes are held under a variety of environmental conditions, and quality and longevity in storage will vary accordingly. The development of Java black rot, caused by *B. theobromae,* was reduced in sweet potato roots that were cured and stored at 13-16°C. However, the ideal conditions for sweet potato storage have been given as curing immediately after harvesting followed by storage at 12-16°C or 13- 16°C and 85-95% relative humidity.Storage of sweet potato roots at lower temperature causes chilling injury characterized by root decay, internal

discolouration and surface pitting. Storage at temperatures above 16°C induces premature sprouting and pathological and physiological deterioration are undesirably accelerated. But temperatures lower than 55 Farenheit degrees will trigger the cold chill injury factor (cause the sweet potatoes to develop a hard center) and reduce their eating quality. At 73°F (23°C), the optimum relative humidity for the initiation of soft rot is in the range of 75–84%. Few infections occur at 93–99% relative humidity, but once an infection has been established the pathogen continues to grow at relative humidity levels of 50–100%.

2.9 Microorganisms Associated with Postharvest Deterioration of Sweet Potato

Harvested sweet potato roots are prone to infection by a wide range of microorganisms resulting in decay. Bacteria and fungi can affect sweet potato in storage, with fungi being the most common. Most viruses do not cause serious postharvest diseases, although symptoms from field infections may be first noticed after harvest (as with russet crack) or may develop in storage (internal cork). Spoilage microorganisms are often specific to location and therefore their identification is an important fundamental step in the formulation of disease control strategies in any particular environment Different genera of fungi have been reported to induce rot and spoilage in stored sweet potato and include *Ceratocystis fimbriata* (Onuegbu, 2002), *Penicillum* species, *Diaporthe, Aspergillus niger, Aspergillus flavus* (Oyewole, 2006), *Fusarium oxysporum, Fusarium solani, Monitochaetes infuscan, Macrophomina phaseolina, Rhizopus stolonifer, Mucor pusillus, Botrytis cinerea and Erisiphe polygoni*. Agu *et al.* (2015) implicated three fungi *Aspergillus fumigatus, Aspergillus niger* and *Rhizopus stolonifer* with the post-harvest loss of sweet potato in Awka South Local Government Area, Anambra State. Amienyo and Ataga (2007) analyzed sweet potato samples from different markets in Port Harcourt and identified six fungi comprising

Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae and *Fusarium solani* as agents of postharvest rot of sweet potatoes. Oladoye *et al.* (2016) in their study titled Biomolecular characterization, identification, enzyme activities of molds and physiological changes in sweet potatoes (*Ipomea batatas*) stored under controlled atmospheric conditions, isolated *Penicillium expansum* from sweet potato and implicated it with causation of sweet potato rot.

From the foregoing, different authors reportedly isolated different species of fungi in association with sweet potato rot, indicating species variation with respect to location. In spite of this, the fungi associated with the postharvest rot of sweet potato in Ebonyi State, a major sweet potato producing area in Nigeria has not been documented.

Aspergillus niger is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found. Aspergilli are ubiquitous in nature. They are geographically widely distributed, and have been observed in a broad range of habitats because they can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil. The taxonomy of *Aspergillus* is primarily based on morphological features, rather than the physiological, biochemical features and genetic characteristics often used to classify bacteria. The genus *Aspergillus* is usually defined as asexual saprophytic fungi that produce large black or brown conidia by phialides that are arranged in a globose head radiating from a vesicle or spherical conidiophore. The colony is initially white and quickly turned black as conidial production starts. Its growth produces radial fissures in the agar. These physical characters such as spore colour and rate of growth on a defined media are subject to change, especially under extended pure culture or selection and mutation. *A. niger* has been isolated from 37 genera of plants. *A. niger* can cause the rotting of

numerous fruits, vegetables, and other food products, thus causing substantial economic losses due to spoilage. For example, black rot of onions associated with A. *niger* is responsible for serious losses of onion bulbs in the field and in storage. Often these reports involve co-isolation with other perhaps more destructive microorganisms or isolation from astored plant product. There are reports of *A. niger* being a plant pathogen in sweet potatoes (Agu *et al*., 2015). The taxonomy of *Aspergillus* has public health implications due to the production of potent mycotoxins by members of this genus. Most notable of these is the association of aflatoxins with members of the *A. flavus* group. *Aspergillus niger* can produce a variety of fungal metabolites, termed mycotoxins, depending upon growth conditions and the strain of the organisms.

Rhizopus oryzae spores are ubiquitous and continually present in the air around us. The fungus can survive in crop debris in the soil, in fruits and vegetables, and to some extent on contaminated equipment. *Rhizopus* species require wounds and necrotic tissue for infection of sweet potato storage roots. These sites are usually the broken tips of sweet potatoes damaged during harvest, holes in the periderm caused by tools or insects such as weevils, or bruises or wounds created by overly rough handling of sweet potatoes after harvest and during processing. Uncured sweet potatoes are more prone to damage than are cured sweet potatoes (Scot, 2009). When either airborne spores or infested soil comes into contact with a wound, the spores germinate, producing hyphae that enter the root. Pectolytic and other enzymes produced by Rhizopus species quickly cause host discoloration and liquefy host tissues. Symptoms may begin in any wounded or damaged area of the sweet potato. Rhizopus rot often begins at the tips of sweet potatoes or in wounds associated with harvesting (wound created where roots are broken from the plants during harvest) and handling. Other sweet potato diseases (for example, Java black rot) can produce the same general symptom initially; therefore, further diagnostic work is needed to accurately identify the cause of the problem. But symptoms may sometimes develop before harvest in fields with heavy or clayey soils after

flooding or heavy, continuous rainfall. Diseased sweet potatoes turn soft and moist, with stringy flesh. The infected tissue of sweet potatoes usually turns brownish to blackish quickly. The colour of the diseased tissue may vary among sweet potato cultivars. A pronounced, fermented odor is often present and may attract egg-laying fruit flies. The entire root usually rots completely within days after infection. The parenchyma (composing the pith or innermost layer) may liquefy, whereas the periderm (skin) and root fibers remain relatively undegraded. As decay progresses, mycelia and whisker-like tufts of sporangiophores and darkly pigmented sporangiospores (spores) grow out through cracks, breaks, wounds, bruises, holes, or natural openings in the sweet potato periderm. Tufts of the plant pathogen Rhizopus emerge from openings in a rotting sweet potato. Fungal "whiskers," typically darkly colored, fuzzy-looking tufts, are diagnostic for Rhizopus soft rot disease. The tufts are composed of fungal mycelia, sporangiophores, and sporangia.

Aspergillus flavus is a saprotrophic and pathogenicfungus with a cosmopolitan distribution. Post-harvest rot typically develops during harvest, storage, and/or transit. *A. flavus* infections can occur while hosts are still in the field (pre-harvest), but often show no symptoms (dormancy) until post-harvest storage and/or transport. In addition to causing pre-harvest and post-harvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins, which when consumed are toxic to mammals. *A. flavus* is also an opportunistic human and animal pathogen causing aspergillosis in immunocompromised individuals (Amaike, 2011). *A. flavus* is unique in that it is a thermo-tolerant pathogen and can survive in temperatures that other pathogens will not. Itcan contribute to the storage rots, especially when the plant material is stored at high moisture levels; grows and thrives in hot and humid climates. *A. flavus* has a minimum growth temperature of 12°C (54°F) and a maximum growth temperature of 48°C (118°F). Even though the maximum growth temperature is around 48°C (118°F), the optimum growth temperature is right at 37°C (98.6°F). With these temperatures in mind, *A. flavus* had rapid growth at 30-55°C, slow growth at 12-15°C and it

almost ceases growth at 5-8°C. *A. flavus* growth occurs at different percentage levels for different crops. For starchy cereals, growth will occur at 13-13.2%. For Soybeans, growth will occur at 11.5-11.8%. For other crops, growth will occur at 14%.

Bortyodiplodiae theobromae induced disease is easily identified by its distinct symptoms. Symptoms usually begin at the root end as a firm, moist decay that turns colour from pale yellow, to brown, to black. Internal symptoms of Java black rot include a clearly demarcated, firm, dark rot. External symptoms of Java black rot are hard black "stroma" protruding out of the sweet potato skin.

Fusarium solani has a very broad host range, causing primarily root rots, stem cankers, and damping off in plant species across 66 families. *F. solani* is present in soil around the world. It is a common soil inhabitant, and can survive in the absence of a host plant for up to five years as chlamydospores. Therefore, wounds occurring during harvest are subject to infection by populations of *F. solani* in the soil. *F. solani* may also survive on contaminated harvesting and packing equipment, allowing for subsequent infections after packing. Recent studies have also shown that *F. solani* can be transmitted from infected slips to sweet potato roots. Upon injury and adequate environmental conditions, Fusarium root rot may develop on these roots. *Fusarium solani* is identified based mainly on the morphology of both the asexual spores and the structures bearing the spores following Booth's division. Colonies grow rapidly, 4.5 cm in 4 days, aerial mycelium white to cream, becoming bluish-brown when sporodochia are present. Macroconidia are formed after 4-7 days from short multi-branched conidiophores which may form sporodochia. They are 3- to 5- septate (usually 3- septate), fusiform, cylindrical, often moderately curved, with an indistinctly pedicellate foot cell and a short blunt apical cell, 28-42 x 4-6 μm. Microconidia are usually abundant, cylindrical to oval, one- to two-celled and formed from long lateral phialides, 8-16 x 2-4.5 μm. Chlamydospores are hyaline, globose, smooth to rough-walled, borne singly or in pairs on short lateral hyphal branches or intercalary.

Fusarium root rot occurs where roots have been injured during harvest or postharvest handling. Harvest conditions that increase injury to roots, thus potentially increasing the incidence of *Fusarium* root rot include harvesting when the soil is cool and damp, harvesting when the soil is overly dry, exposing roots to extreme temperatures before curing. Once *Fusarium* root rot has been established, it will continue to progress throughout storage. Following infection of sweet potato roots, *F. solani* produces characteristic symptoms. Externally, lesions are dark tan and often have a distinct ring pattern of light and dark brown bands. This decay extends into the flesh of the root, showing internal decay with characteristic cavities that may contain white fungal growth. The rot is firm, dry, and dark brown, with internal cavities often filled with white mycelium of the fungus. While fusarium root rot can progress over a wide temperature range (55-95°F), temperatures ranging from 73-84°F results in the most decay. Furthermore, high relative humidities (>90%) also increase progression of this disease and results in higher losses. Having high initial inoculum levels in the field and on harvest and packing equipment also results in higher disease levels. Fusarium rot disease can be managed by minimizing injuries during harvesting and handling, harvesting when soil moisture is optimal, curing promptly after harvest and using resistant cultivars.

Penicillium expansum genus is an ascomycete fungus that is of major importance in the natural environment as well as to the food and drug industry. There are approximately 150 species of *Penicillium* and only a small percentage of these species effect agriculturally significant crops. Species of *Penicillium* are common soil fungi that prefer cool and temperate climates and are ubiquitous wherever organic material is available. Commonly known as moulds, species of *Penicillium* are among the chief causes of food spoilage. Blue mould rot by *P. expansum* is the disease that is most frequently reported on. However, there are a number of other lesser known pathogenic species that are usually also less destructive. All species of *Penicillium* that cause blue mould are primarily wound pathogens that usually gain access to fruit via fresh mechanical injuries.

The pathogen produces hardy spores that survive between seasons on infected objects, on which *Penicillium* has the ability to develop and produce spores in abundance. *Penicillium* rots (blue and green mould rots and core rots) are the most common and usually the most destructive of all postharvest diseases, which account for up to 90% of decay in transit, storage and in the market. *Penicillium expansum* has been identified as being the main cause of these infections in the postharvest context. The pathogen gains entry to tissues through wounds. However, infection can spread from one fruit to another through uninjured skin, the stem end, the open calyx tube and lenticels. *Penicillium expansum* is amongst the species of *Penicillium* that is known to produce a mycotoxin, called patulin, which has been reported to be mutagenic and can have a neurotoxic, immunotoxic and gastrointestinal effect on animals. This mycotoxin can be found mainly in low quality fruit, which is usually used in processed apple products, such as juices and baby food.

2.10 Implications of Microbial Spoilage of Sweet Potato to Food Security

According to Food and Agricultural Organization (FAO) of the United Nations (UN), "Food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life. By implication therefore, any factor that limits the people's physical, social and economic access to sufficient, safe and nutritious food has great adverse implications on food security (FAO, 2008).

In Nigeria, Zimbabwe and many other parts of the world, sweet potato has become an important component of the diet for both urban and rural households. Nigeria population relies on sweet potato as a food security crop. The impacts of microbial spoilage of food, including sweet potato to food security include:

2.10.1 Reduction in Food Produce Availability

The availability of farm produce during off-season has been plagued majorly by problems associated with preservation and storage. By far, postharvest diseases account for the greatest loss in stored sweet potatoes. Fungi found world-wide, attacking and inducing diseases on many kinds of plants has been reported to cause about 60 per cent of plant diseases. They are responsible for billions of Naira worth of damage to crops in Nigeria. In extreme instances, decay losses can run nearly 100 percent. Many countries are documented in history to have suffered famine and financial losses due to these diseases (Bijendra *et al.*, 2017). The total estimates of losses due to fungal diseases are often enormous not only on the field and/or yield but also in storage.

2.10.2 Compromises Food Quality and Nutrition

The quality of any crop depends on the level of nutritional content of such a crop (Markson *et al.*, 2010b) for root and tubers; their value is in the quantity and quality of their nutritional content. Sweet potato is a nutritionally rich crop in which are found various types of nutrients including antioxidants (phenolic acids, anthocyanins and carotenoids), vitamins, minerals, dietary fibre, proteins and non-fibrous carbohydrates. However, microbial spoilage of sweet potato and other foods is a metabolic process brought about by microbial action and causes food to become undesirable or unacceptable for human consumption due to deterioration in quality characteristics (Dayle, 2007) (rotting, discoloration and formation of off-flavours in the food products depending on the types of microorganisms and their corresponding physiological activities). Microbial spoilage of sweet potato is found associated with decrease in starch, total sugar, organic acid (ascorbic and oxalic acid) contents with concomitant increase in polyphenols and ethylene. Several research findings donate evidence on this. Markson *et al*. (2014) in their study titled "Differential Response of *Solanum tuberosum* L. and *Ipomea batatas* to three rot Pathogens" showed that within four weeks of the

experiment, *Botryodiplodia theobromae, Rhizopus stolonifer* and *Penicillium expansum* consistently caused starch grain depletion from the tissues of the two potato species studied. Adeogun *et al.* (2014) investigated healthy and diseased white yams tubers (*Dioscorea rotundata*) from different open markets in Lagos State in a proximate analysis and revealed that real gradual reduction in ash, lipid and crude fibre contents in the diseased yam tubers with reference to healthy yam except protein and moisture that had an insignificant increase in the diseased yam. In their studies, the carbohydrate content of healthy yam was 45.6%±0.1, diseased yam was 42.8%±0.32, protein content of healthy yam was 45.6%±0.1% while the protein content of diseased yam was 42.8%±0.32, fat content of healthy yam was 0.37%±0.01 and diseased yam was 0.24%±0.01, moisture content of healthy yam is 42.7%±0.1 and diseased yam was 44.4%±0.2, ash content of healthy yam is 1.54%±0.01 and the diseased yam was 1.2%±0.02 and fibre of healthy yam is 1.54±0.01% while the diseased yam is 1.2±0.02%. The significant difference ($P \leq 0.05$) between the healthy and diseased in the nutritional composition of the yam was expressive in carbohydrate, moisture and fibre contents of the yam while there was no significance difference in protein, ash and fat contents in the nutritional status of healthy and infected yam. Sanyaolu *et al.* (2014) carried out investigation on the effects of post-harvest mycodeterioration on the proximate composition of *Irvingia gabonensis* seeds and reported that for each of proximate parameter of moisture, ash, fats, proteins, fibre, carbohydrate and energy in the flour of both the healthy and the diseased seeds of *I. gabonensis,* (with the exception of fibre where there was no significant difference ($P \leq 0.05$) between the mean values of flour from both the healthy and diseased seeds (3.8567% and 3.3233% respectively) and the mean value for fats, where the diseased seeds had a significantly higher ($P \leq 0.05$) mean value of 56.2700% compared to those from the healthy seeds with 55.4533%]; the mean values for the composition of the other proximate parameters such as moisture, ash, protein, carbohydrate and energy in the healthy seed samples was significantly higher ($P \leq 0.05$) at 5.0733%, 2.6500%, 10.0300%, 24.8000% and 641.0700%

respectively than in the diseased seeds with a value of 4.4700%, 2.2700%, 8.8600%, 22.9367% and 631.0670% respectively for each of the afore listed parameters"

The impact of fungi infection of on the varieties of sweet potato grown by the farmers in Ebonyi State has not been documented before now. Given that effective disease management strategy is proactive and prophylactic rather than curative (Markson *et al.,* 2014), more convincing evidence on impact of postharvest deterioration on sweet potato can help make the farmers and handlers of sweet potato realize that a crop well produced and less protected is a crop lost and get motivated to take a more serious look at good agricultural practices and storage than is generally done so as to maintain the quality of their harvested produce.

2.10.3 Compromises Food Safety

Ordinarily, sweet potatoes are largely toxin-free. The yellow/orange cultivars contain variable but sometimes large quantities of carotenoids which act as precursors of vitamin A and their consumption is considered an important food-based approach to combat vitamin A deficiency. Beside these, the crop has been noted to provide surprising health benefits including fighting cancer, diabetes, vitamin A deficiency, and inflammation; preventing arteriosclerosis, heart disease, depression, emphysema, arthritis, stroke, muscle cramp and stomach ulcers; reducing arthritis and inflammation and curing bronchitis and stomach ulcers (Alum *et al*., 2013).

Healthy sweet potato roots are toxin free; contains no phytoalexins (Fighera *et al*., 2003). However, when attacked by microbes such as fungi or molds, sweet potatoes generate a variety of food toxins such as phytoalexins and furanotepanoid phytoalexine-like compound. Phytoalexins are naturally induced antibiotics that contribute to a plant's self defense (Lydia *et al*., 2015). The first phytoalexin isolated and identified in the plant kingdom was obtained from diseased sweet potato roots and named ipomeamarone. Sweet potato cultivars may differ in their level of phytoalexin

induction. Phytoalexins make sweet potato roots toxic and unpalatable due to a bitter taste (Lydia *et al*., 2015). There is a well established history of cattle deaths as a direct consequence of consuming diseased sweet potato roots (Fighera *et al*., 2003).

Fusaria are known for their production of mycotoxins, which include zearalenone and trichothecenes, such as deoxynivelenol (DON). These toxins are secondary metabolites and are thought to result in immunosuppression as well as carcinogenesis in mammals (Berek *et al*., 2001).

Unfortunate bovine fatalities occurring after ingestion of mold-damaged sweet potatoes preclude the use of the culled roots in livestock feed. In cattle, mold-damaged sweet potatoes induce an acute respiratory distress syndrome resulting in asphyxiation. Because of esthetic requirements and the potential detrimental health effects, the amount of root diseases that can be tolerated on sweet potato is much lower than it is for non-root crops which generally require multiple infections by rot pathogens before the crop is economically affected.

2.10.4 Adverse Effect on the Economic Value of the Crop

The practice of disposing off everything during the harvesting season to avoid much loss to postharvest deterioration results in a temporary glut in the market leading to low (give away) prices and adversely affecting the economic value of the crop.

2.10.5 A Wasted Investment that Reduces Economic Access to Food

Economically, sweet potato spoilage and loss represent a wasted investment that can reduce farmers' incomes and increase consumers' expenses, thus adversely affecting the people's economic access to it.

2.11 Postharvest Disease Management Strategies

Postharvest diseases can be managed in so many ways. These include but are not limited to:

2.11.1 Good Agricultural Practices

Successful storage starts with high-quality roots. Events occurring during the growing season may later negatively affect postharvest quality. Some factors such as weather are impossible to control, whereas others (such as fertilization) can be manipulated by a grower to ensure that a quality product goes into storage. Following approved Good Agricultural Practices in the field, inspecting sweet potatoes as they are harvested, leaving roots with indications of established disease (lesions) or obvious defects such as growth cracks or excessive skinning in the field. Gentle handling and minimization of environmental stresses can substantially reduce the level of postharvest disease, conducting producer/consumer awareness campaigns about how to mitigate microbial spoilage.

Field practices control some postharvest diseases. *Fusarium* root rot, *Fusarium* surface rot, and black rot are just three diseases that start as infections in the field but develop symptoms in storage. Growers can reduce losses from these diseases by avoiding fields with a known history of disease and by using slips (plant cuttings used as transplants) that have been cut instead of pulled, which avoids transferring disease from the plant bed into the field (Plate 2.2).

Proper curing is also essential to controlling many diseases. This is done above the soil line to avoid contact of the knife blade with soil. A contaminated blade may transfer disease organisms from the soil to the cut ends of slips.

Plate 2.2: Proper cutting of slips

Source: Brooke *et al.* (2003).

2.11.2 Proper Handling

Proper handling of sweet potatoes pays**.** Studies show that significant postharvest losses to spoilage occur because of improper handling and other factors. Providing consumers with an acceptable product demands attention to the unique postharvest requirements of sweet potatoes.

2.11.3 Prestorage Treatment of Sweet Potatoes

Typically, sweet potatoes are stored and eaten fresh. However, there are some simple methods used to increase their storability that can be used in conjunction with other storage methods. Pre-treatment of sweet potato can help to minimize risk of losses. Drying and curing of sweet potatoes are two common methods to prepare them prior to storage. Rapid deterioration in the condition of the produce is a result of exposure to environmental conditions and is exacerbated by mechanical damage during handling and transport. Curing can toughen the skin and heal minor physical damages while drying can reduce spoilage and inactivate metabolic degradation. Sweet potatoes can lose up to 1–2 months of storage time because of wilting and root rot, which can affect 15% to 65% of the crop.

Drying of roots is done on those that are too damaged to be stored fresh but still have edible materials on them. This involves slicing tubers to a thickness of approximately 2–4 mm and then

laying them out in the sun for four days or until they are rid of most of their moisture. During drying the sweet potatoes can be covered in prickly bushes or thorns to ward off animals. Drying removes moisture, reduces bacterial growth, and inactivates metabolic processes and enzymatic decomposition.

Curing is also an important pretreatment of roots. Even with extreme care in harvesting and handling, a wound often occurs at each end of the root as a result of digging. These wounds and other breaks in the skin create areas where disease-producing organisms can enter the root. Therefore, these wounds must be allowed to heal over as soon as possible. Curing is a technique done to toughen up the outer layer of skin and thus excessive moisture loss, entry of microorganisms into the plant and even facilitates healing of mechanically damaged tubers. A common method to cure without use of incubation equipment is to wrap tubers in black polythene sheets and leave out in the sun for 5 days. The black sheet keeps moisture in and collects heat to reach necessary conditions for curing. Immediately following curing the temperature must be dropped quickly. Altenatively, grasses or straw can be used as insulating materials and the pile covered with woven grass mats. Curing requires high temperature and high relative humidity, and this covering will trap self-generated heat and moisture. The stack should be left for about four days. Sweet potatoes can also be cured inside a protected structure at ambient temperature, provided the relative humidity is high in order to provide the warm humid conditions necessary for curing. The most uniform distribution of heat is obtained when heat is introduced near the floor level of a curing structure. Heaters can be placed on the floor near the bins ofproduce, or heat ducted in from outside the curing room. A high relative humidity can be obtained by wetting the floor or by using an evaporative cooler in the room without introducing outside air. A temperature of 80 degrees to 85 degrees and a relative humidity of 80 percent to 90 percent are ideal. These exact conditions will be hard to establish around the home, so a room or building that comes close to these conditions is usually selected.

2.12 Decay Control Treatments

Although every effort should be made to prevent mechanical injury to sweet potatoes during packing, it is impossible to avoid all injuries. Decay-producing organisms, especially those that cause soft rot (such as *Rhizopus stolonifer*), enter through injuries. Bruised or crushed tissue offers a particularly favorable place for decay to develop. For this reason, most sweet potatoes not destined for canneries or further processing are given decay control treatments. These treatments include an approved fungicide and irradiation.

2.12.1 Irradiation

The use of irradiation is a technology which was thought to have the potential to control postharvest diseases. Various types of electromagnetic radiation (UV light, X-rays, and gamma rays) have been studied for their ability to control postharvest diseases by killing the disease-causing postharvest pathogens of various commodities. However, irradiation has some limitations.Some important microorganisms are not killed at the maximum allowed dosage of radiation treatment. Factors such as temperature, atmospheric composition and physiological state of the produce at the time of treatment all play a role in the final outcome of the treatment.

2.12.2 Chemical Strategies

At present, the application of chemical agents remains the primary method of choice for the management of postharvest diseases. The strategies usually take the form of pre-and/or postharvest sprays, dip or drench treatments, and fumigation. Quite often postharvest pathogens infect produce before harvest. In such cases it is necessary to apply the fungicides in the field. In general, preharvest sprays control the surface inoculum, and provide preventative control of contamination and infection during harvest and postharvest. Fungicides applied during the postharvest process need to control latent infections and protect against infections which may occur along the postharvest handling chain,

including during storage. The application of postharvest fungicides is accomplished through the use of dips, sprays, fumigants, treated wraps and box liners or in waxes and coatings. Dips and sprays are commonly used and depending on the compound, these can take the form of aqueous solutions, suspensions or emulsions. The fungicides that are commonly applied as dips or sprays include the benzimidazoles (e.g. benomyl and thiabendazole) and the demethylation inhibitor fungicides (e.g. prochloraz and imazalil). Other fumigants used include carbon dioxide, ozone and ammonia.

Another fungicide is Mancozeb which in turn is classified as a contact fungicide with preventive activity. It inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those involved in the production of adenosine triphosphate (ATP). Mancozeb is used to protect many fruit, vegetable, nut and field crops against a wide spectrum of fungal diseases, including potato blight, leaf spot, scab on apples and pears, and rust on roses. It is also used for seed treatment of cotton, potatoes, corn, safflower, sorghum, peanuts, tomatoes, flax and cereal grains. Dar *et al.* (2013) reported that mancozeb proved to be the best for the growth inhibition of *F. solani* and *F. oxysporum*) and also achieved effective control of *Fusarium solani* using zineb. Combination of that cymoxanil + mancozeb, carbendazim + mancozeb and tricyclazole + mancozeb were proved the most effective and gave 100% growth inhibition of test fungus at lowest concentration of 500 ppm.

Though effective against postharvest fungi, fungicides that are principally used for controlling postharvest diseases have been well scrutinized as posing oncogenic and other major health related risks. Most fungicides can cause acute toxicity, and some cause chronic toxicity as well. The use of chemical pesticides has been known to cause various environmental and health problems (Ray and Ravi, 2005). To prevent losses from Rhizopus soft rot, a post-harvest disease incited by one of the major sweet potato pathogens, the majority of sweet potato packing houses make prophylactic applications of the fungicide dicloran (Botran) as a spray or dip treatment on the packing line.

Because the fungicide is applied so close to the time of consumption, the amount of residue left on the product is of great concern to regulatory agencies and consumers. Other limitations of chemical fungicides include not being readily available, not readily affordable to farmers and pathogen resistance to commonly used fungicides.

2.13 Alternative Control Strategies

Due to the perceived negative effects that chemical fungicides pose and the problem of fungicide resistance, there is an international demand for the discovery of safer alternatives that can control postharvest diseases adequately. The need to reduce the use of fungicides on export fruit has opened the door for innovative alternative measures ("green" alternatives) to control postharvest diseases. The successful development of alternative measures for decay control would provide a more environmentally friendly and consumer-acceptable substitute for the current synthetic fungicides and would provide a competitive advantage to the farmers and exporters in international markets. Biological control is one of the most promising alternatives to the chemical treatment of postharvest diseases. Over the last two decades, a number of biological control agents have been studied for their use on a multitude of pathogens and fruit crops.

2.13.1 Antagonistic Microorganisms

The use of antagonistic microorganisms has proved to be the most promising alternative for chemical control, and can be used either alone or as part of an integrated pest management strategy. An ideal antagonist has been described as being an organism which is genetically stable, can be effective at low concentrations and acts against a broad range of pathogens on various fruit commodities. The antagonist should have simple nutritional requirements, have the ability to survive in unfavourable environmental conditions and should be able to grow on cheap substrates in fermenters. In addition, an ideal antagonist should be one that lacks pathogenicity for the host plant

and does not produce metabolites that are toxic to plants and humans. It should also be resistant to the most frequently used pesticides and should be compatible with other chemical and physical treatments. An effective antagonistic microorganism that possesses the above traits will work against pathogenic organisms by either the production of antibiotics, competing for nutrients and space, parasitism or direct interaction with the pathogen, or by inducing resistance within the host tissue.

Some of the commercially available products for postharvest use, namely: “Biosave” (*Pseudomonas syringae* Van Hall) registered in the USA and used for the control of sweet potato and potato diseases and “Shemer” (*Metschnikowia fructicola*) which is registered in Israel and used commercially for the control of sweet potato and carrot diseases (Droby *et al*., 2009)

2.13.2 Secondary Compounds in Plants

In recent years, plant bioactive substances have been studied as a new approach to postharvest disease management. Plants produce an array of secondary metabolites, which in many cases have been found to be biologically active and a rich source of antimicrobial, allelopathic, antioxidant and bioregulatory properties. Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. These groups of compounds show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms. Phenolic toxicity to microorganisms is due to the site(s) and number of hydroxyl groups present in the phenolic compound. Flavones, flavonoids and flavonols are phenolic structure with one carbonyl group and are synthesized by plants in response to microbial infection and are often found effective in vitro as antimicrobial substance against a wide array of microorganisms. The presence of an active component at a particular time is determined by factors such as environmental conditions, the period during which the plant part was collected, method of drying the plant part, storage condition and isolation methods.

Advantages of plant botanicals over others include:

1. Naturally occurring biologically active compounds from plants are believed to be more adaptable
2. These plant products are biodegradable compounds which could be used in an integrated pest management program.
3. Many have shown low mammalian toxicity.
4. Sustainable solutions in agriculture
5. Reduce crop losses
6. Eco-friendly
7. Organic farming
8. Cheaper

2.13.3 Plant Extracts

Owing to the drawbacks associated with the orthodox fungi/rot control approaches based on chemicals, focus in recent times has shifted toward exploitation of plant extracts as novel fungicides in plant protection (Okigbo and Omodamiro, 2006). Extraction methods involve separation of medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology. Solvents diffuse into the solid plant tissues and solubilize compounds of similar polarity. Quality of plant extract depends on plant material, choice of solvents and the extraction methods. Successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex

or dissociate. As the end product in extraction will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted. Initial screening of plants for possible antimicrobial activities typically begins by using the crude or alcohol extractions and can be followed by various organic solvent extraction methods. Water is universal solvent, used to extract plant products with antimicrobial activity. Variation in extraction methods are usually depend on the length of the extraction period, solvent used, pH of the solvent, temperature, particle size of the plant tissues and the solvent-to-sample ratio. The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction.

2.13.4 Methods for Evaluation of Efficacy of Plant Extract

In vitro antimicrobial susceptibility testing (AST) methods include Diffusion test, Agar well diffusion, Agar disk diffusion, Poison food technique, Bio autography, Dilution methods, Agar dilution, Broth micro dilution assay and Broth macro dilution assay. Many botanicals have been researched on and proved to possess antimicrobial properties; hence myriads of reports have been documented stating the uses of plant extracts to control plant diseases. Some plants tested with antimicrobial properties include *Chromolena odorata, Ocimum gratissimum, Moringa oleifera* and *Zingiber officinale* (Alum *et al*., 2014; Okigbo *et al*., 2009a; 2009b).

Allium sativum: Allium sativum, commonly known as garlic, is native to central Asia. It was known to ancient Egyptians, and has been used for both culinary and medicinal purposes since their time (Harris *et al*., 2001). *Allium sativum* belongs to the family *Alliaceae*. A garlic plant may grow to be 30-90 cm tall. The bulb below ground is the main part of the plant and is divided into segments called cloves, with each bulb containing between 6- 12 cloves. The flower cluster varies in colour from purplish white to pale pink or a reddish white, according to the variety, soil and chemical influences.

Garlic's recognizable smell is derived from its sulphur-containing constituents. Garlic (*Allium sativum* L.) has been used for centuries for culinary purposes and its medicinal properties in traditional and conventional medicine are well documented. In India, it has been used to prevent wound infection and food spoilage. Garlic has proven to be effective against a host of gram-positive, gram-negative and acid-fast bacteria, including *Pseudomonas*, *Proteus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Klebsiella*, *Micrococcus*, *Bacillus subtilis*, *Clostridium*, *Mycobacterium* and *Helicobacter.*

The value of garlic as a deterrent to plant pathogens has been investigated. Extracts of garlic have been shown to have a strong inhibitory effect on the mycelia development of plant pathogenic fungi such as *Fusarium solani*, *Rhizoctonia solani*, *Pythium ultimum* and *Colletotrichum lindemuthianum.* The antibacterial, antifungal, antiviral and antiprotozoal effects of garlic has been ascribed to the constituents of the plant including the non-volatile sulphur containing compounds such as alliin (S-allyl-L-cysteine sulfoxides) (the main active constituent of garlic)which when crushed, converts to allicin (thiosulphinate) through enzymatic reactions (Amagase, 2006), other sulphur-containing compounds such as ajoene, diallylsulfide, dithiin, S-allylcystein; enzymes, B vitamins, proteins, minerals, saponins, flavonoids, and Maillard reaction products (which are non sulphur-containing compounds); phytoalexin (allixin) (non-sulphur compound with a γ-pyrone skeleton structure); other thiosulphinate and oil-soluble components such as ajoenes, vinyldithiins and sulphides such as diallyl sulphide (DAS), diallyl disulphide (DADS), and diallyl trisulphide (DATS) (that also contribute to garlic's characteristic flavour and odour and biological properties. The intracellular effects of allicin are not well understood; however, it is known that allicin has sulfhydryl modifying activity and is therefore capable of inhibiting sulfhydryl enzymes. Garlic extracts have also been shown to decrease oxygen uptake of microbes, reduce the growth of

pathogenic organisms, and to inhibit the synthesis of lipids, proteins and nucleic acids and damage to membranes of microorganisms (Harris *et al*., 2001).

Zingiber officinale: Ginger (ginger rhizome) is the root of the *Zingiber officinale* plant, which can be utilized as a medication or as pleasant condiments. Ginger derived its name from the genus (*Zingiber officinale)* and the family (Zingiberaceae). It is a perennial plant with a slender stem about 24 – 39 inches in height. The underground stem of ginger (fibrous and approximately arid roots if fully developed) is the most familiar part of the plant and it is extensively used for commercial as well as domestic purposes. The plant is reported to have antibacterial, anti-oxidant, antiprotozoal, anti-fungal, anti-emetic, anti-rhinoviral, anti-inflammatory, anti-insecticidal activity. The herbal therapeutic benefits of ginger are mainly due to the presence of volatile oils and the high oleoresin content. A compound known as *gingerol* is an acrid chemical constituent of the ginger and it is responsible for the hot taste of ginger and its stimulating effect on the human body. Investigation was carried out to test the potency of some plant extracts for the control of yam tuber rot caused by *Fusarium oxysporum, Aspergillus niger and Aspergillus flavus*. Hot water extracts obtained from leaf and seed of uda (*Xylopia aethiopica*) and ginger (*Zingiber officinale*) were fungitoxic against the fungi. The extracts suppressed the growth of these fungi in culture and reduced rot development in yam tubers (Okigbo and Nmeka, 2005).The crop contains volatile oil, phenols, alkaloid and mucilage. The herbal therapeutic benefits of ginger are mainly due to the presence of volatile oils and the high oleoresin content. A compound known as *gingerol* is an acrid chemical constituent of the ginger and it is responsible for the hot taste of ginger and its stimulating effect on the human body.

Moringa oleifera: Moringa oleifera Lam commonly referred as Moringa belongs to the family Moringaceae. It is also known as the horseradish tree, drumstick tree, saijhan, or Ben oil tree. It is a highly valued plant, distributed in many countriesof the tropics and subtropics. It is known as ‘sajna’

in Bangladesh; Zogele by the Nigerian Northerners who use the leaves for soup and salad vegetable. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β – carotene, amino acids and various phenolics. In an earlier study, it has been found that lectins isolated from the leaves of this plant possess both antimicrobial and cytotoxic activity. The antimicrobial activity of *Moringa oleifera* plant parts against postharvest pathogens had been demonstrated by several researchers (Alum *et al.,* 2014).

Garcinia Kola: Bitter kola (*Garcinia cola*) is also known as an African wonder nut. It comes from *Garcinia* cola trees, which belong to the family of *Clusiaceae* and it grows in the coastal rainforests in the South Western and South Eastern parts of Nigeria. It is an evergreen tree grown in the tropical rainforest of West Africa, Southern Nigeria and is further distributed by man and often found cultivated around villages. It grows to a height of about 30metres high, and the fruit, which is in the size of an orange, is smooth and reddish yellow with peach-like skin and yellow pulp and contains three or four seeds covered with brown seed coat. The seed is an edible nut, generally known and called Bitter Kola in Nigeria, and commonly called *"Namiji goro"* in Hausa, "*Orogbo"* in Yoruba and *"Aku-ilu"* in Igbo. Traditionally, these nuts were chewed as a masticatory substance, to stimulate the flow of saliva but are now widely consumed as snack in West and Central Africa. Bitter kola is also rich in caffeine and threobromine and is also believed to be an aphrodisiac. Unlike other kola nuts, bitter kola is believed to clean the digestive system, without side effects such as abdominal problems, even when a lot of the nuts are eaten. Many pharmacological effects have been demonstrated for the biflavonoids in *Garcinia kola* extractives. Amongst them are anti-Malarial, anti-viral, anti-inflammatory, anti-diabetic and bronchodilatory properties. Phytochemical and biochemical studies of *Garcinia kola* showed the presence of sterols, terpenoids, flavonoids, glycosides, pseudotannins, saponin, proteins and starch; kolanone, a poly-isoprenyl-benzophenone

compound in fruit pulp. It also contains ascorbic acid, some micro-elements including nitrogen, potassium, phosphorus, magnesium and calcium, and a trace amount of chromium. Hydroxycitric acid (HCA) is another medicinal constituent of *Garcinia kola*. Xanthones, xanthone derivatives, and polyisoprenylated benzophenones have also been isolated from Garcinia *kola.* Other constituents include Ash and Crude protein, Crude Fiber, Crude Lipid, water – soluble oxalate, terpenoids and fat. *Garcinia kola* also contains toxic substances such as tannins, phytic and hydrocyanic acids at a low concentration. From exiting literature, while many studies were shown to have highlighted the antimicrobial action of garlic, bitter kola, moringa and ginger on pathogens, about 99% of the evaluated plant extracts were assayed individually. In majority of the trials, the antimicrobial activity recorded by the plant extracts, though significantly higher than those of non-treated controls, were always far less potent than those of synthetic chemicals employed as treatment checks in such works. Moreover, the antimicrobial activity of plant extracts that is observed in *in-vitro* conditions is quite different from its effect in complex food systems. In most cases antimicrobial activity is decreased due to interactions with food components. This could be a challenge in utilizing plant antimicrobials, as a higher concentration could result in unfavorable changes to the taste and aroma of food. Despite these recognitions, little research has been done relating to the effect of the plant extract combinations on postharvest plant pathogens and specifically the postharvest pathogens of sweet potatoes in Ebonyi State.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Ebonyi State, South Eastern Nigeria with a total area of 5,533km (2,126sqm) located on Latitude $6^{0}15^{I}$N and Longitude $8^{0}05^{I}$E (See Figure 3.1). The state is populated primarily by Igbos with farming as predominant occupation. Ebonyi State is bounded to the North by Benue State, the west by Enugu State, to the south by Imo and Abia States and to the East by Cross River State. There are two distinct seasons, the wet and the dry seasons. The former takes place between April and October, while the latter occurs from November to March (Uneke *et al*., 2009). The state has pseudo-bimodal rainfall pattern spread from April to November with annual rainfall range between 1700mm-2060mm. The maximum mean annual temperature range is 27-36ºC all through the year. Humidity is high (60-94.3%) with lowest levels during the dry season in April before the wet season begins (Longinus, 2015). The state has 13 Local Government Areas (LGAs) out of which 4 LGAs (Ikwo, Ezza North, Izii and Ezza South) with high sweet potato production were selected as sampling location.

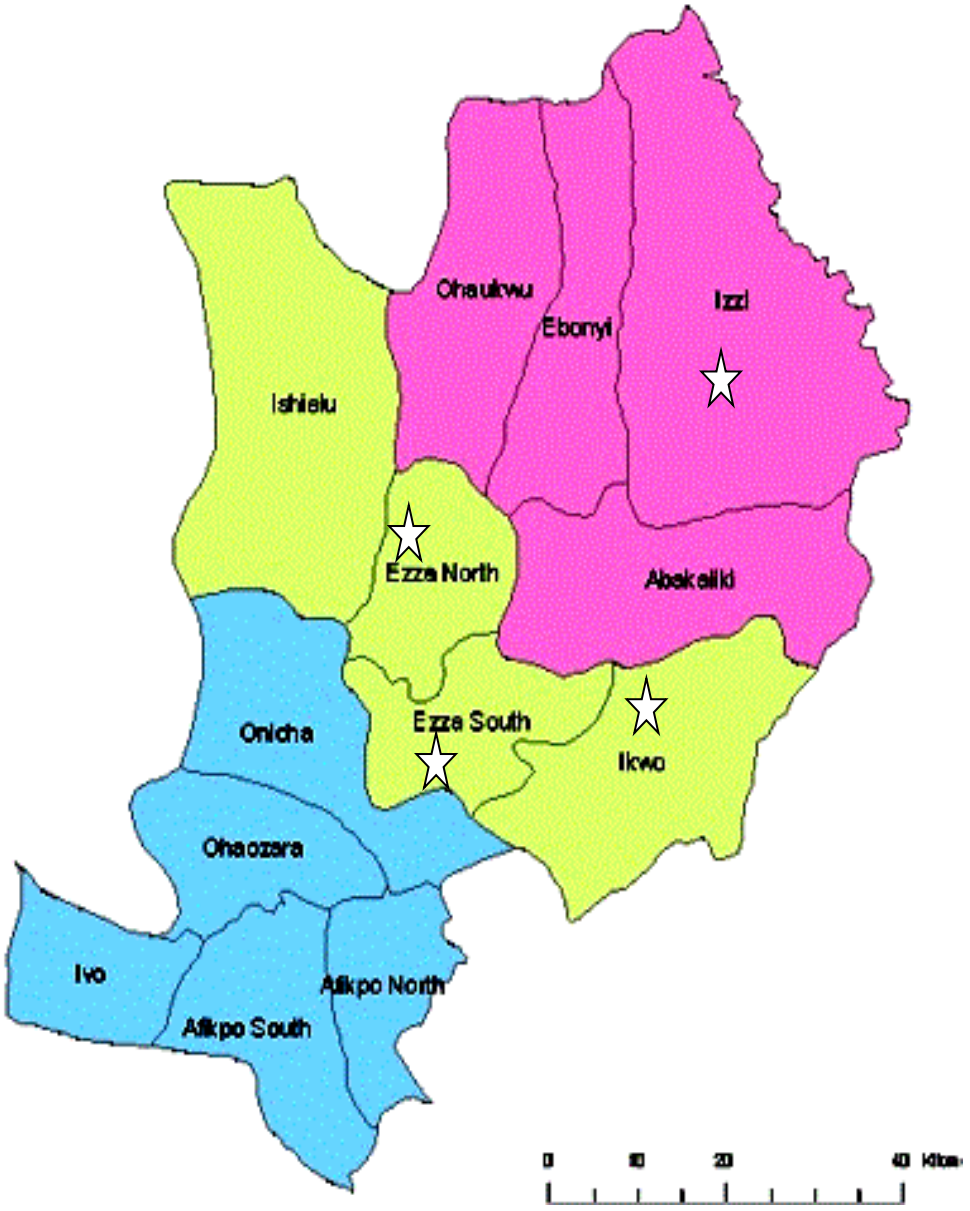

Figure 3.1: A map of Ebonyi State with all the Local Government Areas (LGAs).

The LGAs bearing the same colour are within the same Senatorial zones (the blue, yellow and pink colours represent Ebonyi South, Ebonyi Central and Ebonyi North Senatorial Zones respectively). The star ☆ indicates the sampled location site.

Source: http://www.satellitecitymaps.com/africa-map/nigeria-map/ebonyi-state-map/

Coordinates: $6^{0}15^{I}N\ 8^{0}05^{I}E$

3.2 Collection of Sweet potato Samples

A hundred fungi-infected sweet potato roots in four LGAs (25 samples from each LGA) were collected from Ebonyi sweet potato farmers in the dry season of 2015 (December). This was repeated in the wet season of 2016 (June), giving a total sample size of 200 fungi infested roots. In each of the seasons, healthy roots of two sweet potato cultivars ('Tupiaochi' and 'Oyorima') most commoly cultivated by the Farmers in Ebonyi State were bought from the farmers. In each case, collected sweet potatoes with fungal rot and the healthy sweet potato roots were packaged in clean polyethylene bags and jute bags respectively. The samples were labeled with date, collection site and sample number and taken to the Laboratory for analysis.

3.3 Preparation of Growth Medium

Thirty-nine grams (39g) of Potato Dextrose Agar (PDA) (Oxoid CM0139, Hamphire, England) were weighed and suspended in 1litre of distilled water, stirred to obtain a uniform mixture and autoclaved at 121^0C for 15 minutes. When the medium cooled to about 45^0C, it was amended using a broad spectrum antibiotic, Ampicillin (250mg); two capsules of which were dissolved in 2ml of autoclaved distilled water and added to 500ml molten PDA. This was gently shaken to obtain a fine mixture before pouring was done. The resulting mixture was then dispensed into 9mm diameter Petri dishes (autoclaved) when cooled and the poured medium stored at 4^oC and used when needed.

3.4 Study Design

The design employed in the study was Experimental Research Design. The set up were arranged in a Completely Randomized Design (CRD). Experiments were replicated three times. In pathogenicity evaluation and rot severity, the different fungal isolates served astreatments. For sweet potato cultivar susceptibility to fungal rot, the two sweet potato cultivars- '*Tupiaochi'* and '*Oyorima'* served as treatments because they were the most commonly preferred and cultivated varieties by

farmers in Ebonyi State. For effect of fungi rot on the root proximate compostion, the healhty and fungi infected roots of sweet potato served as treatments. For the control studies, the single and two by two combined extracts of *Allium sativum, Zingiber officinale, Garcinia kola* and *Moringa oleifera* served as treatments while mancozeb served as a positive control.

3.5 Fungal Isolation

The fungi associated with the rotten sweet potato roots were isolated using the point inoculation method described by Anukworji *et al.* (2012). Sweet potato samples were washed with clean water and the root tissues cut into sections of approximately 2mm cubes from the tissue at the junction between healthy and infested portion of root with knife, surface sterilized in 70% ethanol and then rinsed twice in sterile distilled water. The root piece was placed on sterile paper towels in a Laminar Flow Hood chamber for 10 minutes to dry. With sterile forceps, four to five-point plating was done in which the sweet potato sections were inoculated onto PDA plates (Plate 3.1) and the plates incubated at room temperature (28^0C) for five days and then examined daily for the development of fungi growth. When growth was established, different fungi colonies shown on a three to five day old cultures were sub-cultured through point inoculation in which agar plugs were taken from the growing tips of different colonies of fungi observed, with sterile needle and then transferred unto new plates. Plugs were placed downwards with the growing surface touching the PDA and the plates incubated at 27^0C. The Petri dishes of pure cultures of the fungi were then sealed with masking tape to prevent contamination and the pure isolates were used for further investigations.

Plate 3.1: Isolation of Fungi from Rot-Infested Sweet Potato Fractions

3.6 Identification of Fungal Isolates

3 6.1 Macroscopic and Microscopic Characterization of Isolates

Fungi identification was based on morphological characteristic described by Mathur and Kongsdal (2003) and Scot (2009). Macroscopic features such as texture of mycelia, spore or conidia-producing structures, type of pigmentation were observed from fungal tissues grown on PDA. Microscopic characteristics (spore and mycelium shape and colour) of the isolates were examined by Lactophenol Cotton Blue (LPCB) wet mount preparation widely used in staining and observing fungi. The preparation had three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures and cotton blue which stains the chitin in the fungal cells. A drop of Lactophenol cotton blue stain was placed on a clean slide and a small portion of each mycelial colony aseptically taken using a sterile inoculating needle, placed and teased in a drop of lactophenol cotton blue and covered with a cover slip. The slide was finally examined under the microscope at low and high power objectives for colony characteristic such as colour and texture of mycelia, type of pigmentation and microscopic characteristics of spores such as shape and colour.

3.6.2 Molecular Identification of Confirmed Pathogenic Fungi

After confirmation of fungi pathogenicity on sweet potatoes, some representative pathogens were subjected to molecular identification through the Partial Internal Transcribed Spacer Ribosomal Deoxyribonuceic Acid Sequencing Analysis and a Basic Local Alignment Search Tool (BLAST) search using the GenBank Sequence Database. Methodology was based on Polymerase Chain Reaction (PCR) and Sanger Sequencing Analysis (Obeninrader, 2003).

3.7 Evolutionary Relationships of Taxa

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the

evolutionary history of the taxa analyzed. In *Aspergillus flavus*, branches corresponding to partitions reproduced in less than 62% bootstrap replicates were collapsed. In *Aspergillus awamori*, branches corresponding to partitions reproduced in less than 38% bootstrap replicates were collapsed. In *A. flavus*, branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. In *R. oryzae*, branches corresponding to partitions reproduced in less than 33% bootstrap replicates were collapsed. The evolutionary distances were computed using the Kimura 2-parameter method. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2015).

3.8 Determination of Fungi Pathogenicity

Fungi pathogenicity was evaluated through the methods of Amienyo and Ataga (2006) (Plate 3.2) with healthy roots of '*Oyorima'* sweet potato cultivar obtained from the famers in Ebonyi State. Fresh, healthy sweet potato roots were washed with tap water, rinsed with distilled water, weighed and surface sterilized with 70% ethanol. Cylindrical discs were removed from the root with a sterile 4mm cork borer. A disc of a five-day old pure culture of each test isolate was inoculated into the hole created in the roots with the aid of another cork borer (4mm diameter). After inoculation, the parts of the tissue bore out were carefully replaced, sealed with sterile vaseline to prevent contamination and labeled accordingly.

The inoculated roots were placed in clean polyethylene bag (one root per bag) each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated for 7 days at room temperature (28 ± 2°C). The same procedure was used for the control except that discs of un-inoculated PDA were placed in the holes created in the tubers. After the incubation period, the sweet potato roots were weighed, incised horizontally with sterile knife and examined for infection and disease development. The fungi were re-isolated from the infected sweet potato roots and compared with the original isolates. This experiment was replicated three times.

Rots were categorized using the descriptions of Amusa *et al.* (2003) and Brooke *et al.* (2003).

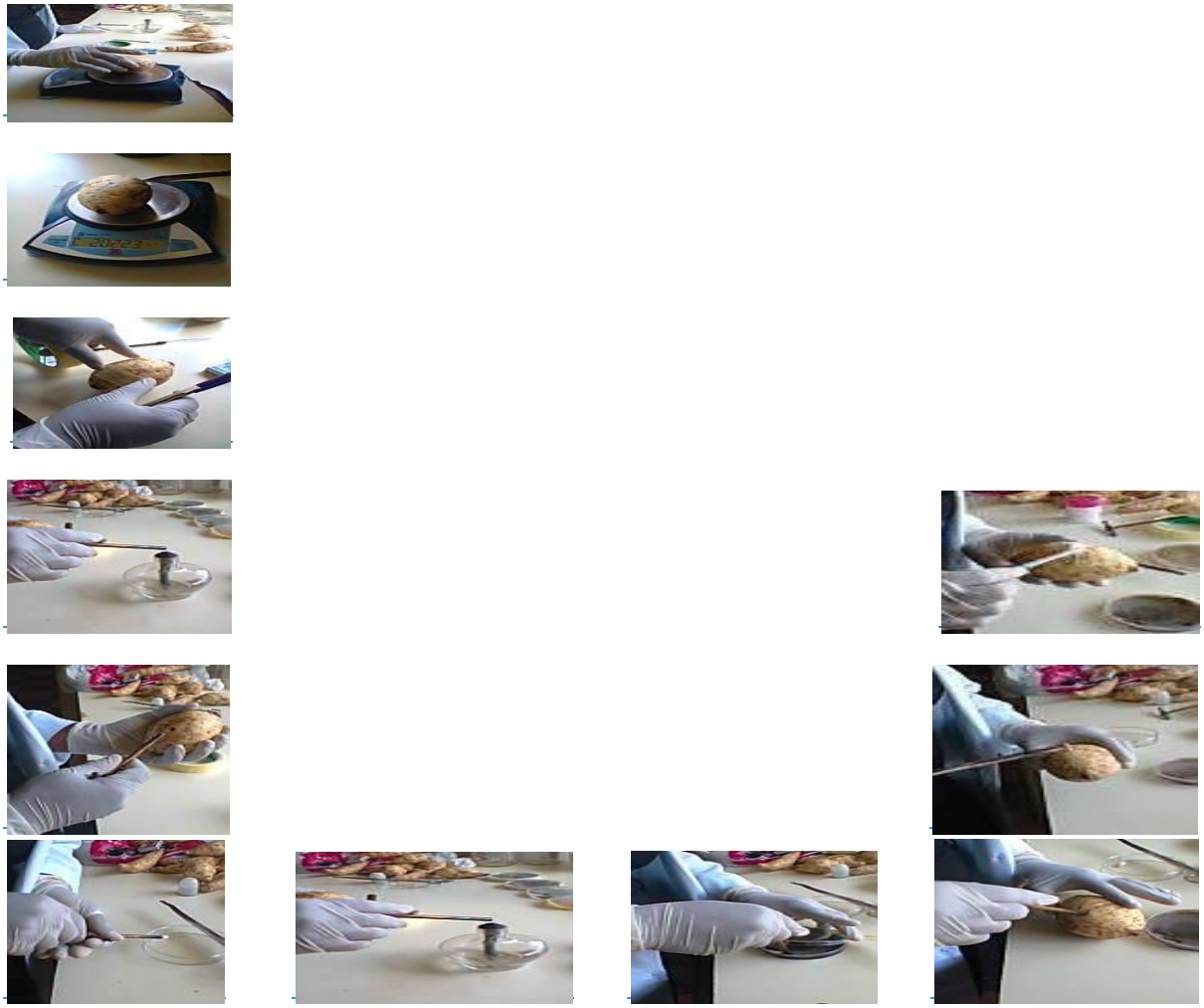

Figure 3.2: Pathogenicity Evaluation of Fungi Isolated from Sweet Potato Roots

A) The weighing of the sweet potato root. However, prior to this, the roots were washed with tap water, rinsed with distilled water, weighed and surface sterilized with 70% ethanol.
B) The labeling of the root
C) Heat sterilization of the cork borer
D) Removal of cylindrical disc from the sweet potato root
E) Placement of part of the tissue bored out from the sweet potato root in a sterile petri dish
F) Heat sterilization of the cork borer
G) Collection of inocula from the culture plate using a sterile cork borer
H) Inoculation of test fungus into the cylindrical hole made on the sweet potato roots
I) Replacement of the part of the tissue earlier bored out while creating the cylindrical hole
J) The sealing of the inoculation point with sterile vaseline to prevent contamination

3.9 Determination of Postharvest Fungi Occurrence

The occurrence of the fungi isolated from rotted sweet potatoes roots was computed as Percentage Frequency of Isolation (PFI) using the method of Ilondu (2013):

$$\% \text{ PFI } = \frac{\text{No. of times a fungus is encountered}}{\text{Total no. of times all fungi were encountered}} \times 100$$

3.10 Determination of Fungi Severity

Healthy roots of two sweet potato cultivars ('*Tupiaochi'* and '*Oyorima'*) most commonly cultivated by Ebonyi sweet potato farmers were collected from the farmers and subjected to rot induction using the test fungi -*Botryodiplodia theobromae, Fusarium solani, Aspergillus flavus, Aspergillus niger, Penicillium expansum, Rhizopus oryzae* and *Aspergillus awamori* according to the method of Amienyo and Ataga (2006) as already outlined in Sub-Section 3.9 (Fungi Pathogenicity); after which fungi severity and sweet potato cultivar susceptibility was determined as described by Ogbo and Agu (2014) and Lewthwaite *et al.* (2013) respectively. Fresh, healthy sweet potato roots were washed with tap water, rinsed with distilled water. The initial weight of the storage roots was determined. The roots were surface sterilized with 70% ethanol. Cylindrical discs were removed from the root with a sterile 4mm cork borer. A disc of a five-day old pure culture of each test isolate was inoculated into the hole created in the roots with sterile cork borer (4mm diameter), after the inoculation the parts of the tissue bore out were carefully replaced and sealed with sterile. The inoculated roots were placed in clean polyethylene bag (one root per bag) each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated for 7 days at room temperature (28 ± 2°C).

After incubation, the roots were individually weighed and cut open through the point of inoculation. The interface between rotted and healthy tissue was well defined in all infected roots, the rotted portions were removed from the whole roots and the final weight of the individual sweet potato roots taken.

The percentage severity of rot (Sr %) was calculated as described by Ogbo and Agu (2014) :

$$\text{Sr (\%)} = \frac{W - w \times 100}{W}$$

Where: W = Initial weight of healthy Sweet potato root; w = final weight of rotted tuber portion.
Pathogen Severity was ranked based on:

0 = none pathogenic fungi (no rots); 1= Very mild pathogen severity (1-5%); 2= Mild pathogen severity (10%); 3= Moderate pathogen severity (>10-25%), 4= High pathogen severity (>25-50%); 5 =Very high pathogen severity (> 50%)

3.11 Determination of Sweet Potato Cultivar Susceptibility

The susceptibility of the two varieties of sweet potato was determined using the Mean Weight of rotted tissue per cultivar (Lewthwaite *et al.,* 2013) (determined as the difference between the initial root weight after inoculation and the weight after rot removal).

Cultivar susceptibility was ranked based on:

0 = Resistant cultivar (no rots); 1= Very mild cultivar susceptibility (1-5g/root);

2= Mildcultivar susceptibility (>5-10g/root); 3= Moderate cultivar susceptibility (>10-25g/root);

4= Highcultivar susceptibility (>25-50g/root); 5 =Very high cultivar susceptibility (> 50g/root)

3.12 Effect of Fungi Rot on the Nutritional Quality of Roots of Sweet Potato Cultivars Grown in Ebonyi State

Healthy roots of two sweet potato cultivars (‘*Tupiaochi’* and ‘*Oyorima’*) most commonly grown by Ebonyi sweet potato farmers were collected from the farmers and subjected to rot induction using the method of Amienyo and Ataga (2006) outlined in Sub-Section 3.8 (Fungi Pathogenicity) with modification involving incubation of the pathogen-inoculated roots for two weeks instead of one week. The test fungi included *Botryodiplodia theobromae, Fusarium solani, Aspergillus flavus, Aspergillus niger, Penicillium expansum, Rhizopus oryzae* and *Aspergillus awamori*. Samples of the healthy roots of the same two sweet potato cultivars were stored for two weeks (within the same period the pathogen-inoculated roots were being incubated). Thereafter, the samples of the healthy and fungi infected roots of the two sweet potato cultivars were analyzed for nutritional quality including moisture, crude fibre, fat, ash, protein and carbohydrate and also dry matter.

3.12.1 Moisture Content Determination

This involves measurement of the weight loss due to the evaporation of water and was determined using the methods described by Chinedu and Nwinyi (2012). Roots were peeled, wiped and chopped into small pieces. Empty crucibles were washed, dried in oven, cooled in the desiccators and the weight of each crucible taken. Twenty grams of the undried sample were weighed into the pre-labeled sterile crucible of constant weight and the crucible with the sample placed in an oven set at 80°C for 2hr. This was removed and cooled in desiccators. After drying, using a measuring analytical balance, the weights were measured every one hour until a constant weight was obtained.

The loss in weight was taken as the moisture content and calculated thus:

$$\textbf{Moisture Content} = \frac{Loss\ in\ weight\ (W_2 - W_3)(g)}{Original\ weight\ of\ sample\ \ (W_2 - W_1)} \times 100$$

Where:

W_1= initial weight of empty crucible,

W_2= weight of crucible + food before drying,

W_3= final weight of crucible + food after drying.

3.12.2 Dry Matter Determination

Dry matter content (%) was calculated by 100 – Moisture content (%).

3.12.3 Ash Content Determination

The ash of a foodstuff is the inorganic component (minerals) of the sample after all moisture has been removed as well as the organic material burnt away. The method of Association of Official Analytical Chemist (AOAC) (2000) was used in determining the ash content. Three grams (3g) of the sample was weighed into a crucible of known weight and placed in a furnace which was ignited for about 24 hours until grey ash was obtained. The crucible containing the ash sample was removed from the furnace, cooled in the dessicator and then weighed using the analytical balance.

$$\%\ \textbf{Ash} = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

3.12.4 Protein Content Determination

Protein content was determined in the form of Total nitrogen using the method of AOAC (2000).Two grams of dried and powdered samples were weighed in duplicate, digested with H_2SO_4 and K_2SO_4/Se catalyst tablets, using the block digestion method in a Foss Tecator Auto Digestor. The resulting digest was steam distilled into boric acid using a Labconco Rapid Still II. Titration of the distillate with standard HCl was used to estimate crude protein.

3.12.5 Fat Content Determination

The acid hydrolysis method of AOAC (2000) was used to determine the fat content. Eight grams (8g) of samples were weighed in duplicate and digested in acid and the digests transferred to flasks where the fat was extracted with ethers. The ether extract was transferred into previously dried and weighed flasks and the ethers evaporated. The remaining fat was dried and weighed and determination made for the % fat content which was calculated as weight of extracted lipid over weight of dry sample multiplied by 100.

$$\% \, Fat = \frac{Weight \, of \, extracted \, lipid}{Weight \, of \, dry \, sample} \times 100$$

3.12.6 Crude Fibre Determination

The method of the AOAC (2000) was adopted in determination of crude fibre content of the samples. Defatted sample (3.00g) was put into a beaker (250 ml) and boiling water (200ml) with 1.25% H_2SO_4 added into the 250ml beaker containing the sample. The content in the beaker was heated and kept boiling for 30 minutes with constant stirring of the mixture using the glass stirring rod to remove all particles from the sides and top up with boiling water. The mixture while still hot was filtered with ashless filter paper fitted to a Buckner funnel. The beaker was rinsed with 50ml boiling water and washed through Buchner funnel to collect the precipitate. The precipitate in the funnel was washed until it was neutral to litmus paper. The residue was scrapped off from the ashless filter paper back into the 250 ml beaker and 200ml boiling water with 2.5% NaOH and was boiled for 30 minutes and during this period, boiling water was added from time to time to make up the 200 ml level and rewashed through Buchner funnel to collect the precipitate, after which the residue was washed with twice with 95% ethanol after which the final residue was dried in an oven to a constant weight and thereafter cooled in a dessicator.

The crude fibre content was calculated using the formula: % Crude Fibre=(X_1-X_2)/X_3

$$Crude\ Fibre = \frac{X1 - X2}{X3}$$

Where

X_1 = weight before,
X_2 = weight after,
X_3 = weight of sample

3.12.7 Carbohydrate Content Determination

The method of AOAC (2000) was used to determine the Carbohydrate content; which was done by subtracting the sum of total crude fibre, total fat content, total protein, ash and moisture from 100.

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

3.13 *In Vitro* Effect of Plant Botanicals against Sweet Potato Postharvest Fungi

3.13.1 Collection and Authentication of Plant Materials for Antimicrobial Evaluation

Plant materials used in this study were selected based on previous reports of their antimicrobial activities and are given in Plate 3.3 and Table 3.1 Bulbs of *Allium sativum*, roots of *Zingiber officinale*, and seeds of *Garcinia kola* were bought from Ndoro local markets in Ikwuano LGA, leaves of *Moringa oleifera* collected from Ishiadu, Ibeku, Umuahia North LGA of Abia state and their identification confirmed by Dr. Emeka Ohaeri, a Plant Taxonomist with the Department of Horticulture, NRCRI, Umudike.

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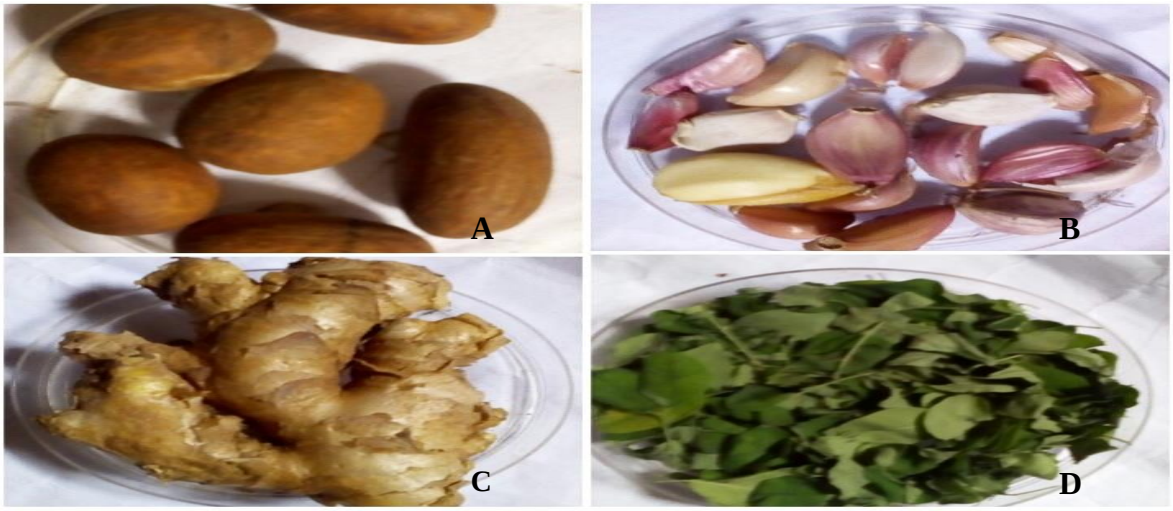


Plate 3.3: Plant Botanicals Used in the Experiment

A) Garcinia kola (Bitter kola) B) *Allium sativum* (Garlic) *C) Zingiber officinale* (Ginger)

D) *Moringer oleifera* (Moringa)

Table 3.1: Plants Used in the Experiment

S/N	Plant name	Botanical name	Family	Part used
1.	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
2	Bitter kola nut	*Garcinia kola*	Glusiaceae	Fruits
3	Garlic	*Allium sativum*	Liliaceae	Bulb
4	Moringa	*Moringer oleifera*	Moringacea	leaves

3.13.2 Processing and Preparation of Plant Extracts

After de-husking bulbs of *A. sativum* and peeling off the skin of *Z. officinale*, Bulds of *A. sativum,* fruits of *G. kola*, leaves of *Moringa oleifera* and rhizome of *Z. officinale* were washed in clean water, air dried at room temperature for two weeks and 100g of each weighed out separately and crushed in a blender. Powdered plant materials were suspended separately in methanol (1 L) in conical flasks, thoroughly shaken, and allowed to stand for 24 hours at room temperature. The contents were first filtered through cheese cloth and then through Whatman filter paper No.4. The filtrates were concentrated in a water bath, dried, weighed and stored in sterile beaker in the refrigerator until use.

The percentage yield was obtained using this formula W_2-$W_1/W_0 \times 100$.

Where W_2 is the weight of the extract and the container, W_1 the weight of the container alone and W_0 the weight of the initial dried sample

3.13.3 Preparation of Extracts Concentrations

The concentrate considered to be the 100% concentrate was diluted down to make up the required concentrations of 50mg/ml and 100mg/ml used for antifungal testing. This was carried out according to the method described by (Cheesbrough, 2006). Stock solution of the garlic, ginger, bitter kola and moringa methanol extracts were prepared by dissolving 0.6g of each plant extracts in 6ml of sterile distilled water in glass vial bottles. Therefore, each stock solution had a concentration of 100000µg/mL (100mg/mL). The stock solution was again diluted once to get one more varied extracts concentration in addition to it, making them two different concentrations of 100mg/ml and 50mg/ml.

3.13.4 Preparation and Standardization of Inoculum

These were done according to the Hematocytometer Spore Counting Method described by Eva *et al.* (2001). The test fungi included the confirmed postharvest fungal pathogens of sweet potato including *A. flavus, Penicillium expansum, A. awamori, A. niger, Rhizopus oryzae, Fusarium solani* and *Botrydiplodiae theobromae*. Fresh, mature (3- to 5-day-old) cultures of test fungi grown on Potato Dextrose Agar slants were suspended in sterile distilled water and sub-cultured from the stock water suspensions on Potato Dextrose Agar and incubated at room temperature (28^0C). The colonies were covered with 5ml sterile distilled water and tween 20 (5%) was added to facilitate the preparation of fungi inocula. For *Aspergillus* spp., the inocula were achieved by carefully rubbing the colonies with a sterile loop; the isolates were then shaken vigorously for 15sec with a Vortex mixer and then transferred to a sterile tube. For *Fusarium* that is a slowly sporulating fungus, the suspensions were obtained by exhaustive scraping of the surface with a sterile loop. Then, the inoculum was transferred to a sterile syringe attached to a sterile filter with a pore diameter of 11 mm (Millipore, Madrid, Spain). The suspension was filtered to remove the majority of the hyphae, producing inocula that were mainly composed of spores and collected in a sterile tube. The size of the inoculum was adjusted to 1.0 x 10^6 spores/ml by microscopic enumeration with a cell counting hematocytometer (Neubauer Chamber; Merck, S.A., Madrid, Spain).

3.13.5 *In vitro* Antimicrobial Screening

The antifungal activities of the plant extracts were determined by Food Poison/Radial Growth Techniques described by Hanafey *et al.* (2012) with a modification in fungi inoculation. Extracts were evaluated singly and in their combinations. For the purpose of assessing antifungal efficacy of single plant extracts, 50 and 100 mg/ml concentrations of each plant extract were assayed. For the

purpose of combined plant extract evaluation, only 100mg/ml of each plant extract was mixed to provide extract combinations at equal mixing ratio of 1:1 extracts.

The treatments evaluated are listed below:

i) Negative control (sterile distilled water)

ii) Positive control (mancozeb)

iii) 50mg/ml and 100mg/ml of *Z. officinale* alone

iv) 50mg/ml and 100mg/ml of *M. oleifera* alone

v) 50mg/ml and 100mg/ml of *G. kola* alone

vi) 50mg/ml and 100mg/ml of *A. sativum* alone

vii) Combination of 100mg/ml of *A. sativum* and 100mg/ml of M. *oleifera*

viii) Combination of 100mg/ml of *A. sativum* and 100mg/ml of *Z. officinale*

ix) Combination of 100mg/ml of *A. sativum* and 100mg/ml of *G. kola*

x) Combination of 100mg/ml of M. *oleifera* and 100mg/ml of *Z. officinale*

xi) Combination of 100mg/ml of M. *oleifera* and 100mg/ml of *G. kola*

xii) Combination of 100mg/ml of *Z. officinale* and 100mg/ml of *G. kola*

In the *in vitro* antifungal assay, a four equidistant section was created on each Petri dish by drawing two perpendicular lines at the reverse bottom of the plate, the point of intersection indicating the centre of the plate. An aliquot of 1 ml each of the extracts was separately introduced into the Petri dish containing 9ml PDA, carefully rotated to ensure even distribution of extract and allowed to set. A 4-mm cork borer was used to creat a well on the medium containing extract just at the point of intersection of the two previously drawn lines at the bottom of the Petri dish in three replicates. The standardized fungal inoculum (0.1 ml) was inoculated into the well. Negative and positive control experiments were set up with the addition of water and mancozeb (Mancozeb 75% WP) respectively,

each seeded in 1ml into 9ml PDA. The inoculated plates were left to stand overnight before incubating at room temperature (28^0C) for 3 days. After the incubation, the diameters of fungal growth in control and sample plates were measured. The Inhibitory effect of the extract was expressed as percentage inhibition and calculated using the formula:

$$\% \textbf{ Mycelial Inhibition } = \frac{(XC - YT) \times 100}{XC} \quad (1)$$

Where: XC = Average diameter of fungal colony in control plate (without plant extract)

YT = Average diameter of fungal colony with treatment (plant extract or mancozeb)

Extract Pathogen Interactions Rating: Single extracts were rated for their inhibitory effects using the scale: < 0% inhibition (not effective); >0-20% inhibition (slightly effective); >20-50% inhibition (moderately effective); >50-<100% inhibition (effective) and 100% inhibition (highly effective).

For Combined extracts, the synergism ratio for percentage inhibition was based on the Abbott formula as described in Burtram *et al.* (2015):

$$\textbf{Cexp} = (A + B + \ldots.. + n) - (AB \ldots n/100) \quad (2)$$

Where Cexp = expected efficacy of the mixture,

A and B and ….n = the control levels given by the individual single extracts from A to the last one (n) respectively making up the combination.

The synergy ratio (SR) between the observed (Cobs) and expected (Cexp) efficacies of the mixture was calculated as:

$$SR = Cob/Cexp \quad (3)$$

An SR >1.5 indicates a synergistic interaction between compounds;

0.5–1.5 indicates an additiveinteraction between compounds;

<0.5 indicates an antagonistic interaction between compounds.

3.14 *In Vivo* Effect of Plant Extract Combinations on Sweet Potato Rot

To determine if the antimicrobial-producing plant extracts combinations have a potential application in bio-preservation of sweet potato roots, a food trial was undertaken using the methods of Tijjani *et al.* (2013). Fresh, healthy sweet potato roots of the Tupiaochi cultivar were purchased from Ebonyi sweet potato farmers and used for the evaluation.

3.14.1 Treatments and Treatment Application

The treatments include negative control (sterile distilled water), positive control (Mancozeb), *A. sativum* and *M. oleifera* extract combination and *A. sativum* and *Z. officinale* extract combination. The healthy sweet potato roots were washed with tap water, rinsed with distilled water and surface sterilized with 70% ethanol. Cylindrical disc of 0.8cm were removed from each tuber with a sterile cork borer and treatments were applied as demanded by the method of assay. Evaluation was done via two methods- preventive and the curative methods.

3.14.2 Preventive Method of Fungal Rot Control on Healthy Sweet Potato Roots

The method of Tijjani *et al.* (2013) was adopted for this evaluation. Using a sterile cork borer, cylindrical disc of 4mm was removed from each root and the roots treated with plant extract concentrates, distilled water and mancozeb where applicable. After thirty minutes, the roots were then inoculated through transfer of a disc of five days old culture of the isolated fungus into the holes created into sweet potato roots.

3.14.3 Curative Method of Fungal Rot Control on Healthy Sweet Potato Roots

This evaluation was based on the method of Tijjani *et al.* (2013) with modification in the quantity of applied inocula (3×10^4 sporangia/ml in sterile distilled (Suleiman and Emua, 2009)

instead of a disc of pathogen lifted with the cork borer. In the curative method, cylindrical disc of 4mm was removed from each root using a sterile cork borer. The roots were first inoculated with 1ml pathogen sporangia suspension (3 x 10^4 sporangia/ml of sterile distilled water) and then later (after 12hrs) treated with the plant extracts and mancozeb. Control setups were two- positive and negative control. The negative control set up consisted non-treated, wounded and inoculated while the positive control setup was sweet potato roots treated with Mancozeb 80WP (2%). Vaseline jelly was used to completely seal each hole. The inoculated sweet potato roots were placed in sterile sealed containers and incubated at room temperature (28 ± 2^{o}C) for 14 days. After the incubation period, the tubers were incised horizontally with sterile knife. The length of rotted portion from each hole was measured over the total surface length with a metre rule and fungitoxicity determined in form of percentage growth inhibition which was calculated according to the formula of Okigbo and Nmeka (2005)

$$\text{Growth inhibition (\%)} = \frac{(LC - LT) \times 100}{LC}$$

Where: LC = average length of unrotted portion of control and LT = average length of unrotted portion with treatment.

3.15 Statistical Analysis

All data were analyzed by Analysis of Variance (ANOVA) using SPSS 20.0 Version. Differences between the treatment means were tested using Duncan's New Multiple Range Test. Significance was considered at P=0.05.

CHAPTER FOUR

RESULTS

All the 200 (100%) fungi-infested sweet potato samples yielded viable postharvest fungal pathogen. Isolated fungi and their colony and microscopic characteristics are presented in Table 4.1. Based on colony morphology and microscopic characteristics, five fungi genera (*Aspergillus, Fusarium, Rhizopus, Penicillium* and *Botrydiplodiae*) comprising seven fungi species (*Aspergillus flavus, Aspergillus niger, Fusarium solani, Rhizopus oryzae*, *Penicillium expansum, Botrydiplodiae theobromae* and *Aspergillus awamori)* were identified. One representative isolate of fungi from each species were selected for further investigation.

R. oryzae*:* The colonies of *R. oryzae*on PDA were fast growing with tendency to collapse, white and cottony at first (after 24 hours of incubation), then became heavily speckled with the appearance of sporangia and finally became brownish-grey to blackish-grey depending on the amount of sporulation (Plate 4.1).

Plate 4.1: Pure Culture of *R oryzae* Isolated from Sweet Potato from Ebonyi State

When viewed under the microscope, the fungi had broad hyphae with rhizoids and stolons bearing brown sporangiophores which smooth walled, septate, simple, at other times, branched. Sporagioa are globose, often with a flattened base, grayish black, powdery in appearance and heavily spored. Spores were lemon shaped, unlike *R. stolonifer* that is usually striated, elongated to polyhedric.

Furthermore, results of amplification and sequencing of isolate's Partial Internal Transcribed Spacer (ITS) rDNA generated a sequence 1028 bp long. The BLASTn analysis against the NCBI database of this nucleotide sequence resulted in 100% homology with *Rhizopus oryzae* (GenBank database Accession No. KJ439050). Moreover, the evolutionary relationship of taxa is presented in Figure 4.1. The bootstrap values are indicated by numbers above the branches while Bars indicate number of nucleotide substitutions per site. The present isolate infecting sweet potatoes in Ebonyi State is 2nd to the topmost *R. oryzae* strain.

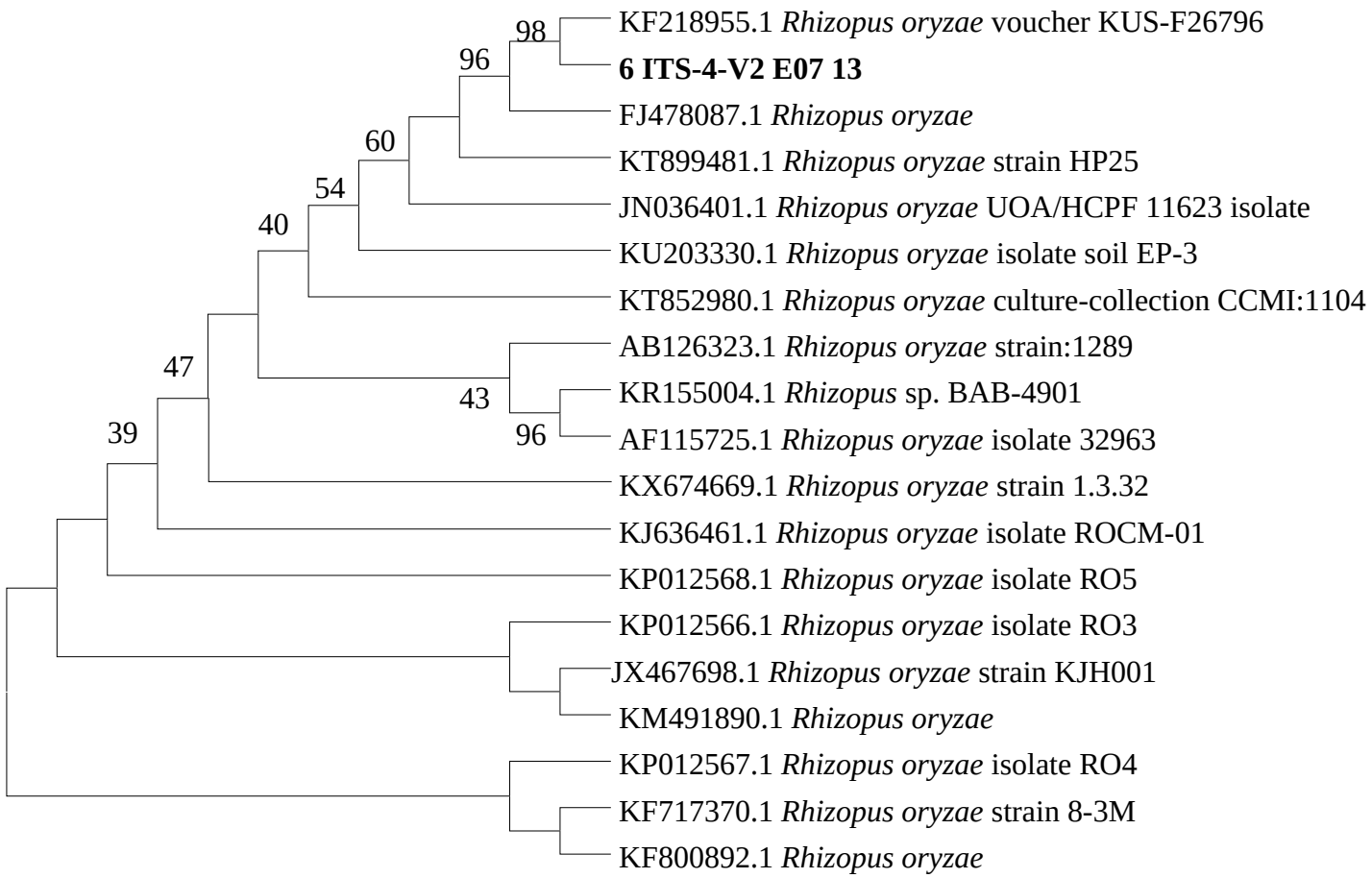


Figure 4.1: Phylogenetic Tree using Internal Transcribed Spacer Sequences showing Closest Known Relatives of *Rhizopus oryzae* Isolated from Sweet Potato from Ebonyi State

Aspergillus flavus: The colonies of *A. flavus*were effuse in nature and grew rapidly on potato dextrose agar. Old cultures were olive to lime green (Plate 4.2). When viewed under the microscope, the Spores produced were numerous, globose to subglobose, pale green and smooth in nature; conidiophores were hyaline and finely roughened.

Furthermore, results of amplification and sequencing of isolate's Partial Internal Transcribed Spacer (ITS) rDNA generated a sequence 1095 bp long. The BLASTn analysis against the NCBI database of this nucleotide sequence resulted in 100% homology with *Aspergillus flavus* (GenBank database Accession No KF908788). The evolutionary relationship of taxa is presented in Figure 4.2. The bootstrap values are indicated by numbers above the branches while Bars indicate number of nucleotide substitutions per site. The present isolate infecting sweet potatoes in Ebonyi state is the last isolate on the tree.

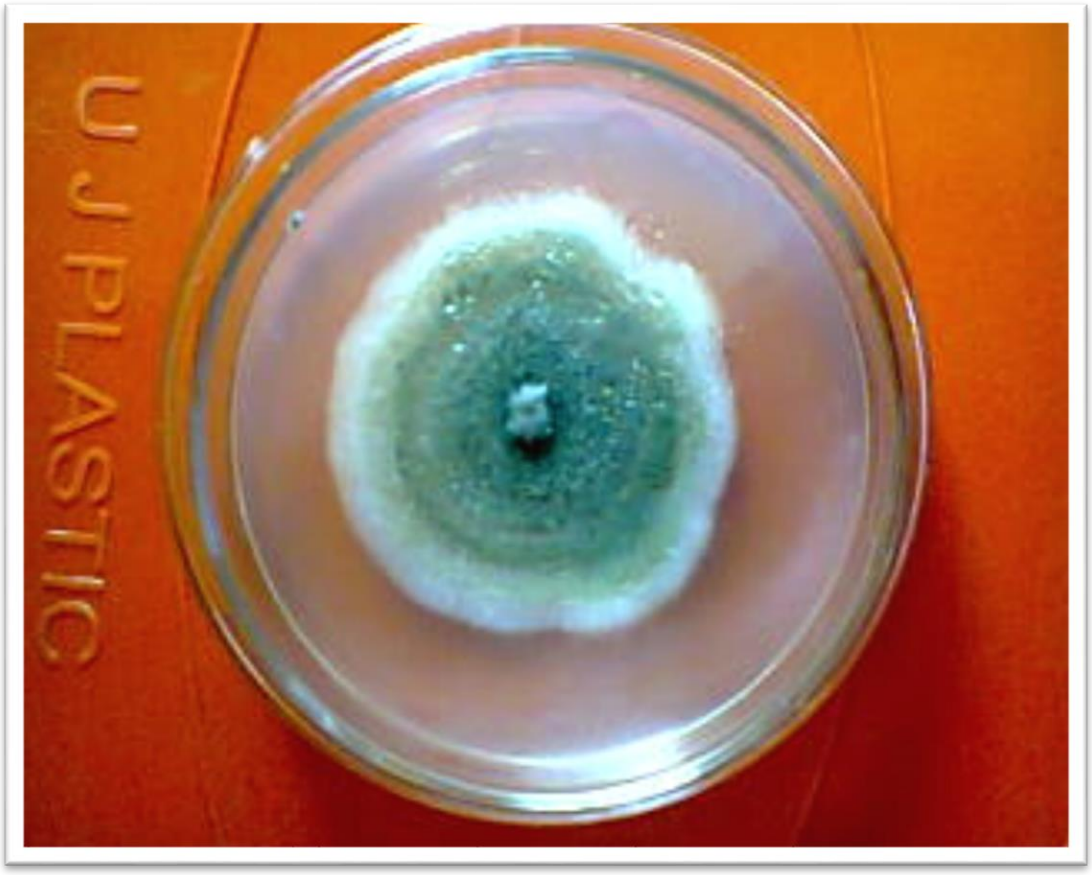


Plate 4.2: Pure Culture of *A. flavus* Isolated from Sweet Potato from Ebonyi State

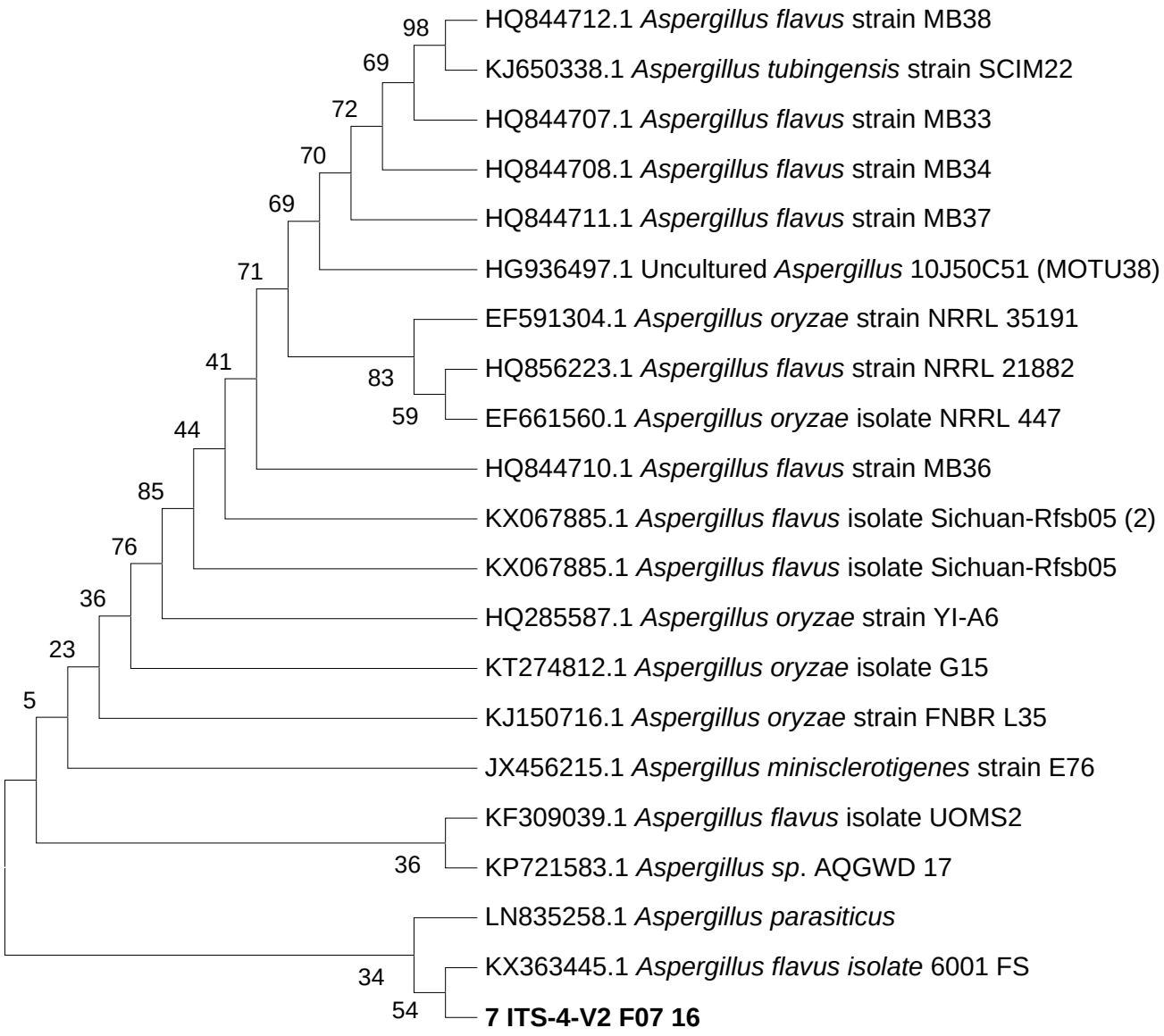


Figure 4.2: Phylogenetic Tree using Internal Transcribed Spacer Sequences showing Closest Known Relatives of *Aspergillus flavus* Isolated from Sweet Potato from Ebonyi State

Fusarium solani: Colonies of *F. solani* grew rapidly, aerial mycelium was white to cream, becoming bluish-brown when sporodochia are present (Plate 4.3). When viewed under the microscope, Spores were 3- to 5- septate, fusiform, cylindrical, and often moderately curved, with an indistinctly pedicellate foot cell and a short blunt apical cell.

Furthermore, results of amplification and sequencing of isolate's Partial Internal Transcribed Spacer (ITS) rDNA generated a sequence 842 bp long. The BLASTn analysis against the NCBI database of this nucleotide sequence resulted in 100% homology with *Fusarium solani* (GenBank database Accession No KJ863521). Moreover, the evolutionary relationship of taxa is presented in Figure 4.3. The bootstrap values are indicated by numbers above the branches while Bars indicate number of nucleotide substitutions per site. The present isolate infecting sweet potatoes was marked in bold font.

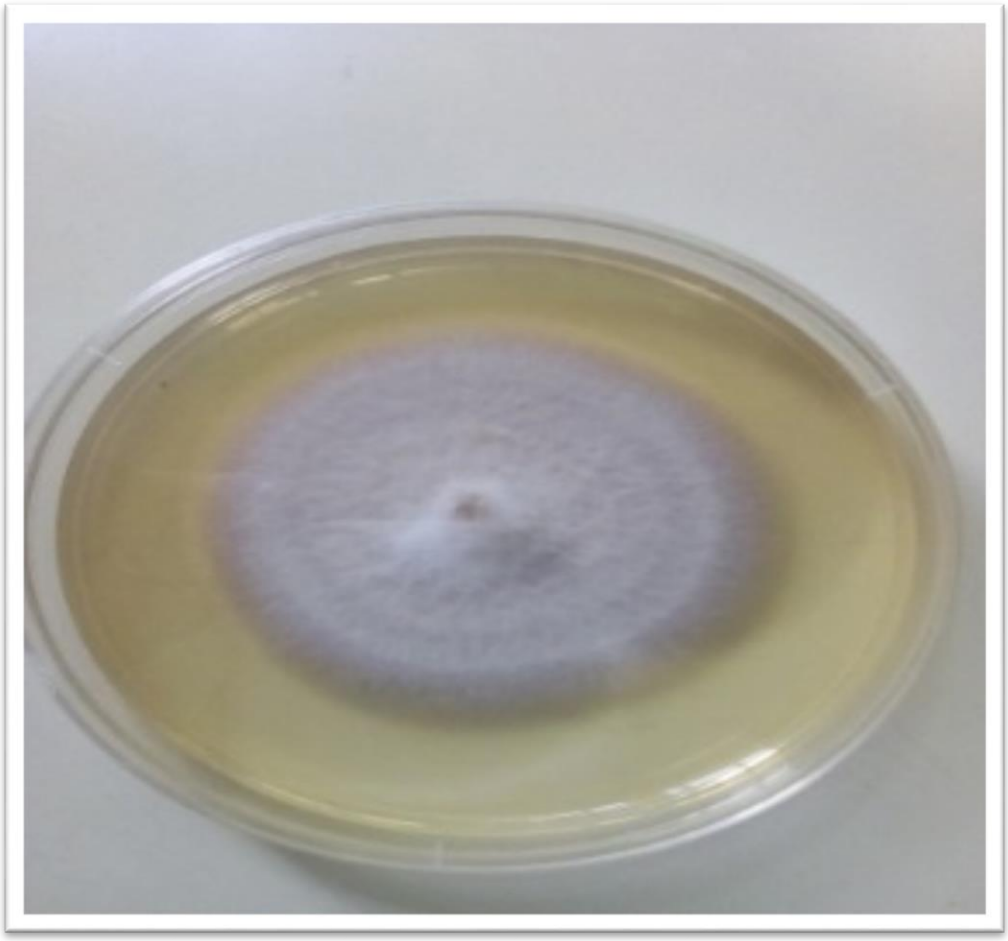


Plate 4.3: Pure Culture of *F. solani* Isolated from Sweet Potato from Ebonyi State

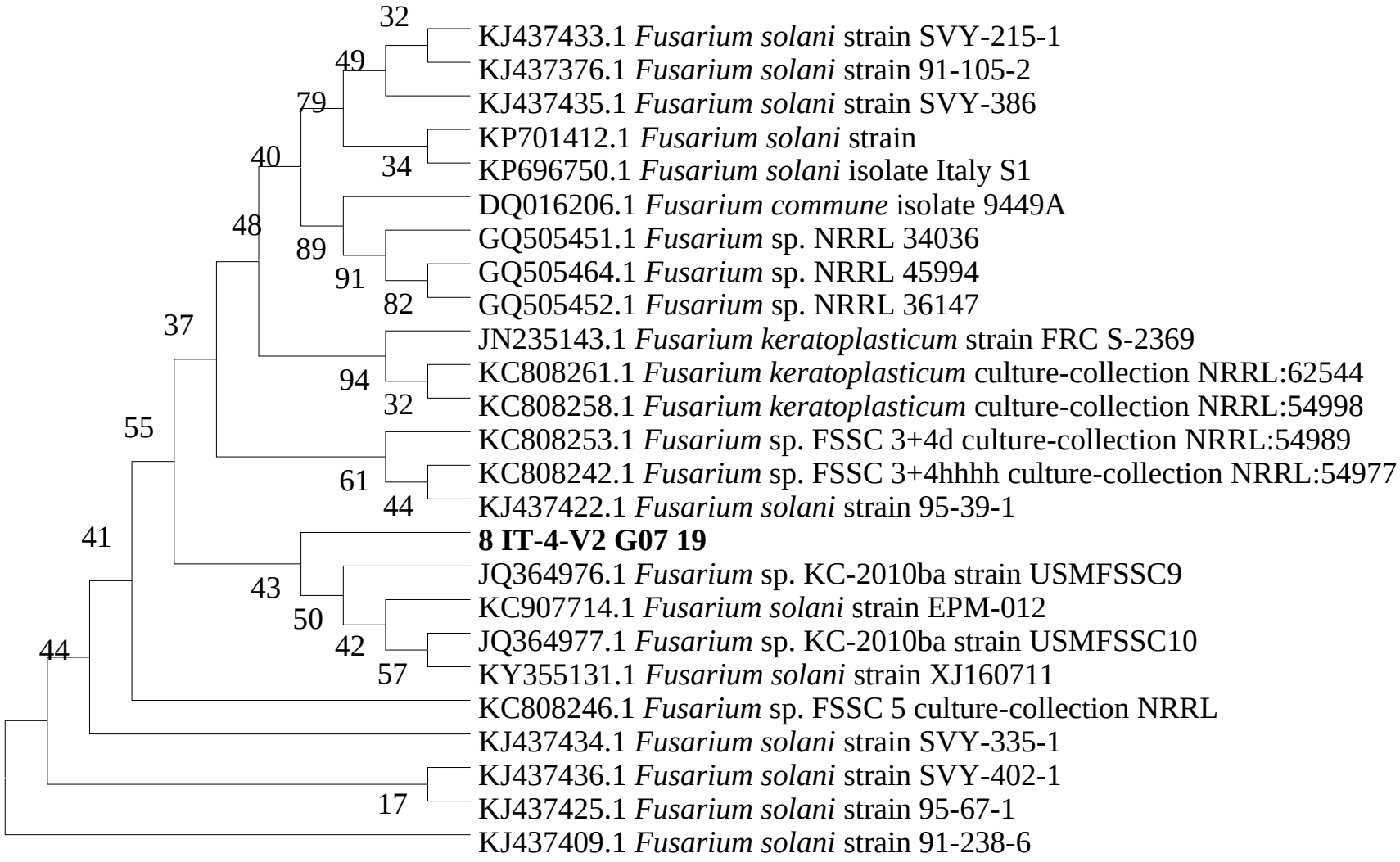


Figure 4.3: Phylogenetic Tree using Internal Transcribed Spacer Sequences Showing Closest Known Relatives of *Fusarium solani* Isolated from Sweet Potato from Ebonyi State.

Aspergillus awamori: Colonies of *A. awamori* were velutinous to floccose; conidial heads radiate or splitting into columns, light brownish olive to brownish olive, or dark brownish olive, mycelium white to dull yellow; exudate clear to dark diamine brown; reverse colourless to baryta yellow (Plate 4.7). When viewed under the microscope, Conidia globose to subglobose, 2.8-5.6 μm long, smooth to conspicuously rough, or with irregular ridges.

Furthermore results of amplification and sequencing of isolate's Partial Internal Transcribed Spacer (ITS) rDNA generated a sequence 960 bp long. The BLASTn analysis against the NCBI database of this nucleotide sequence resulted in 100% homology with *Aspergillus awamori* (GenBank database Accession No FJ441004). Moreover, the evolutionary relationship of taxa is presented in Figure 4.4. The bootstrap values are indicated by numbers above the branches while Bars indicate number of nucleotide substitutions per site. The present isolate infecting sweet potatoes in Ebonyi State is the last fungi down the tree.

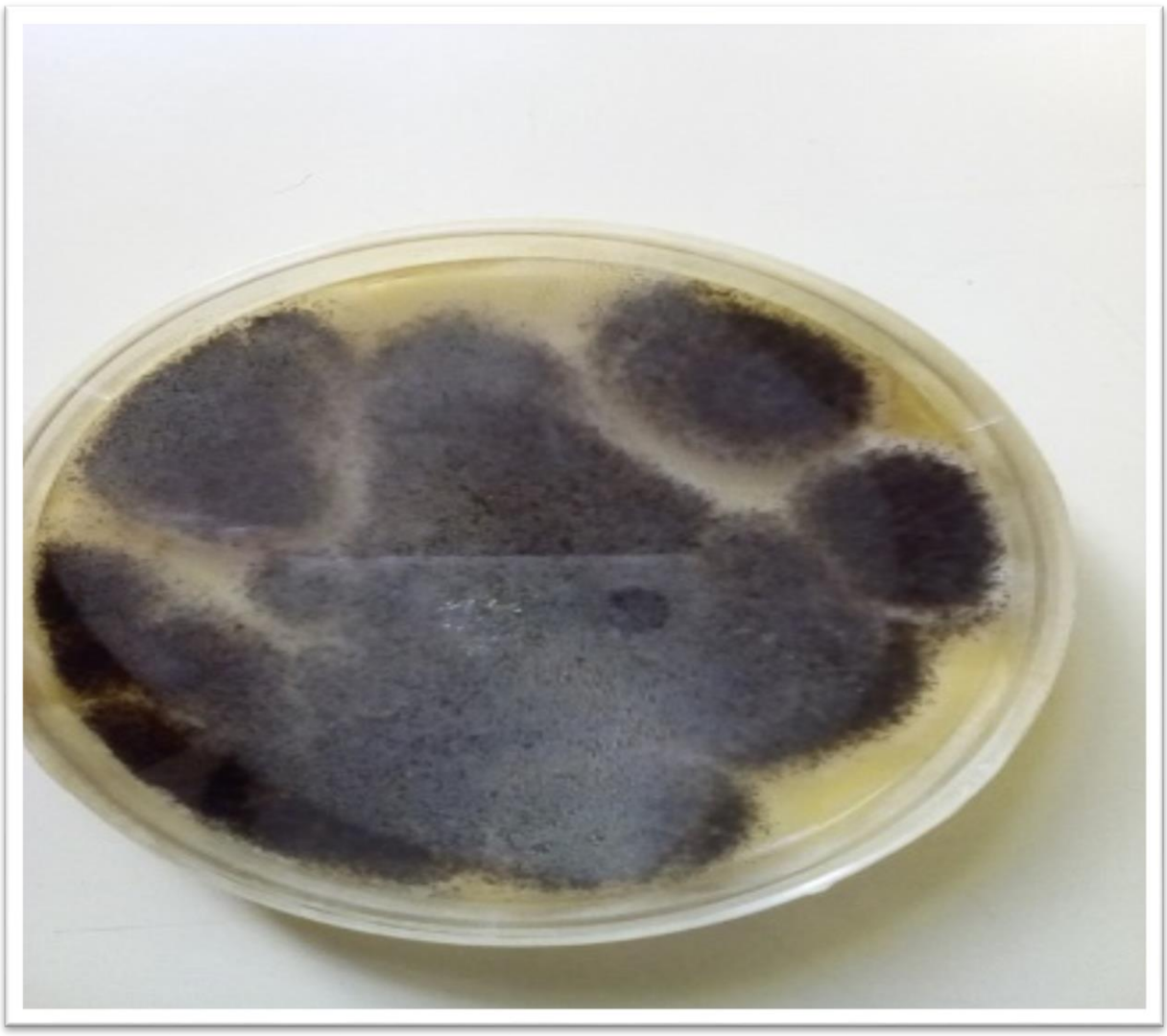


Plate 4.4: Pure Culture of *Aspergillus awamori* Isolated from Sweet Potato from Ebonyi State

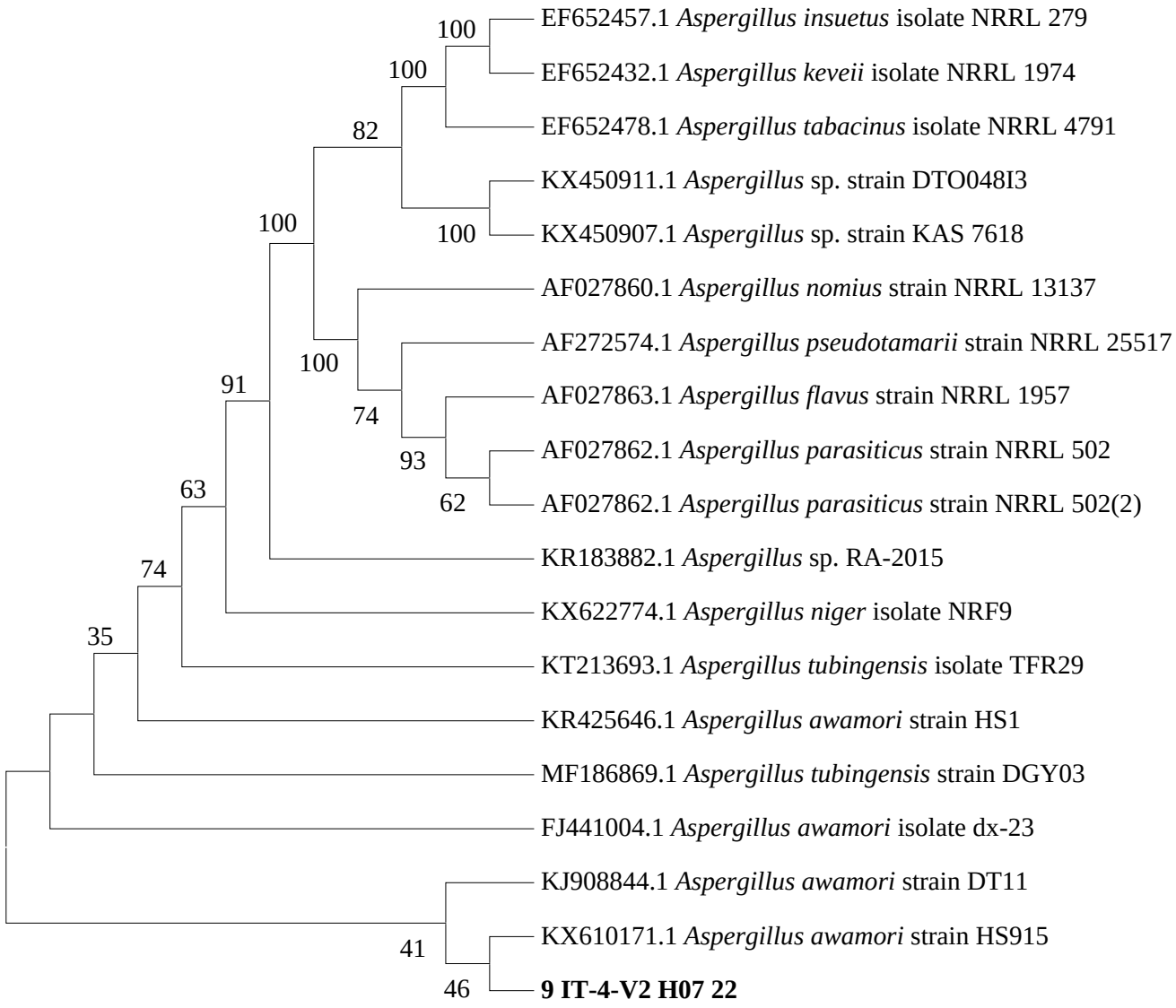


Figure 4.4: Phylogenetic Tree Using Internal Transcribed Spacer Sequences Showing Closest Known Relatives of *Aspergillus awamori* Isolated from Sweet Potato from Ebonyi State

Penicillium expansum: Isolate 5 failed DNA Amplification. However, macroscopic and microscopic characterization revealed it to be *Penicillium expansum* (Plate 4.5). Colonies were radilysulcate, moderately deep, texture floccose to weakly fasciculate; conidiogesis moderate, blue green to moderate bluish green; exudate clear to pale orange brown. Microscopically, conidiophores were borne from subsurface or aerial hyphae, single or in fascicles, appressed, stipes usually smooth walled, occasionally very finely rough walled, terverticillate, less common y biverticillate; rami cylindrical, phialidsampulliform; conidia ellipsoidal to subglobose, smooth-walled.

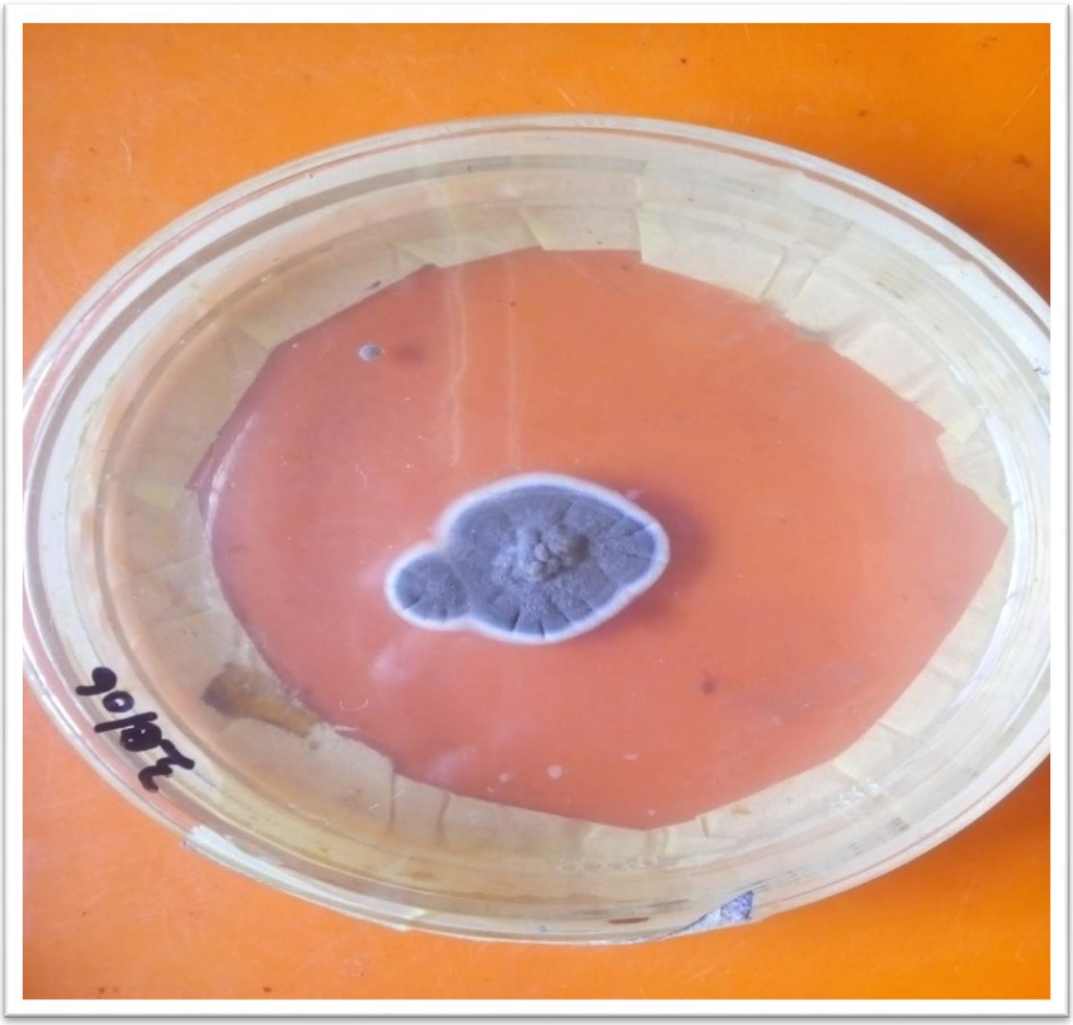


Plate 4.5: Pure Culture of *Penicillium expansum* Isolated from Sweet Potato from Ebonyi State

Botrydiplodiae theobromae: Macroscopically, the fungus had fluffy mycelia and grew rapidly. Young cultures were snow-white in nature, turning grayish with time. Older cultures turned black and produced pycnidia (black projections on culture) which were visible to the naked eye (Plate 4.6). Moreover, spores were big and oval. Mature spores were dark and 1-septate (single septum) when viewed under the microscope (Table 4.1).

Plate 4.6: Pure Culture of *B. theobromae* Isolated from Sweet Potato from Ebonyi State

Aspergillus niger: Macroscopically, the colonies of *A. niger* were initially white and quickly turned black with white edges as conidial production started. Its growth was rapid, produced radial fissures in the agar (Plate 4.7) with downy to powdery textures. Microscopically, conidia present were numerous, globose and very rough. Immature spores were brown and older spores turn black.

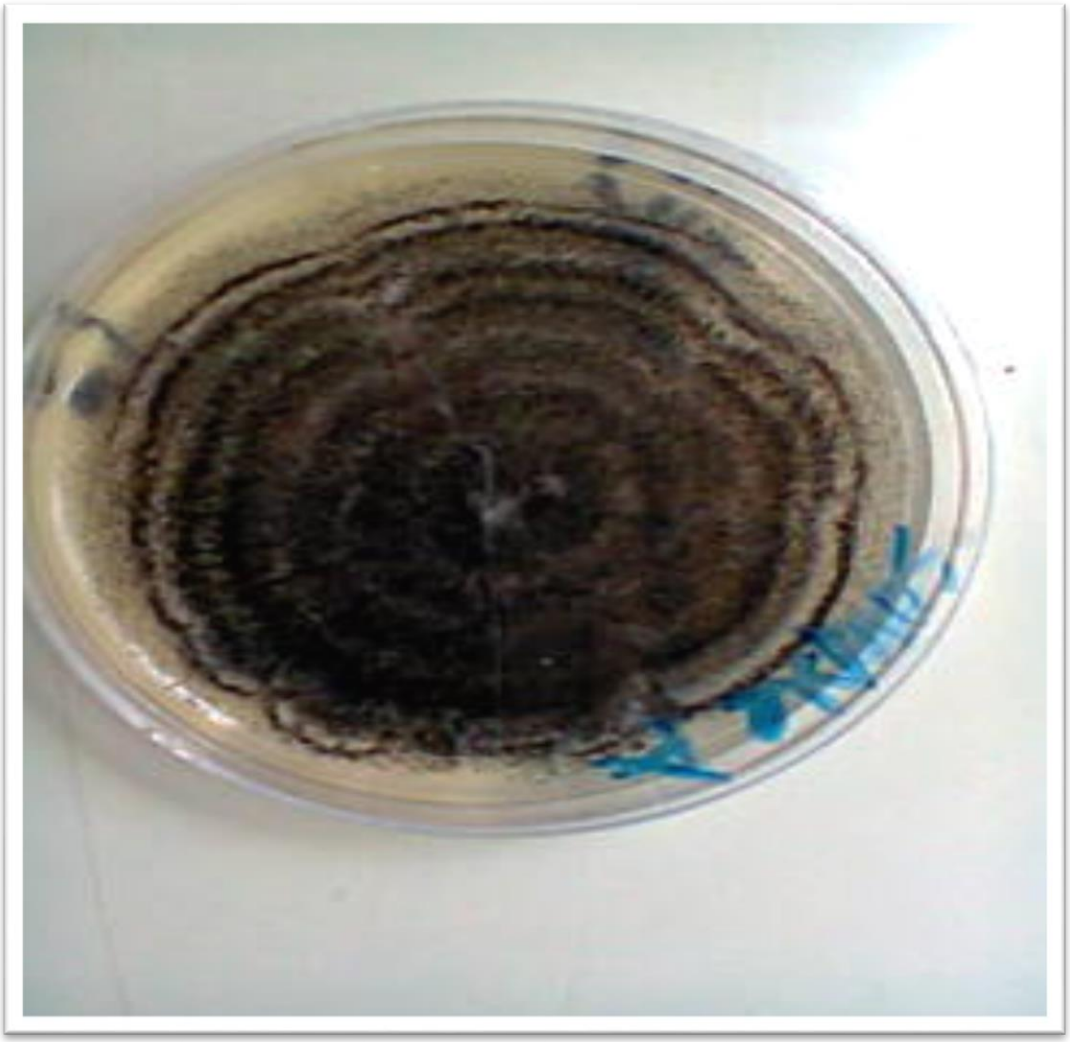


Plate 4.7: Pure Culture of *Aspergillus niger* Isolated from Sweet Potato from Ebonyi State

Table 4:1 Phenotypic and Microscopic Properties of Fungi Isolated from Rotted Sweet potato from Ebonyi State

Fungi	Colony Characteristic on PDA	Microscopic Characteristics
A. flavus	The fungus was effuse in nature and grew rapidly on Potato Dextrose Agar. Old cultures were olive to lime green	Spores produced were numerous, globose to subglobose and smooth in nature under microscope
A.niger	On PDA, the colony was initially white and quickly turned black as conidial production started; with pale yellowreverse.	Hyphae were septate and hyaline. Conidia head were radiate initially, splitted intocolumns at maturity. Conidia present were numerous, globose and very rough. Immature spores were brown and older spores turn black (Mathur and Kongsdal, 2003; Scot, 2009).
B. theobromae	The fungus had fluffy mycelia and grew rapidly. Young cultures were snow-white in nature, turning grayish with time. Older cultures turned black and produced pycnidia (black projections on culture) which were visible to the naked eye	Spores were big, ovoid to elongate. Mature spores were dark and 1-septate (single septum) when viewed under the microscope (Mathur and Kongsdal, 2003; Scot, 2009).
R.oryzae	The colonies were white and cottony at first, then became heavily speckled with the appearance of sporangia and finally became brownish-grey to blackish-grey and spread rapidly	Had broad hyphae, scarely septate with rhizoids and stolons bearing brown sporangiophores sometimes occurring alone, at other times in tufts and which diverged from the point of rhizoid formation. Spores were elongated to lemon shaped, unlike R. stolonifer that is usually striated, elongated to polyhedric (Mathur and Kongsdal, 2003; Scot, 2009). .
F. solani	Colonies growing rapidly, aerial mycelium white to cream, becoming bluish-brown when sporodochia are present	Spores were 3- to 5- septate, fusiform, cylindrical, often moderately curved, with an indistinctly pedicellate foot cell and a short blunt apical cell.
P. expansum	Colonies were radilysulcate, moderately deep, texture floccose to weakly fasciculate; conidiogesis moderate, blue green to moderate bluish green; exudate clear to pale orange brown; reverse light to vivid yellow.	Conidiophores borne from subsurface or aerial hyphae, single or in fascicles, appressed, stipes usually smooth-walled, occasionally very finely rough-walled, terverticillate, less commonly biverticillate; rami cylindrical, phialidsampulliform,; conidia ellipsoidal to subglobose, smooth-walled,
A. awamori	Colony was velutinous to floccose; conidial heads radiate or splitting into columns, light brownish olive to brownish olive, or dark brownish olive, mycelium white to dull yellow; exudate clear to dark diamine brown; reverse colorless to baryta yellow	Conidia globose to subglobose, 2.8-5.6 µm long, smooth to conspicuously rough, or with irregular ridges (Mathur and Kongsdal, 2003; Scot, 2009).

The percentage seasonal/quantitative occurrence of Fungi isolated from rotted sweet potatoes is presented in Figure 4.5 and is shown tovary with respect to pathogen and season of isolation. In the dry season of 2015 (December), out of the 100 sweet potato samples, 194 fungal isolates (55.11%) were obtained. Conversely in the wet season of 2016 (June), out of the 100 rotted sweet potato roots sampled, 158 fungi (44.89%) were isolated. Thus, in both seasons, 352 fungi were isolated from 200 symptomatic sweet potato root samples, with a mean percentage occurrence of 176.

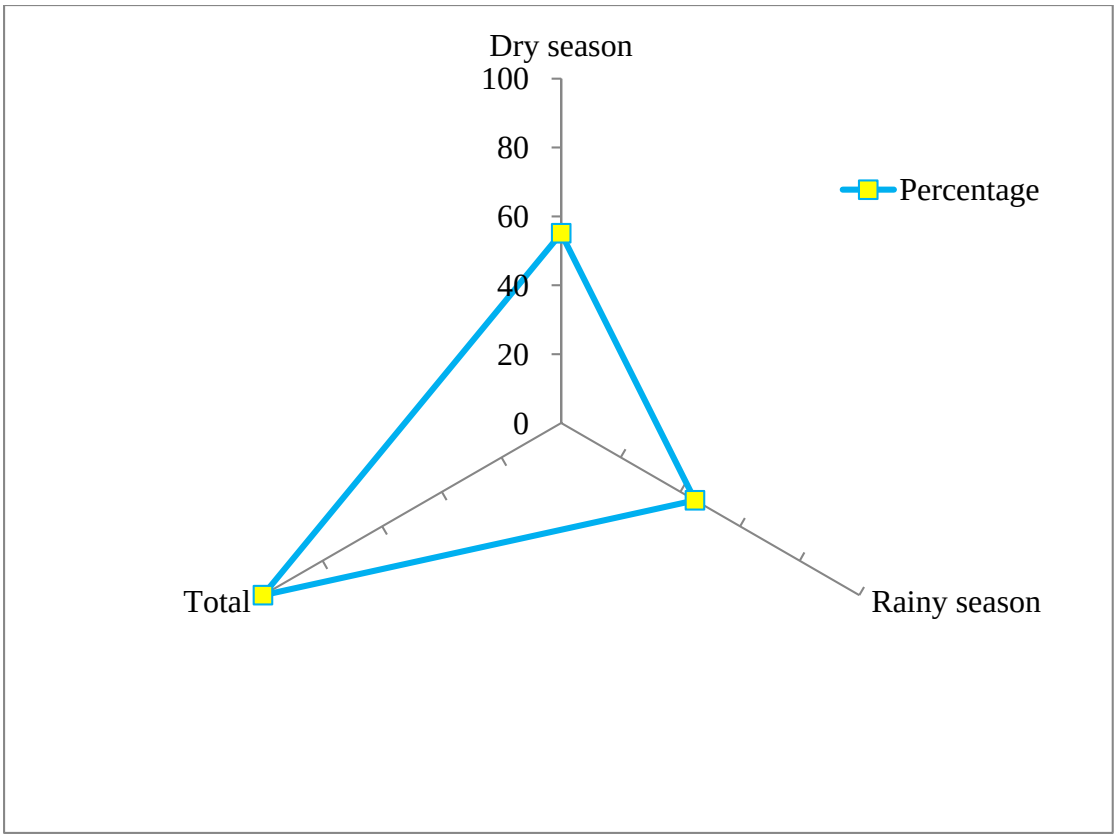


Figure 4.5: Seasonal/Quantitative Percentage Occurrence of Fungi Isolated from Rotted Sweet Potatoes

Figure 4.6 shows the seasonal qualitative occurrence of the fungi isolates obtained from post-harvest rot of sweet potato in the study area in the dry season and rainy seasons. In the dry season of 2015, seven fungal species were isolated and included 26 *Aspergillus flavus* (13.4%), 42 *Aspergillus niger* (21.65%), 26 *Fusarium solani* (11.86), 36 *Rhizopus oryzae* (18.55%), 5 *Penicilium expansum* (2.58%), 56 *Botryodiplodiae theobromae* (28.87%) and 6 *Aspergillus awamori* isolated (3.09%). Conversely, in the rainy season of 2016, six fungal species (158 isolates) were obtained. These included 20 *Aspergillus flavus* (12.66%), 29 *Aspergillus niger* (17.72%), 16 *Fusarium solani* (10.13%), 42 *Rhizopus oryzae* (26.56%), 9 *Penicillium expansum* (5.69%) and 43 *Botrydiplodiae theobromae* (27.22%).

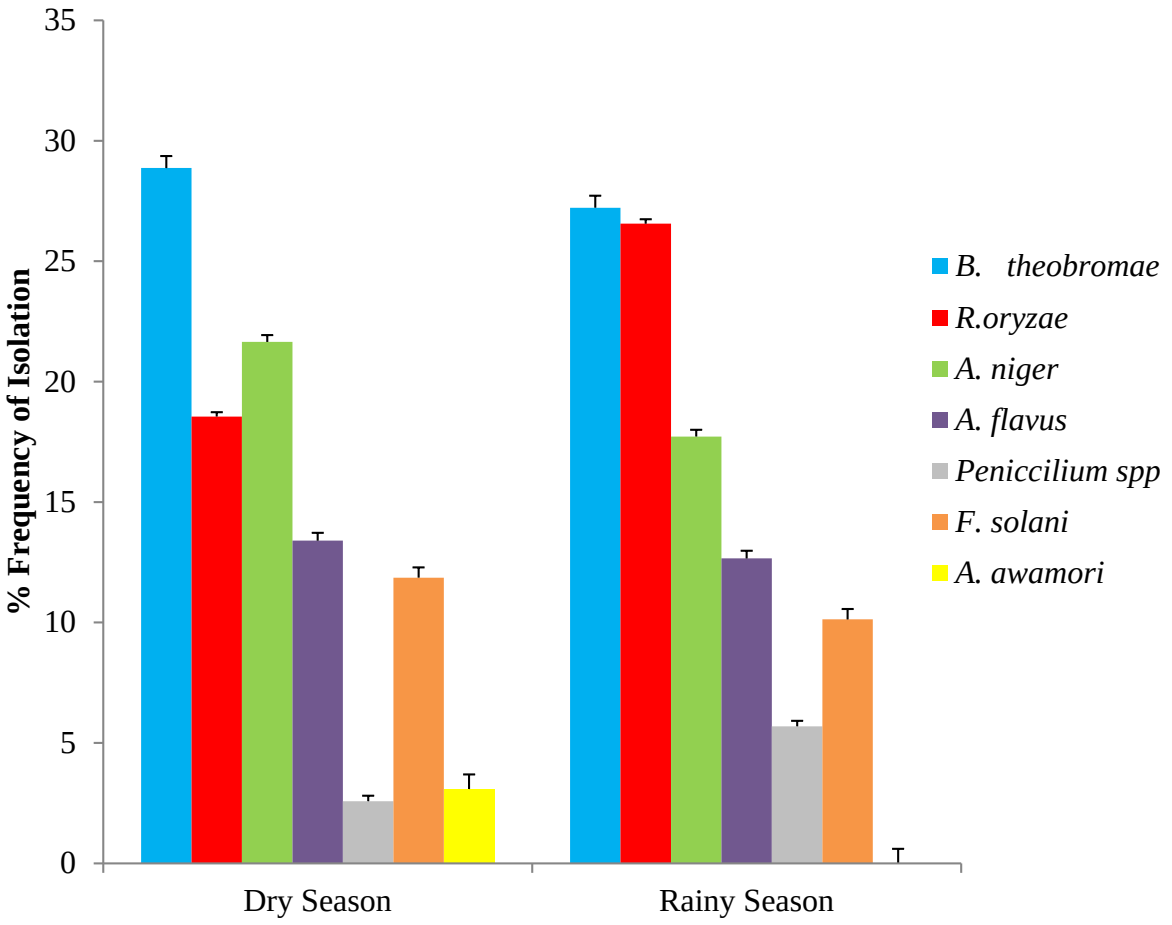


Figure 4.6: Seasonal/Qualitative Percentage Occurrence of Fungi Isolated from Rotten Sweet Potato from Ebonyi State

Figure 4.7 presents the Mean percentage qualitative occurrence of fungi isolates obtained in both seasons. In both seasons, the 352 fungi isolated from the 200 sweet potato samples consisted of *Aspergillus flavus* (13.03%), *Aspergillus niger* (19.58%), *Fusarium solani* (10.99%), *Rhizopus oryzae* (22.56%), *Penicillium expansum* (4.08%), *Botrydiplodiae theobromae* (28.45%) and *Aspergillus awamori* (1.54%).

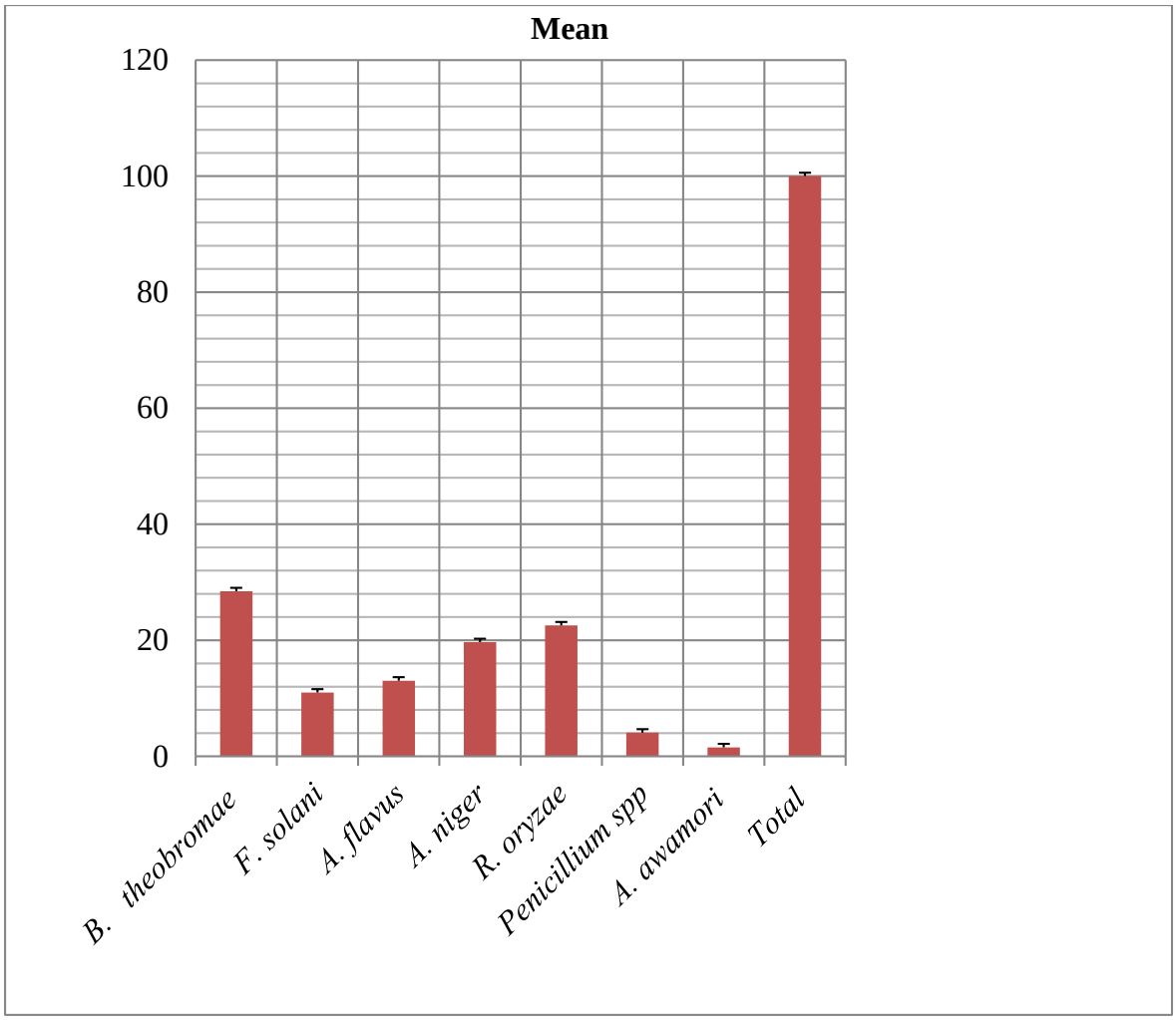


Figure 4.7: Mean Percentage Occurrence of Fungi Isolated from Rotten Sweet Potato in Both Seasons

Furthermore, the qualitative distribution of fungal pathogens associated with root rot of sweet potato is presented in Table 4.2. The results showed that *A. niger* and *B. theobromae* were isolated from all the studied LGAs and in both seasons; *A. flavus* and *Rhizopus oryzae* were isolated in both seasons and in three LGAs and none from Izii LGA. *F. solani* was isolated in both seasons and from two LGAs, namely Ikwo and Ezza North and Penicillium also in both seasons and from two LGAs – Ezza North and Izii while *A. awamori* was isolated only in the dry season and from only one LGA.

Table 4.2: Distribution of Fungal Pathogens Associated with Root Rot of Sweet Potato across the LGAs

Locality	Fungal pathogens isolated
Ikwo	
RS	*A. niger, B. theobromae, A. flavus, Rhizopus oryzae, F. solani,*
DS	*A. niger, B. theobromae, A. flavus, Rhizopus oryzae, F. solani,*
Ezza South	
RS	*A. niger, B. theobromae, A. flavus, Rhizopus oryzae*
DS	*A. niger, B. theobromae, A. flavus, Rhizopus oryzae*
Ezza North	
RS	*A. niger, B. theobromae, A. flavus, Rhizopus oryzae, F. solani, P. expansum*
DS	*A. niger, B. theobromae, A. flavus, Rhizopus oryzae, F. solani, P. expansum*
Izii	
RS	*A. niger, B. theobromae, Penicillium expansum*
DS	*A. niger, B. theobromae, Penicillium expansum, A. awamori*

DS=Dry season RS=Rainy season

Result of the pathogenicity test is shown in Table 4.3 and Plate 4.8. Results showed that all the seven fungal isolates induced rot in sweet potatoes after 7 days of inoculation. Roots artificially inoculated with test fungi showed typical rot symptoms with the root tissue rotting around the inoculation point within seven days and when cut longitudinally into two halves through the inoculation point, showed variation in tissue degradation while the control root showed no tissue degradation. All the fungi were successfully re-isolated and on re-isolation, exhibited morphological characteristics and growth patterns similar to those earlier observed on axenic cultures, confirming their pathogenicity.

Categories of rot incited by isolates are presented in Table 4.3. They included soft rot, dry rot, java rot and blue green mold.

Table 4.3: Type of Rots Induced on Mechanically Wounded and Artificially Pathogen Inoculated Sweet Potato Roots after 7 Days of Storage

S/N	Inoculum	Pathogenicity status	Charateristic of rot	Rot Type
	Control	-	Nil	Nil
1	*Aspergillus niger*	+	Infected tissues became light brown, hard and dry	Dry rot
2	*Aspergillus flavus*	+	Infected tissues became light yellow, hard and dry	Dry rot
3	*Botryodiplodiae theobromae*	+	Infected tissue clearly demarcated, firm and dark surrounded by a brown portion	Java rot (dry rot)
4	*Aspergillus awamori*	+	Infected tissues became light brown, hard and dry	Dry rot
5	*Fusarium solani*	+	Infected tissues became firm, dry and dark brown, with internal cavities filed with white fungus mycelium	Dry rot
6	*Rhizopus oryzae*	+	The infected tissues became brownish, soft and moist with stringy flesh	Soft rot
7	*Penicillium expansum*	+	Infected tissues became blue-green, hard and dry	Blue green mold

- =did not produce rot symptom ;+ = Produced rot symptom with successful re-isolation

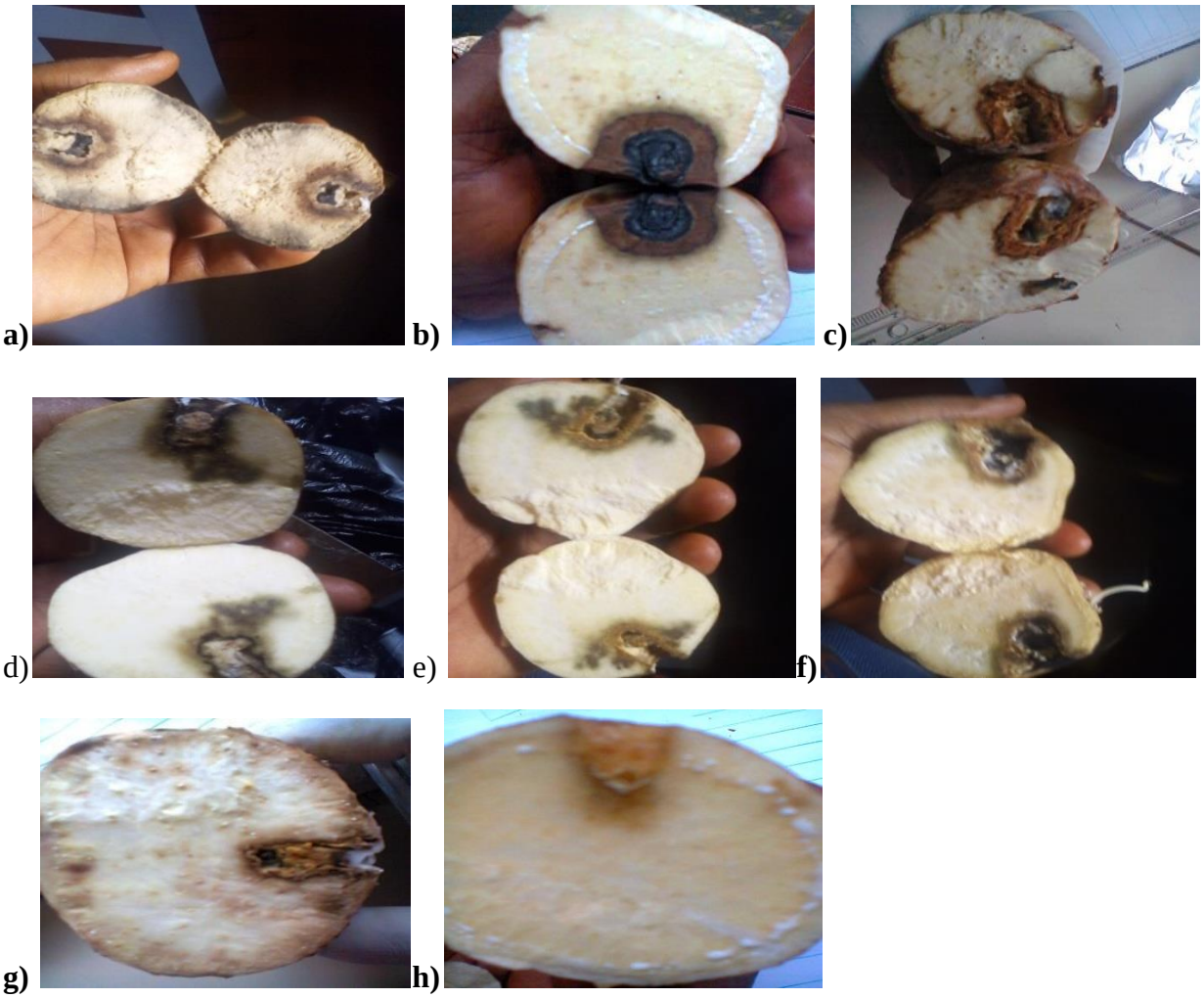


Plate 4.8: Sweet Potato Roots Showing Lesions of Postharvest Rot at the Point of Inoculation with Fungi

(a) Sweet potato root showing lesions of soft rot at the point of inoculation with *R. oryzae*
(b) Sweet potato root showing lesions of java rot at the point of inoculation with *B. theobromae*
(c) Sweet potato root showing lesions of dry rot at the point of inoculation with *A. niger*
(d) Sweet potato root showing lesions of dry rot at the point of inoculation with *Penicilium expansum*
(e) Sweet potato root showing lesions of dry rot at the point of inoculation with *A. flavus*
(f) Sweet potato root showing lesions of dry rot (blue mold) at the point of inoculation with *fusariun solani*
(g) Sweet potato root showing lesions of dry rot at the point of inoculation with *A. awamori*
(h) Control showing absence of rot

Table 4.4 presents results on the severity of fungi associated with sweet potato spoilage in Ebonyi State. In this study, no disease symptoms developed on roots wounded but inoculated with disc of sterile PDA. As posits in Table 4.4, the fungi/sweet potato cultivar interaction varied among the seasons, being higher in dry season (49.51% and 41.41% respectively for cultivar Tupiaochi and Oyorima) and lower in the wet season. The results revealed that in the first phase, the severity of the test fungi on both sweet potato cultivars was significantly different except for those of *P. expansum* and *A. awamori*. *R. oryzae* recorded the highest mean rot severity index of 76.36% on both sweet potato cultivars (80.87% and 71.84% respectively for tupiaochi and Oyorima) followed by *A. niger* with mean rot severity index of 68.39% on both sweet potato cultivars (78.52% and 58.25% respectively for tupiaochi and Oyorima); *B. theobromae* (mean rot severity index of 64.06% on both sweet potato cultivars (74.20% and 53.91% respectively for tupiaochi and Oyorima); *F. solani* (mean rot severity index of 34.29% on both sweet potato cultivars (34.65% and 33.92% respectively for tupiaochi and Oyorima); *A. flavus*, *A. awamori* and *P. expansum* with mean rot severity index of 32.03%, 22.73% and 20.39% respectively on both sweet potato cultivars within the test period (dry season).

Table 4.4: Severity of Fungi Implicated with Postharvest Spoilage in the Two Varieties of Sweet Potato in Ebonyi State

Treatment/ Pathogen	Pathogen Severity (mean %)		
	Dry Season		
	Tupiaochi	Oyorima	Total Pathogen Means
	Mean rot severity index	Mean rot severity index	Mean rot severity index
Control	(0)	(0)	(0)
B. theobromae	74.20_{a} (5)	53.91_{ab} (5)	64.06 (5)
A. flavus	34.65_{a} (4)	29.61_{ab} (4)	32.03 (4)
R. oryzae	80.87_{a} (5)	71.84_{ab} (5)	76.36 (5)
F. solani	27.89_{a} (4)	33.92_{ab} (4)	34.29 (4)
A. niger	78.52_{a} (5)	58.25_{ab} (5)	68.39 (5)
P. expansum.	21.02_{a} (3)	19.76_{a} (3)	20.39 (3)
A. awamori	22.85_{a} (3)	22.60_{a} (3)	22.73 (3)
Total Cultivar Means	49.51_{a} (4)	41.4_{ab}	
	Rainy Season		
Control	0	0	0
B. theobromae	70.65_{a} (5)	54.12_{ab} (5)	62.39 (5)
A. flavus	34.44_{a} (4)	26.21_{ab} (4)	30.33 (4)
R. oryzae	77.89_{a} (5)	54.39_{ab} (5)	66.14 (5)
F. solani	23.60_{a} (3)	32.22_{ab} (4)	27.91 (4)
A. niger	74.1_{a} (5)	53.91_{ab} (5)	64.01 (5)
P. expansum	20.98_{a} (3)	16.99_{ab} (3)	18.99 (3)
Total Cultivar Means	50.28_{a} (5)	39.64_{b} (4)	

Values are means of two replicates.
Pathogen Means with different subscript are significantly different
(Figures in Parenthesis) = Pathogen Severity ranking viz:
0 = none pathogenic fungi (no rots);
1= Very mild pathogen severity (1-5%);
2= Mild pathogen severity (10%);
3= Moderate pathogen severity (>10-25%),
4= High pathogen severity (>25-50%);
5 =Very high pathogen severity (> 50%)

The susceptibility of the sweet potato cultivars to postharvest fungi is presented in Figure 4.8. None of the sweet potato cultivars evaluated under laboratory conditions appeared immune to the pathogens; that is, both sweet potato cultivars showed typical rot symptoms with the root tissue rotting around the inoculation point within two weeks of inoculation. However, the mean weights (g/root) of rotted tissue varied significantly among the 3 evaluated cultivars in both the 1st and 2nd trials. The control roots showed no tissue degradation. The Cultivar x pathogen interaction for cultivar 'Tupiaochi' (38.08 and 38.67g/root in the dry and rainy season respectively) was significantly higher than that of 'Oyorima' (29.80 and 29.53g/root). 'Tupiaochi' clone exhibited 'very high susceptibility' (>50g/root) towards three pathogens in both trials: *R. oryzae*, *A. niger* and *B. theobromae* showed significantly different responses (65.86, 60.80 and 56.97g/root in first trial and 59.25, 55.16 and 52.30g/root respectively in the second trial), 'high susceptibility' (>25-50g/root) towards *A. flavus* (30.45g/root) and 'moderate susceptibility (>10-25g/root) towards *F. solani*, *P. expansum* and *A. awamori* with statistically significantly different (P=0.05) responses (23.82, 15.29 and 12.81g/root respectively). '*Oyorima'* cultivar exhibited a 'very high susceptibility' (>50g/root) towards *R. oryzae*, (54.02g/root respectively), 'high susceptibility' (>25-50g/root) towards *A niger*, *B. theobromae* and *F. solani wi*ith statistically significantly different (P<0.05) responses (45.17, 34.03 and 27.03g/root respectively) and 'moderate susceptibility (>10-25g/root) towards *A. flavus*, *P. expansum* and *A. awamori* with significantly different (P<0.05) responses (22.38, 14.05 and 11.93g/root respectively).

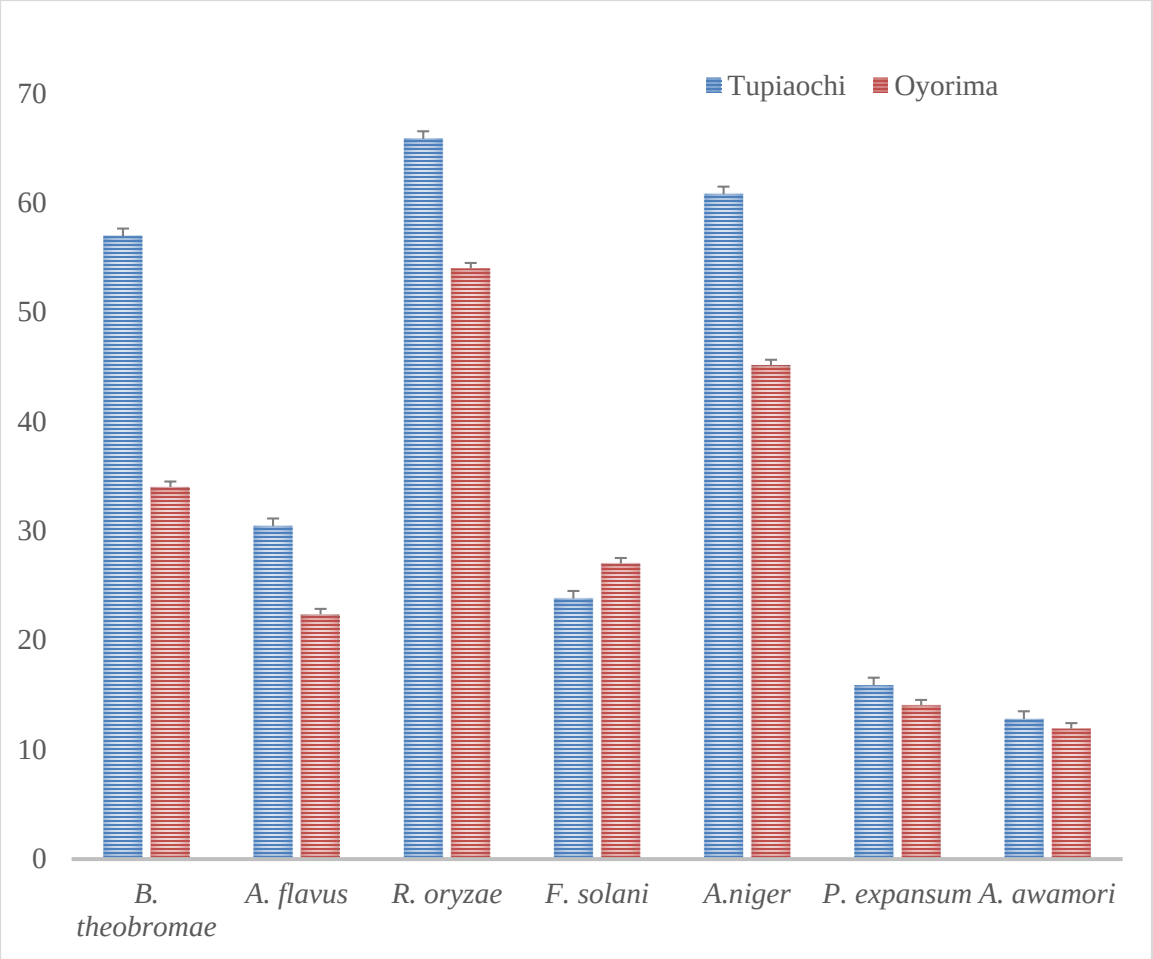
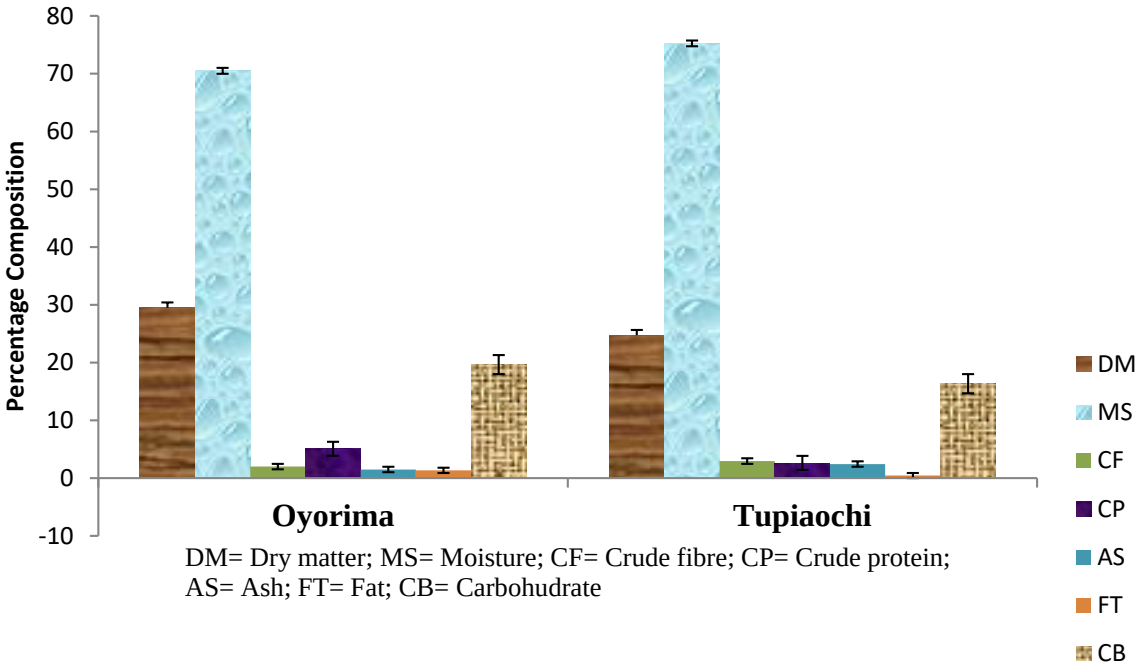


Figure 4.8: Mean Weights (gram/root) of Rotted Tissue in Storage Roots of Sweet Potato (*Ipomoea batatas*)

The result of the proximate analysis of the healthy sweet potato roots is presented in Figure 4.9. Results revealed the corresponding nutritiomal values for the sweet potato cultivars- Tupiaochi and Oyorima to be 75.25% and 70.50% Moisture, 2.92 and 1.99% Crude Fibre, 2.62 and 5.05% Protein, 2.24 and 1.48% Ash, 0.44 and 1.34% Fat and 16.33 and 19.64% Carbohydrates respectively.



DM= Dry matter; MS= Moisture; CF= Crude fibre; CP= Crude protein; AS= Ash; FT= Fat; CB= Carbohudrate

Figure 4.9: Nutritional Composition of Healthy Sweet Potato Cultivars Most Commonly Grown by Ebonyi Farmers

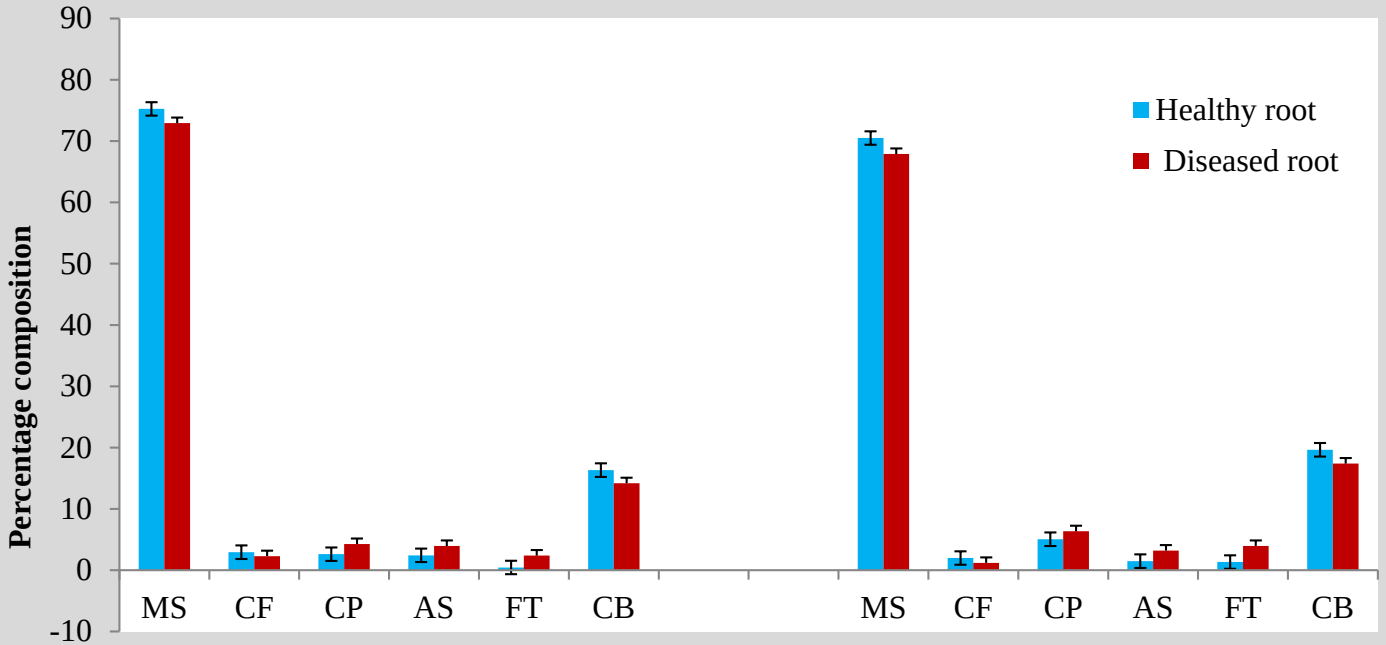
The comparative results of the nutritional compositions of the roots (both healthy and diseased) are presented in Table 4.5 and Figures 4.10- 4.16. A one-way ANOVA conducted to compare the means of the proximate composition of healthy and fungi rotted roots of the sweet potato cultivars showed significant differences (P =0.05) among the treatment groups (healthy and fungi rotted roots of both tested sweet potato cultivars) in moisture content, crude fibre, crude protein, ash, fat and carbohydrate contents. A Duncan post hoc test revealed that there was a general significant reduction in the moisture, crude fibre and carbohydrate content and statistically different increase in the crude protein, ash and fat contents of the artificially phytopathogen inoculated/ infected roots irrespective of cultivar vis avis the healthy roots. The rate of variation between these parameters was dependent on the infecting pathogens and the sweet potato cultivar.

Table 4.5: A Comparative Assessment of the Percentage Nutritional Composition of Flour from Diseased and Healthy Roots of *Ipomoae batatas*

			Nutritional Compostion					
			M	CF	P	A	F	CARB
Tupiaochi	Healthy root	Control	75.2500e	2.9400e	2.6200a	2.4200a	0.44a	16.3300d
	Diseased root	Ro	72.9250c	2.2850a	4.2800d	3.9450d	2.3800c	14.1850a
		Bt	71.8200a	2.3100b	4.6200f	4.4600f	2.3250c	14.4650a
		Fs	72.9950c	2.4050c	4.2100c	3.8500c	1.2800b	15.2600c
		An	71.9600b	2.2500a	4.4350e	4.1050e	2.8200c	14.4300a
		Af	73.3450d	2.4400c	4.2350c	3.8300c	1.2450b	14.9050b
		Ps	73.0150c	2.8550d	4.0650b	3.4350b	1.1300b	15.5000c
		Aa	73.0050c	2.8350d	4.2250c	3.4500b	1.1000b	15.4850b
Oyorima	Healthy root	Control	70.5000_{a}	1.9900_{a}	5.0500_{c}	1.4800_{d}	1.3400_{g}	19.6400_{a}
	Diseased root	Bt	67.4100_{e}	1.3300_{d}	6.1150_{b}	3.1850_{a}	3.9200_{c}	18.0400_{c}
		Ro	67.9000_{d}	1.1950_{f}	6.3450_{a}	3.2000_{a}	3.9750_{b}	17.3850_{c}
		Fs	68.4100_{b}	1.3500_{d}	6.0600_{b}	3.0900_{b}	2.3250_{d}	18.8450_{b}
		An	67.8000_{d}	1.2950_{e}	6.2650_{a}	3.1800_{a}	4.0650_{a}	17.3950_{c}
		Af	68.6500_{b}	1.6050_{c}	6.0450_{b}	2.9700_{d}	1.8250_{f}	18.9050_{b}
		Ps	68.4550_{b}	1.6400_{b}	6.0800_{b}	3.0800_{c}	1.8950_{e}	18.8500_{b}
		Aa	68.4100_{b}	1.6650_{b}	6.0550_{b}	3.1050_{c}	1.9200_{e}	18.9800_{b}

Values are means of two replicates. Mean values with different subscripts along the column are significantly different at P=0.05. Bt= *B. theobromae*; Ro= *R. oryzae*; Fs= F solani; An= *A. niger*; Af=*A. flavus;* Aa= *A. awamori;* Ps=*Penicillium spp;* M= Moisture; A= Ash; CF= Crude fibre; F= Fat; P= Protein; Cab= Carbohydrate.

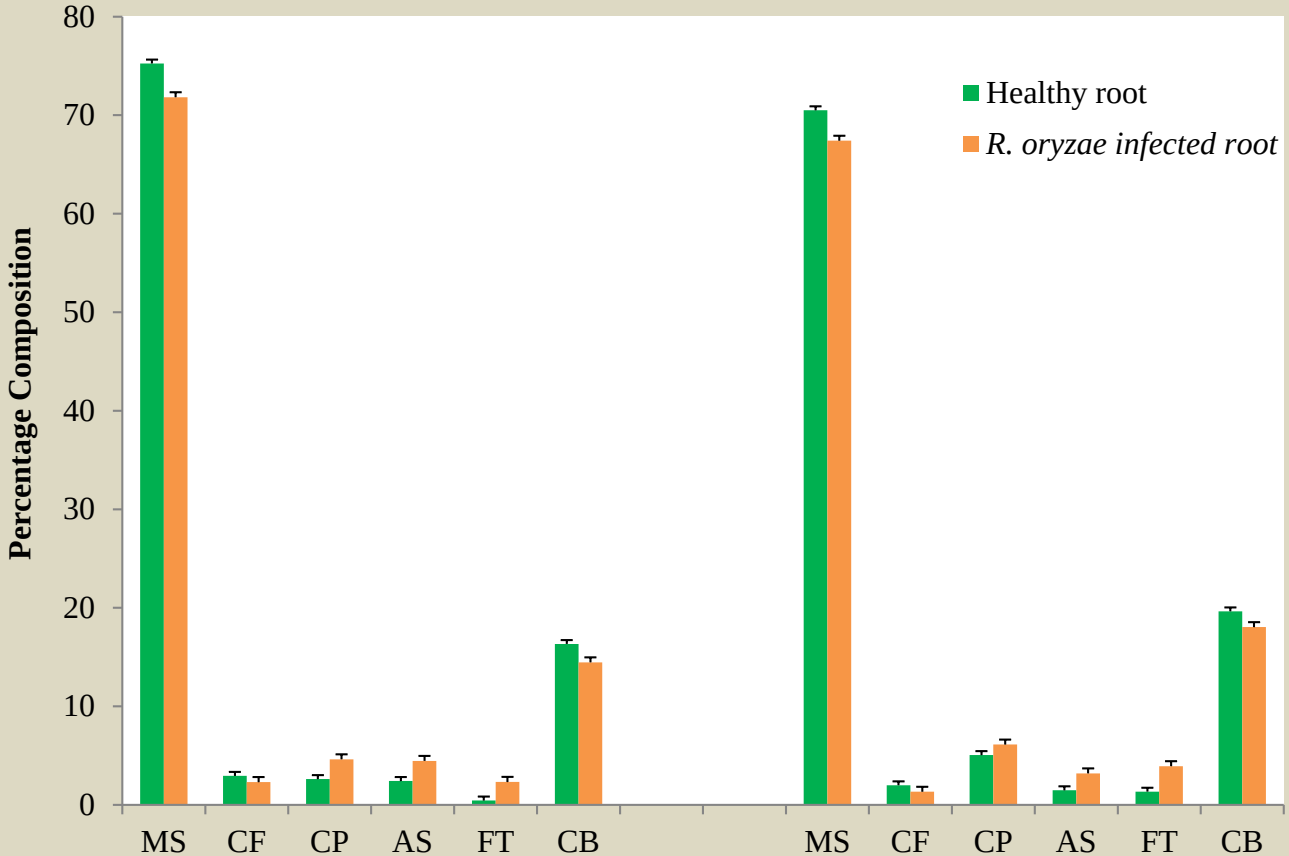
Results on the comparative assessment of the nutritional composition of both healthy and *B. theobromae* infected roots are presented in Figure 4.10. As shown in the figure, roots of both sweet potato cultivars infected by *R. oryzae* showed highest value in carbohydrate reduction (16.33 to 14.19% and 19.64 to 17.39% respectively for Tupiaochi and Oyorima varieties) with mean difference of 2.14% and 2.25% which were significantly different from the control (healthy). The same pathogen was shown to induce significant reduction in the crude fibre content of both infected sweet potato cultivars (2.92 to 2.29% and 1.99 to 1.20% respectively for Tupiaochi and Oyorima varieties) with a significant mean difference of 0.63% and 0.79%. There was also a significant reduction in the value of moisture content of the roots (75.25 to 72. 93% and 70.50 to 67.90 respectively for Tupiaochi and Oyorima varieties witha significant mean difference of 2.32% and 2.6%. Contrarily, crude protein, ash and fat contents of *R. oryzae* infected Tupiaochi sweet potato roots recorded significant (p= 0.05) increase (4.28%, 3.97% and 2.28% respectively) vis avis healthy roots (2.26%, 2.42% and 0.44% respectively). Similarly, the crude protein, ash and fat contents of *R. oryzae* infected roots of Oyorima cultivar recorded significant (P=0.05) increase (6.35%, 3.207% and 3.96% respectively) *vis avis* healthy roots (5.05%, 1.48% and 1.34% respectively).



MS= Moisture; CF= Crude fibre; CP= Crude protein; AS= Ash; FT= Fat; CB= Carbohydrate

Figure 4.10: Effect *of R. oryzae* Induced Rot on the Nutritional Composition of the Roots of Ebonyi Most Commonly Grown Sweet Potato Cultivars

Results of the comparative assessment of the nutritional composition of both healthy and *B. theobromae* infected roots are presented in Figure 4.11. Root samples of Tupiaochi cultivar infected *B. theobromae* recorded significantly lower (P=0.05) mean values for moisture, crude fibre and carbohydrate proximate parameters at 72.93%, 2.29% and 14.19% respectively than in the healthy root samples where each of the parameters recorded a value of 75.25%, 2.94%, and 16.33% respectively with corresponding mean difference of 2.32%, 0.65% and 2.14%. In the same vein, Root samples of Oyorima cultivar also infected with *B. theobromae* recorded significantly lower (P=0.05) mean values for moisture, crude fibre and carbohydrate proximate parameters at 67.41%, 1.33% and 18.04% respectively than in the healthy root samples where each of the parameters recorded a value of 70.5%, 1.99% and 19.64% respectively with corresponding mean difference of 3.09%, 0.66% and 1.24%. On the contrary, the mean values for the composition of crude protein, ash and fat proximate parameters in *B. theobromae* roots of both sweet potato cultivars was significantly higher (P=0.05) at 4.28%, 3.95% and 2.38% as against 2.62%, 2.42% and 0.44% recorded by healthy roots, with mean difference of -1.66%, -1.53% and -1.99% respectively (for 'Tupiaochi' cultivar) and at 6.12%, 3.19% and 3.92% as against 5.05%, 1.48% and 1.34% recorded by healthy roots, with mean difference of -1.07%, -1.71% and -2.58% respectively (for 'Oyorima' cultivar).



MS= Moisture; CF= Crude fibre; CP= Crude protein; AS= Ash; FT= Fat; CB= Carbohydrate

Figure 4.11: Impact of *B. theobromae*- Induced Rot on the Nutritional Composition of Roots of Ebonyi Most Commonly Grown Sweet Potato Cultivars

Results of the comparative assessment of the nutritional composition of both healthy and *F. solani* infected roots are presented in Figure 4.12. The mean values for the composition of the moisture, crude fibre and carbohydrate in the *F. solani* diseased roots samples of 'Tupiaochi' cultivar was significantly lower (P=0.05) at 71.96%, 2.25% and 14.43% respectively than in the healthy roots with a value of 75.25%, 2.94% and 16.33% respectively with corresponding mean difference of 3.29%, 0.69% and 1.9%. Similarly, statistically significantly lower (P=0.05) values for moisture, crude fibre and carbohydrate composition at 67.80%, 1.3% and 17.4% was recorded for the root samples of 'Oyorima' variety infected with *F. solani* than that of healthy roots (70.5%, 1.99 and 19.64 respectively with respective mean differences of 2.7%, 0.69% and 2.24%). On the contrary, the mean values for the composition of crude protein, ash and fat proximate parameters in the *F. solani* infected roots of both sweet potato cultivars was significantly higher (P=0.05) at 4.21%, 3.85% and 1.28% repcetively as against 2.62%, 2.42% and 0.44% recorded by healthy roots (for 'Tupiaochi' cultivar) and at 6.06%, 3.09% and 2.33% as against 5.05%, 1.48% and 1.34% recorded by healthy roots (for 'Oyorima' cultivar).

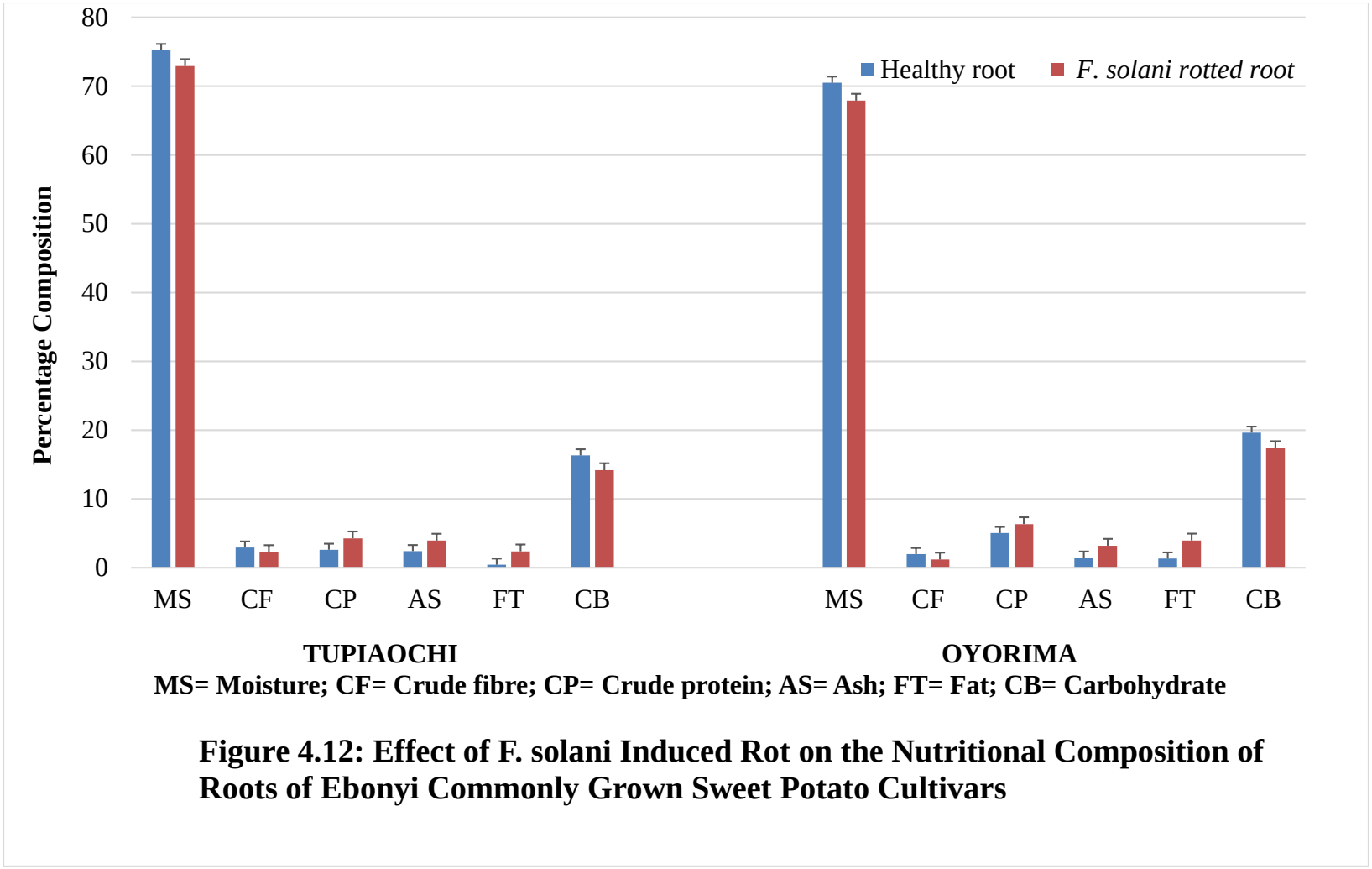


Figure 4.12: Effect of F. solani Induced Rot on the Nutritional Composition of Roots of Ebonyi Commonly Grown Sweet Potato Cultivars

Results of the comparative assessment of the nutritional composition of both healthy and. *A. niger* infected roots is presented in Figure 4.13. *A. niger* infected Sweet potato samples of Tupiaochi cultivar roots showed significant (p=0.05) reduction in moisture, crude fibre and carbohydrate at values of 73.35%, 2.44% and 14.91% respectively vis avis 75.25%, 2.94% and 16.33% recorded by the healthy roots. Likewise, Oyorima cultivar infected with *A. niger* exhibited significantly (P= 0.05) less moisture, crude fibre and carbohydrate content (73.34%, 2.25% and 14.43%) compared to healthy roots (75.25%, 2.94% and 16.33%). However, the levels of crude protein, ash and fat were significantly (P= 0.05) higher in roots that were infected with *A. niger* (4.24%, 3.83% and 2.82% respectively) than those that were healthy (2.62%, 2.42% and 0.44% respectively).

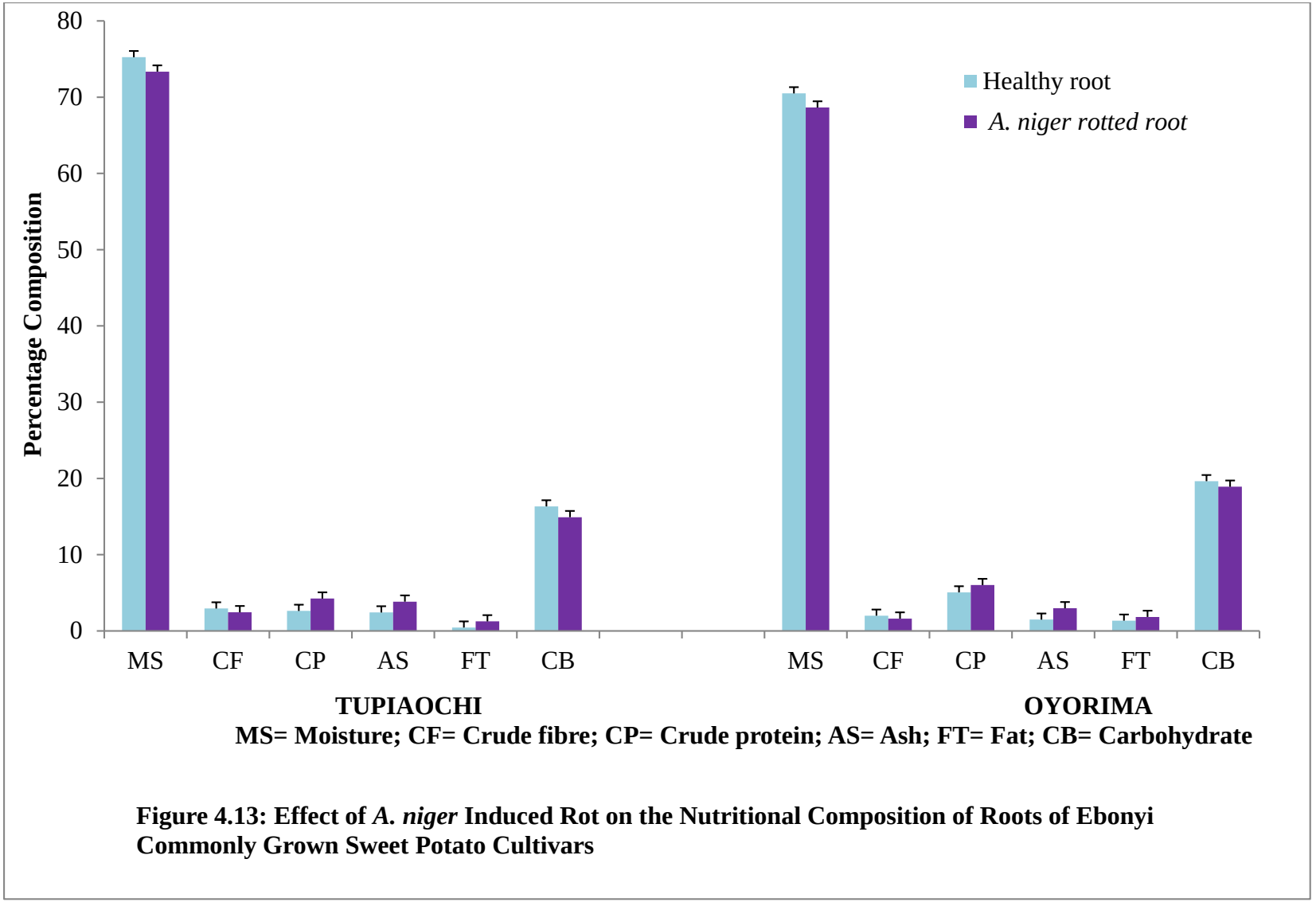
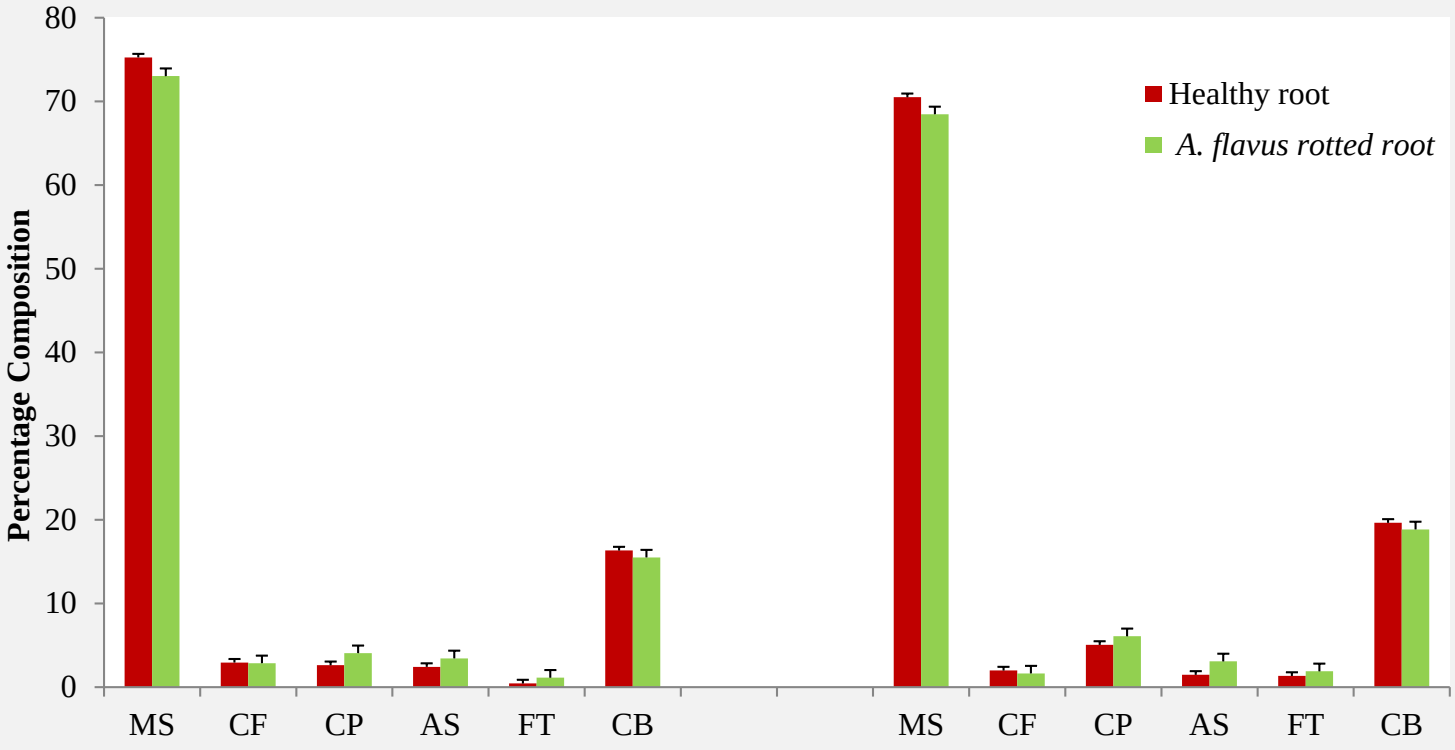


Figure 4.13: Effect of *A. niger* Induced Rot on the Nutritional Composition of Roots of Ebonyi Commonly Grown Sweet Potato Cultivars

Figure 4.14 presents the mean values of the nutritional composition of the healthy and *A. flavus* diseased sweet potato roots of Tupiaochi and Oyorima cultivar.As shown in the figure, Tupiochi sweet potato cultivar, significant (P=0.05) reduction in moisture, Crude fibre and carbohydrate contents in *A. flavus* infected roots from 75.2 to 73.35%, 2.94 to 2.44% and 16.33 to 14.91% respectively while crude protein, ash and fat contents of infected roots recorded significant (P=0.05) increase (4.24%, 3.83% and 1.23% respectively) vis avis healthy roots (2.62%, 2.42% and 0.44% respectively). Similarly, in Oyorima cultivar, a significant (P=0.05) reduction in moisture, Crude fibre and carbohydrate contents from70.50 to 68.65%, 1.99 to 1.61% and 19.64 to 18.91% respectively while crude protein, ash and fat contents of infected roots recorded significant (P=0.05) increase (6.05%, 2.97% and 1.83% respectively) vis avis healthy roots (5.05%,1.48% and 1.34% respectively).



MS= Moisture; CF= Crude fibre; CP= Crude protein; AS= Ash; FT= Fat; CB= Carbohydrate

Figure 4.14: Effect of *A. flavus* Induced Rot on the Proximate Composition of Roots of Ebonyi Commonly Grown Sweet Potato Cultivars

Figure 4.15 shows the nutritional composition of both healthy and *Penicillium expansum* infected roots of both Oyorima and Tupiaochi cultivars. The result on impact of *P. expansum* on the sweet potato proximate compositions with respect to moisture, crude fibre and carbohydrate showed significant (P=0.05) decrease from 75.25%, 2.94% and 16.33% in healthy root to 73.01%, 2.84% and 15.94% in *P. expansum* infected roots of Tupiaochi cultivar while the mean values for crude protein, ash and fat of healthy roots were 2.82%, 2.42% and 0.44% respectively but found to exhibit significant increase to 4.26%, 3.45% and 1.11% respectively following infection by *P. expansum*. Results revealed similar trend on the impact of the fungi on Oyorima cultivar, with statistically significant decrease in moisture, crude fibre and carbohydrate (from 70.05%, 1.99% and 19.64% respectively in healthy roots to 68.41%, 1.67% and 18.98% respectively in diseased roots) and a significant increase in mean values for crude protein, ash and fat (from 5.05%, 1.48% and 1.34% in healthy roots to 6.06%, 3.1% and 1.94% in diseased roots)

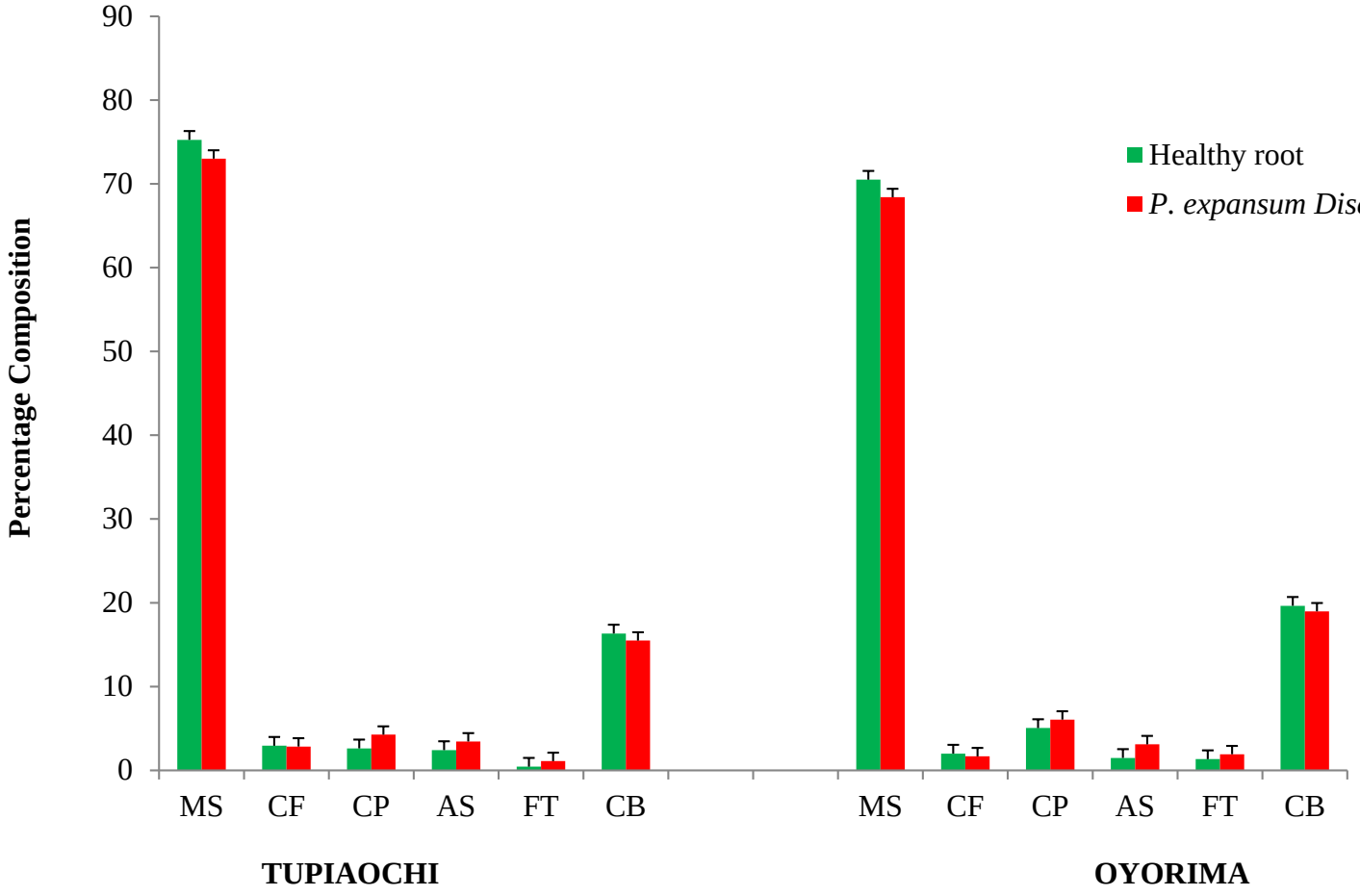


Figure 4.15: Effect of *Penicillium expansum* induced rot on the Nutritional Composition of Roots of Ebonyi Commonly Grown Sweetpotato Cultivars

The effects of *A. awamori* infection on nutritional composition of sweet potato cultivars 'Tupiaochi' and 'Oyorima' are shown in Figure 4.16. There was significant ($p<0.05$) reduction in the amount of moisture content, crude fibre and carbohydrates (73.01%, 2.94% and 15.49% in Tupiaochi cultivar and 68.41%, 1.67% and 18.98% respectively in Oyorima cultivar) in *A. awamori* infected roots compared to the healthy roots (75.25%, 2.94% and 15.49% in Tupiaochi cultivar and 70.5%, 1.99% and 19.64% in Oyorima cultivar respectively). On the contrary, significant ($p=0.05$) increase was observed in crude protein, ash and fat between the *A. awamori* infected and healthy roots. For the infected roots, the crude protein, ash and fat proximate parameters were at 4.26%, 3.45% and 1.11% as against 2.62%, 2.42% and 0.44% respectively for healthy roots of Tupiaochi cultivar and at 6.06%, 3.11% and 1.92% as against 5.05%, 1.48% and 1.34% recorded respectively by healthy roots of Oyorima cultivar.

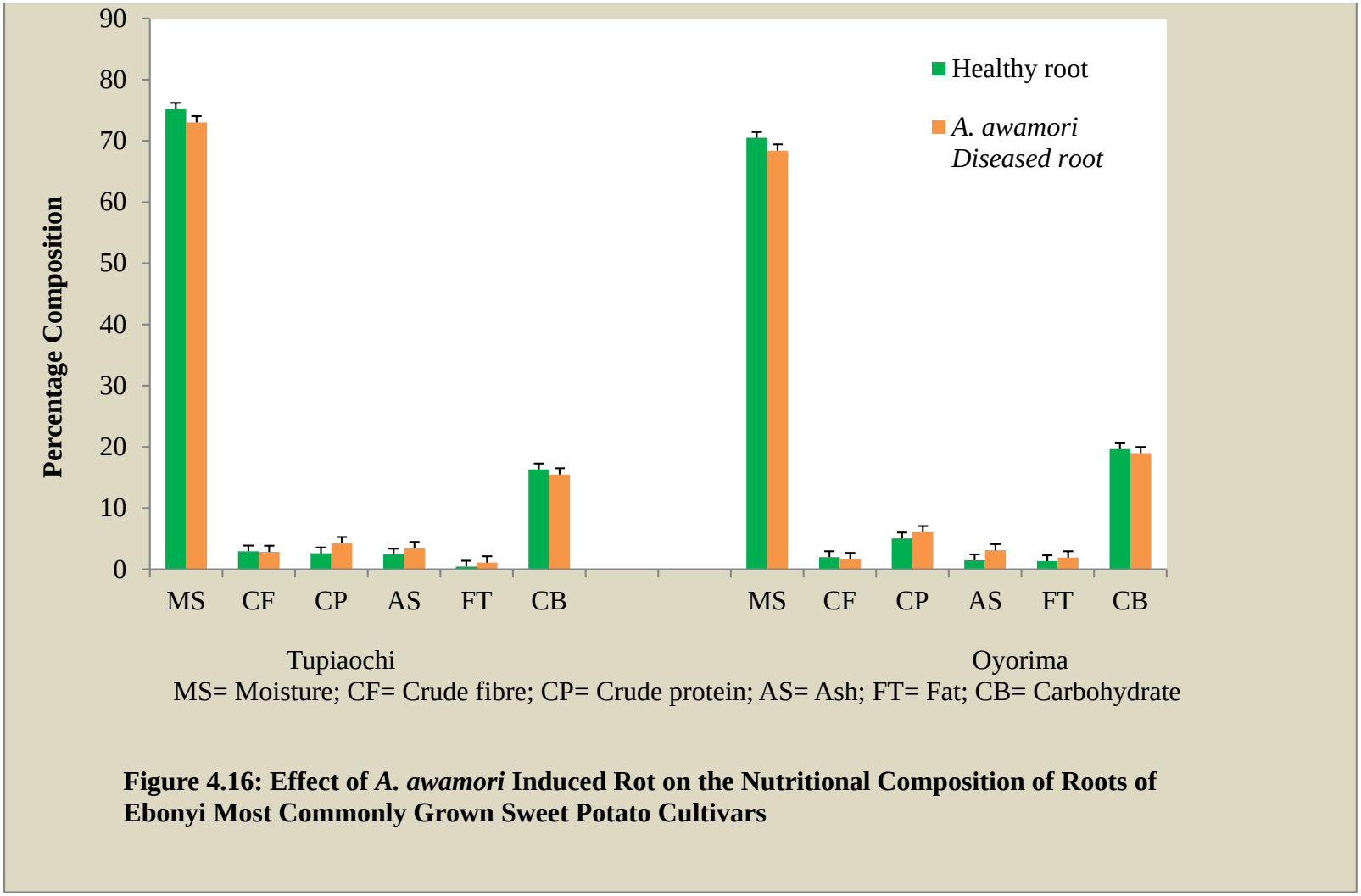


Figure 4.16: Effect of *A. awamori* Induced Rot on the Nutritional Composition of Roots of Ebonyi Most Commonly Grown Sweet Potato Cultivars

The antifungal effects of four plant extracts viz: *Allium sativum, Moringa oleifera, Zingiber officinale* and *Garcinia kola* were evaluated singly and in their combinations against seven postharvest fungi of sweet potato and compared with the effect of mancozeb. Table 4.6 showed the percentage yield of extracts of *Allium sativum, Moringa oleifera, Zingiber officinale* and *Garcinia kola* in methanol as solvent. In methanol, *Moringa oleifera* extract gave the highest yield of 21.09%, followed by *G. kola* (14.31%), *Allium sativum* (9.11%) and *Zingiber officinale* (5.7%)

Table 4.6: Percentage Yield of Different Plant Extracts in Methanol

Plants used	Yield (%)
Allium sativum	$9.11 \pm 0.11_c$
Moringa oleifera	$21.09 \pm 0.42_a$
Zingiber officinale	$5.7 \pm 0.88_d$
Garcinia kola	$14.31 \pm 0.27_b$

Values are means of three replicates± S.E.;
Yield means with different subscript are significantly different (P =0.05)

The antifungal activities of single plant extracts against postharvest pathogens of sweet potato are shown in Figure 4.17. All the extracts had activity against all the fungi. However, the inhibitory effect was found to vary with extract plant type, extract concentration, pathogen and extract constitution. Higher single plant extract concentrations exhibited stronger inhibition of all the test fungi than their lower concentrated counterparts. No plant extracts when used alone, irrespective of the concentration exhibited percentage mycelia growth inhibition that was up to 50% with the exception of *Allium sativum.*

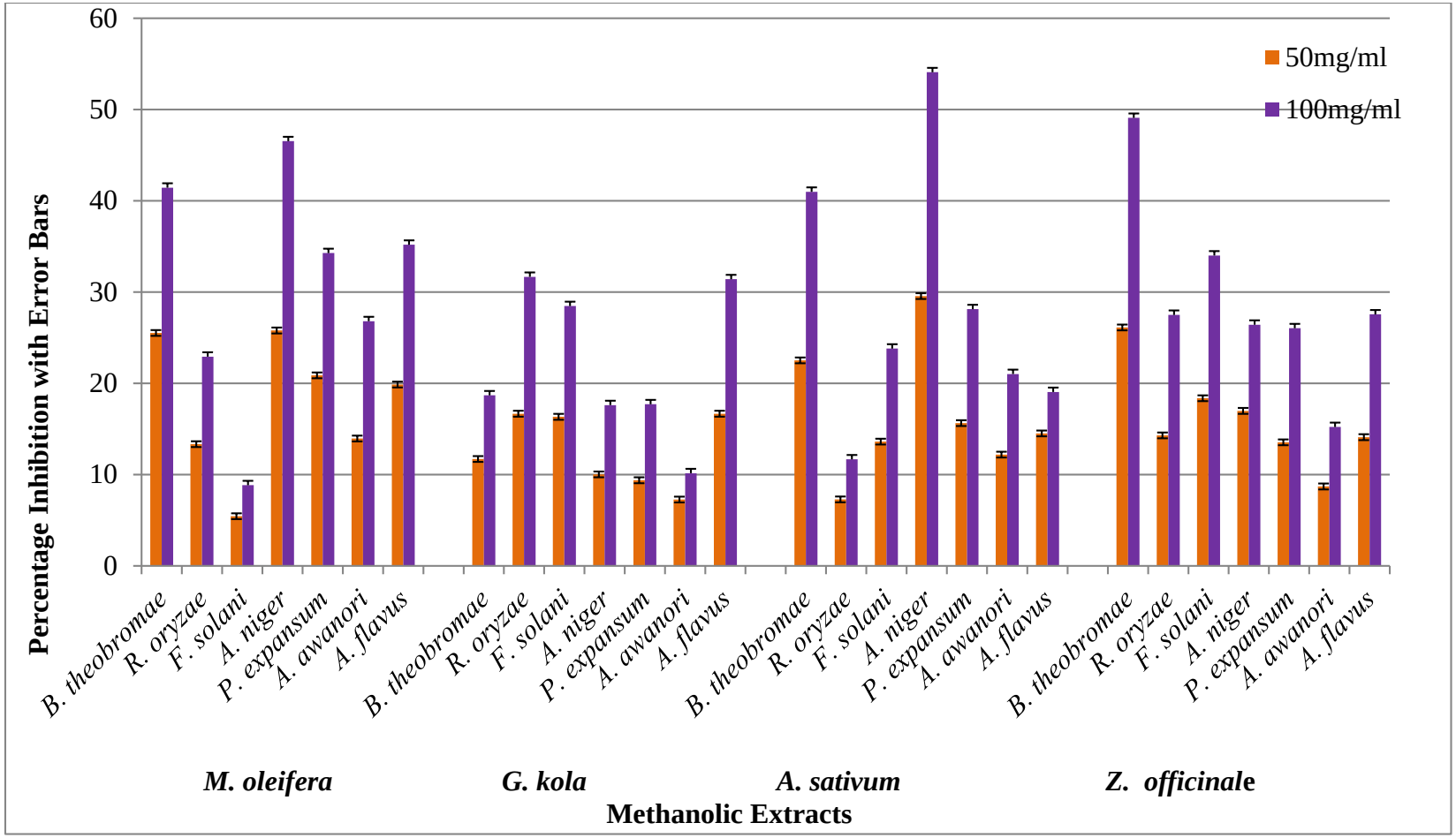


Figure 4.17: Percentage Inhibition of Mycelial Growth of Sweet Potato Postharvest Fungi at Different Concentrations of Methanolic Extracts of *Moringa oleifera, Garcinia kola, Allium sativum* and *Zingiber officinale*.

Figure 4.18 presents the effects of plant extracts on the mycelial growth inhibition of *R. oryzae*. At 50mg/ml extract concentration, each of the plant extracts exhibited significantly different percentage inhibition against all the test fungi compared to the negative control; with inhibitions ranging from 7.48, 13.33 and 14.29 to 16.67% in *Allium sativum, Moringa oleifera, Zingiber officinale* and *Garcinia kola*. Increasing the concentration level for each tested plant extract gave significantly higher percentage inhibitions ranging from 11.67±0.42, 22.92±0.72, 27.50±0.00 to 31.67±0.42% in the respectively aforementioned extracts. From the mean values, *R. oryzae* was most sensitive to *Garcinia kola*, followed by *Zingiber officinale*, *Moringa oleifera* and lastly *Allium sativum.*

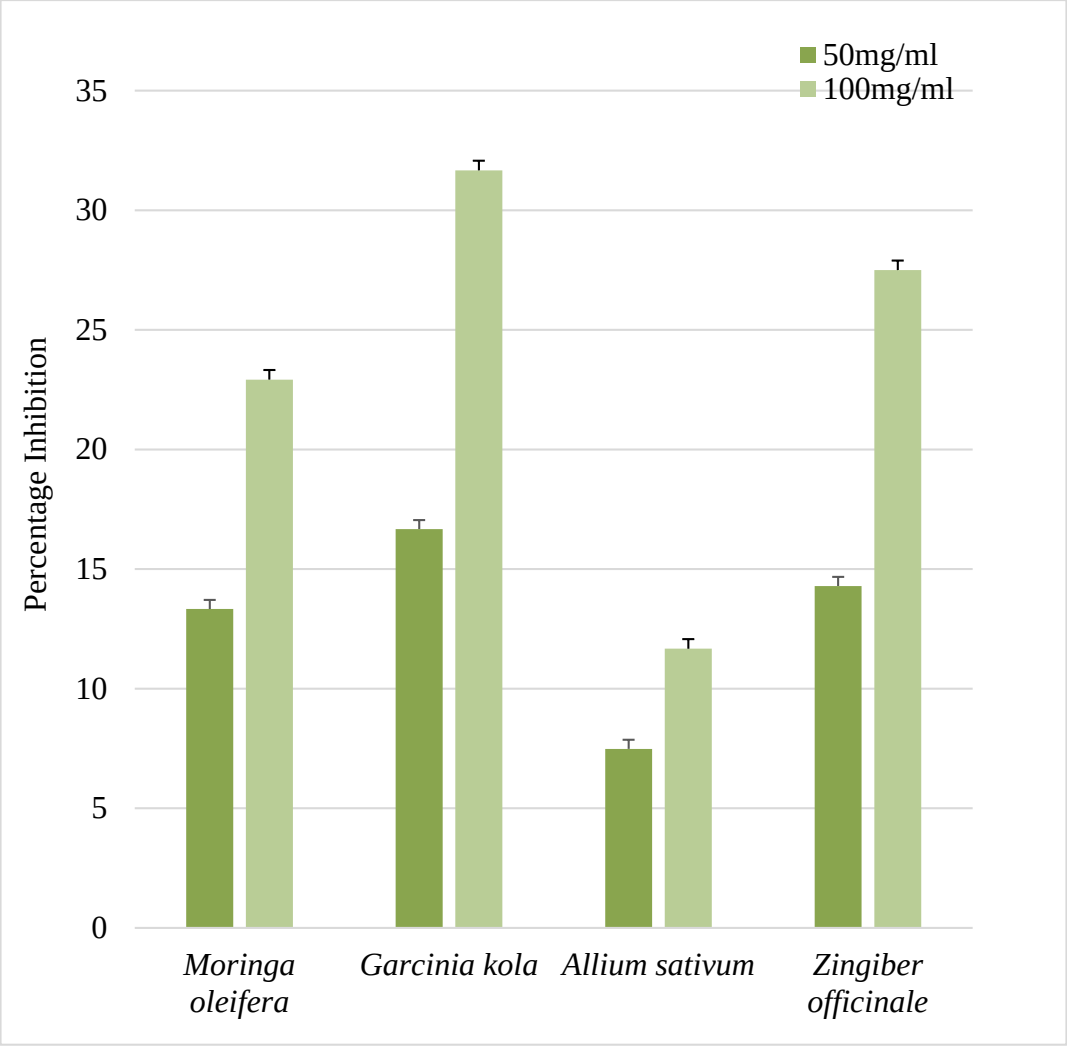


Figure 4.18: Percentage Inhibition of Mycelial Growth of *R. oryzae* in Different Concentrations of Methanolic Extracts of *Moringa oleifera, Garcinia kola, Allium sativum* and *Zingiber officinale*.

Figure 4.19 presents the effects of plant extracts on the mycelial growth inhibition of *B. theobromae*. There was a general increase in percentage inhibition with an increase in extract concentration. Radial growth inhibition percentages at 50mg/ml extract concentration ranged from 11.71, 22.52 and 25.52 to 26.12% *in Garcinia kola, Allium sativum, Moringa oleifera* and *Zingiber officinale*. Conversely, at 100mg/ml extract concentration, inhibition percentages were significantly higher (18.69±0.22, 40.99±0.45, 41.44±0.78 to 49.09±0.45%) in *Garcinia kola, Allium sativum, Moringa oleifera* and *Zingiber officinale*) than those of the corresponding 50mg/ml extract concentration; with *B. theobromae* being most sensitive to *Zingiber officinale, followed by Moringa oleifera, Allium sativum and Garcinia kola* extracts.

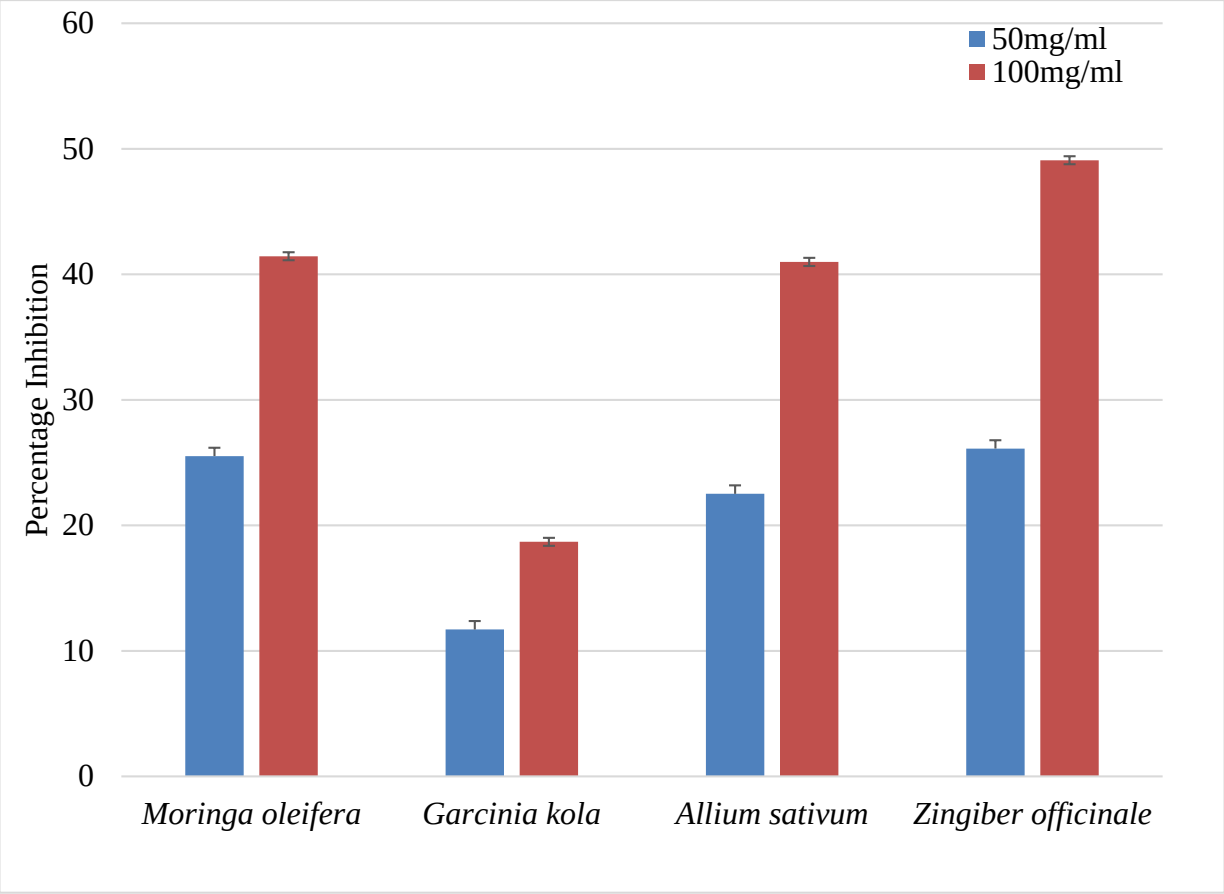


Figure 4.19: Percentage inhibition of mycelial growth of *B. theobromae* in Different Concentrations of Methanolic Extracts of *Moringa oleifera, Garcinia kola, Allium sativum* and *Zingiber officinale*.

Figure 4.20 presents the effects of plant extracts on the mycelial growth inhibition of *F. solani.* As shown in Figure 4.20, extract of *Zingiber officinale* caused maximum mycelia growth inhibition percentages of 18.37% and 34.04% at 50mg/ml and 100mg/ml extract concentration respectively. This was followed by *Garcinia kola* with statistically significantly lower mycelia growth inhibition percentages of 16.33% and 28.47% at 50mg/ml and 100mg/ml extract concentration respectively. The inhibitory effects of *Allium sativum* followed that of *Garcinia kola* with percentage inhibitions of 16.33% and 28.47±0.0% respectively at 50mg/ml and 100mg/ml extract concentration respectively and then *Moringa oleifera* with 5.44% and 8.84±0.68% percentage inhibitions at respective extract concentration of 50mg/ml and 100mg/ml.

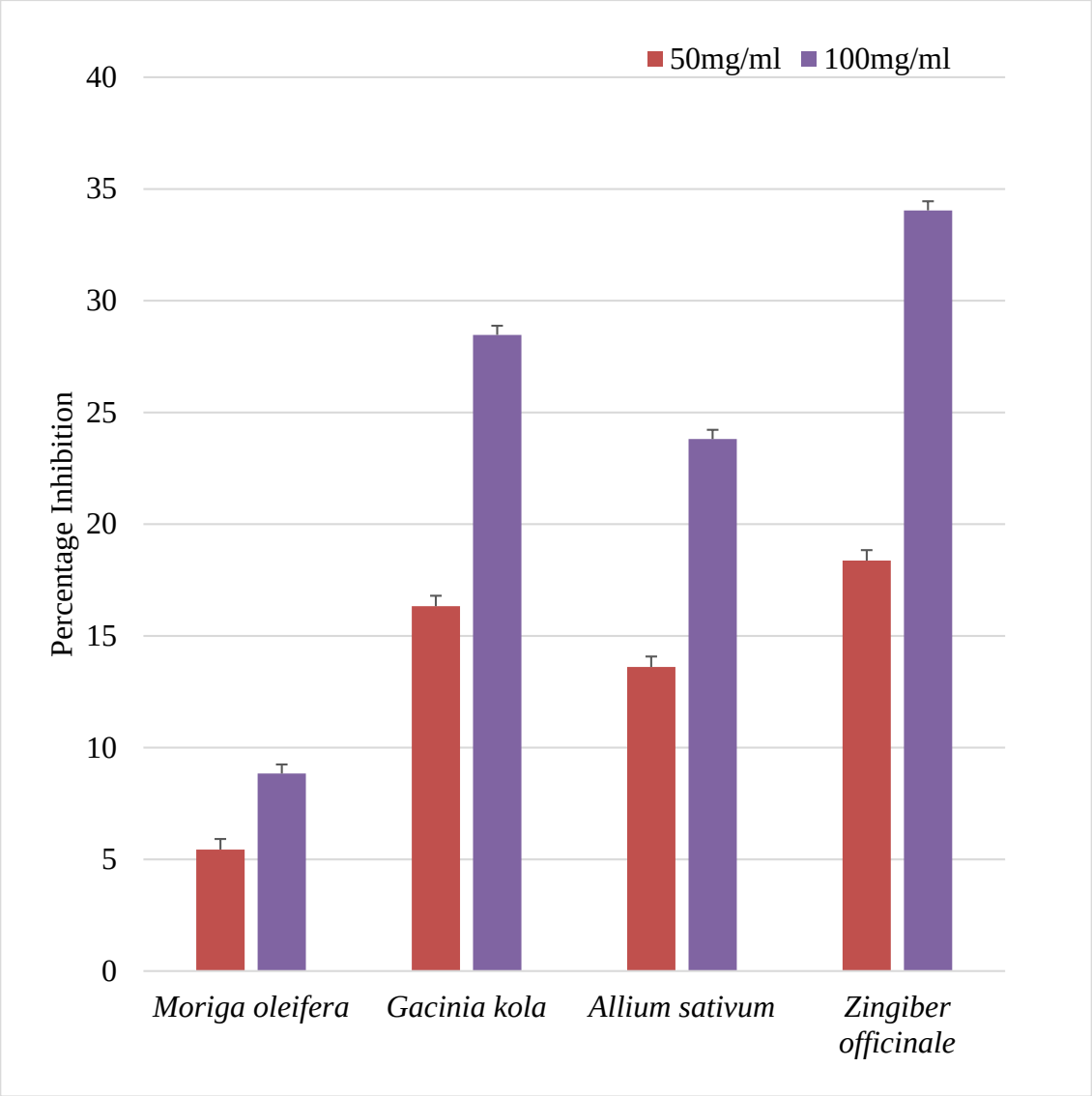


Figure 4.20: Percentage inhibition of mycelial growth of *Fuasrium solani* in different concentrations of Methanolic Extracts of *Moringa oleifera, Garcinia kola, Allium sativum* and *Zingiber officinale.*

Figure 4.21 presents the effects of plant extracts on the mycelial growth inhibition of *A. niger*. At 50mg/ml extract concentration, each of the plant extracts exhibited significantly different percentage inhibition against all the test fungi compared to the negative control; with inhibitions ranging from, 10.02, 16.98, and 25.79 to 29.09% in *Garcinia kola, Zingiber officinale, Moringa oleifera* and *Allium sativum*. At increased concentration level of 100mg/ml for each tested plant extract, significantly higher percentage inhibitions ranging from 17.61, 26.42, 46.54 to 54.09% in the respectively aforementioned extracts were obtained. From the mean values, *A. niger* was most sensitive to *Allium sativum* (highest percentage inhibtion over 50% (precisely 54.04%) followed by *Zingiber officinale, Moringa oleifera* and lastly *Garcinia kola.*

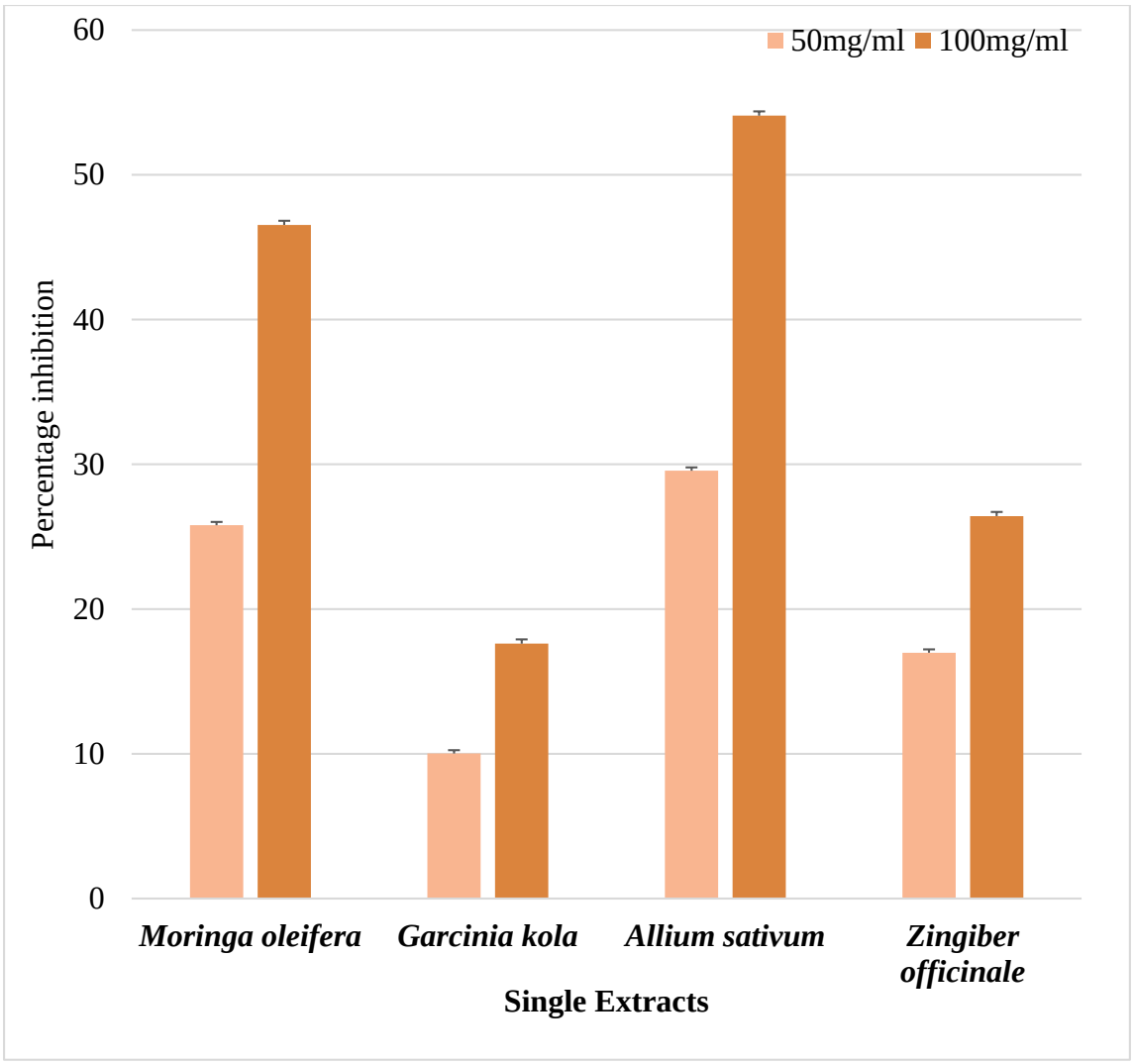


Figure 4.21: Percentage inhibition of Mycelial Growth of *A. niger* in Different Concentrations of Methanolic Extracts of *Moringa oleifera, Garcinia kola, Allium sativum* and *Zingiber officinale*

Figure 4.22 presents the effects of plant extracts on the mycelial growth inhibition of *A. flavus.* There was a general increase in percentage inhibition with an increase in extract concentration. Radial growth inhibition percentages at 50mg/ml extract concentration ranged from 14.1, 14.51, and 16.87 to 19.87% *in Zingiber officinale*, *Garcinia kola*, *Allium sativum* and *Moringa oleifera.* Conversely, at 100mg/ml extract concentration, inhibition percentages were significantly higher (19.05, 27.56, 31.41 and 35.19% respectively) in *Allium sativum, Zingiber officinale, Garcinia kola* and *Moringa oleifera*) than those of the corresponding 50mg/ml extract concentration; with *Moringa oleifera* extracts being the most potent against *A. flavus.*

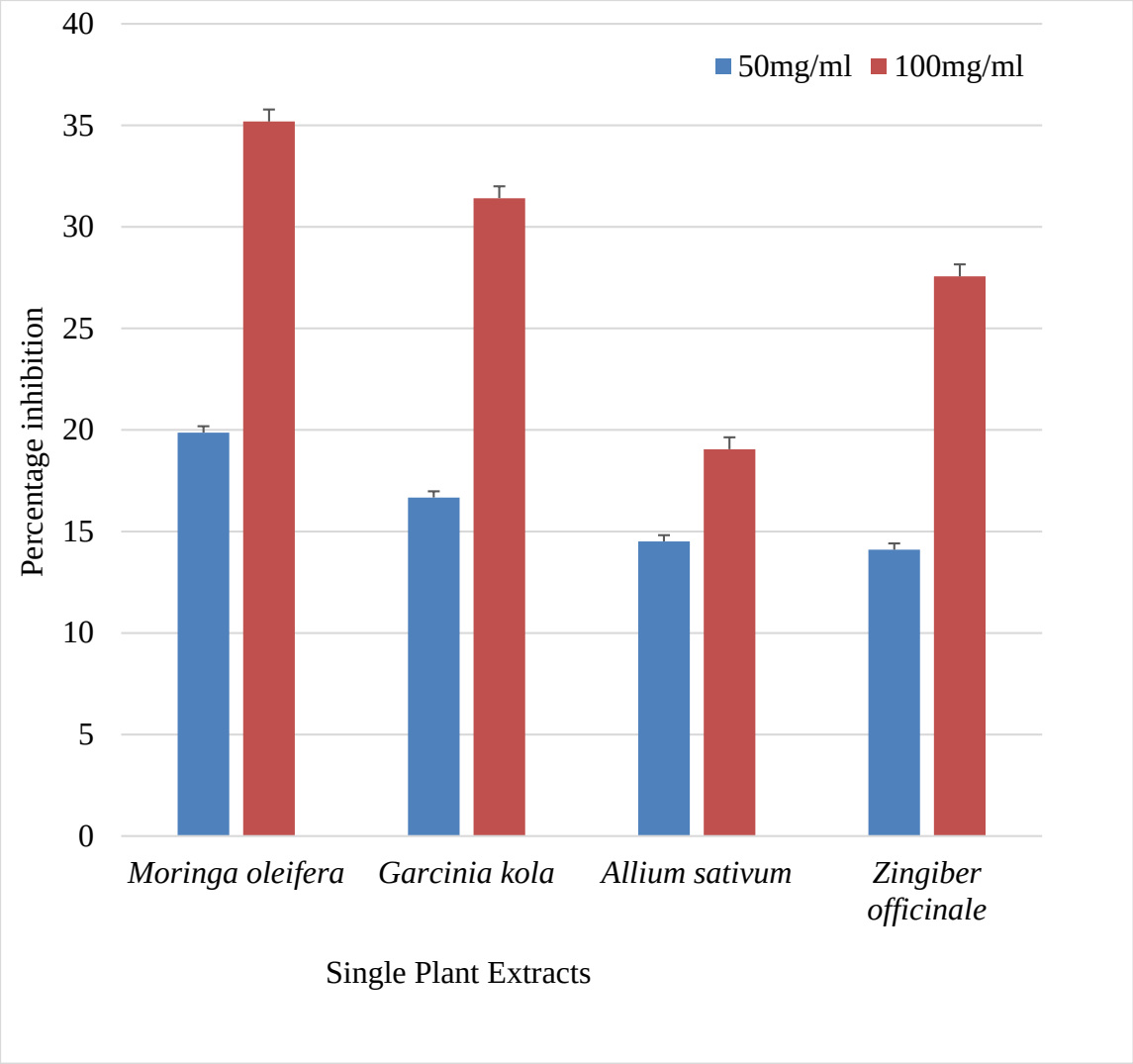


Figure 4.22: Percentage Inhibition of Mycelial Growth of *A. flavus* in Different Concentrations of Methanolic Extracts of *Moringa oleifera, Garcinia kola, Allium sativum* and *Zingiber officinale*

Figure 4.23 presents the effects of plant extracts on the mycelial growth inhibition of *P. expansum.* Figure 4.23 revealed that the radial growth inhibition percentages at 50mg/ml extract concentration ranged from 9.38, 13.54 and 15.64.52 to 20.87% *in Garcinia kola, Zingiber officinale, Allium sativu and Moringa oleifera*. Conversely, at 100mg/ml extract concentration, inhibition percentages were significantly higher (17.71, 26.04 and 28.13 to 34.28% in *Garcinia kola, Zingiber officinale, Allium sativum and Moringa oleifera*) than those of the corresponding 50mg/ml extract concentration; with *P. expansum* being most sensitive to *Moringa oleifera, followed by Allium sativum, Zingiber officinale and Garcinia kola.*

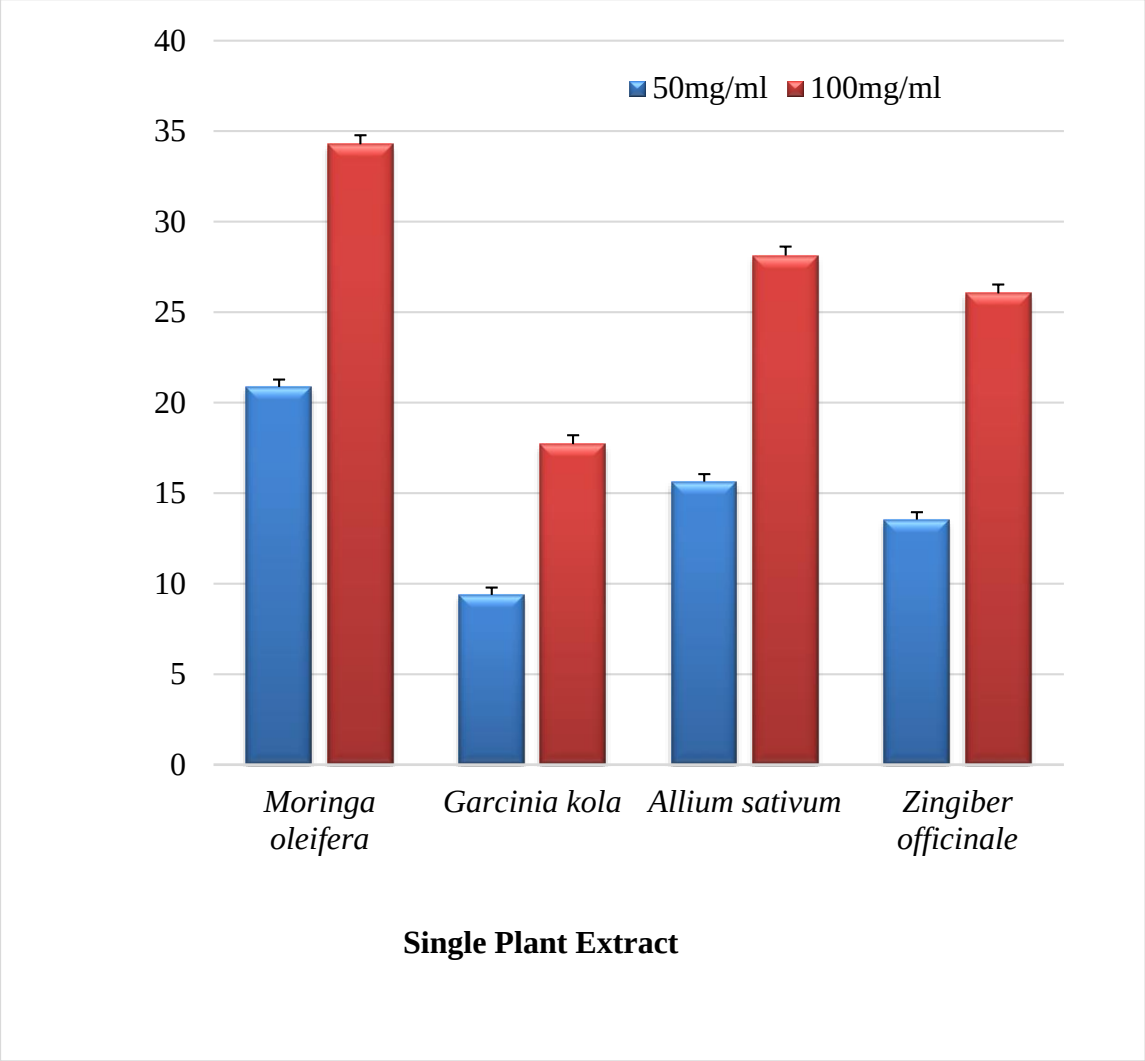


Figure 4.23: Percentage inhibition of mycelial growth of *P. expansum* in Different Concentrations of Methanolic Extracts of *Moringa oleifera, Garcinia kola, Allium sativum* and *Zingiber officinale*

As shown in Figure 4.24, extract of *Moringa oleifera* caused maximum mycelia growth inhibition percentages of 13.95% and 26.81% at 50mg/ml and 100mg/ml extract concentration respectively against *A. awamori*. This was followed by *Allium sativum* with statistically significantly lower mycelia growth inhibition percentages of 12.19% and 21.02% at 50mg/ml and 100mg/ml extract concentration respectively. The inhibitory effects of *Zingiber officinale* followed that of *Allium sativum* with percentage inhibitions of 8.7% and 15.22% respectively at 50mg/ml and 100mg/ml extract concentration respectively and then *Garcinia kola* with 7.27% and 10.15% percentage inhibitions at respective extracts concentration of 50mg/ml and 100mg/ml.

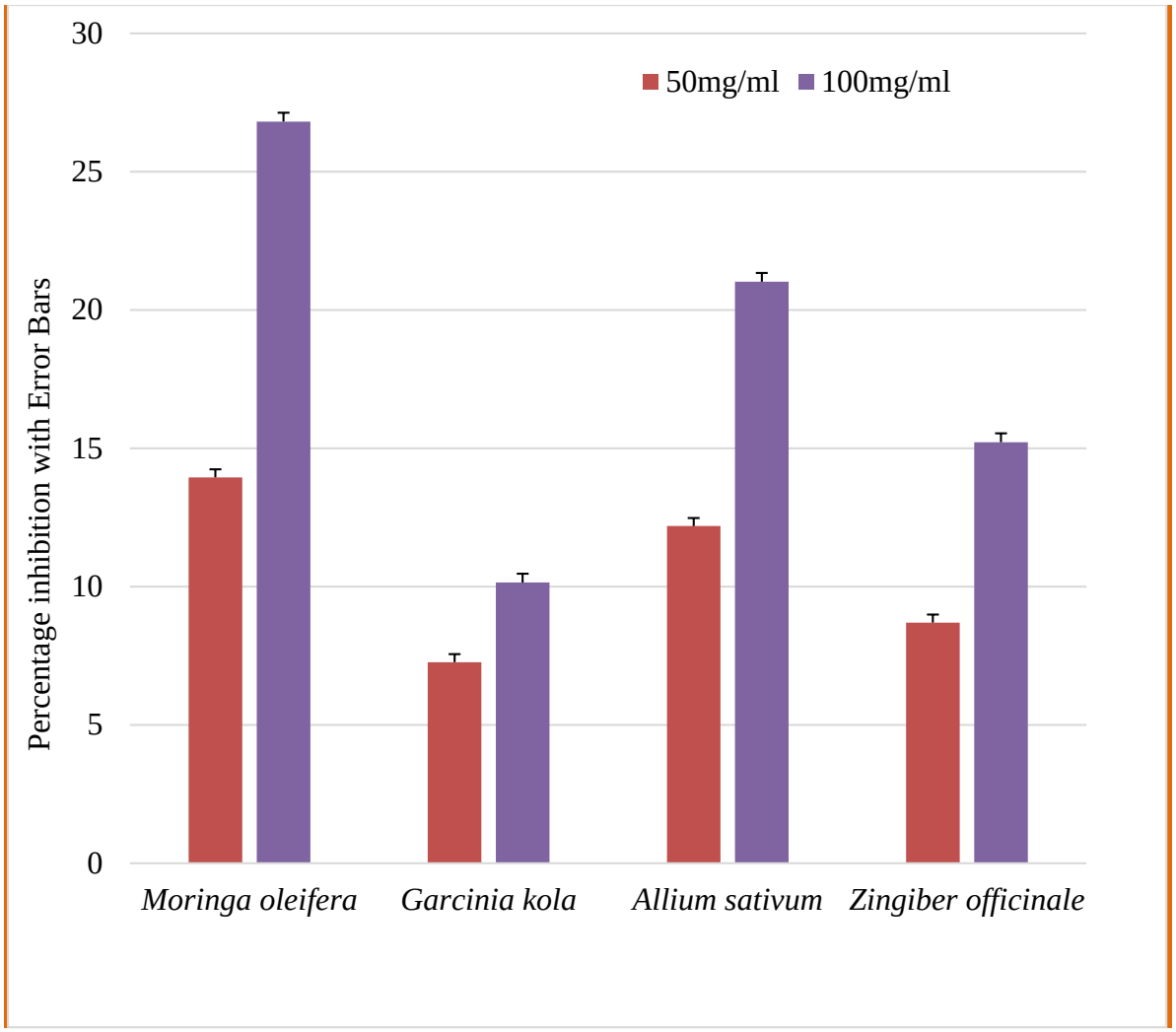


Figure 4.24: Percentage inhibition of mycelial growth of *A. awamori* in Different Concentrations of Methanolic Extracts of *Moringa oleifera, Garcinia kola, Allium sativum* and *Zingiber officinale*

The comparative effect of macozeb, single and combined plant botanicals against the seven postharvest fungi isolated from symptomatic sweet potato roots from Ebonyi state is presented under this section. The inhibition percentage exhibited by Mancozeb on the test fungi is presented in Figures 4.24 to 4.30. As posits in the figures, mancozeb exhibited a very high inhibition (all above 50% against all test fungi) that was significantly higher than those of plant extract and that include 91.41%, 96.01%, 93.88%, 100%, 100%, 100% and 85.91% respectively against *B. theobromae, R. oryzae, F. solani, A. niger, P. expansum, A. awamori* and *A. flavus.*

The percentage (%) mycelial growth inhibition of garlic extract against food-associated fungi by poisoned food technique is presented in Figure 4.25. *Moringa oleifera* at the concentration of 100mg/ml showed significant but less than 50% inhibition of 41.44%, 22.92%, 8.84%, 46.54%, 35.15%, 34.28% and 26.81% against *B. theobromae, R. oryzae, F. solani, A. niger, A. flavus, P. expansum* and *A. awamori* respectively while individual use of *Z. officinale* at same concentration also exhibited less than 50% inhibition against all the tested fungi (*B. theobromae, R. oryzae, F. solani, A. niger, A. flavus, P. expansum* and *A. awamori* with percentage inhibitions of 49.09%, 27.50%, 34.01%, 26.42%, 27.56%, 26.04% and 15.22% respectively. However, when *Moringa oleifera* was combined with *Zingiber officinale*, results obtained showed additive interaction with only two fungi (*B. theobromae* and *P. expansum* with observed values of synergy ratio trending towards antagonism: 0.72 and 0.93 respectively) and antagonism against five isolates (a significantly lower inhibition of 20.00% as against expected value of 44.12%; 19.05% as against 40.33% expected value; 23.27% as against 60.66% expected value, 21.83% against expected value of 53.05% and 0.00% against 37.95% expected value).

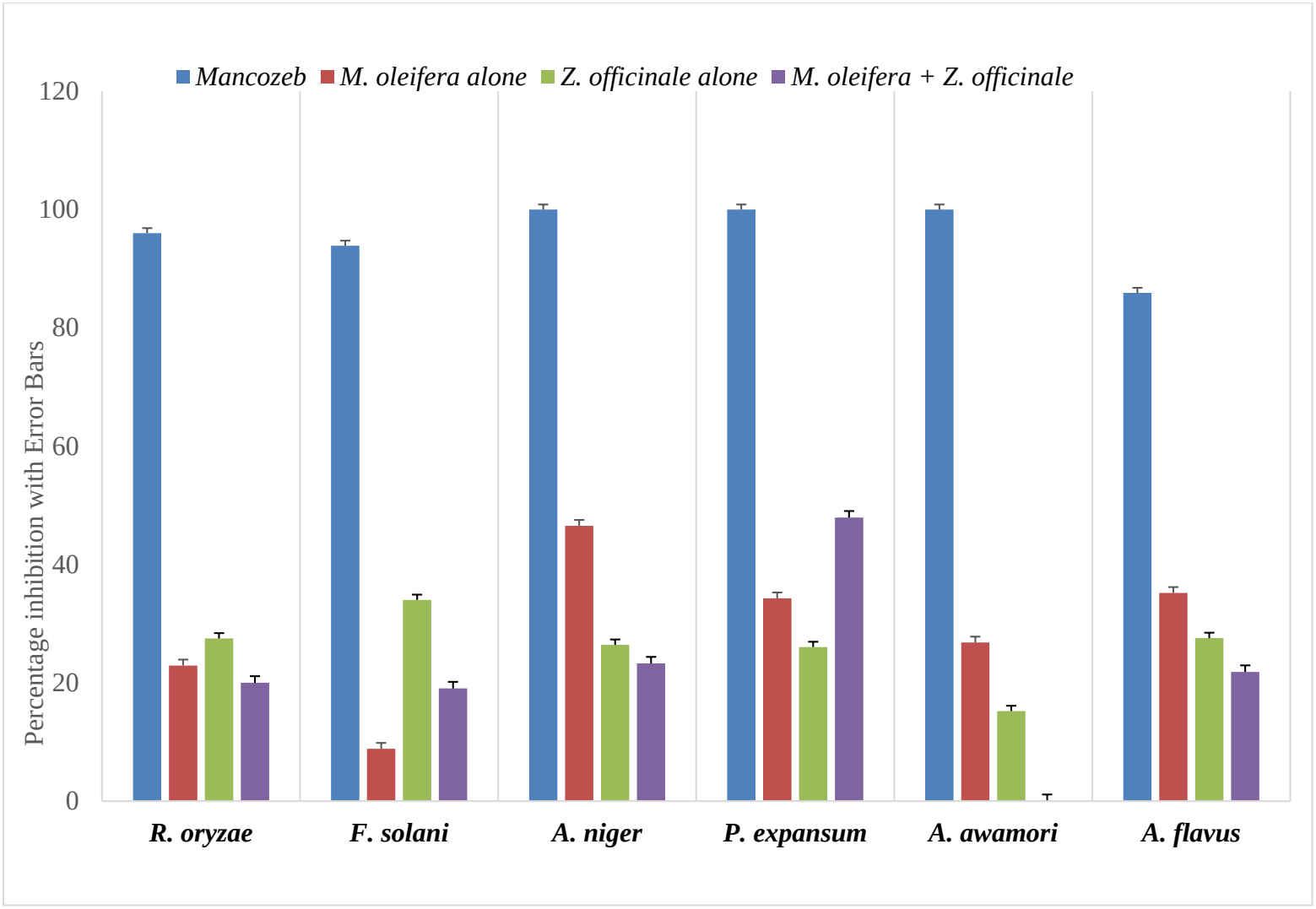


Figure 4.25: Comparative Percentage Mycelial Growth Inhibition of Mancozeb, Single and Combined Extracts of *Moringa oleifera* and *Z. officinale* against Postharvest Fungi of Sweet Potato..

Figure 4.26 presents the percentage (%) mycelial growth inhibition of single and combined extracts of *Garcinia kola* and *Moringa oleifera* against food-associated fungi by poisoned food technique. The inhibition percentage exhibited by Mancozeb on the test fungi has been reported in section 4.6.2.1. The percentage inhibition of *Moringa oleifera* when used alone at the concentration of 100mg/ml is given in Section 5.2.2 above. At same concentration, *Garcinia kola* showed percentage inhibitions that were significantly different from the control but less than 50% inhibition and includes 18.64%, 31.67%, 28.47%, 17.61%, 31.41%, 17.71% and 10.15% against *B. theobromae, R. oryzae, F. solani, A. niger, A. flavus, P. expansum* and *A. awamori* respectively. However, the combination of *Garcinia kola*extract with *Moringa oleifera*extract led to an additive effect *Penicillium expansum* (observed value of 47.927% as against expected value of 51.39%) and *R, oryzae* (observed value of 47.33% as against expected value of 47.08%) while antagonistic effects were observed in other fungi.

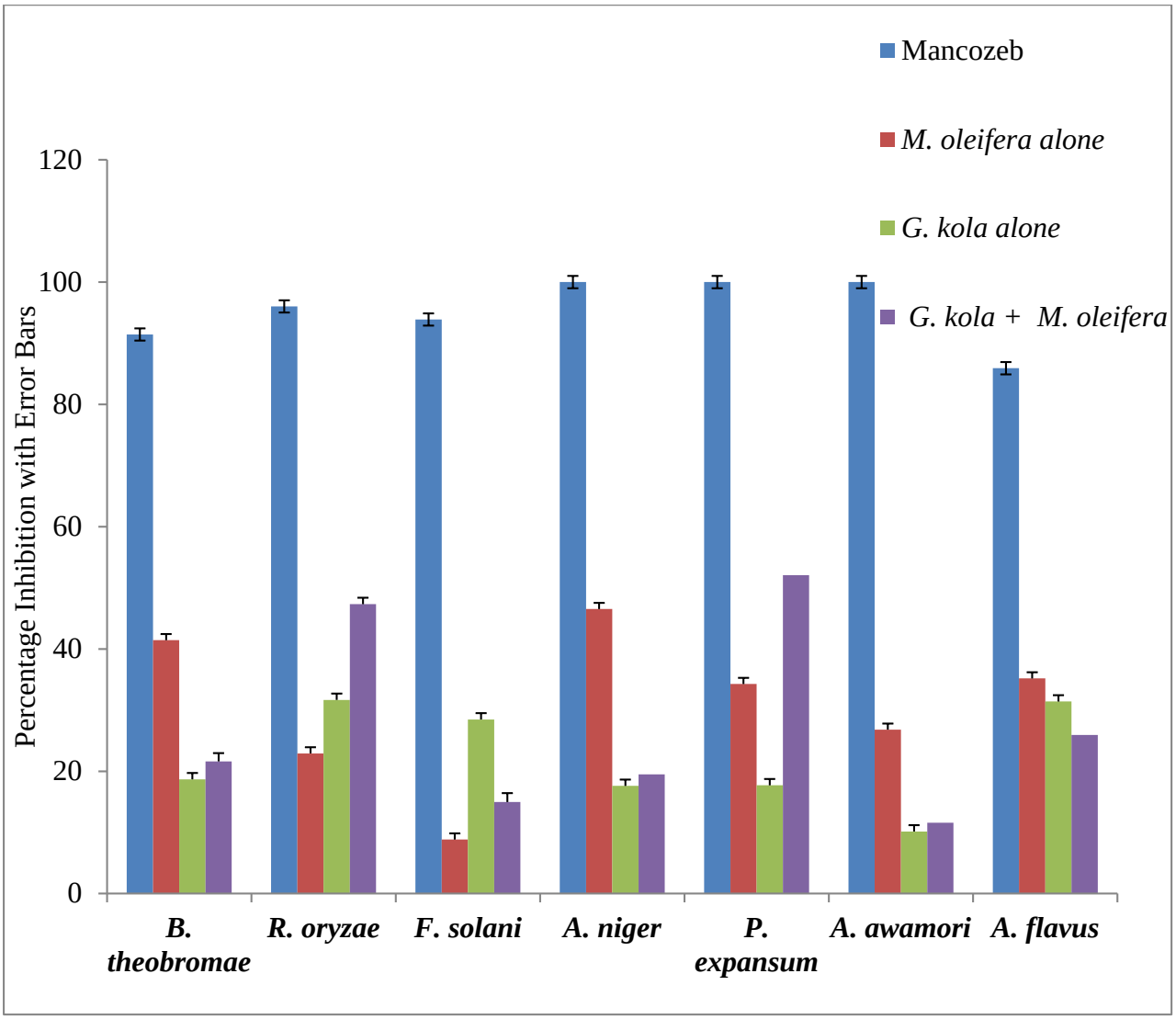


Figure 4.26: Comparative percentage mycelial growth inhibition of Mancozeb, Single and Combined extracts of *Garcinia kola* and *Moringa oleifera* against postharvest fungi of sweet potato

Figure 4.27 presents result of the percentage inhibition exhibited by Mancozeb and *Moringa oleifera* and *Allium sativum* extract combinations on the test fungi. The percentage inhibition of *Moringa oleifera* when used alone at the concentration of 100mg/ml is given in Section 4.6.2.2 above. At same concentration, *Allium sativum* showed maximum percentage inhibitions of 54.09% on *A. niger* and others below 50% inhibitions against other fungi (*B. theobromae, R. oryzae, F. solani, A. flavus, P. expansum* and *A. awamori* with respective inhibition of 40.99%, 11.67%, 28.81%, 19.05%, 28.13% and 21.02%) that were significantly different from the control but less than 50% inhibition. When *Moringa oleifera* was combined with*Allium sativum* (Figure 4.27), a synergistic effect was observed on two fungi (*R. oryzae* and *P. expansum* with observed values of 55.42% and 100% against expected values of 31.91% and 52.77% respectively) and additive effects were observed on the other five tested fungi (*B. theobromae, F. solani, A. niger, A. flavus* and *A. awamori*) with respective observed percentage inhibition values of 75.22%, 36.74%, 83.65%, 52.56% and 60.15% as against respective expected values of 65.45%, 30.55%, 75.46%, 47.54% and 42.21%.

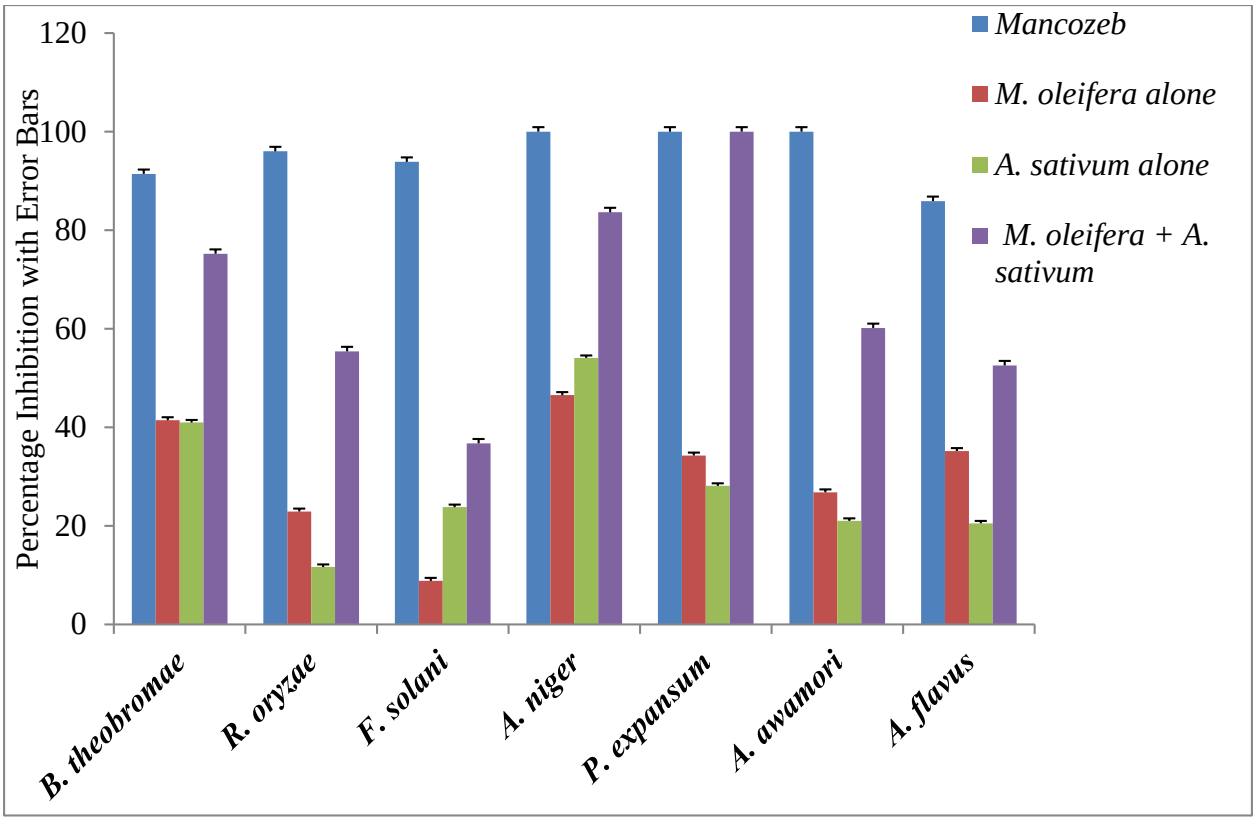


Figure 4.27: Comparative percentage mycelial growth inhibition of Mancozeb, Single and Combined extracts of *Moringa oleifera* and *Allium sativum* against postharvest fungi of sweet potato;

Figure 4.28 presents result of the percentage inhibition exhibited by Mancozeb and *Zingiber officinale* and *Allium sativum* extract combinations on the test fungi. *Zingiber officinale* extract had pathogen dependent inhibition that was significantly different from the control but not above 50% inhibition when used alone at a concentration of 100mg/ml. They included inhibitions of 49.09%, 27.50%, 34.01%, 26.42%, 27.56%, 26.04% and 15.22% respectively against *B. theobromae, R. oryzae, F. solani, A. niger, A. flavus, P. expansum* and *A. awamori.*As shown in Figure 4.28, the combined extract of *Zingiber officinale* and *A. sativum* exerted a synergistic effect against four fungi viz: , *F. solani,* , *A. flavus, P. expansum* and *A. awamori* (with respective observed percentage inhibition values of 88.44%, 76.92%, 69.18% and 78.11% as against respective expected values of 49.72%, 41.36%, 33.06% and 45.84%) and additive effect against the other fungi: *B. theobromae, R. oryzae* and *A. niger* (with respective observed percentage inhibition values of 51.25%, 94.17% and 98.74% as against expected values of 35.97%, 71.99% and 66.22% when it was combined with *Allium sativum*.

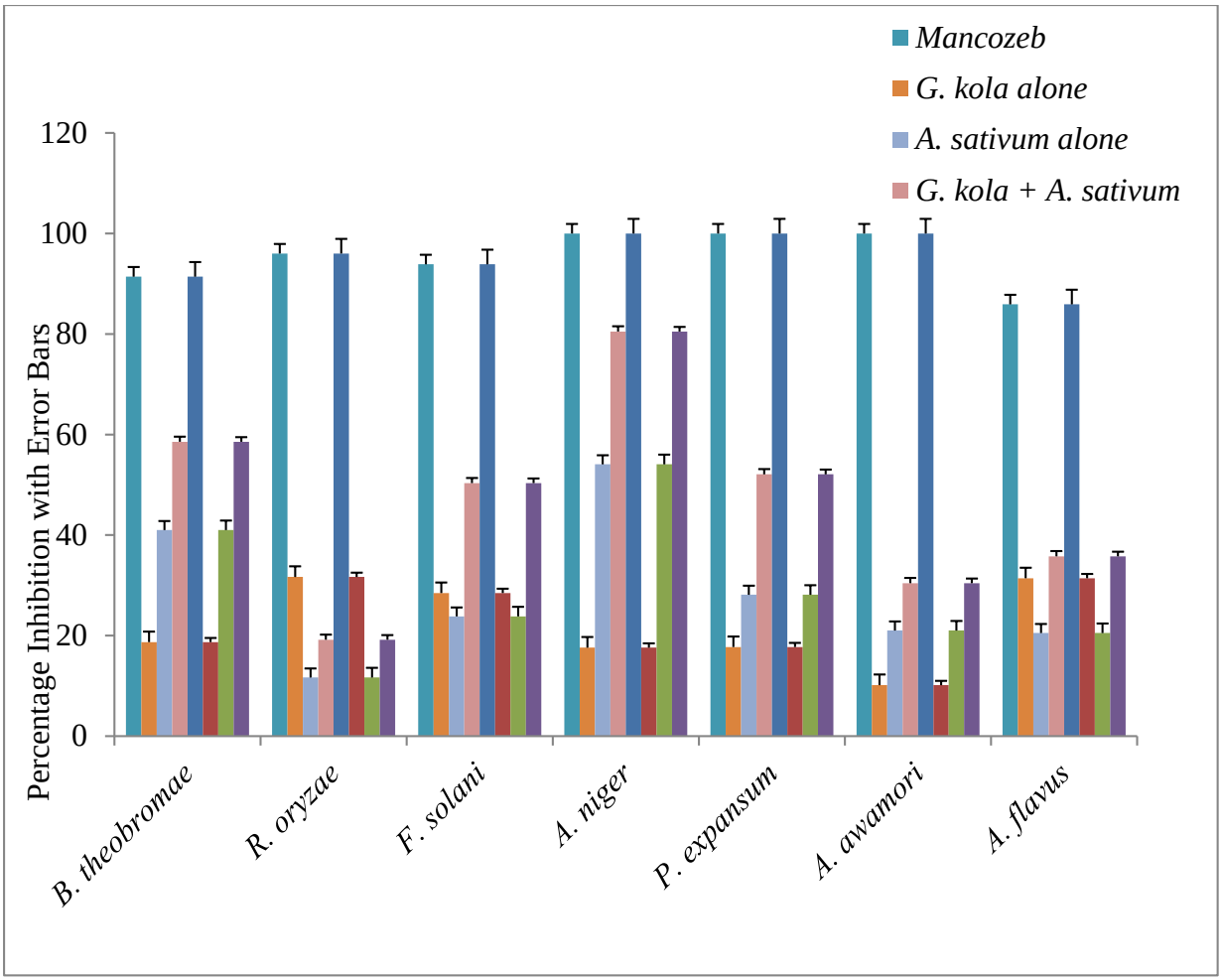


Figure 4.28: Comparative percentage mycelial growth inhibition of Mancozeb, Single and Combined extracts of *Zingiber officinale* and *Allium sativm* against Postharvest Fungi of Sweet Potato

Figure 4.29 presents result of the percentage inhibition exhibited by Mancozeb and *Garcinia kola* and *Allium sativum* extract combinations on the test fungi. *Garcinia kola* showed percentage inhibitions that were statistically significantly different from the control but less than 50% inhibition and includes 18.64%, 31.67%, 28.47%, 17.61%, 31.41%, 17.71% and 10.15% against *B. theobromae, R. oryzae, F. solani, A. niger, A. flavus, P. expansum* and *A. awamori* respectively. However, the combination of *Garcinia kola* extract with that of *Allium sativum* (Figure 4.29) led to antagonistic effect on *R. oryzae* (observed value of 19.17% as against expected value of 39.64%). The extract combination also gave additive effect on other tested fungi (*B. theobromae, F. solani, A. niger, A. flavus, P. expansum* and *A. awamori*) with respective observed percentage inhibition values of 58.55%, 50.34%, 80.50%, 35.77%, 52.09% and 30.43% as against respective expected values of 55.27%, 45.5%, 62.17%, 44.48%, 40.86%, and 29.05%).

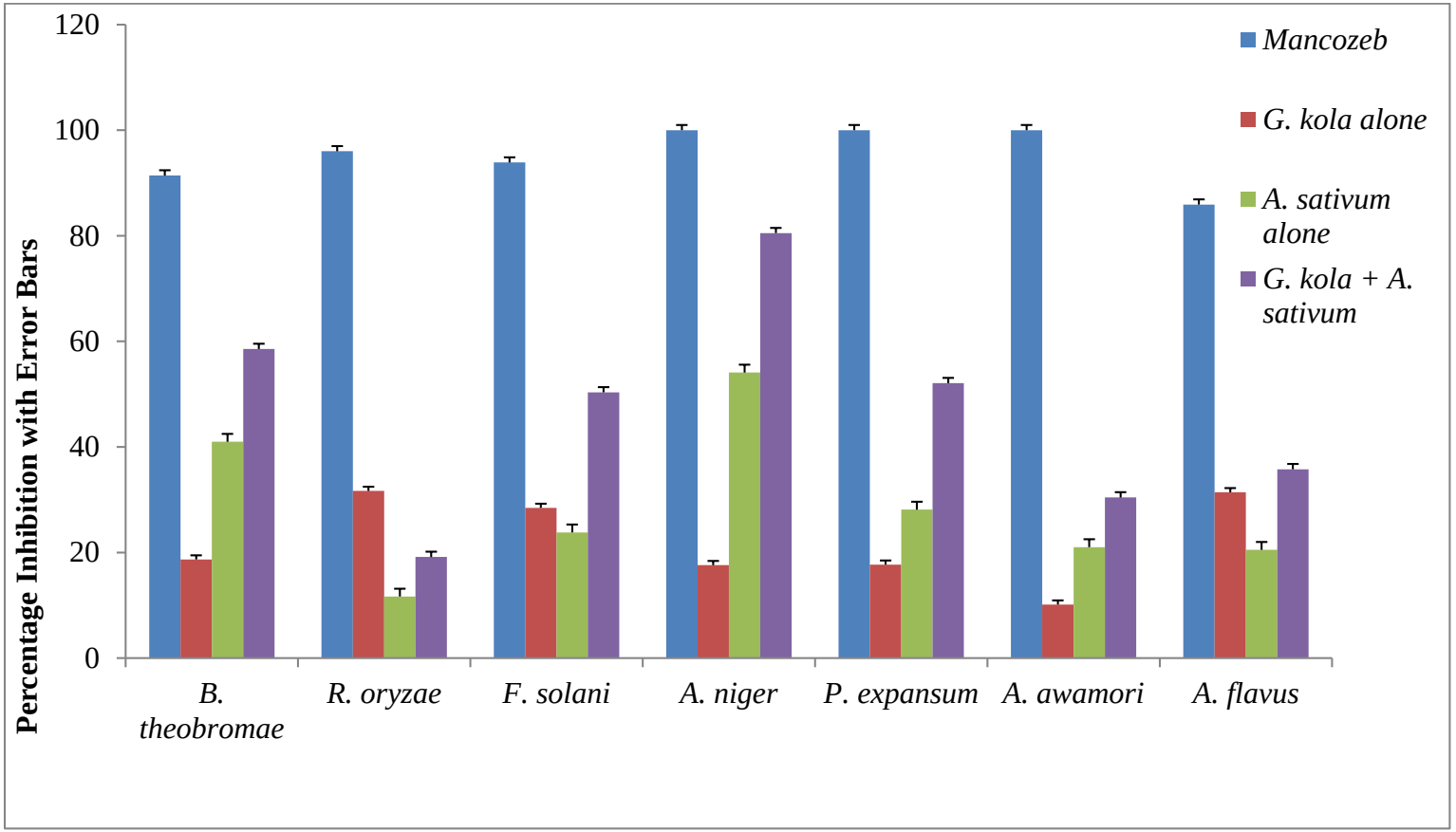


Figure 4.29: Comparative Percentage Mycelial Growth Inhibition of Mancozeb, Single and Combined Extracts of *Garcinia kola* and *Allium sativum* against Postharvest Fungi of Sweet Potato

Figure 4.30 presents result of the percentage inhibition exhibited by Mancozeb and *Garcinia kola* and *Zingiber officinale* extract combinations on the test fungi. On application as single extracts, neither *Garcinia kola* nor *Zingiber officinale* could exhibit up to 50% mycelia growth inhibition. However, the combination of *Garcinia kola* with *Zingiber officinale* (Figure 4.30) had a synergistic effect in *R. oryzae* (76.12% observed value against 50.46% expected value) an additive effect in *B. theobromae, F. solani, A. niger and A. flavus (*with respective observed percentage inhibition values of 75.13%, 71.43%, 39.62% and 75.00% as against respective expected values of 58.61%, 52.8%, 39.38%, and 50.30%) and an antagonistic effect in two fungi (*A. awamori* and *P. expansum* with observed percentage inhibitions of 10.87% and 18.75 as against expected values of 23.83% and 39.14% respectively).

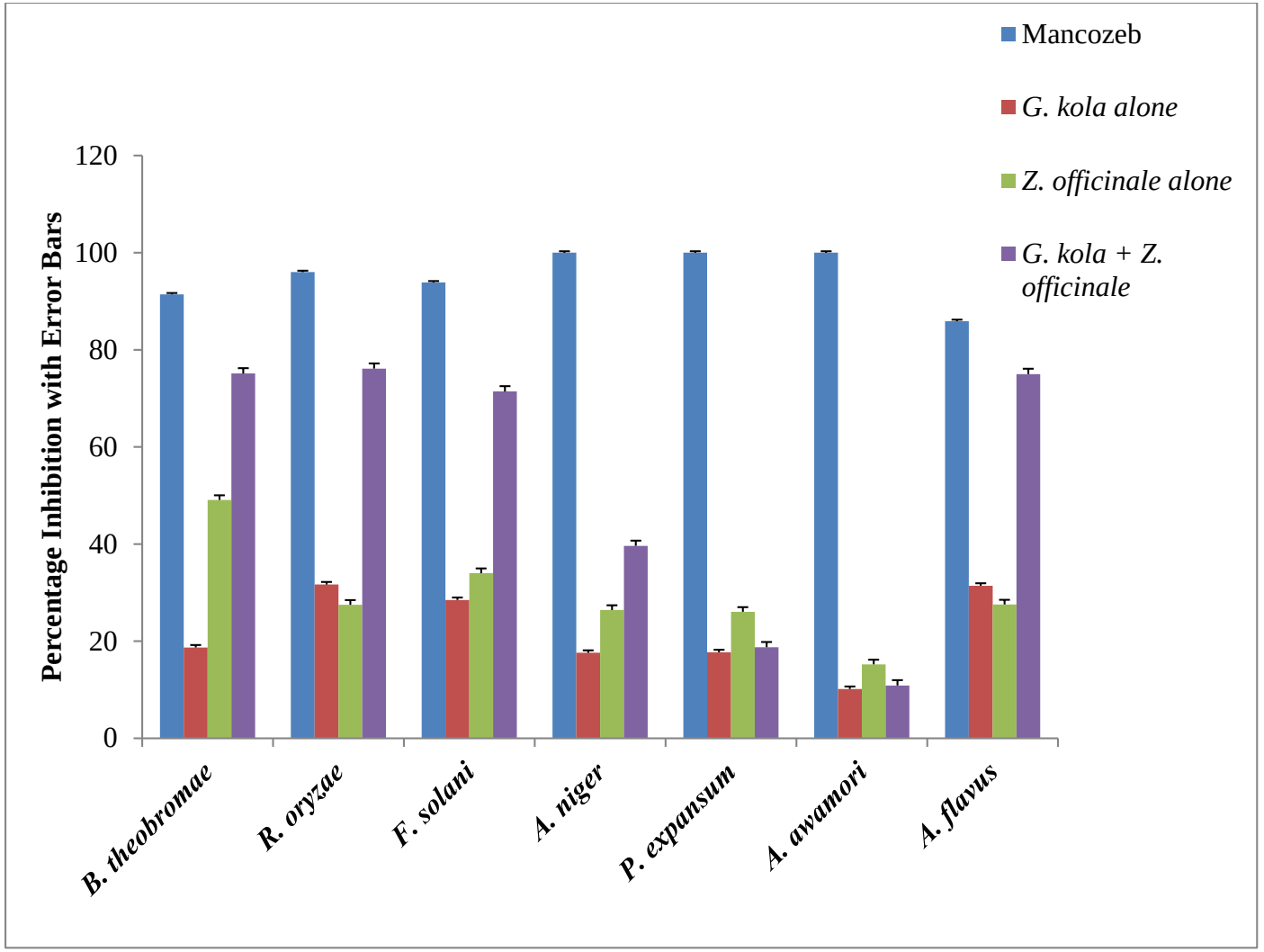


Figure 4.30: Comparative Percentage Mycelial Growth Inhibition of Single and Combined Extracts of *Garcinia kola* and *Zingiber officinale* against Postharvest Fungi of Sweet Potato

Table 4.7 presents results on the effect that mancozeb and the combined plant extracts exhibited against the postharvest rot caused by the test fungi on sweet potatoes. The results showed that all treatments significantly inhibited rot development by all the fungi compared to the negative control (non-treated, wounded and inoculated root) that gave zero inhibition. Furthermore, the results showed significant (P=0.05) interaction between method (preventive and curative) and treatments for all the pathogens. Inhibition of rot exhibited by all the treatments was significantly (9=0.05) higher with the preventive method than with the curative method across all postharvest pathogens except in only *A. niger* where percentage inhibition (100%) recorded by mancozeb was the same in both preventive and curative methods. Mancozed, *Allium sativum/Zingiber officinale* extract combination and *Allium sativum/Moringa oleifera* extract combination exhibited rot inhibitions that ranged from 85.9333±0.4099 to 100%, 39.4133±0.3099 to 97.8367±0.0876 % and 31.9400±0.0971 to 99.0467±0.5374% respectively. The highest percentage rot inhibition (100± 0.00%) was recorded by mancozeb against *A. niger*; however, inhibition of *B. theobromae* by mancozeb (88.8267±0.0807%) was significantly lower than that of *Allium sativum/Zingiber officinale* extract combination (91.1233 ±0.1510%) on the same pathogen.

Table 4.7: Comparative Evaluation of *In Vivo* Inhibitory Effect of Plant Extract Combinations on Postharvest Rot on Sweet Potato

Fungi	Treatments/ Percentage Inhibition					
	Treatment Method	**Positive Control**	**ASZO**	**ASMO**	**Total**	**Negative Control**
B. theobromae	PVT	$^{b}88.8267 \pm 0.8087_{b}$	$^{a}91.1233 \pm 0.1510_{a}$	$^{d}70.1167 \pm 0.1692_{c}$	83.3556 ±3.3350	0.00
	CRT	$^{c}86.6533 \pm 0.3267_{a}$	$^{e}67.7267 \pm 0.3670_{b}$	$^{f}65.6967 \pm 0.4489_{c}$	73.3589 ±3.3420	0.00
R. oryzae	PVT	$^{b}91.1233 \pm 0.1510_{a}$	$^{c}53.2267 \pm 0.1746_{b}$	$^{d}49.9667 \pm 0.1052_{c}$	64.9589 ±6.7100	0.00
	CRT	$^{a}90.0867 \pm 0.0867_{a}$	$^{e}39.4133 \pm 0.3099_{c}$	$^{d}48.8600 \pm 0.5311_{b}$	59.4533 ±7.7808	0.00
F. solani	PVT	$^{a}97.4333 \pm 0.2963_{a}$	$^{b}85.1900 \pm 0.4356_{b}$	$^{d}35.3133 \pm 0.1878_{c}$	72.6458 ±9.5003	0.00
	CRT	$^{a}97.4167 \pm 0.3950_{a}$	$^{c}64.6033 \pm 0.3967_{b}$	$^{d}31.9400 \pm 0.0971_{c}$	64.6533 ±9.4520	0.00
A. niger	PVT	$^{a}100 \pm 0.00_{a}$	$^{b}83.3767 \pm 0.3232_{b}$	$^{c}81.9500 \pm 0.1266_{c}$	88.4423 ±2.8981	0.00
	CRT	$^{a}100 \pm 0.00_{a}$	$^{e}67.9433 \pm 0.5904_{c}$	$^{d}80.30 \pm 0.3881_{b}$	82.7478 ±4.6717	0.00
P. expansum	PVT	$^{a}100 \pm 0.00_{a}$	$^{c}97.8367 \pm 0.0876_{b}$	$^{b}99.0467 \pm 0.5374_{a}$	98.9611 ±0.3502	0.00
	CRT	$^{c}98.0400 \pm 0.3469_{a}$	$^{d}74.0200 \pm 0.0814_{b}$	$^{d}73.3567 \pm 0.3273_{b}$	81.8056 ±4.0621	0.00
A. awamori	PVT	$^{a}94.8333 \pm 0.6880_{a}$	$^{c}64.1367 \pm 0.5216_{b}$	$^{e}53.0600 \pm 0.35346_{c}$	70.6767 ±6.2530	0.00
	CRT	$^{b}89.5400 \pm 0.2573_{a}$	$^{d}57.2800 \pm 0.6248_{b}$	$^{f}44.9400 \pm 0.0600_{c}$	63.9200 ±6.6512	0.00
A. flavus	PVT	$^{a}86.9967 \pm 0.01453_{a}$	$^{b}77.1400 \pm 0.59408_{b}$	$^{d}50.7467 \pm 0.3047_{c}$	71.6278 ±5.4141	0.00
	CRT	$^{a}85.9333 \pm 0.4099_{a}$	$^{c}61.8600 \pm 0.5816_{b}$	$^{e}48.7467 \pm 0.3307_{c}$	65.5133 ±5.4493	0.00

Data represent average means of three replicates ± S.E.; Mean values with different subscripts along the row are significantly different at P=0.05 and Mean values with different superscripts along the column are significantly different at P=0.05.
PVT= Preventive; CRT= Curative; ASZO= *A. sativum* plus *Z. officinale*; ASMO= *A. sativum* plus *M. oleifera*

CHAPTER FIVE

DISCUSSION

The present study investigated the occurrence and biocontrol of postharvest fungi responsible for sweet potato spoilage in Ebonyi State, South Eastern Nigeria. In this study, 352 fungal isolates were obtained from 200 rotted sweet potato roots, suggesting that postharvest rots of sweet potatoes in Ebonyi State occur together as a complex rot involving many fungi. Results indicated that postharvest rots were more prevalent in the dry season than in the wet season. Such evidence of decreased percentage occurrence of isolated fungi during the rainy season indicates an improved environmental status, less favourable to fungi proliferation.

Furthermore, results showed that five genera (*Aspergillus, Fusarium, Botryodipladea, Rhizopus* and *Penicillium*) and seven species (*A. niger, A. flavus, A. awamori, F. solani, B. theobromae, R. oryzae* and *Penicillium expansum*) were the postharvest fungi responsible for the spoilage of sweet potato in Ebonyi State. A similar study in Anambra State by Agu *et al.* (2015) examined fungi associated with the post-harvest loss of sweet potato using a total of ten tubers obtained from Eke-Awka market, Awka South Local Government Area, Anambra State. The spoilage molds they identified were three species: *Aspergillus fumigatus, Aspergillus niger* and *Rhizopus stolonifer.* Though Anambra and Ebonyi States are both in South East of Nigeria, the reason for the variation in occurrence of up to seven different species of fungi in the present study and those (3 species) of Agu *et al* (2015) may be due to several factors such as sample size and sampling location. A different report buttressing this point can be seen with the findings of Amienyo and Ataga (2007) who analyzed sweet potato samples collected from different markets

in Port Harcourt and identified six fungi comprising four of the fungi genera (*Fusarium, Rhizopus, Aspergillus* and *Botryodiplodea*) and species (*Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Fusarium solani*). In a similar vein, four of the genera of fungi (*Fusarium, Rhizopus, Aspergillus and Penicillium*) reported in this work were also implicated sweet potato rot by Salami (2007) though with different species composition with the exception of *A. niger (Fusarium roselens, Rhizopus stolonifer, Aspergillus fumigatus, Penicillium,* and *Aspergillus niger*)

Botryodplodia theobromae was the mostprevalent fungi (99 in number with percentage mean of 28.45%) isolated from the rotten sweet potato rootsin this study. This finding is similar to observations made in Portharcot, Rivers State by Amienyo and Ataga (2007). Out of six storage fungi (*B. theobromae*, *R. stolonifer*, *A. niger*, *A. flavus*, *F. oxysporum* and *F. solani*) that were isolated from the rot infested sweet potato roots, the authors reported *Botryodplodia theobromae* as most abundant fungi. *Rhizopus oryzae* was the second most frequently isolated fungi (78 in number with mean percentage of 22.56 %) from sweet potato rot in both seasons. Agu *et al* (2015) reported *Rhizopus spp (*though *R. stolonifer)* as one of the most frequently isolated fungus from spoilt sweet potato tubers in Anambra State. Similar scenario was found between two studies done by Amienyo and Ataga (2006) and Salami (2007) where Amionye reported that *Rhizopus* Spp (*R stolonifer*) was the most frequently isolated fungus from spoilt sweet potato tubers in South western, Nigeria. Soft, Rot, caused by *Rhizopus stolonifer* (Ehr. ex Fr.) Lind and several other species of *Rhizopus*, principally affects edible roots. Soft rot probably is widely distributed where ever sweet potatoes are grown, but apparently causes greater losses in more temperate areas (Brooke *et al*., 2003). The pathogen is soil and air borne and harvested sweet potato roots usually get contaminated with fungal spores, development of which requires

wounds for penetration and establishment of the fungus. Therefore, control measures are based on prevention of wounding sweet potatoes (to avoid creating portals of entry for the fungus), and proper curing of roots before storage. *Aspergillus niger* was the third most isolated fungi (70 in number for both seasons, mean frequency of 35 with percentage mean of 19.68%) from the rotted sweet potato roots. This lends credence to report by Person *et al*. (2010) that possessing the ability to grow on a wide variety of substances. *A. niger* is a common contaminant of food, soil and indoor environment. *A. Flavus* was the fourth most isolated fungi (46 in number with percentage mean of 13.03%) from the decaying sweet potato roots. Oyewole (2006) too reported *A. flavus* as one of the fungi associated with postharvest fungal rots. *F. solani* was the fifth most isolated fungi (39 in number with percentage mean of 10.99%). Amienyo and Ataga (2007) also isolated *F. solani* from rotting sweet potatoes. In addition, *F. solani* was also reported to have been recovered from corn (Nur *et al*., 2011). *Penicillium expansum* was the sixth most isolated fungi in this present study. This is in keeping with the finding by Oladoye *et al.* (2016). In their study titled Biomolecular characterization, identification, enzyme activities of molds and physiological changes in sweet potatoes (*Ipomea batatas*) stored under controlled atmospheric conditions, the authors isolated *Penicillium expansum* from sweet potato and implicated it with causation of sweet potato rot. Our findings regarding fungi responsible for rot causation in sweet potato is also in accord with that of Onuegbu (2002) who also implicated *Penicillium species*, *Aspergillus niger* and *Aspergillus flavus* as fungi responsible for decay of sweet potato roots. *Aspergillus awamori* was the seventh most isolated fungi in this study.

Several other fungi (*Monilochaetes infuscans, Fusarium oxysporum, Ceratocysts fimbriata, Rhizopus stolonifer, Macrophomina phaseolina, Diaporthe batatalis, Mortierella ramanniana, Mucor pusillus, Botrytis cinerea and Erysiphe polygoni*) implicated with Sweet

potato rotting by other investigators (Oyewole, 2006) were not isolated in this study. This buttressed the report that complexity of postharvest fungi occurrence vary from place to place. High moisture content of sweet potato makes storage difficult and roots vulnerable to microbial attacks, resulting in high losses (Agu *et al*., 2015). Several other reports (Kihurani, 2004; Eleazu and Ironua, 2013) also have it that moisture content and nutritional compositions of sweet potato roots make the roots susceptible to infection by fungi.

A test of pathogenicity confirmed that all the isolated fungi *(Aspergillus flavus, Aspergillus niger, Fusarium solani, Rhizopus oryzae, Penicillium expansum, Botrydiplodiae theobromae* and *Aspergillus awamori)* were responsible for rot induction in sweet potatoes evidenced by typical rot symptoms shown by roots artificially inoculated with the test fungi within 7 days of incubation and the fact that the isolates on re-isolation, exhibited morphological characteristics and growth patterns similar to those earlier observed on axenic cultures. The isolated fungi have been found to incite different categories of rot, with dry rot being the most prevalent amongst java rot caused by *B. theobromae*, soft rot caused by *R. oryzae* and dry rot observed with root infection by *A. niger*, *A. flavus*, *A. awamori*, *F. solani* and *P. expansum.* Though the mechanisms of fungi action were not experimentally elucidated in the present study, many researchers have discussed the mechanisms of some of the fungi implicated with rot causation in Ebonyi Satae the present study. In a study conducted by Oladoye *et al.* (2016) tittled "Biomolecular characterization, identification, enzyme activities of molds and physiological changes in sweet potatoes (*Ipomea batatas*) stored under controlled atmospheric conditions", fungi were isolated from both the surface peels and the deep tissue cuts of stored sweet potatoes by which virtue the authors proffered the suggestion that the spoilage fungi, once they successfully colonize the surface of the tuber, that the infections can easily proceed into the deep

tissue to cause tissue spoilage. Several reports show that the Phytopathogenic microorganisms are assisted by the enzymes they secrete. Ray (2004) has also reported the production of extracellular hydrolytic enzymes and in particular, cellulolytic and pectinolytic enzymes capable of breaking down storage tubers and that the cellulase degrades cell walls during pathogenesis and inhibition of this enzyme ultimately affects the disease development.

Findings on fungi severity showed that the rot severity exhibited by the fungi ranged from very moderate severity to very high severity. All fungal pathogens were more severe in the dry season than in the wet season. *R. oryzae* exhibited the highest percentage severity of rot in both cultivars and seasons while *P. expansum* displayed the lowest severity. The highest percentage severity recorded by *R. oryzae* in this study lends credence to report by Clark *et al.* (2009) that soft caused by *Rhizopus* species rot is internationally considered one of sweet potato's most important postharvest diseases. As reported by Scot (2009), pectolytic and other enzymes produced by *Rhizopus spp* quickly cause host discoloration and liquefy host tissues. Uncured sweet potatoes are more prone to damage by all the postharvest fungi than are cured sweet potatoes. Therefore there is the need to educate the farmers on the need for adequate and proper curing of their sweet potato produce before storage.

The relative susceptibility of two most commonly grown sweet potato cultivars by Ebonyi farmers was determined and results revealed that the twosweet potato varieties known as 'Tupiaochi' and 'Oyorima' were susceptible to the phytopathogens prevalent in the area, cultivars showing a significant range of responses. Sweet potato cultivar Tupiaochi was considered more susceptible to fungal infection in the dry season than in the rainy season. Several factors can affect root susceptibility to infection. Sweet potato root susceptibility to some fungal diseases may change during storage and with wound type. Sweet potatoes stored for

long periods of time after harvest are more susceptible to rot, especially soft rot disease. Other causes and implications of variation in susceptibility to pathogenic infections have been suggested and discussed by several authors (Laine *et al*., 2011 and Arash *et al.,* 2013). According to May and Anderson reported by Arash *et al.* (2013), variation in susceptibility to pathogenic infections is a function of the host genetic structure. Arash *et al.* (2013) added that constant genotype-by-environment interactions can influence the degree of disease expression in host plants; they gave examples of the environmental variables including, but not limited to, ambient incubation temperature, host nutrient status and host age. The cultivar variability in susceptibility to the postharvest fungi exhibited by the tested sweet potato cultivars in this study also showed that it is possible to pursue host resistance as a means of controlling postharvest rot in some sweet potato cultivars. It will be rational to infer that because of their susceptibility and consequent poor storability, most farmers sell their produce just after harvest to avoid losses, resulting in low income or reduced profits; a practice that also affects farmers' food security particularly in the lean season. The results show that all the cultivars were very susceptible to infection by both pathogens in the first trial (dry season) than in the second trial (wet season). This might be due to differences in the prevailing temperature during incubation. During the first trial, the ambient temperature ranged from 22 to 28^0C and from 20 to 25^0C in the second trial, thus the temperature during the first trial may have provided a more favourable environment for pathogen activity, thus enhancing infection.

The nutritional composition of the sweet potato cultivars commonly grown by Ebonyi farmers - Tupiaochi and Oyorima were moisture (water), crude fibre, protein, ash, fat and carbohydrate. The moisture content of any food is an index of its water activity and is used as a measure of the stability and susceptibility to microbial contamination. The moisture contents of

both sweet potato cultivars were high (75.25%for Tupiaochi and 70.50% for Oyorima). According to Agu *et al.* (2015), high water content of sweet potato makes storage difficult and the roots vulnerable to microbial attacks, resulting in high losses. The high percentage moisture content of the screened roots was indicative of poor shelf life characteristics and high chances of being attacked by microbes. The crude fibre contents of both sweet potato cultivars were 2.92 for Tupiaochi and 1.99% for Oyorima. Dietary fiber serves as a useful tool in the control of oxidative processes in food products and as functional food ingredient (Mandalari *et al.*, 2010); decreases the absorption of cholesterol from the gut in addition to delaying the digestion and conversion of starch to simple sugars, an important factor in the management of diabetes and functions in the protection against cardiovascular disease, colorectal cancer and obesity. Results on values of crude fibre recorded for both sweet potato varieties can be said to be low and agrees with data ((0.65±0.03%) for TIS/87/0087 (fresh wet basis) by Eleazu and Ironua (2014). The protein content of Oyorima was 5.05% and significantly higher than that recorded by Tupiaochi cultivar (2.24%). This supports report that sweet potatoes contain unique proteins called sporamins that account for more than 80% of the total proteins. Protein is required for proper body building and replenishment of worn out tissue in human beings. Going by the WHO's recommendation of 2-9 mg protein per day for an adult, the screened sweet potato cultivars possess useful protein values suggesting they could serve as a promising source of protein. The values obtained for protein in Oyorima cultivar is comparable with the data reported by Ukom *et al*. (2009) for TIS 87/0087 sweet potato cultivar grown without fertilizer application, a value that was significantly higher than those the authors reported for other sweet potato cultivars (CIP-Tanzania, Ex-Igbariam and TIS 8164 with mean protein value of 3.28±0.04, 4.16±0.85 and 3.94±0.03 respectively). According to findings by Ukom *et al*. (2009), higher levels of protein

may occur in sweet potato following application of Nitrogen fertilizer. The importance of lipids in food substances cannot be over-emphasized as it contributes significantly to the energy value of foods. Fat also promotes the absorption of fat soluble vitamins (Atasie *et al*., 2009). The assay of the lipid content of the flour showed that it had low total lipid content (1.44 and 1.34% respectively for Tupiaochi and Oyorima) and this could be of benefit to those that suffer from atherosclerosis or related problems. The ash content varied from 1.48% for 'Oyorima to 2.42% for 'Tupiaochi. This suggests that the roots contain important minerals such as potassium, phosphorus and magnesium.The carbohydrate content was moderately high in the two cultivars (16.33 and 19.64% respectively for Tupiaochi and Oyorima). Though not elucidated in this study, the main components of sweet potato have been asserted to be the carbohydrates components (complex carbohydrates called starches, which make up 53% of the carbohydrate content, simple sugars, such as glucose, fructose, sucrose and maltose making up another 32% of the carbohydrate). The high carbohydrate content of the sweet potatoes cultivars could make them good sources of energy as well as substrates for the production of aromatic amino acids and phenolic compounds through the Shikimic acid pathway. From the foregoing, the nutritional attributes of healthy sweet potato cultivars grown in Ebonyi State can be praised; having proximate components with potential nutritional and health benefits, which if continually available in the roots should enable the sweet potatoes to contribute significantly to a healthy and balanced diet in Ebonyi State and beyond.

Following the evalution of the nutritional composition of the sweet potato roots infected with the phytopathogens and subsequent comparison with those of the healthy root, it was sad to note that infection of roots by phytopathogens can lead to nutritional loss beside other inferred grave consequences. Proximate analysis of the two sweet potato cultivars showed significant

reduction (P= 0.05) in the amount of moisture, crude fibre and carbohydrate in the fungi infected roots when compared to the healthy and control roots of both varieties. The significant decrease in crude fibre recorded in the present study is in agreement with the finding by Adeogun *et al.* (2014). In their studies titled "Studies on Fungi Isolated from *Dioscorea rotundata* (Yam) collected from Open Markets in Lagos Nigeria", the authors reported a gradual reduction in crude fibre of diseased yam tubers with reference to healthy yam. Losses are in the form of reduction in the quality of the tubers through breakdown of tissues resulting in anatomical aberrations and depletion of the nutrient component such as protein, lipids and the major one – starch (Markson *et al.,* 2010a). The observed general significant decrease in the moisture, carbohydrate and crude fibre contents of the roots infected with fungal pathogens when compared with that of healthy roots samples may be attributed to the fact that the fungi utilize the water and carbohydrate for metabolic activities and growth, lending credence to report by Amadioha (2001) that attack by pathogenic fungi on plant products is aimed at absorbing the nutrients available in such stored products for their cellular growth, survival and reproduction.

On the contrary, the crude protein, ash content and fat contents of the fungi infected sweet potato cultivars were shown by the analysis of variance to exhibit significant increase (P= 0.05) when compared to the healthy and control roots of both varieties. This corroborates report by Rodolfo *et al.* (2000) that fungi increase the protein content of samples on which they grow. The increase in the fat content of fungi-infected roots from that of the healthy roots (Table 4.5) is in keeping with reports of Sanyaolu *et al*. (2014) that there was significant increase in the fat content in diseased *Irvingia gabonensis*. The implications of these findings are on several levels. The direct effect of these pathogens is of concern because it revealed that sweet potato roots attacked by these test fungi beyond two weeks are not fit for consumption as they will no longer

provide the major food nutrients they were supposed to have supplied to the body, due to the activity of these pathogens. Adequate and proper care and handling of the roots prior to harvest, during harvest and postharvest and mitigation of fungal spoilage of the roots at the aforementioned stages is critical and will ensure the conservation of these nutritional components. Additionally, the potential impact on human and animal health (Berek *et al.*, 2001) should also be considered.

To establish a precedence for control of the fungi pathogen and rot, the in vitro antifungal effects of four plant extracts viz: *Allium sativum, Moringa oleifera, Zingiber officinale* and *Garcinia kola* were, in the present study, evaluated singly and in their combinations against seven postharvest fungi of sweet potato. Mancozeb solution was used as a standard fungicide to compare its effects on the fungi with those of the single and combined plant extracts. The result of the percentage yield of the plant extracts suggested that 75% methanol was a better solvent for the extraction of *Moringa oleifera* and *Garcinia kola* than it was for *Zingiber officinale* and *Allium sativum*.

The outcome of the single extract antifungal evaluations made it clear that all the tested plant extracts when used individually had activity against all the tested fungi, indicating and as well confirming earlier reports (Ijato, 2011) that all of the extracts possess anti-fungal properties. Ethanolic extracts of garlic was revealed by results of an investigation by Akinmusire *et al.* (2014) to inhibit the growth of *Aspergillus ustus; Aspergillus niger* and *Penicillium* species. The results of the present study also confirmed earlier observations of Tagoe *et al.* (2011), who had reported that ethanol extract of garlic is active against *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum*. The antimicrobial activity of the Moringa extract was in keeping with the previous studies done by Busani *et al.* (2012) and Foidl *et al*. (2001) who reported on the

antibacterial properties of *M. oleifera* seed and leaf. The inhibitory effects of aqueous extracts of moringa leaf on mycelia growth of *B. theobromae, A. niger, F. solani* and *R. stolonifer* and their attendant rots on sweet potato had earlier been reported by Alum *et al*. (2014).

The inhibitory effects of the extracts tested in the present study was shown by results to vary with extract plant type, extract concentration, pathogen and extract constitution. On the basis of pathogen sensitivity, all the tested fungi were more or less sensitive to the four plant extracts, though sensitivity was generally low with the exception of *A. niger*.*R. oryzae* was most sensitive to *Garcinia kola* and showed least susceptibility towards *A. sativum* at both concentrations. *B. theobromae* was most susceptible to *Z officinale* and least sensitive to *Garcinia kola* extract. *F. solani*in turn was most and least susceptible to *Z. officinale* and *M. oleifera* respectively. *A. niger* was the most susceptible of all the tested fungi; was most sensitive to *A. sativum* followed by *M. oleifera* and least sensitive to *G. kola*. *A. flavus* was most sensitive to *M. oleifera* and least susceptible to *A. sativum*. *A. awamori* and *P. expansum* were most sensitive to *M. oleifera* and least sensitive to *G. kola*.

With respect to extract concentration, results in the study, using individual extracts alone showed that at a low extract concentration (50mg/ml), percentage inhibition was significantly better than the control but generally low (below 30% i.e inhibition from 5.44 to 29.56%) across the tested fungi. However, increasing the extract concentrations to 100mg/ml caused Significantly higher percentage inhibition against all the test fungi than their lower concentrated counterparts. This finding is in aggreement with findings of several researchers (Amionye and Ataga, 2007; Alhussaen *et al.,* 2011). Alhussaen *et al.* (2011) obtained results that indicated that garlic extract had a concentration dependent activity against *Pythium ultimum* isolated from tomato seedlings. Alhussaen *et al., (*2011) in their study, reported that undiluted garlic extract

showed a high control activity with no growth as compared to the biotic control without the extract whereas diluted garlic extracts 10% and 5% reduced the fungal growth to 15.5% and 41% respectively.

Increasing concentrations of these extracts implied an increase in the active ingredients of the solutions which acts on the fungi thereby affecting their physiological processes and consequently lowering the growth of the fungi. That not withstanding, none, apart from garlic extract against only *A. niger* exhibited percentage mycelia growth inhibition that was up to 50%. This is in keeping with findings of several researchers, for instance, results obtained by Okigbo and Nmeka (2015) in their investigation of hot water extracts of *Z. officinale* seeds via radial growth technique for antimicrobial activity against *Fusarium sp*ecies., *A. flavus* and *A. niger* showed that *Z. officinale* exhibited 33.3%, 31.5%, and 18.2% mycelial growth inhibition respectively. Possible explanations for the generally low percentage inhibition recorded in this study may be due to the fact that the concentrations were low.

The concomitant increases in percentage inhibition attendant with increase in extract concentration observed in this work and corroborated by other works, chances are that further increase in single extract concentration could produce correspondingly increase in percentage inhibition of phytopathogen but that may trigger unfavorable outcome on the organoleptic properties of food produce or worse still triger emergence of resistance as was the case with overuse of chemical fungicides. From the forgoing and based on a survey report harnessed from some of Ebonyi sweet potato farmers who reported to have in time past used neem leaf extracts on a postharvest context but had to stop as they found the extracts impacting on the organoleptic characteristics of their food, there is need for development of a more potent control agent that will possess high activity at low dose rate. Moreover, the result of the antifungal action of the

individually used plant extracts showed that none of the extracts had broad spectrum activity against the test fungi. The outcomes of susceptibility experiment depicted that whereas extract of garlic showed the highest inhibitory effect at both concentrations (29.56 and 54.09%) against *A. niger*, it gave inhibitions as low as 7.28% and 11.67% against R. oryzae. *Allium sativum* exhibiting the highest percentage inhibition amongst *Zingiber officinale* and other extracts in the present studies is in keeping with findings by Joe *et al. (*2009), Iram *et al*. (2012) and Skrinjar and Nemet (2009). Iram *et al*. (2012) reported a higher inhibitory effects of garlic extract at different concentrations against bacterial strains of S. typhi, *E. coli*, and *S. aureus.* The antimicrobial activity of garlic is believed to be due to the effect of allicin, the main ingredient in garlic, generated by the phosphopyridoxal enzyme allinase and ajoene (Harris *et al*., 2001). Previous studies reported that allicin is a pure, bioactive and the most powerful medicinal compound isolated from garlic. It has strong antimicrobial and antifungal activities. Thus, inhibition of fungi observed in this study may be related to allicin or ajoene which curbs the performance of some enzymes that are important to fungi.

The second highest percentage inhibition (26.12% and 49.09%) was exhibited by *Zingiber officinale* extract against *B. theobromae* but the same plant extract could exhibit only 8.7% and 15.22% inhibitions against *A. awamori.* Similarly, *Moringa oleifera* which exhibited the third highest inhibition percentage (25.79% and 46.54%) shown against *A. niger* barely inhibited (5.44% and 8.84%) *F. solani*. Given the fact that none of the single extracts exhibited broad spectrum antimicrobial activity and considering also the broad spectrum of pathogens involved in the post harvest food spoilage complex; for any alternative control methods to be efficient, such alternative control methods should not be too specific.

From the forgoing, the need to seek for control agents with high biological activity against the fungi at low rate application and a broad spectrum activity became obvious. Based on expositions by Wagner and Merzenich (2009) that identifying synergistic combinations of the natural plant compounds could result in control strategies with high biological activity (enhanced antimicrobial activity), low dose rate application and a low risk of pathogen-resistance development and the paucity of information on the activity of combined plant extracts against sweet potato postharvest fungi, the present study comparatively evaluated the plant extracts (*M. oleifera*, *G. kola*, *A. sativum and Z. officinale*) in their two by two combinations and mancozeb used as a positive control.

Mancozeb exhibited appreciable inhibition that was above 50% against all the test fungi. The result of the present study agrees with several reports on the antifungal activity of mancozeb. Suleiman and Sule (2016) had earlier reported an inhibition as high as 92.22% against *P. expansum*. Singh *et al.* (2000) and Dar *et al.* (2013) in like manner reported after their investigation that mancozeb proved to be the best for the growth inhibition of *F. solani* and *F. oxysporum*. Chirag (2014) reported 100% inhibition of *F. solani* by mancozeb at different concentrations including 1000, 1500, 2000 and 2500ppm. This study wasnot able to verify the mechanism of action of mancozeb but according to reports in literature, Mancozeb inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those involved in the production of adenosine triphosphate (ATP).

As aforementioned, single extracts of the four plant species exhibited weak percentage inhibition (below 50%) against all the text fungi except for *Allium sativum* extract and *A. niger* interaction. The results of evaluation of the two-by-two combination 100mg/ml concentration each) of plant extracts showed three different kinds of interactions viz: synergism, additivity and

antagonism; with additive interaction predominating, followed by synergistic interactions, both of which resulted in increased inhibition (above 50%) across majority of the pathogens. Of the plant extract combinations of *A. sativum and Z. officinale* was found most effective followed by *A. sativum + M. oleifera*; both extract combinations proving the most potent with broadened spectra of activities and inhibition values slightly comparable to those of Mancozeb. While *Zingiber officinale* and *Allium sativum* exerted a synergistic effect against four fungi viz: *F. solani, A. flavus, P. expansum* and *A. awamori* and additive effect against the other fungi (*B. theobromae, R. oryzae* and *A. niger*), *Moringa oleifera* and *Allium sativum* extract combination gave synergistic effects against two fungi and additive effects on the other five fungi, with no record of antagonism. The cause of increased efficacy is unknown, although possession of the different modes of action by the active phytochemical of the constituent plant botanicals might be a factor resulting to additive and synergistic effects.

On the contrary, combination of extract of *Moringa oleifera* and *Zingiber officinale* and that of *Garcinia kola* and *Moringa oleifera* exhibited the poorest antifungal interaction, giving antagonistic interaction against five fungi and additive effect against only two fungi. The poor antifungal interaction (antagonism) shown in this study by *Moringa oleifera* and *Zingiber officinale* and *Garcinia kola* and *Moringa oleifera* extact combinations is in agreement with findings reported by Emad *et al.* (2012). *Moringa oleifera* and *Allium sativum* gave synergistic effects against two fungi and additive effects on the other five fungi. *Garcinia kola* and *Allium sativum* led to an antagonistic effect on *R. oryzae* and an additive effect was observed in the other tested fungi (in spite of which inhibition against two fungi (*P. expansum* and *A. awamori*) were less than 50%. *Garcinia kola* and *Zingiber officinale* exhibited a synergistic effect in *R. oryzae*, additive effect in *B. theobromae, F. solani, A. niger* and *A. flavus* and an antagonistic

effect in two fungi (*A. awamori* and *P. expansum).* The reason for the reduced fungi sensitivity to the combinations that exhibited antagonism was not elucidated in the present study. However, our speculation is that that could be indicative of competitive inhibition resulting from constituents in the plant extracts competing for the mode of action sites of the active ingredients.

The observed synergistic as well as additive interactions provided increased antimicrobial activity using lower concentration when used together while antagonist interaction provided decreased antimicrobial activity. The result obtained with the combined plant extracts suggests that plants antimicrobial agent's combination can modify the antimicrobial activity. The synergism and additivity should be developed to enhance antimicrobial potentiation while antagonistic should be avoided due to emerging resistance microorganisms. The two different extract combinations viz: *A. sativum* and *Z. officinale* and *A. sativun* and *M. oleifera* that depicted better spectra of antimicrobial activity characterized majorly by additivity (SR 0.5–1.5) and synergism (SR > 1.5; $p < 0.5$) were selected and subjected to in vivo evaluation for efficacy in protecting mechanically wounded storage roots of sweet potato. The extract combinations (*A. sativum* and *Z. officinale* and *A. Sativum* and *M. oleifera*) had a significant reduction on the rot development on sweet potato by the test fungal pathogens that were comparable to the effects of mancozeb. This finding is in agreement with earlier reports on the antimicrobial properties of combined treatments. In a similar vein, Amaeze *et al*. (2009) in an investigation titled "Evaluation of Garlic (*Allium sativum*) and Uziza (*Piper guineense*) on the Control of Tuber Rot Fungi of Potato and Carrot" reported both synergism against *F. oxysporum* and antagonism against *A. niger, A. flavus, F. solani* and *G. candidum* exhibited by the combination of *Allium sativum* and *Piper guineense*. A glean from their result revealed interactions that varied with

respect to food substance; where a combination of *A. sativum* and *P. guineense* gave antagonistic effect on *F. solani* rot in carrot and synergistic effect when controlling *F. solani* rot in potatoes.

The in vivo effects of the single plant extracts was not evaluated in this study, therefore there were no values with which to compare the in vivo effects of the combined extracts so as to ascertain potentials for synergism or otherwise. Nevertheless, when comparing the in vivo effects of combined plant extracts to them in vitro effects as well as the in vivo effects of Mancozeb, a parallel may be drawn between results from the in vivo study and those achieved by the in vitro study, in that the results of the in vivo effects showed a similar trend (above 50% inhibition) with those in the in vitro assay.

Moreover, the effects of the combined botanicals exhibited percentage rot inhibitions that were comparable to that of mancozeb, with *A. sativum* and *Zingiber officinale* extract combination showing a significantly higher rot inhibition against *B. theobroma.* The *in vivo* effect of the combined plant botanicals was evaluated by preventive and curative application. Results of this study demonstrated that while all the treatments were effective in reducing fungi activity in vivo when applied through both methods, control treatments were more effective when applied as a preventive measure than in curative form. This was evidenced by the significantly higher rot inhibition exhibited by the sweet potato pre-inoculated and pre storage treatment test in all the different combinations of plant botanical extracts and some mancozeb than those of post inoculation treatments in 14 days of storage at room temperature.

5.1 Conclusion

The fungi associated with the spoilage of sweet potato in Ebonyi State include *A. niger, A. flavus, B. theobromae, R. oryzae, F. solani, Penicillium expansum and A. awamori,* with

percentage occurrence quantitatively and qualitatively greater in the dry season than in the rainy season and varies with pathogen. The fungi on infection of the food crop, cause root decay ranging from soft rot, dry rot, java rot to root rot and also deplete important nutritional components of the food crop, indicating that sweet potato roots affected by these fungi beyond two weeks are not fit for consumption as they will no longer provide the major food nutrients they were supposed to have supplied to the body and may constitute health hazards due to the presence and activity of these pathogens. Therefore, it is not enough to plant and harvest high yielding sweet potato roots but it is also pertinent and most important to protect these crop produce in storage to circumvent postharvest attack and rot on them by fungi. While some treatment combinations perform better than their individual counterparts when used alone, not all extract combinations were effective despite the potency of their individual components, thus suggesting that empirical formulation and application of plant botanical combinations as protectants without prior verification may not yield good outcomes. Combinations of *A. sativum* extracts with either *Z. officinale* or *M. oleifera* extract hold promising prospect for use in the development of novel biofungicide for management of postharvest fungi and rot of sweet potatoes.

5.2 Recommendations

Based on our findings, documenting postharvest fungi occurrence, evidence of the impact of the postharvest fungi and the promising potential of the novel plant extract combinations as fungi and decay control agents will only be of use if acted upon. Collaborations between the farmers, Agricultural policy markers, Plant Protection/Health research and the Extension Services should be established if crop including sweet potato loss to postharvest pathogens is to be reduced.

The Government and NGOs that seem to champion courses on crop production in disfavour to crop protection should be made to know or reminded that the panacea to food security is not just crop production but rather crop protection given that a crop well produced and less protected is a crop lost which in turn is worse than a crop not cultivated as the labour cost, time, energy and the land cultivated to the crop at that time space become a waste.

The Ministry of Agriculture should intensify education of farmers on the use of good agricultural practices to reduce disease inoculum levels in the field and thereby minimize the incidence of postharvest fungi and rot disease on sweet potato.

Furthermore, farmers should also be educated on the need to take a more serious look at storage than is generally done now; they should be encouraged to give the issue of storage equal attention as is allotted yield improvement if the quality of their harvested produce is to be maintained and fungi infection/rot mitigated.

Of the extract combinations, by virtue of the optimal and broad spectrum antifungal effects displayed in vitro and in vivo, *A. sativum* and *Z. officinale* and *A. sativum* and *M. oleifera* extract combinations could be considered as useful plant combinations for the production of botanical fungicides. However, they need to be subjected to further evaluations.

Moreover studies on the molecular basis of synergistic interactions to understand the synergistic mechanism which is fundamental to the development of alternatives to synthetic fungicides for management of postharvest fungi/rot is very imperative. Hence, research should be focused towards this direction.

5.3 Contributions to the Subject and Body of Knowledge

The contributions from this study are as follows:

1) Important Knowledge gap filled: Spoilage microorganisms are often specific to location and their identification is an important fundamental step in the formulation of disease control strategies in any particular environment. Before now, there was paucity of information on the postharvest fungi associated with the spoilage of sweet potato in Ebonyi State despite it being one of the major sweet potato producing areas. This study has established and to the best of my knowledge, gives the first report onthe natural occurrence of the postharvest fungi in naturally infected sweet potato roots sampled from Ebonyi State.
2) Discovery and report on the susceptibility of Ebonyi farmers' most preferred and commonly grown sweet potato cultivars to the prevailing postharvest fungi: The study has led to the discovery that none of the sweet potato cultivars most cherished and commonly cultivated by Ebonyi Farmers is immune to the pathogen; that is, all isolates expressed virulence to all cultivars which in turn varied in their susceptibility to the postharvest pathogens.
3) Provision of additional evidence on the detrimental impact of microorganisms on the nutritional composition of root and tuber crops.
4) Substantiation of previous findings on antimicrobial activity of plant botanicals: This study substantiates previous findings on the antimicrobial activity of plant botanicals; particularly, extracts of *A. sativum, M. oleifera, Z. officinale* and *G. kola* and contributes additional evidence for requirement of high doses of extracts of single plant botanicals for optimum antimicrobial action.

5) This is the first use of extract combinations against postharvest fungi of sweet potato especially in South Eastern Nigeria. Before now, plant botanical potency studies on postharvest pathogens and rot of sweet potato had been on single plant botanicals. The present study has evaluated the botanicals (extracts) in their combinations and has identified plant extract combinations that hold promising prospect than single extracts and could be sufficiently optimal when considering a natural alternative to synthetic fungicides for management of the studied fungi on sweet potato.
6) The study offers theoretical contribution: This dissertation offers an innovative analytical and methodological approach in the in vitro and in vivo assessment or use of plant botanicals as antimicrobials against sweet potato pathogens. It combined the simultaneous examination of the in vitro effects of the plant extract- singly and in their two-by-two combinations and in vivo effects of only some extract combinations via preventive method vis-à-vis curative methods to identify their effects respectively on the mycelia growth and rot development of the sweet potato postharvest fungi and focuses on broadening the antimicrobial spectra of the plant botanicals.

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APPENDICE

APPENDIX A:

Procedure for Molecular Identification of Sweet Potato Postharvest Pathogens

The fungi identification methods used include:

1: Culture on agar slant

2: DNA Extraction ZR Fungal/Bacterial DNA MiniPrep™50 Preps. Model D6005

3: PCR Amplification and DNA sequencing by Sanger Sequencing Method

4: BLASTn ANALYSIS: NCBI Blast Online Sequencing: Sanger Sequencing (for more details visit: http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Obenrader/sanger_method_page.htm), http://www.protocol online.org/prot/Molecular_Biology/DNA_Sequencing/DNA_Sequencing_by_the_Dideoxy_Method/index.html BLASTn. (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

Primer used: 27F.1 Forward 5'AGRGTTTGATCMTGGCTCAG 3 and 1492R reverse 5'GGTTACCTTGTTACGACTT 3'

DNA Extraction

DNA extraction was from a 24 hours growth of microbial isolates in BHI broth harvested by centrifugation at 14, 000 x g for 10 minutes. The cells were washed three times in 1 ml of Ultra-pure water by centrifuging at 12,000 rpm for 5 min. DNA extraction and purification was done using ZR Fungal/Bacterial DNA MiniPrep™50 Preps. Model D6005 (Zymo Research, California, USA). 50-100 mg of bacterial cells was resuspended in 200 µl of sterile water. This was transferred into a ZR BashingBead™ Lysis Tube. Exactly 750 µl Lysis solution was added to the tube. The bead containing the solution was secured in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 5 minutes. The ZR BashingBead™ Lysis Tube was centrifuged in a micro-centrifuge at 10,000 x g for 1 minute. 400 µl of the supernatant was pipeted into a Zymo-Spin™ IV Spin Filter in a collection tube and centrifuged at 7,000 x g for 1 minute. This was followed by the addition of 1,200 µl of Fungal/Bacterial DNA binding

buffer into the filtrate in the collection tube. After this 800 µl of the mixture was transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuge at 10,000 x g for 1 minute.

The flow through was discarded from the collection Tube and the process was repeated to obtain the remaining products. The 200 µl DNA pre-wash buffer was added into the Zymo-Spin™ IIC Column in a new collection tube and centrifuge at 10,000 x g for 1 minute. This was followed by the addition of 500 µl Fungal/Bacterial DNA Wash Buffer into the Zymo-Spin™ IIC column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IIC column was transferred into a clean 1.5 ml micro-centrifuge tube and 100 µl of DNA Elution Buffer was then added directly to the column matrix. This was centrifuged at 10,000 x g for 30 seconds to elute the DNA. The Ultra-pure resulting filtrate (DNA) obtained was used as a template during the assay. This was transported in ice the laboratory for sequencing.

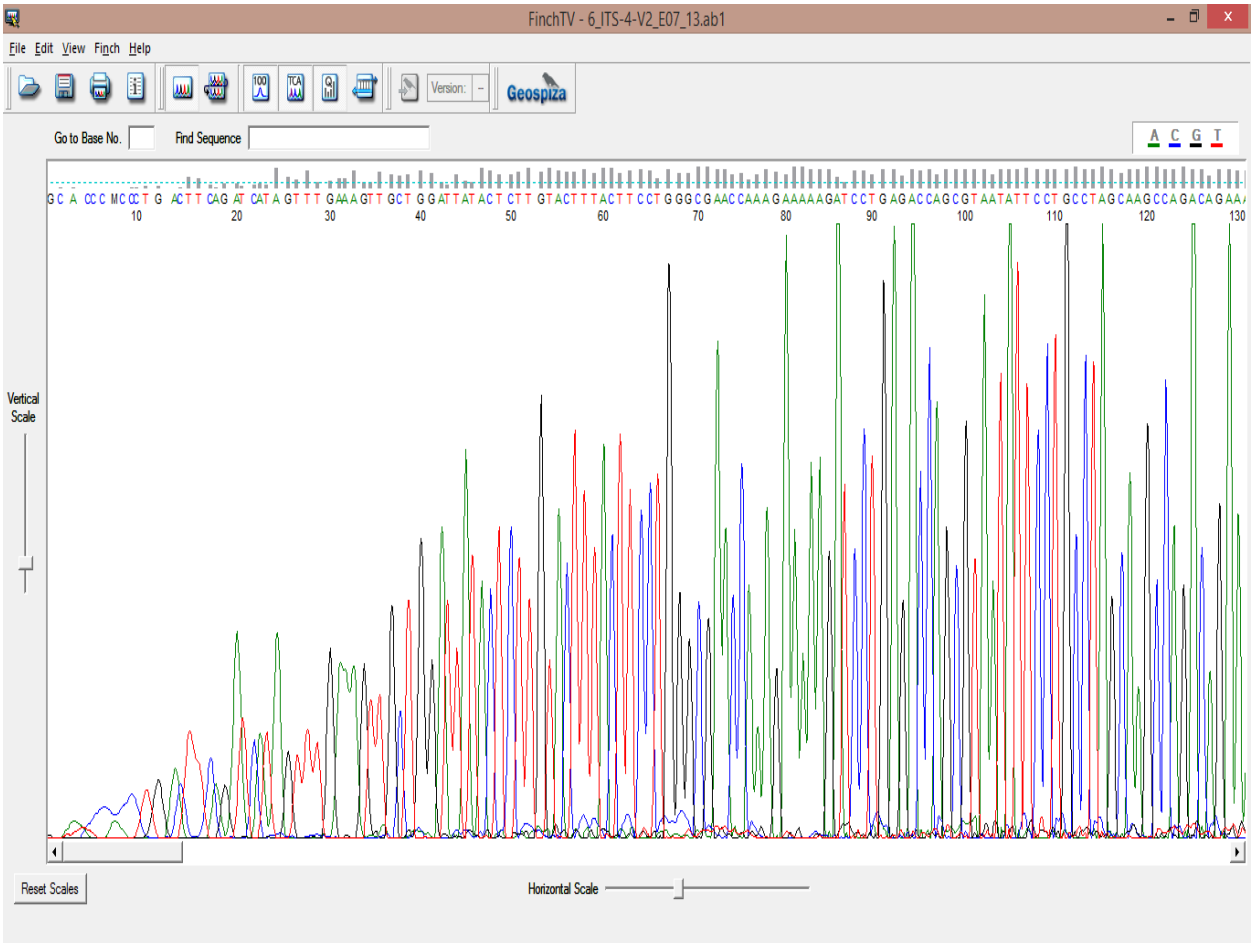
PCR Reaction Amplification of DNA

The PCR reaction was performed on the extracted DNA samples using universal degenerate primers 27F.1 Forward 5'AGRGTTTGATCMTGGCTCAG 3 and 1492R reverse 5'GGTTACCTTGTTACGACTT 3' (De Santis et al.,2007) that amplifies the entire 16s Variable region at annealing temperature of 58. Each PCR reaction contained 5 µl of 10 × Taq buffer, 2 mM MgCl 2,1.5 U Super-Therm DNA Polymerase (Southern Cross), 0.25 mM dNTP's, 0.1 µM of each primer, 1 µl of extracted DNA and Nuclease Free Water (NFW) up to the final reaction volume of 50 µl. The PCR cycle started with an initial denaturation step at 94°C for 10 min. This was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 5 min that was then followed by cooling to 4°C. Few microliters of the samples were run on a 1% agarose gel at 90 V for 30 min in order to verify amplification. The gel image is as shown on figure 1. The entire PCR reaction was loaded onto a 1% agarose gel and the correct band size (approximately 1500 bp) was excised. The DNA was recovered from the gel slices by using the GeneJET™ gel extraction kit (Fermentas).

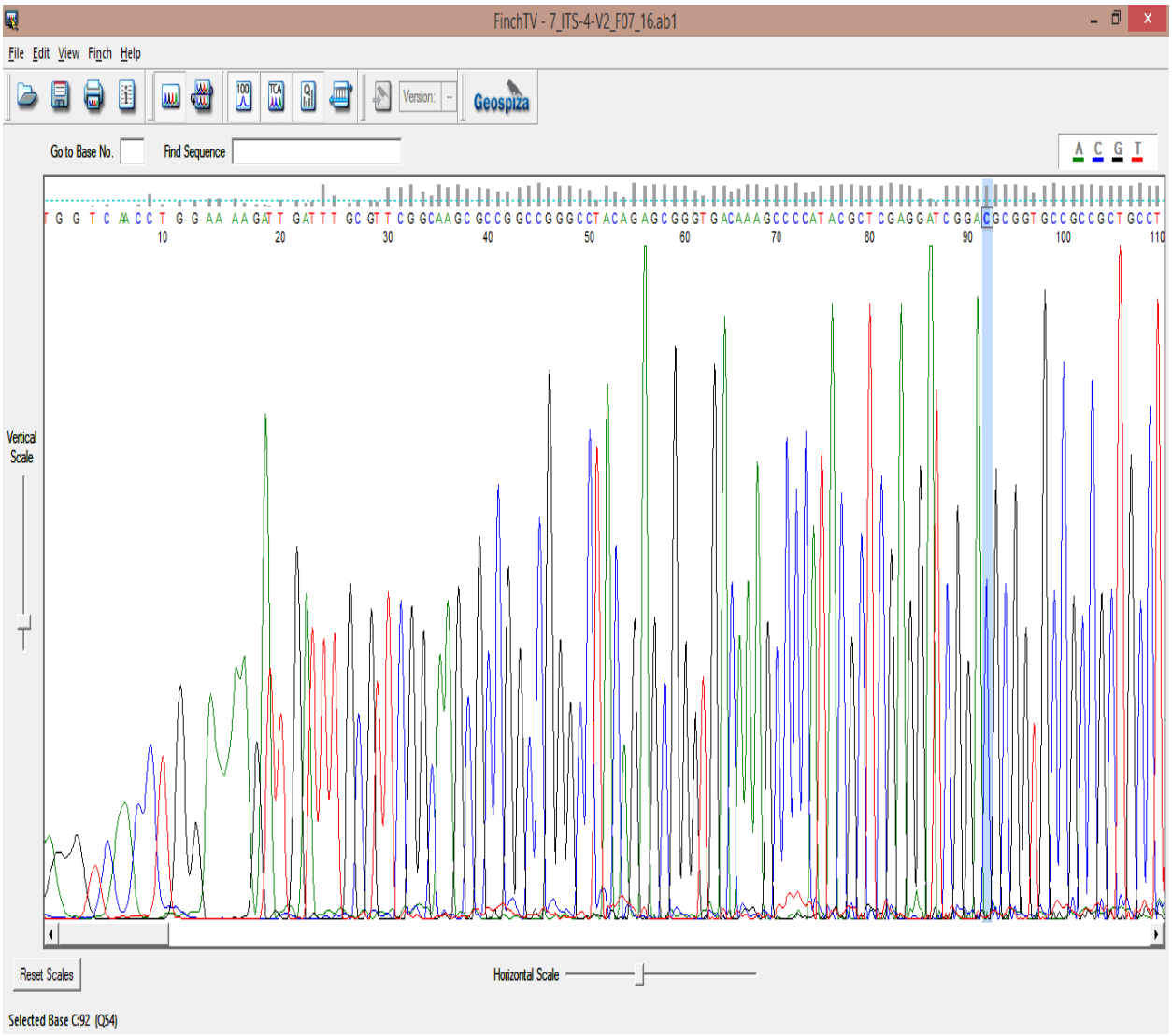
DNA Sequencing

DNA sequencing was performed by Sanger (dideoxy) sequencing Technique to determine the nucleotide sequence of the specific microorganism isolated using automated PCR cycle- Sanger Sequencer™ 3730/3730XL DNA Analyzers from Applied Biosystems (Russell, 2002; Metzenberg 2003). This result was obtained as nucleotides. Sequence analysis from resultant nucleotides base pairs was performed by BLAST analysis by direct blasting on American data base http://blast.ncbi.nlm.nih.gov. For every set of isolate, a read was BLASTED and the resultant top hits with minimum E-score for every BLAST result showing species name was used to name the specific organism.

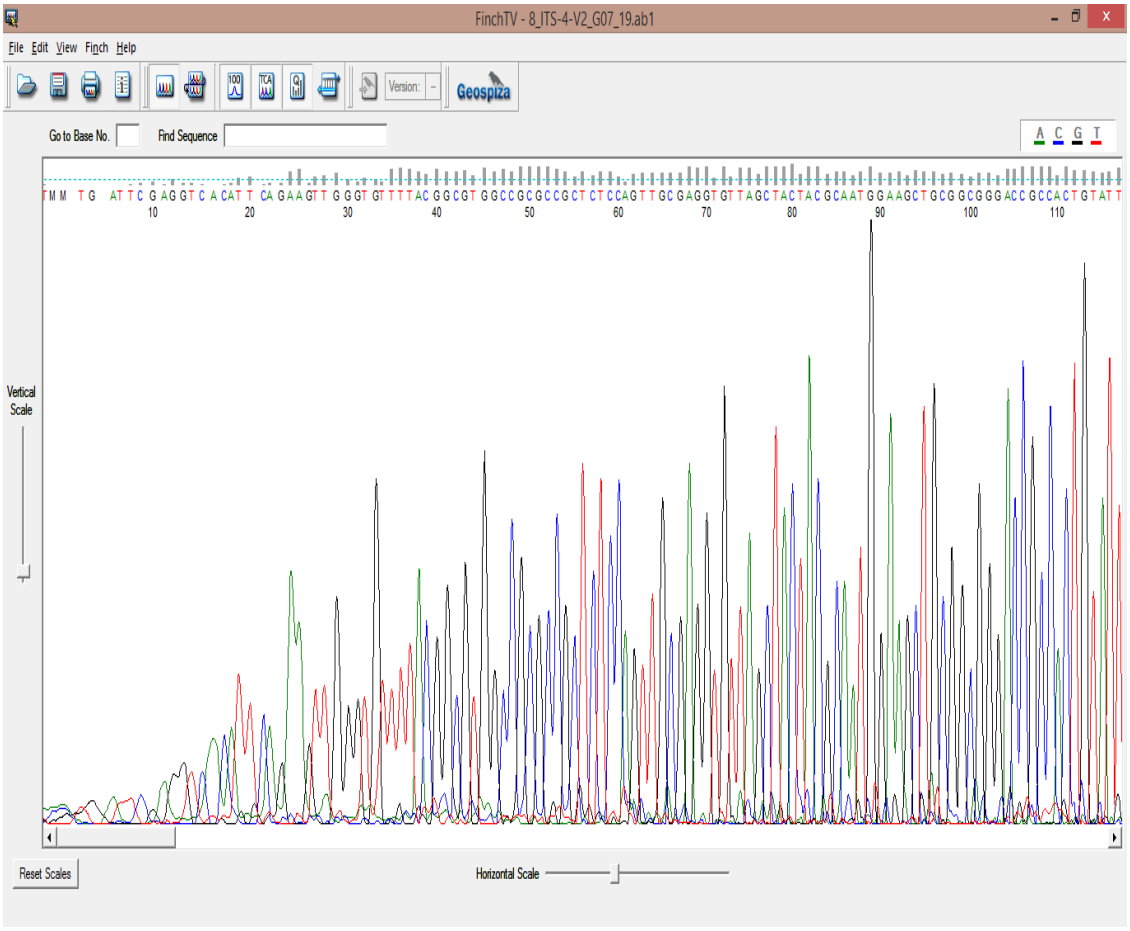
APPENDIX B: Chromatogram of *R. oryzae* Obtained from Sweet Potato



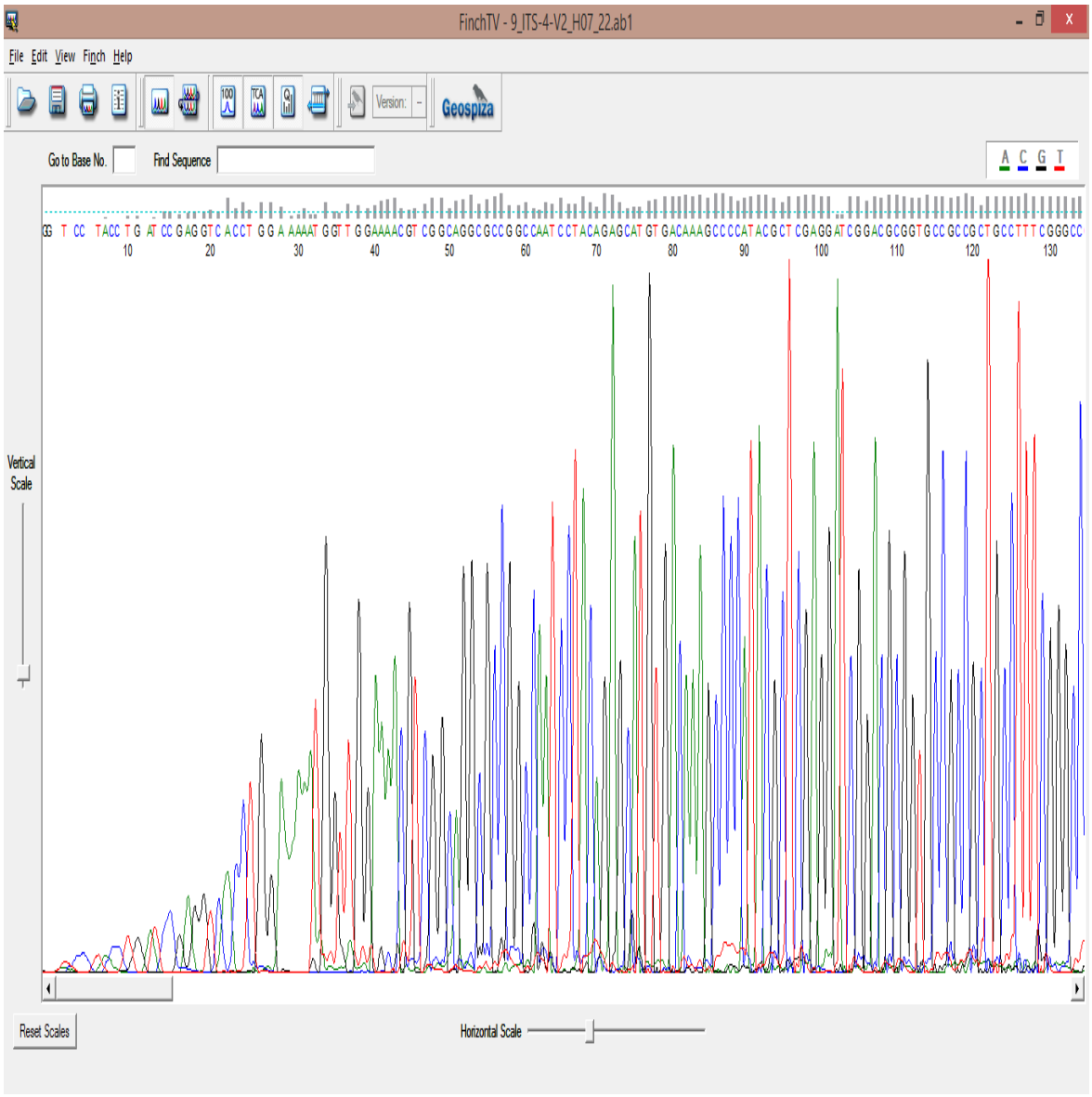
APPENDIX C: Chromatogram of *A. flavus* Obtained from Sweet Potato



Appendix D: Chromatogram of *F. solani* Obtained from Sweet Potato



Appendix E: Chromatogram of *Aspergillus awamori* Obtained from Sweet Potato



APPENDIX F:

Sequencing Result in Fasta Format and Corresponding ID after Blast Analysis on NCBI Website

Sample No	Type	Query Length	Sequence Nucleotides obtained (FASTA format)	Similarity/Gene Bank Accession Number	Identity Of Isolate Obtained
FM1	Fungi Isolate/ Genomic DNA	1028	>6_ITS-4-V2_E07_13 GCACCCCCCTGACTTCAGATCATAGTTTGAAAGTTGC TGGATTATACTCTTGTACTTTACTTCCTGGGCGAACCA AAGAAAAAGATCCTGAGACCAGCGTAATATTCCTGCC TAGCAAGCCAGACAGAAAATCACACACATTTTAGGTG CTCACTGTAATAAAACAGCGATGCGACCCATTACCAC ATAAACAAATGTTATGTGTGGGTTTGTGATGATACTG AAGCAGGCGTACTCTATAGAAAAACCATAGAGTGCA AGCTGCGTTCAAAGACTCGATGATTCACTGAATATGC AATTCACACTAGTTATCGCACTTTGCTACGTTCTTCAT CGATGCGAGAACCAAGAGATCCATTGTTAAAAGTTGT TTTTTATTAAACTTTATAATACTGAATTTCTAGGTTTA TTATGAAGGGTGCTCCTGAAACCAGGAGTGGCATCGA TCAAACCCCAGATAGGTCTACCCATGACCAGTCTGAG TCTCTCAGCCAAATTTTCACAGTGTAGAAGCAATCAC TTACCCCAGAGGAAACCCTAAGAGGTAAGGCGCTTTA ACATAATTAATGATCCTTCCGCAGGTCACCCGGAAGG AGTCATTATTTATCTTCGCGCGCCTACTCTTAAGGTTT CCCTCGGGTTGTTATTGTTCTCCGGAATTTGGCTAAGA CCACACGGTCAGGGTGACTATTGGGTTGATCGATCCT CGGTGTCGGAGCACCTTCAAAGACTGGATTCATATTA	*100% /*KJ439050	*Rhizopus oryzae*

TAGTTTAAAGAGACTTAATGACTTGATCTGCAGTAAA
AGTACAAGTGATACGTGTGATGCAATCCACAGTCTTA
GCACTGCATCTTGTTTTGGTACGCGCTCATATACAACC
CACAATTTTTGTTGGGACCGTTTTATACGCCAAAGGA
GTGGATGTTCTCTGTGGGGCGGATAACTGCTCAGACC
GTTCTTGGTGCGGGTAGAAGATACCACTCACTTATAT
CGTGATAGCTCATACA

FM2	Fungi Isolate/ Genomic DNA	1095	>7_ITS-4-V2_F07_16 TGGTCAACCTGGAAAAGATTGATTTGCGTTCGGCAAG CGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCC ATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCT TTGGGGCCCGTCCCCCCCGGAGAGGGGACGACGACC CAACACACAAGCCGTGCTTGATGGGCAGCAATGACG CTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCG CAATGTGCGTTCAAAGACTCGATGATTCACGGAATTC TGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTT CATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGT TTTAACTGATTGCGATACAATCAACTCAGACTTCACT AGATCAGACAGAGTTCGTGGTGTCTCCGGCGGGCGCG GGCCCGGGGCTGAGAGCCCCCGGCGGCCATGAATGG CGGGCCCGCCGAAGCAACTAAGGTACAGTAAACACG GGTGGGAGGTTGGGCTCGCTAGGAACCCTACACTCGG TAATGATCCTTCCGCAGGTCCCCGGAGGTATTAACCG AGTGTAGGGTTCCTAGCGACCCCTCCCAGTTCGTACC TTAATTGCTTCGCGGGCCGCCTTTCAGGGCCCCGGGG CTCTTAACCCGGAAAAACACAAATTTTTTTAAATGGG	100%/ KF908788 100% /KT274812	*Aspergillus flavus* *Aspergillus oryzae*

		GAACCAAAAAAATGGGGGGGATCCTAAAAATCTCAA TGGGAATTTGGGGGTTCCGCCCCATAAAAAAGAAGA ATGGTTACGGGTGATGGAAAAACTGAATCCGCTCTTT AAAGCGTGGCCGCCGCTTCCGGGGGGGCGGCCCAATT TCCCCCCCCGGGAGGTTGTGGTTTGGCCCCCCCCCCCC CCCCGGGGGGGAGGGCCAGCCCCCACCCCCCCACGG GGGTGAGTCTTCTCCTCTCTCGGCGGGGGGGGGGGAA TTATTTATTTCTCTTGCTGGGGGGCGGGGTCCTCTCTA TATCGAGAAAGAGAAGAAACTCGC		
FM3	842	>8_IT-4-V2_G07_19 TTGATTCGAGGTCACATTCAGAAGTTGGGTGTTTTAC GGCGTGGCCGCGCCGCTCTCCAGTTGCGAGGTGTTAG CTACTACGCAATGGAAGCTGCGGCGGGACCGCCACTG TATTTGGGGGACGGCGTTGTGCCCACAGGGGGCTTCC GCCGATCCCCAACGCCAGACCCGGGGGCCTGAGGGTT GTAATGACGCTCGAACAGGCATGCCCGCCAGAATACT GGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCA CTGAATTCTGCAATTCACATTACTTATCGCATTTCGCT GCGTTCTTCATCGATGCCAGAGCCAAGAGATCCGTTG TTGAAAGTTTTGATTTATTTGCTTGTTTACTCAGAAAA AACATTATAGAAACAGAGTTAGGGGGTCCTCTGGCGG GGGCGGCCCGTGTTACGGGGCCGTCTGTTCCCGCCGA GGCAACGTTTTAGGTATGTTCACAGGGTTGATGAGTT GTATAACTCGGTAATGATCCCTCCGCAGGTCCCCCGG AGTTTTACCGAGTTAACTACAACCTGACATAGTTCCC GCGGACGACGCCCTTACAGGGCCGCCCCCCCAACCCC TTTTTTTTTTTATGTTTTTTCTAGTCCCATATATATTCT CCAGATTTGGGCCTGCCTATAAAAGCACGCATGATAG TATGTGATTGAATATGGACACACTTACAATGGGCGCG TATCGCGGATGCTTAAGGTATACCTGCCTTGTTTGAG ACGAAGACACCTCTGCACACTCTCCACAATTGTGCG	99% / KJ863521	*Fusarium solani*

FM4	960	9_IT-4-V2_H07_22 GGTCCTACCTGATCCGAGGTCACCTGGAAAAATGGTT GGAAAACGTCGGCAGGCGCCGGCCAATCCTACA GAGCATGTGACAAAGCCCCATACGCTCGAGGATCGG ACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCC CCCGGAGAGGGGGACGGCGACCCAACACACAAGCCG GGCTTGAGGGCAGCAATGACGCTCGGACAGGCAT GCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAA AGACTCGATGATTCACTGAATTCTGCAATTCACATTA GTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAA CCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCA TTCAATCAACTCAACTGCACGTTTCAACAGTGTTCGT GTTGGGGTCTCCGGCGGGCACGGGCCCGGGGGGCAA AGGCGCCCCCCCGGCGGCCGACAAGCGGCGGGCCCG CCGAAGCAACAGGGTATAATAGACACGGATGGGAGG TTGGGCCCAAAGGACCCGCACTCGGTAATGATCCTTC CGCAGTCCCCTTCAAGGATATATGGTGGTGGTGCCCC CCCCTGTCTTAAAACCCTGTTGCTTCGG CGGCCCCCTTGTCGGCGCCGGGGGGGCGCCTTTTCCC CGGCGGCCCGGAACCCCAAATTTAAGTGGTCCGATTG ATTGATGCCCTTAAACTTTTCATAGAGTTTGGGCCGCT CATAAAAGCACAATGTACGAGGTGATTGCAATTGAAC ACAGTCTTACATGCTCGATATTCGAGCAGACTACAAT CTCTCCCGCGGGTGTGAGCCCTCCTCGGGGGTCAGGC CGCGCATCAGGGTAGGATTTCTAGTCTGATACCCCGC CCATTCCATGCGTATCGAGGATCTATAGACGAAAA	99%/ FJ441004	*Aspergillus awamori*
FM6		FAILED PCR AMPLIFICATION		

APPENDIX G

Descriptive Statistics of Proximate Composition of Two of the Most Commonly Grown Sweet Potato Cultivars In Ebonyi State

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Dry Matter	Tupiaochi	2	24.7500	.01414	.01000	24.6229	24.8771	24.74	24.76
	Oyorima	2	29.5000	.70711	.50000	23.1469	35.8531	29.00	30.00
	Total	4	27.1250	2.77265	1.38632	22.7131	31.5369	24.74	30.00
Moisture	Tupiaochi	2	75.2500	.01414	.01000	75.1229	75.3771	75.24	75.26
	Oyorima	2	70.5000	.70711	.50000	64.1469	76.8531	70.00	71.00
	Total	4	72.8750	2.77265	1.38632	68.4631	77.2869	70.00	75.26
Crude fibre	Tupiaochi	2	2.9400	.02828	.02000	2.6859	3.1941	2.92	2.96
	Oyorima	2	1.9900	.00000	.00000	1.9900	1.9900	1.99	1.99
	Total	4	2.4650	.54873	.27436	1.5919	3.3381	1.99	2.96
Crude protein	Tupiaochi	2	2.6200	.00000	.00000	2.6200	2.6200	2.62	2.62
	Oyorima	2	5.0500	.07071	.05000	4.4147	5.6853	5.00	5.10
	Total	4	3.8350	1.40356	.70178	1.6016	6.0684	2.62	5.10
Fat	Tupiaochi	2	.4400	.01414	.01000	.3129	.5671	.43	.45
	Oyorima	2	1.3400	.02828	.02000	1.0859	1.5941	1.32	1.36
	Total	4	.8900	.51994	.25997	.0627	1.7173	.43	1.36
Ash	Tupiaochi	2	2.4200	.01414	.01000	2.2929	2.5471	2.41	2.43
	Oyorima	2	1.4800	.02828	.02000	1.2259	1.7341	1.46	1.50
	Total	4	1.9500	.54302	.27151	1.0859	2.8141	1.46	2.43
Carbohydrate	Tupiaochi	2	16.3300	.01414	.01000	16.2029	16.4571	16.32	16.34
	Oyorima	2	19.6400	.77782	.55000	12.6516	26.6284	19.09	20.19
	Total	4	17.9850	1.96310	.98155	14.8613	21.1087	16.32	20.19

APPENDIX H

Analysis of Variance (Anova) for Proximate Composition of Two of the Most Commonly Grown Sweet Potato Cultivars in Ebonyi State

		Sum of Squares	Df	Mean Square	F	Sig.
Dry Matter	Between Groups	22.563	1	22.563	90.214	.011
	Within Groups	.500	2	.250		
	Total	23.063	3			
Moisture	Between Groups	22.563	1	22.563	90.214	.011
	Within Groups	.500	2	.250		
	Total	23.063	3			
Crude fibre	Between Groups	.902	1	.902	2256.250	.000
	Within Groups	.001	2	.000		
	Total	.903	3			
Crude protein	Between Groups	5.905	1	5.905	2361.960	.000
	Within Groups	.005	2	.002		
	Total	5.910	3			
Fat	Between Groups	.810	1	.810	1620.000	.001
	Within Groups	.001	2	.001		
	Total	.811	3			
Ash	Between Groups	.884	1	.884	1767.200	.001
	Within Groups	.001	2	.001		
	Total	.885	3			
Carbohydrate	Between Groups	10.956	1	10.956	36.207	.027
	Within Groups	.605	2	.303		
	Total	11.561	3			

APPENDIX I:

Anova for Effect of Fungi Inoculation/Rot on the Proximate Composition of Oyorima Sweet Potato Cultivar

		Sum of Squares	df	Mean Square	F	Sig.
M	Between Groups	12.199	7	1.743	27.770	.000
	Within Groups	.502	8	.063		
	Total	12.701	15			
CF	Between Groups	.968	7	.138	1105.800	.000
	Within Groups	.001	8	.000		
	Total	.969	15			
PRO	Between Groups	2.251	7	.322	203.392	.000
	Within Groups	.013	8	.002		
	Total	2.264	15			
ASH	Between Groups	4.674	7	.668	1369.692	.000
	Within Groups	.004	8	.000		
	Total	4.678	15			
FAT	Between Groups	18.515	7	2.645	10322.031	.000
	Within Groups	.002	8	.000		
	Total	18.517	15			
CARB	Between Groups	9.911	7	1.416	17.714	.000
	Within Groups	.639	8	.080		
	Total	10.551	15			

APPENDIX J:
ANOVA OF PERCENTAGE MYCELIAL GROWTH INHIBITION OF SWEET POTATO PHYTOPATHOGENS BY SINGLE PLANT EXTRACTS AT DIFFERENT CONCENTRATIONS

		Sum of Squares	df	Mean Square	F	Sig.
BOT 50mg/ml	Between Groups	1547.364	3	515.788	1039.022	.000
	Within Groups	3.971	8	.496		
	Total	1551.335	11			
BOT 100mg/ml	Between Groups	350.460	3	116.820	192.296	.000
	Within Groups	4.860	8	.607		
	Total	355.320	11			
RO 50mg/ml	Between Groups	669.141	3	223.047	571.000	.000
	Within Groups	3.125	8	.391		
	Total	672.266	11			
RO 100mg/ml	Between Groups	137.030	3	45.677	75.223	.000
	Within Groups	4.858	8	.607		
	Total	141.888	11			
FS 50mg/ml	Between Groups	1049.560	3	349.853	336.268	.000
	Within Groups	8.323	8	1.040		
	Total	1057.884	11			
FS 100mg/ml	Between Groups	290.054	3	96.685	138.714	.000
	Within Groups	5.576	8	.697		
	Total	295.630	11			
AN 50mg/ml	Between Groups	2604.598	3	868.199	972.200	.000
	Within Groups	7.144	8	.893		
	Total	2611.742	11			
AN 100mg/ml	Between Groups	696.562	3	232.187	270.179	.000
	Within Groups	6.875	8	.859		
	Total	703.437	11			
PS 50mg/ml	Between Groups	421.954	3	140.651	86.416	.000
	Within Groups	13.021	8	1.628		
	Total	434.975	11			
PS 100mg/ml	Between Groups	204.262	3	68.087	41.698	.000
	Within Groups	13.063	8	1.633		
	Total	217.325	11			
AA 50mg/ml	Between Groups	467.461	3	155.820	132.362	.000
	Within Groups	9.418	8	1.177		
	Total	476.879	11			
AA 100mg/ml	Between Groups	460.965	3	153.655	.655	.602
	Within Groups	1875.409	8	234.426		
	Total	2336.373	11			
AF 50mg/ml	Between Groups	429.886	3	143.295	118.334	.000
	Within Groups	9.688	8	1.211		
	Total	439.573	11			
AF 100mg/ml	Between Groups	79.808	3	26.603	21.593	.000
	Within Groups	9.856	8	1.232		
	Total	89.664	11			

APPENDIX K:

ANOVA OF PERCENTAGE MYCELIAL GROWTH INHIBITION OF SWEET POTATO PHYTOPATHOGENS BY SINGLE AND COMBINED PLANT EXTRACTS

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	19750.438	10	1975.044	2530.417	.000
B. theobromae	Within Groups	17.171	22	.781		
	Total	19767.610	32			
	Between Groups	20936.989	10	2093.699	785.104	.000
R. oryzae	Within Groups	58.669	22	2.667		
	Total	20995.658	32			
	Between Groups	26222.937	10	2622.294	1289.647	.000
F. solani	Within Groups	44.734	22	2.033		
	Total	26267.671	32			
	Between Groups	30538.454	10	3053.845	3535.858	.000
A. niger	Within Groups	19.001	22	.864		
	Total	30557.454	32			
	Between Groups	27356.925	10	2735.692	1113.903	.000
P. expansum	Within Groups	54.031	22	2.456		
	Total	27410.956	32			
	Between Groups	28779.977	10	2877.998	2431.264	.000
A. awamori	Within Groups	26.042	22	1.184		
	Total	28806.020	32			
	Between Groups	17447.020	10	1744.702	1925.095	.000
A. flavus	Within Groups	19.938	22	.906		
	Total	17466.959	32			

APPENDIX L:

Antifungal Activity of Ethanolic Single and Combined Extracts of Garlic, Ginger, Moringa and Bitter Kola (Percentage Inhibition of Mycelial Growth) against Sweet potato-Associated Fungi

Extract	BOT		RO		FS		AN		PS		AA
	Concentrations (Mg/ml)										
	50	100	50	100	50	100	50	100	50	100	50
M. oleifera	25.52 ±0.45	41.44 ±0.45	13.33 ±0.42	22.92 ±0.42	±	±	±	±	±	±	±
G. kola	11.71 ±0.45	18.69 ±0.23	16.67 ±0.42	31.67 ±0.42	±	±	±	±	±	±	±
A. sativum	22.52 ±0.45	40.99 ±0.45	7.48 ±0.68	11.67 ±0.42	±	±	±	±	±	±	±
Z. officinale	26.12 0.45±	49.09 ±0.45	14.29 ±0.00	27.50 ±0.00	±	±	±	±	±	±	±
Total	20.71 ±1.64	37.55 ±3.43	12.94 ±1.04	23.44 ±2.25	±	±	±	±	±	±	±

APPENDIX M:

Antifungal Activity of Single and Combined Extracts of Garlic, Ginger, Moringa and Bitter kola (Percentage)

Fungi	Treatments/Percentage inhibition										
	SF	Single Plant extracts				Two by Two Extract Combinations					
	MnCzb	MO	GK	AS	ZO	MOZO	ASZO	MOGK	ASMO	ASGK	ZOGK
BT	91.42±0.37	41.44±0.78	18.69±0.22	40.99±0.45	49.09±0.45	50.29±0.83 Ad	94.17±0.42 Ad	21.62±0.78 Ant	75.22±0.45 Ad	58.55±0.43 Ad	75.13±0.45 Ad
RO	96.01±2.7	22.92±0.72	31.67±0.42	11.67±0.42	27.50±0.00	20.00±0.72 Ant	51.25±0.72 Ad	47.08±0.42 Ad	55.42±0.42 Sy	19.17±0.42 Ag	76.12±0.45 Sy
FS	93.88±1.20	8.84±0.68	28.47.±0.0	23.81±0.68	34.01±0.68	19.05±0.68 Ag	88.44±0.68 Sy	14.97±1.36 Ag	36.74±0.01 Ad	50.34±0.68 Ad	71.43±1.18 Ad
AN	100.00±0.0	46.54±0.63	17.61±0.63	54.09±0.63	26.42±0.00	23.27±0.63 Ag	98.74±0.63 Ad	19.50±0.63 Ag	83.65±0.00 Ad	80.50±0.63 Ad	39.62±0.00 Ad
PE	100.00±0.0	34.28.±0.0	17.71±1.04	28.13±0.00	26.04±1.04	47.92±1.04 Ad	78.11±0.57 Sy	52.07±1.04 Ad	100.00±0.0	52.09±1.04 Ad	18.75±1.80 Ag
AA	100.00±0.0	26.81±0.72	10.15±0.72	21.02±0.72	15.22±0.00	0.00±0.00 Ag	69.18±.0.39 Sy	11.59±1.45 Ag	60.15±0.72 Ad	30.43±0.00 Ad	10.87±0.00 Ag
AF	85.91±0.63	35.19±0.57	31.41±0.64	20.51±0.64	27.56±0.64	21.83±0.63 Ag	76.92±0.00 Sy	25.95±0.47 Ag	52.56±0.64 Ad	35.77±0.57 Ad	75.00±0.00 Ad

Values are represented as mean ± SEM; Ad: Additive; Sy: Synergy; Ag: Antagonism; SF: Synthetic fungicide; MnCzd: Mancozeb
MO: *Moringa oleifera*; GK: *Gacinia kola*; AS: *Allium sativum*; ZO: *Z. officinale*; MOZO: *M. oleifera* and*Z. officinale*; ASZO: *A. sativum* and*Z. officinale*; MOGK: *M.oleifera*and*G. kola*; ASMO: *A. sativum* and*M. oleifera*; ASGK: *A. sativum* and*G. kola*; ZOGK: *Z. officinale* and*G. kola*; BT: *B. theobromae*; RO: *R. oryzae*; FS: *F. solani*; AN: *A. niger*; PE: *P. expansum*
AA: *A. awamori*; AF: *A. flavus*

APPENDIX N:

Antimicrobial Activities/ Interaction of combined Plant Extracts Against Postharvest Fungi of Sweet Potato

Combined Extracts	Expected Percentage Inhibition	Observed Percentage Inhibition	Synergy Ratio	Interaction
		R. oryzae		
MOGI	44.12	20.00	0.45	Antagonistic
GAGI	35.97	51.25	1.43	Additive
MOBK	47.33	47.08	1.0	Additive
GAMO	31.91	55.42	1.74	Synergistic
GABK	39.64	19.17	0.48	Antagonistic
GIBK	50.46	76.12	1.51	Synergistic
		B. theobromae		
MOGI	70.19	50.29	0.72	Additive
GAGI	71.99	94.17	1.31	Additive
MOBK	52.38	21.62	0.41	Antagonistic
GAMO	65.45	75.22	1.15	Additive
GABK	55.27	58.55	1.06	Additive
GIBK	58.61	75.13	1.28	Additive
		F. solani		
MOGI	40.33	19.05	0.47	Antagonistic
GAGI	49.72	88.44	1.78	Synergistic
MOBK	34.79	14.97	0.43	Antagonistic
GAMO	30.55	36.74	1.20	Additive
GABK	45.5	50.34	1.11	Additive
GIBK	52.8	71.43	1.35	Additive
		A. Niger		
MOGI	60.66	23.27	0.38	Antagonistic
GAGI	66.22	98.74	1.49	Additive
MOBK	55.95	19.50	0.35	Antagonistic
GAMO	75.46	83.65	1.11	Additive
GABK	62.17	80.50	1.29	Additive
GIBK	39.38	39.62	1.00	Additive
		A. flavus		
MOGI	53.05	21.83	0.41	Antagonistic
GAGI	41.36	76.92	1.86	Synergistic
MOBK	55.05	25.95	0.47	Antagonistic
GAMO	47.54	52.56	1.11	Additive
GABK	44.48	35.77	0.80	Additive
GIBK	50.30	75.00	1.49	Additive
		A. awamori		
MOGI	37.95	0.0000	0.00	Antagonistic
GAGI	33.06	69.18	2.09	Synergistic

MOBK	34.17	11.59	0.34	Antagonistic
GAMO	42.21	60.15	1.43	Additive
GABK	29.05	30.43	1.05	Additive
GIBK	23.83	10.87	0.46	Antagonistic
		P. expansum		
MOGI	51.39	47.92	0.93	Additive
GAGI	45.84	78.11	1.70	Synergistic
MOBK	45.92	52.07	1.13	Additive
GAMO	52.77	100.00	1.90	Synergistic
GABK	40.86	52.09	1.27	Additive
GIBK	39.14	18.75	0.48	Antagonistic

APPENDIX O:

Descriptive Statistics of *In Vivo* Antifungal Activities of Mancozeb and Plant Extract Combinations (Preventive Method)

		N	**Mean**	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
BOT	Mncz pvt	3	**88.8267**	1.40076	.80873	85.3470	92.3064	87.40	90.20
	ASZO pvt	3	**91.1233**	.26160	.15103	90.4735	91.7732	90.88	91.40
	ASMO pvt	3	**70.1167**	.29297	.16915	69.3889	70.8445	69.90	70.45
	Total	9	**83.3556**	10.00532	3.33511	75.6648	91.0463	69.90	91.40
RO	Mncz pvt	3	**91.6833**	1.14823	.66293	88.8310	94.5357	90.41	92.64
	ASZO pvt	3	**53.2267**	.30238	.17458	52.4755	53.9778	53.00	53.57
	ASMO pvt	3	**49.9667**	.18230	.10525	49.5138	50.4195	49.77	50.13
	Total	9	**64.9589**	20.10196	6.70065	49.5072	80.4106	49.77	92.64
FS	Mncz pvt	3	**97.4333**	.51316	.29627	96.1586	98.7081	97.00	98.00
	ASZO pvt	3	**85.1900**	.75445	.43558	83.3158	87.0642	84.57	86.03
	ASMO pvt	3	**35.3133**	.32532	.18782	34.5052	36.1215	34.98	35.63
	Total	9	**72.6456**	28.50077	9.50026	50.7379	94.5532	34.98	98.00
AN	Mncz pvt	3	**100.0000**	.00000	.00000	100.0000	100.0000	100.00	100.00
	ASZO pvt	3	**83.3767**	.55985	.32323	81.9859	84.7674	83.00	84.02
	ASMO pvt	3	**81.9500**	.21932	.12662	81.4052	82.4948	81.71	82.14
	Total	9	**88.4422**	8.69552	2.89851	81.7583	95.1262	81.71	100.00
PS	Mncz pvt	3	**100.0000**	.00000	.00000	100.0000	100.0000	100.00	100.00
	ASZO pvt	3	**97.8367**	.15177	.08762	97.4597	98.2137	97.70	98.00
	ASMO pvt	3	**99.0467**	.93088	.53744	96.7342	101.3591	98.14	100.00
	Total	9	**98.9611**	1.05072	.35024	98.1535	99.7688	97.70	100.00
AA	Mncz pvt	3	**94.8333**	1.19304	.68880	91.8697	97.7970	94.00	96.20
	ASZO pvt	3	**64.1367**	.90787	.52416	61.8814	66.3919	63.19	65.00
	ASMO pvt	3	**53.0600**	.61221	.35346	51.5392	54.5808	52.48	53.70
	Total	9	**70.6767**	18.75911	6.25304	56.2571	85.0962	52.48	96.20
AF	Mncz pvt	3	**86.9967**	.02517	.01453	86.9342	87.0592	86.97	87.02
	ASZO pvt	3	**77.1400**	1.02898	.59408	74.5839	79.6961	76.00	78.00
	ASMO pvt	3	**50.7467**	.52776	.30470	49.4356	52.0577	50.14	51.10
	Total	9	**71.6278**	16.24231	5.41410	59.1428	84.1127	50.14	87.02

APPENDIX P:

ANOVA of In Vivo Antifungal Activities of Mancozeb and Plant Extract Combinations (Preventive Method)

		Sum of Squares	*df*	*Mean Square*	*F*	*Sig.*
BOT	*Between Groups*	*796.619*	*2*	*398.309*	*564.604*	*.000*
	Within Groups	*4.233*	*6*	*.705*		
	Total	*800.852*	*8*			
RO	*Between Groups*	*3229.823*	*2*	*1614.912*	*3357.172*	*.000*
	Within Groups	*2.886*	*6*	*.481*		
	Total	*3232.709*	*8*			
FS	*Between Groups*	*6496.475*	*2*	*3248.238*	*10384.761*	*.000*
	Within Groups	*1.877*	*6*	*.313*		
	Total	*6498.352*	*8*			
AN	*Between Groups*	*604.173*	*2*	*302.087*	*2506.711*	*.000*
	Within Groups	*.723*	*6*	*.121*		
	Total	*604.896*	*8*			
PS	*Between Groups*	*7.053*	*2*	*3.526*	*11.893*	*.008*
	Within Groups	*1.779*	*6*	*.297*		
	Total	*8.832*	*8*			
AA	*Between Groups*	*2809.989*	*2*	*1404.995*	*1607.321*	*.000*
	Within Groups	*5.245*	*6*	*.874*		
	Total	*2815.234*	*8*			
AF	*Between Groups*	*2107.824*	*2*	*1053.912*	*2363.091*	*.000*
	Within Groups	*2.676*	*6*	*.446*		
	Total	*2110.500*	*8*			

APPENDIX Q:

ANOVA of *In vivo* Antifungal Activities of Mancozeb and Plant Extract Combinations (Curative Method)

		Sum of Squares	Df	Mean Square	F	Sig.
BOT	Between Groups	801.521	2	400.761	904.856	.000
	Within Groups	2.657	6	.443		
	Total	804.179	8			
RO	Between Groups	4356.664	2	2178.332	5648.388	.000
	Within Groups	2.314	6	.386		
	Total	4358.978	8			
FS	Between Groups	6430.802	2	3215.401	11134.088	.000
	Within Groups	1.733	6	.289		
	Total	6432.535	8			
AN	Between Groups	1568.407	2	784.204	1570.990	.000
	Within Groups	2.995	6	.499		
	Total	1571.402	8			
PS	Between Groups	1186.667	2	593.334	2533.689	.000
	Within Groups	1.405	6	.234		
	Total	1188.072	8			
AA	Between Groups	3182.143	2	1591.072	3210.611	.000
	Within Groups	2.973	6	.496		
	Total	3185.117	8			
AF	Between Groups	2134.333	2	1067.167	1733.570	.000
	Within Groups	3.694	6	.616		
	Total	2138.027	8			