

Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis* L) and banana (*Musa sapientum* L) in Sokoto Metropolis

Abstract

Mycological studies on fungi in apparently diseased sweet orange (*Citrus sinensis*) and banana (*Musa sapientum*) sampled from various points in Kara Market in Sokoto Metropolis was carried out between August and September, 2016. The samples were surfaced sterilized with ethanol and the homogenates were cultured on Potato Dextrose Agar and incubated aerobically at room temperature for 7 days at 30°C. The pure cultures obtained were identified morphologically and microscopically. The investigation revealed that the samples were infected with several fungal species. The most predominant fungi isolated from sweet orange was *Cladosporium* spp (40%), *Fusarium* spp (30%), *Alternaria* spp (20%) and *Chrysonilia* spp (10%) while the most predominant fungi isolated from banana was *Fusarium* spp (50%), *Mucor* spp (30%) and *Rhizopus* spp (20%). The pathogenicity test result shows that *Cladosporium* spp and *Fusarium* spp were the most active in sweet orange with rot length of 74mm and 70mm respectively and the least active fungi were *Alternaria* spp and *Chrysonilia* spp with rot length of 52mm and 48mm respectively while *Fusarium* spp and *Mucor* spp were the most active in banana with rot length of 84mm and 75mm respectively and the least active fungus is *Rhizopus* spp with rot length of 54mm.

Keywords: isolation, identification, fungi, fruits spoilage

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Introduction

Fruit refers to the fleshy seed-associated structures of certain plants that are sweet and eaten in the raw state. They constitute the natural sources of vitamins and mineral nutrients to the body. They are used as nutritional remedies for many patients suffering from different ailments such as diabetes, constipations and stroke.¹

Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help keep a good and normal health. They are widely distributed in nature and can play an important role in health through the prevention of heart disease and cancer. Fruits and vegetables are different from most foods that we buy because they are not cooked and they are usually eaten raw. One of the limiting factors that influence the fruit economic value is the relatively short shelf life period caused by pathogen attack. It is estimated that about 20–25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries.²

Microorganisms are associated, in a variety of ways with all the food we eat. They may influence the quality, availability and quantity of our food such as fruits and vegetables which normally contain some microorganisms and maybe contaminated with additional organisms during handling.³ Food can serve as the medium for the growth of microorganisms and as a result transmit diseases. Other microorganisms, if allowed to grow in certain food products, produce toxic substances that result in food poisoning when ingested.⁴ Different disease problems arise when crops are harvested, because seed, fruit or other storage organs are essentially dormant structures and their cells are physiologically unlike those of growing plant.⁵

Over the years there has been an increasing need to identify and isolate the fungi associated with the fruit spoilage. Thus, spoilage refers

to any change in the condition of food, in which the food becomes less palatable or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture.⁶ The occurrence of fungal spoilage of fruits is also recognized as a source of potential health hazard to man and animals. This is due to their production of mycotoxins (naturally occurring toxic chemical often of aromatic structure), compounds which are capable of including mycotoxicoses in man following ingestion and inhalation. They differ in their manner and degree of toxicity.⁷

The contamination of fruits and vegetables by fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing, practices and transportation.

Large quantities of fruits are lost due to spoilage caused by pathogenic microorganisms particularly fungi. These pathogenic fungi limit the economic value of these fruits as well as their shelf life.

Over the years there has been an increasing need to identify and isolate the fungi associated with fruit spoilage. The occurrence of fungal spoilage of fruits is recognized as a source of potential health hazard to man and animals. This is due to their production of mycotoxins (naturally occurring toxic chemical often of aromatic structure), compounds which are capable of including mycotoxicoses in man following ingestion and inhalation.⁷ Hence, the need to isolate and identify these microorganisms responsible for spoilage.

Aim and objectives

The aim of the study is to isolate and identify the microorganisms responsible for the contamination of Sweet orange and banana fruits in Sokoto metropolis. The specific objectives are

- To isolate and identify the fungal organisms responsible for the post-harvest spoilage of sweet orange and banana fruits.

- b) To determine the frequency of occurrence of these microorganisms.

Materials and methods

Materials and reagents

The material and reagents used in the laboratory for this research work includes; Potato dextrose agar (PDA), ethanol, petri dishes, conical flasks, syringes, cotton wool, lacto phenol cotton blue, sterilized knife, glass rod, test tubes, streptomycin, nutrient agar, aluminum foil, masking tape, distilled water, face mask, hand gloves, filter paper, microscope, Cork borer, slide and cover slip.

Specimen collection

Two different fruits; sweet orange and banana both fresh and spoilt were purchased from Kara market within the Sokoto metropolis. A total of thirty fruits comprising fifteen fruits from each fruit type were purchased from three different points designated A₁–A₃ for sweet orange and B₁–B₃ for banana and transported to the laboratory in an ethanol sterile polythene bag for analysis (Table 1).

Table 1 Nutrient composition of sweet orange

Composition	Amount
Energy	197kj (47 kcal)
Sugars	9.35g
Dietary fibre	2.4g
Fat	0.12g
Protein	0.94g
Water	86.75g
Vitamin A equiv.	11 µg (1%)
Thiamine (vit. B1)	0.087mg (8%)
Riboflavin (vit. B2)	0.04mg (3%)
Niacin (vit. B3)	0.282mg (2%)
Pantothenic acid (B5)	0.25mg (5%)
Vitamin B6	0.06mg (5%)
Folate (vit. B9)	30µg (8%)
Choline	8.4mg (2%)
Vitamin C	53.2mg (64%)
Vitamin E	0.18mg (1%)
Calcium	40mg (4%)
Iron	0.1mg (1%)
Magnesium	10mg (3%)
Manganese	0.025mg (1%)
Phosphorus	14mg (2%)
Potassium	181mg (4%)
Zinc	0.07mg (1%)

Source: USDA Nutrient Database¹⁵

Sterilization of glassware

All the glass wares were first soaked and washed thoroughly with tap water and detergent solution and then rinsed with several changes of distilled water in order to completely remove traces of detergent and air dried completely before sterilizing them in steam oven at temperature of 121°C for 15minutes and then allowed to cool down at room temperature before usage. The entire working surface was also disinfected with ethanol to reduce contamination.

Isolation of fungi

Isolation of the fungi was carried out as described by Baiyewu et al.⁸ Segment (3–5cm) of tissues from the spoilt fruits was cut with sterile scalpel and placed on potato dextrose agar containing streptomycin (to prevent growth of bacteria) in petri dishes and incubated at room temperature for 5 days. Pure cultures were obtained from the isolation.

Identification of fungi

Identification of the fungi was done according to Fawole & Oso.⁹ A drop of lactophenol cotton blue stain was placed on a clean slide and with the aid of a mounted needle, a small portion of the mycelium from the fungal cultures was removed and placed in the drop of the stain. The mycelium was spread very well on the slide with the aid of the two mounted needles and a cover slip was gently lowered on it. The slide was then examined under the microscope.

The observation was done at high power objective (×40) of the microscope. Morphological characteristics of the fungi such as type of hyphae and asexual reproductive structure were observed.

Pathogenicity of isolated fungi

Pathogenicity or decay test was carried out in order to know if the isolated fungi were really responsible for the spoilage of citrus and banana fruits. Healthy fruits were surface sterilized with ethanol. Cylindrical plug tissues were cut out from the fruits using a sterilized 2mm sized cork borer. Agar plate containing a week old fungal culture were aseptically placed in these holes, then covered and sealed off by means of petroleum jelly. The procedure was repeated separately across each of the fungal isolates. The inoculated samples and the control were placed in sterile polythene bags and incubated in an oven for 5days. The point of inoculation of each type of fungus was examined and recorded. The diameter of the rotten portion of the orange and banana fruits was measured. The fungi were later re-isolated from the inoculated fruits and compared with the initial isolates.

Data collected

The following parameters were recorded;

- Frequency of occurrence of fungal pathogens associated with the spoilt fruits
- Pathogenicity test result by measuring the diameter of spoilage.

Data generated were subjected to analysis by means of descriptive statistics.

Literature review

Sweet orange (*Citrus sinensis*)

The sub-genus *Citrus*, family Rutaceae and subfamily

Aurantioideae is of three types: *Citrus*, *Fortunella* and *Poncirus Trifoliata*. There are three genera and eighteen defined species, but other natural mutations exist resulting to numerous hybrids which are widely spread throughout the world.¹⁹ Citrus is widely grown in Nigeria and many other tropical and subtropical regions.²⁰ In terms of volume in production, citrus ranks after banana as the world second fruit crop with more than 108million tons.²¹ Sweet orange (*Citrus sinensis*) commonly called orange is a member of this family and a major source of vitamins, especially vitamin C, sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium.²² Economically, oranges are important fruit crops, with an estimated 60million metric tonnes produced worldwide as at 2005 for a total value of 9billion dollars. Of this total, half came from Brazil and the United States of America.^{23,24} The global citrus acreage according to FAO statistics in 2009 was nine million hectares with production put at 122.3 million tons, ranking sweet oranges first among all the fruit crops.²⁴

Oranges probably originated from south East Asia, and were cultivated in China by 2500 BC,²⁶ where it was referred to as ‘‘Chinese’’ apple.²⁷ Today, it is grown almost all over the world as a source of food for humans because of its high nutritional value, source of vitamins and other uses. Propagation through seed is associated with problems like poor pollen production, self-incompatibility and muscular embryo.²⁸ Therefore, budding onto appropriate rootstocks is the common means of assuring the reproduction of cultures of known quality.

Botany

Sweet orange (*Citrus sinensis*) is a small evergreen tree 7.5m high and in some cases up to 15m. It originated from southern China where it has been cultivated for many years, but is today grown commercially worldwide in tropical, semi-tropical and some warm temperate regions to become the most widely planted fruit tree in the world.^{26,27} Orange produces leathery and evergreen leaves of different shapes, ranging from elliptical to oblong to oval, 6.5–15cm long and 2.5–9.5cm wide, often bearing narrow wings on the petioles. It bears fragrant white flowers either singly or in whorls of 6, about 5cm wide, with 5petals and 20–25 yellow stamens. The small, white or purple scented hermaphroditic flowers produce nectar for pollination by insects. The fruit, which may be globose to oval is 6.5 to 9.5cm wide, and ripens to orange or yellow. Anatomically, the fruit consists of two distinct regions, the pericarp also called the peel, skin or rind, and the endocarp, or pulp and juice sacs. The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell.

The flesh or pulp of the fruit is typically juicy and sweet, divided into 10 to 14 segments (although there are seedless varieties) and ranges in color from yellow to orange to red. The ripe fruit is classified as a hesperidium which is a type of berry with multiple seeds and is fleshy.

The centre of origin of most citrus cultivars is perhaps unknown, but the ancient relatives of citrus are native to China, the Southeast Asia, the Malay Archipelago, New Caledonia and Australia.²⁹ The genetic origin of sweet orange is not clear, although it is believed to be derived from the interspecific hybridization of some primitive citrus species.²⁵

Climate

All cultivated citrus species grow very well in both tropical and subtropical parts of the world provided there is sufficient moisture and the temperature does not drop below freezing point. The main produce from citrus is water-based and, therefore, adequate supply of moisture especially during fruit development is very important. Citrus fruits tend to be small (undersized) when grown under dry or semi-dry conditions. Alternatively, excessive moisture is not conducive to optimum productivity of citrus. Citrus grows best under 1100 to 1500mm of rain per annum, distributed over nine months of the year.

The temperature range for good growth and productivity varies from 13°C to 37°C with the optimum at 28°C. The optimum temperature for maturation of best quality fruits is 13°C to 17°C. This special temperature requirement for maturation explains why citrus fruits which mature under subtropical and Mediterranean conditions are superior in quality to those that mature under high tropical temperature. Citrus requires a period of dry but cool weather for final ripening of the fruits. Excessive sky overcast is not favourable for good growth and productivity of citrus.

Site selection

It grows on a wide range of soils. As the taproot plays an important role, the soil must be deep, free from iron concretions, well-drained and high in fertility. High organic matter is known to favour good growth and production. Any site selected should be level or only slightly sloping. It should also be protected from strong winds either naturally or by establishment of windbreaks.

Land preparation

Land preparation for planting citrus consists of clear felling of all vegetation. Any excess trash should be burnt. If felling can be arranged a year ahead of establishing the citrus orchard, a leguminous fallow can be established already at that time. Otherwise, this is to be lined out with planting sites at 6m×6m or 7m×7m. The usual size of the planting holes is 60cm×60cm×60cm.

Plant propagation

Seed collection: plants which are to serve as a source of seeds for planting should be selected on the basis of high yield, good growth, freedom from pests and diseases, especially virus infections, and should give good quality produce e.g. juicy fruits with good flavour. When fruits are harvested for the purpose of extraction of the seeds for planting, the fruits should be selected on the basis of size, fullness and conformation. Seeds should also be selected on the basis of size—the larger the seed, the larger generally the embryo and the food store and the more vigorous the germination and early establishment.

Freshly extracted seeds should be sown immediately, as citrus seeds loss viability on extraction, and during storage under normal conditions.³⁰

Sowing: Although citrus seeds can be sown at stake, it has now become an accepted practice to raise citrus seedlings in the nursery either for transplanting to the field as seedlings or for use as stocks in budding. Citrus seeds are sown either in seed boxes or in nursery beds as single seeds, spaced at 3cm×6cm. To allow good growth, seedlings should be pricked when they are 3cm to 6cm in height into either

15x24cm cane baskets or 15cmx24cm polybags, which have been filled with fertile topsoil. Seedlings should be selected for vigour of growth while weak seedlings should be discarded. Seeds should be provided with light shade until time of pricking.³⁰

Vegetative propagation: The most common method of vegetative propagation used with citrus is budding. Two methods are used extensively. The seedlings to be used as root stocks are raised in the same manner to the age of 12 to 15 months after pricking to a height of at least 45cm. When the root stock seedlings have attained this size or when budding is due, they are prepared for the budding operation. This consists of pruning the seedlings to a height of 30cm a week before the budding operation. The bud wood is normally collected on the morning of the day of budding and used immediately. Bud wood is usually taken from branches which are 8 to 10 months old. Budding is carried out in the cool hours of the morning or in the evening at a height of 20 to 25cm from the ground level on the prepared seedling rootstocks.³⁰

Post-planting maintenance operations

The essential post planting maintenance operations that should be carried out in a citrus orchard are as follows:

- i. Establishment of a ground cover of legumes. The ground cover could be established before citrus seedlings are transplanted into the field or immediately after transplanting.
- ii. Mulching of each seedlings towards the end of the rains.
- iii. Removal of undecomposed mulch during the early rains.
- iv. Maintenance of the leguminous ground cover, weeding and reseedling of gaps.
- v. Watering/ irrigation as necessary.
- vi. Pruning the trees to shape and removals of suckers and laterals.
- vii. Application of fertilizer. Fertilizer application should be carried out in May and September. Appropriate fertilizer rates should be used.
- viii. Regular control of pests and diseases through farm sanitation, use of resistant varieties and application of insecticides and fungicides.
- ix. Regular weed control either by hand weeding or use of herbicides.

Production and harvesting

Citrus begins to bear from three to five years after planting in the field depending on the species, variety and method of propagation. Vegetatively propagated material comes into bearing earlier than seedlings. The main commercial product of citrus is the fruit juice. The citrus industry of the world is principally aimed at the production of fruits for juice extraction in various forms for consumption. In addition to the juice, the following by products are obtainable from citrus;

- I. The rind is used in pharmaceutical industries.
- II. Fruit pulp is the mesocarp tissue, which is left as a residue after the juice has been extracted. Where available in large quantities, it is used as valuable livestock feed ingredient. It is also used as fuel.

III. The wood of citrus plants is an excellent source of firewood.

In citrus the colour of the fruit rind is green when the fruits are not mature. On maturity, the fruits begin to ripen during which period the rind colour turns yellow. The quality of citrus is determined primarily by the taste, juiciness and size of the fruit. In the world market, however, the marketing value is determined to a large extent by the uniformity of the yellowness of the rind on ripening.

Citrus fruits are easily perishable after ripening. To ensure that fruits are not lost through over-ripening, they should be harvested immediately they are ripe and delivered to consumers, processors or retailers.

Health benefits

The human diet contains important micronutrients namely vitamins C and E, carotenoids and flavonoids, essential for maintenance of human health. Multiple dietary sources of these compounds are present virtually in all plant material.³¹ The nutritional importance of foods is due to the presence of these functional food ingredients and antioxidant nutraceuticals or phytochemicals. Phytochemicals are present in edible fruits and vegetables and when eaten potentially modulate human metabolism in a favourable manner, thereby preventing chronic and degenerative diseases.³² Increase in fruits and vegetables consumption protect against degenerative pathologies such as cancer andtherosclerosis,³³ as epidemiological surveys had shown an inverse relationship between dietary flavonoid intake from citrus and cardiovascular diseases.^{31,34} Citrus fruits are the main source of important phytochemical nutrients and for long have been valued for their wholesome nutritious and antioxidant properties. It is scientifically proven that oranges being rich in vitamins and minerals have many health benefits. Moreover, it is now appreciated that other biologically active, non-nutrient compounds found in citrus fruits such as phytochemical antioxidants, soluble and insoluble dietary fibres are known to be helpful in reducing the risk for cancers, many chronic diseases like arthritis, obesity and coronary heart diseases (Crowell, 1999).

As antioxidant: The biological activity and the health effects of citrus flavonoids as antioxidants have been reported.³² These group of pigments as found in plants and together with anthocyanin play a role in flower and fruit colouration. Also, they are present in dietary fruits and vegetables (Macheix *et al.*, 1990), and exercise their antioxidant activity in several ways, including the activities of metal chelation.³⁵ Studies indicate that flavonoids are excellent radical-scavengers of the hydroxyl radical,^{36,37} due to their ability to inhibit the hydroxyl radical and donate hydrogen atom.^{31,32} Oranges as excellent source of vitamin C, contain powerful natural antioxidant, folate, dietary fibre and other bioactive components, like carotenoids and flavonoids that prevent cancer and degenerative diseases.³⁸ Consumption of foods rich in vitamin C improves body immunity against infectious agents and scavenging harmful, pro-inflammatory free radicals from the blood. Sweet orange contains a variety of phytochemicals like hesperidin and naringenin. Naringenin has a bioactive effect on human health as antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator.

Anti-inflammatory: Citrus flavonoids contain compounds with anti-inflammatory activity due to the presence of regulatory enzymes (protein kinase C, phosphodiesterase, phospholipase, lipoxigenase, and cyclooxygenase) that control the formation of the biological

mediators, responsible for the activation of endothelial cells and specialized cells involved in inflammation. Flavonoid inhibition of the immune and inflammation responses can be associated with their inhibition of these enzymes.³² Indeed, citrus flavonoids are able to inhibit the kinases and phosphodiesterases essential for cellular signal transduction and activation. They also affect the activation of a number of cells involved in the immune response, including T and B lymphocytes.³⁹ Citrus flavonoids also prevent atherosclerosis, inhibiting the formation of atheroma.³⁴ Tripoli et al.,³² reported that hesperidin obtained from citrus cultures may have a potential therapeutical use as a mild anti-inflammatory agent, being also useful as a precursor of new flavonoids endowed with this activity (Da Silva et al., 1994).

Anti-cancer and anti-Arteriosclerosis: Citrus flavonoids can prevent cancer through selective cytotoxicity, ant proliferative actions and apoptosis.⁴⁰ (Elangovan et al., 1994. Flavonoids are anti mutagenic, thus protects the DNA from damage by their ability to absorb ultraviolet light.⁴¹ They neutralize free radicals that promote mutations when they are generated near DNA. This has been shown in mice body irradiated with c-ray.⁴² The ability to function as such by citrus flavonoids are based on cell mobility inhibition.⁴³ Oranges are also rich in iron, chlorine, manganese, zinc, sodium, phosphorous, iodine, calcium, folic acid, potassium, pectin, beta-carotene and amino acids and fibre. A single orange is said to have about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory, blood clot inhibiting and antioxidant properties. All these properties help to promote overall health.⁴⁴

Anti-obesity: Sweet oranges contain low calories and no saturated fats or cholesterol, but is rich in dietary fibre, pectin which is very effective in persons with obesity. Pectin as bulk laxative protects the mucous membrane from exposure to toxic substances, as well as by binding to cancer causing chemicals in the colon. Pectin has also been shown to reduce blood cholesterol levels by decreasing its re-absorption in the colon by binding to bile acids in the colon.⁴⁵ Orange peels contain the alkaloid synephrine, which reduces the production of cholesterol in the liver. The antioxidant elements in oranges combat oxidative stress that oxidizes the LDL (low-density lipoprotein) in the blood.

Wholesome health: Oranges also contain very good amount of vitamin A, and other flavonoid antioxidants such as alpha and beta carotenes, beta-cryptoxanthin, zeaxanthin and lutein, compounds that have antioxidant properties. Vitamin A is necessary for maintaining healthy mucus membranes, skin and essential for vision. It is also a very good source of B-complex vitamins such as thiamine, pyridoxine and folates. These vitamins are essential in the sense that body requires them from external sources to replenish. Orange fruit also contains a very good amount of minerals like potassium and calcium. Potassium in an important component of cell and body fluids helps control heart rate and blood pressure. Vitamin A also required for maintaining healthy mucus membranes and skin and is also essential for vision. Consumption of natural fruits rich in flavonoids helps body to protect from lung and oral cervical cancers. Orange fruit also contains a very good amount of minerals like potassium and calcium. Potassium is an important component of cell and body fluids and helps to control heart rate and blood pressure. The alkaline properties in the orange stimulate the digestive juices, thus, reliving constipation. Regular intake of orange juice reduces the chances in the formation calcium oxalate which causes kidney stones. Polyphenols present in oranges prevents viral infections. Oranges protect the skin

from damage caused by free radicals, thereby helping you look young and keeps the skin fresh and glowing.⁴⁶

Fungal diseases of *Citrus sinensis*

Sweet orange is susceptible to a large number of diseases that can cause severe economic losses.

Sweet orange scab (SOS disease): Sweet orange scab is caused by the fungus *Elsinoë australis*.^{47,48} Sweet orange scab occurs on sweet oranges, limes, lemons, mandarins, satsumas, kumquat, grapefruit, tangerines and tangerine hybrids. The disease is common in South America, mainly Brazil,⁴⁹ Argentina and Paraguay. Symptom development starts a few days after infection and is dependent on environmental conditions that promote disease development like warm temperatures and moist plant tissues. The incubation period is at least 5 days. Artificially inoculated seedlings develop scab symptoms after 7 to 14days.⁵⁰ The best time to detect the disease in the field is during early spring which coincides with the formation new of tissues and fruits. Symptoms generally develop one week after tissue infection.⁵¹ Lesions start on the underside of leaves as water soaked spots, typically forming along the edge of the leaf or the mid-vein. There is a dramatic increase in the resistance of the leaves and fruits to infection in later stages.⁵² Once established in an area, *E. australis* can spread readily to nearby hosts in the natural environment with adequate rainfall, temperatures and inoculum. Long distance dissemination of *E. australis* is most likely through the movement of infected nursery stock, including budwood.⁵³ Leaves are susceptible to infection when young (flush stage), primarily in the early spring as they emerge from the bud and 'petal fall' commences; thereafter they become immune to infection.⁵⁴ Infected fruit readily express symptoms after infection, but tissue susceptibility decreases rapidly as fruit mature.⁵¹ Fruits are highly susceptible to *E. australis* during the 6 to 8weeks after petal fall. Fruits infected in the very early stages of their development are subjected to premature fall. The initial scab forms on immature fruit is slightly raised and pinkish to light brown in colour. As the lesion expands, it takes on a cracked or warty appearance and may change color to a yellowish-brown and eventually to dark-grey. The scabs typically form a pattern on the fruit like water splashes. Although there is little effect on internal fruit quality, fruit are severely blemished rendering them unsellable in the fresh produce market. Further, the disease can cause premature fruit drop and stunted young nursery trees on newly established field plantings.

Citrus Black Spot (CBS) disease: First discovered in Australia some 80 years ago, CBS disease is now present in South Africa, Zimbabwe, Swaziland, Mozambique, China, Indonesia, Japan and Brazil.⁵⁵ The climatic conditions in Brazil, a leading producer of citrus favours the disease. CBS produces lesions on infected citrus fruits which do not cause postharvest decay, but affect consumers' acceptability. In addition to *Citrus sinensis*, other citrus species (except sour orange) are affected by the disease and heavy losses may occur in 'Valencia' and navel orange varieties.⁵⁵ The disease may be present for many years before producing symptoms and this affects disease management strategies. Symptoms on fruits have been classified into three categories as hard spot or shot-hole, freckle spot and virulent or spreading spot.⁵⁶ Disease spread is enhanced by fallen citrus leaves that serve as source of inoculum for the production of ascospores and pycnidiospores.⁵⁵ In Brazil, many attempts to control CBS by grass mulching were unsuccessful and at least five fungicide applications are necessary to reduce disease symptoms on fruit (Rossêto, 2009).

Powdery mildew: This disease is caused by *Acrosporium tingitaninum*. A very important disease which causes the shoots to collapse and dry with a white colour. Under severe conditions, affected leaves drop off and the twigs show die-back symptoms. Young fruits also get covered by the fungus and they drop off prematurely, leading to reduction in yield. Suckers running from the base to the centre of the tree are usually affected. Damp mornings with a few hours of sunshine are the most favourable conditions for the onset of the disease (Sastra-Hidayat, 1992). Other fungal diseases of *C. Sinensis* are Gummosis (*Phytophthora species*), pink disease (*Pellicularia salmonicolor*), twig blight or citrus (*Diplodia natalensis*) and anthracnose or wither tip (*Colletotrichum gloeosporioides*).

Control measures

Chemical method: Post-harvest treatments with chemicals effectively reduce the viability of pycnidiospores in lesions on symptomatic citrus fruits with black spot fungal disease that causes lesions on citrus fruit.⁵⁶ Routine packinghouse treatments with chlorine dips, warm water bath, or chemical tank dip and combination of these treatments and wax treatments reduces the viability of conidia.⁵⁶ Imazalil (IMZ) and thiabendazole (TBZ) as systemic fungicides also have a trans-laminar effect as post-harvest surface treatments for several citrus fungi including: *Diplodia*, *Alternaria*, *Fusarium*, *Botrytis*, *Molinia*, *Rhizoctonia*, and apple scab.⁵⁷ Thiabendazole has a range of activity similar to that of benomyl.⁵⁷ Studies on the distribution of postharvest fungicides on citrus fruit show that residues are predominantly found on the surface of the fruit and in the peel and only small amounts migrate into the flesh.⁵⁸ Systemic fungicides (benomyl, carbendazim) are commonly used before flushing and after petal fall.⁵⁹ Benomyl has been used to effectively control *Elsinoe australis* that causes common old scab lesion disease in citrus fruits.⁶⁰ Its use was however discontinued because of the emergence of resistant strains of the causal pathogen.⁶¹ Benomyl is still effective in many locations and is still recommended for scab control.⁶² No chemicals are presently recommended for post-plant control of the citrus nematode.⁶³

Use of bio-control agents: Strains of *Pseudomonas syringae* are very effective in controlling postharvest diseases of citrus fruits, and antagonistic activity has been correlated with *in vitro* production of lipodepsipeptides. Additionally, biocontrol agents can induce a range of defence mechanisms of resistance in citrus tissue that result in a broad spectrum of metabolic modifications, such as systemic acquired resistance, induced systemic resistance and production of reactive oxygen species.⁶⁴

Packaging: Grade standards for sweet oranges are based on maturity, colour intensity and uniformity, firmness, shape, size, smoothness, freedom from decay, as well as freedom from defects (bruises and abrasions), insects, fungal attack, growth cracks, chemical burns and physiological disorders. Well-vented polyethylene and plastic mesh bags of various sizes are also used to market oranges. Oranges may be singly sealed or wrapped with various plastic films, a practice that has not been widely adopted.⁶⁵

Storage conditions: Under normal weather conditions, fruits are better stored on the tree than in cold storage. Cold storage should not be attempted if the fruit storage potential has been expended by prolonged tree storage. Once harvested, fruit quality will not improve. Before placing into storage, fruit should be pre-cooled to slow respiration and treated with an approved fungicide to reduce decay. Oranges can be stored for up to 12 weeks under optimum storage

conditions. Ultimate storage-life depends on cultivar, maturity, pre-harvest conditions, and postharvest handling. During storage, fruit should be inspected often for signs of decay or disorders. Such problems will advance rapidly once the fruits are removed from cold storage.⁶⁵

Banana (*Musa sapientum*)

Banana is a monocotyledonous plant of the order Zingiberales. It makes up the genus *Musa* of the family Musaceae. It is named botanically as *Musa sapientum*. It is native to South-East Asia but currently cultivated extensively in all tropical countries for their fruit, fibre or foliage. The commercial bananas are mutants of wild *Musa* species which produce seedless and therefore edible fruits. Two wild species considered to give rise to the commercial bananas are *M. acuminata* and *M. balbisiana* and there are diploids, triploids and tetraploids with chromosome number 22, 33 and 44. The diploids are usually smaller plants than the triploids and tetraploids and generally have smaller branches and fruits. Tetraploids are the largest but there are not many of these; a number, however have been produced by artificial breeding.

Botany

The vegetative stem (pseudostem) of the plant is made up of interfolded leaf sheaths which grow from the corm. The true stem appears only when the inflorescence is formed. This true stem grows up inside the pseudostem until it emerges and bears the bunch. The inflorescence is formed only after a certain number of leaves have grown and there is a high enough rate of photosynthesis to form the bunch. Nitrogen fertilization is important in promoting leaf development, early bunch production and high yields. When the inflorescence is formed, no more leaves develop on the shoot and after the bunch is ripe the shoot dies. The plant continues growth by producing side shoots.

Bananas are planted from the side shoots or pieces of the corm and under tropical conditions take from 8–12 months to produce bunch. The bunch is formed from the inflorescence which is made up of successive layers of flowers each surrounded by a bract in the young stage. The bracts curl back one after another revealing the layers of flowers which later grow into the ‘hands’ of bananas. The first formed flowers are always female and those formed towards the top of the inflorescence are male. However, in commercial bananas the fruits generally develop without fertilization and without seeds.

The banana bunch may hang vertically downwards or project sideways (diatropic) or hang at an intermediate angle. Generally heavy bunches such as those of ‘Gros Michel’ hang vertically and light bunches such as ‘Sucrier’ tend to be horizontal.

Climate and Soil

Bananas grow best in the humid tropics where bunches are produced in the shortest time; that is after 8–12 months depending clone. For optimal growth the monthly rainfall must be at least 100mm and can be as high 250mm. Where dry seasons occur maturity is delayed unless the crop is irrigated. The region of South-East Asia centred on Malaysia, and the wetter parts of South and Central America are the best regions for growing bananas and for banana industries.⁶⁶

High temperature is the other requirement for banana growth and temperatures between 25 and 30°C are optimal. Temperatures lower than this delay maturity and temperatures below freezing destroy

the crop. Strong winds also affect the banana crop since the shallow roots and absence of tap root, combined with the large leaf and soft pseudostem, make the plant very susceptible to wind damage. Winds above 20kph cause damage to bananas and winds reaching 80kph may completely destroy the crop. The shorter types of bananas such as 'Dwarf Cavendish' and certain hardy clones such as 'Mysore' are best suited to withstand adverse conditions.⁶⁶

Bananas can be grown on many types of soil provided drainage is good. Hill soils are generally adequately drained but alluvial clayey soils generally require drains to about 60cm depth at suitable intervals. Very heavy clay soils need special treatment to raise the level of organic matter and improve drainage. In heavy soils deep cultivation to break up the clay should be carried out at the time of field preparation and laying of the drains.⁶⁶

Less acid soils are best for bananas especially for sensitive clones such as 'Gros Michel' that suffer from Panama disease. The best pH range is 5.5–6.5. soils more acidic than this will benefit from liming with dolomitic limestone.⁶⁶

Cultivation and management

Planting material and planting: Bananas are not planted from seed but from various types of side shoot or from the corm, or pieces of corm which will have buds in various stages of development. Side shoots are obtained from old banana clumps and when many shoots are required for planting, cutting back of the large shoots before bunch formation will encourage more of the side shoots to grow. Only healthy shoots or corms should be chosen for planting.⁶⁶

There are several types of shoots: maidens, swords, peepers and water suckers. Maidens are large shoots which have not yet produced a bunch inflorescence. Swords and peepers are different stages of strong side shoots. Peepers are young with up to about 30 cm of the shoot showing above the soil and swords are larger, producing a number of thin sword-like leaves, which precede the expanded foliage leaves. Of these various kinds of shoots, swords, peepers and maidens are the best for planting.⁶⁶

In planting, holes are dug about 30cm deep (rather larger holes are recommended for heavy clay soils) and the planting material is planted at this depth. Pieces of corm should be planted on the slant with eyes on the upper side. Maidens should be planted upright and swords, peepers and water suckers should be planted whole with the ends protruding if they are taller than the hole.⁶⁶

Spacing: The spacing adopted for banana varies considerably but there has been a tendency for closer spacing in recent years. For pure stand, the spacing is 3.6x4.5m apart for tall varieties. In dry areas, the spacing recommended is 3.6x2.7m. For dwarf varieties, the spacing is 2.4x2.4m which can give a population of 1,680 plants per hectare.⁶⁶

Weed control: Normally weed control is achieved by means of herbicides. For weed control around the stems the following herbicides may prove useful, sprayed in 250litres of water per hectare: sodium chlorate (v) (10–20 kg) followed by diuron (3kg). Sodium chlorate should be sprayed in clear weather since it kills weeds by desiccation. Under wet conditions paraquat (gramoxone), at 1litre per hectare should be used to replace sodium chlorate (v). sodium chlorate (v) and paraquat are contact weed killers which destroy standing weeds; diuron gives lasting control by preventing weeds from growing. If climbing weeds are present, 1.5litre of 2:4-D amine may be added

to the sodium chlorate (v) when the banana plants have become tall. Great care must be taken not to spray the banana foliage but only the soil between the plants. Simazine and atrazine may replace diuron for weed control in bananas.⁶⁶

Fertilization: Bananas require heavy application of fertilizers for optimal yield. In particular, high nitrogen and potassium fertilization is necessary. Nitrogen is required early in the growth stage when canopy is forming. Fertilizers should be applied in fractional doses at least 4 times in a year; more frequent application of smaller doses makes for more economic fertilizer use with continuous cropping with bananas.⁶⁶

When legume covers are established at planting, the nitrogen fertilizer may be greatly reduced or omitted over the first year. The fertilizer should be broadcast in the raining season 0.6m away from the base of the plant. Single super phosphate per plant is needed and should be applied before planting the sucker.⁶⁶

Harvesting: Banana fruits can be harvested green and will ripen after harvesting. Even when harvested for local marketing, particularly for weekly markets, it is normal to harvest the bunch while it is still green just before yellowing occurs, because at the green stage it can stand rougher handling. When bananas are harvested for transport over long distances, for example by ship, they are cut even sooner, when they are about three-quarters mature, and the fruits are not yet fully expanded. For the purposes of marketing, green bananas are sometimes ripened artificially using ethane (ethylene) known as 'Ethrel', or ethyne (acetylene).⁶⁶

Health benefits of banana

Reduced risk of high blood pressure: Bananas are one of the best sources of potassium, an essential mineral for maintaining normal blood pressure and heart function. A medium-sized banana provides 350 mg of potassium. The effectiveness of potassium-rich foods in lowering blood pressure has been demonstrated by a number of studies. The US Food and Drug Administration have allowed the banana industry to make official claims for the fruit's ability to reduce the risk of blood pressure and stroke. According to the FDA, "Diets containing foods that are good sources of potassium and low in sodium may reduce the risk of high blood pressure and stroke." Plus, potassium helps your body maintain normal fluid and electrolyte balances in the cells. Scientists report that natural compounds in bananas act in a manner similar to antihypertensive drug (Sampath-Kumar *et al.*, 2012).

Reduced risk of stroke

Scientists suggest that people with a low amount of potassium in their diet may have an increased risk of stroke. A study of 5,600 people aged over 65 found that those with the lowest intake of the potassium were 50% more likely to suffer a stroke. High-potassium foods, like bananas, may lower the risk of stroke, but researchers say that more studies are needed to confirm whether increasing potassium in the diet can prevent strokes (Sampath-Kumar *et al.*, 2012).

Restore normal bowel activity

Because the banana is rich in non-digestible fibres (including cellulose, hemicellulose, and alpha glucans) it can help restore normal bowel activity and help with both constipation and diarrhoea. Bananas normalize the colon's function to absorb large amounts of water for regular bowel movements. Their usefulness is due to their

richness in pectin, which is water-absorbent and this gives them a bulk producing ability. Bananas are an exceptionally rich source of fructooligosaccharide, a compound called a prebiotic because it nourishes probiotic (friendly) bacteria in the colon. These beneficial bacteria produce vitamins and digestive enzymes that improve our ability to absorb nutrients,⁶⁷ plus compounds that protect us against unfriendly microorganisms. When fructooligosaccharides are fermented by these friendly bacteria, not only do numbers of probiotic bacteria increase, but so does the body's ability to absorb calcium (Sampath-Kumar *et al.*, 2012).

Protection from ulcers & heartburn remedy

Bananas have long been recognized for their antacid effects that protect against stomach ulcers and ulcer damage. A flavonoid⁶⁸ in the banana, leucocyanidin, has been found to significantly increase the thickness of the mucous membrane layer of the stomach. Since bananas help to neutralize acidity, they are also a great way to get rid of heartburn.⁶⁹ In an animal study, a simple mixture of banana and milk significantly suppressed acid secretion (Sampath-Kumar *et al.*, 2012).

Cholesterol-lowering effect

Animal studies have shown that banana has the potential to lower cholesterol. It was suggested that the dietary fibre component in banana pulp was responsible for its cholesterol-lowering effect. The amount of dietary fibre in banana is relatively constant during banana ripening (Sampath-Kumar *et al.*, 2012).^{70,71}

Kidney health

Bananas promote an overall improvement of the functional efficiency of kidneys. Benefits to the kidneys are again due to the high potassium content of bananas. A normal intake of potassium suppresses calcium excretion in the urine and minimizes the risk of kidney stones (Sampath-Kumar *et al.*, 2012).

Energy booster

Bananas contain three natural sugars—sucrose, fructose and glucose combined with fibre. Potassium is also essential for helping muscles to contract properly during exercise and reduces cramping up. A banana gives an instant, sustained and substantial boost of energy. Research has proven that just two bananas provide enough energy for a strenuous 90-minute workout (Sampath-Kumar *et al.*, 2012).

Immunity booster

Bananas contain 25percent of the recommended daily allowance (RDA) for vitamin B6, necessary for producing antibodies and red blood cells as well as aiding in the metabolism of fat. In addition, vitamin B6 serves as an immunity booster. So, this fruit strengthens your armour against infectious diseases. With each average-sized banana, you ingest about 15% of the RDA for vitamin C, one of the strongest antioxidants (Sampath-Kumar *et al.*, 2012).

Fungal diseases of banana

The two most important diseases of bananas are Panama disease and leaf spot.

Panama disease: Panama disease is caused by a variety of the fungus *Fusarium oxysporum*, strains of which cause disease in many plants. Gros Michel is particularly susceptible and this disease

causes widespread losses in banana production. The disease attacks the roots giving rise to wilting and collapse of the leaves. The most effective control is to grow resistant clones, although increasing soil pH by liming and improving drainage also reduces the disease. This disease is known to occur particularly after wind damage which ruptures root tissues. Leaf spot: Leaf spot is caused by another fungus, *Mycosphaerella musicola*. It attacks leaves, first causing pale yellow spots which later becomes dark and necrotic. Older leaves first show the symptoms. The best method of control is by growing resistant strains but the disease can also be controlled by spraying with copper fungicides such as Bordeaux mixture. Spraying with mineral oils with an ULV sprayer also controls leaf spot.

Results and discussion

Results

Table 2 shows the quantity of sweet orange and banana fruits bought from the various selling points in Kara market. The table also shows the number of samples infected which is also expressed in percentages. Fifteen fruits were obtained for each of the samples with 5 at each selling point (fruit trader) with each of the samples having a total of 5 healthy fruits and 10 infected fruits and 66.67% total infected fruits for each sample (Table 3 & 4).

Table 5 & 6 shows the pathogenicity of the isolated fungi from the rotten *Citrus sinensis* and *Musa sapientum* fruit after seven days of incubation by measuring the rot length.

Table 2 Samples of apparently diseased oranges and bananas expressed in percentages

Sampling points	Total samples obtained	Total samples infected	% samples infected
A ₁	5	3	60%
A ₂	5	2	40%
A ₃	5	5	100%
B ₁	5	2	40%
B ₂	5	4	80%
B ₃	5	4	80%
Total	30	20	66.67%

Table 3 Frequency of occurrence of various fungal isolates in diseased oranges

S/N	Organisms identified	Frequency (%)
1	<i>Cladosporium</i> spp	40%
2	<i>Fusarium</i> spp	30%
3	<i>Chrysonilia</i> spp	10%
4	<i>Alternaria</i> spp	20%

Table 4 Frequency of occurrence of various fungal isolates in diseased banana

S/N	Organisms identified	Frequency (%)
1	<i>Fusarium</i> spp	50%
2	<i>Mucor</i> spp	30%
3	<i>Rhizopus</i> spp	20%

Table 5 Decay rate of fungi isolated from *Citrus sinensis* fruits after 7days of incubation

	Fungal isolates	Length of rot (mm)
1	<i>Cladosporium</i> spp	74mm
2	<i>Fusarium</i> spp	70mm
3	<i>Chrysonilia</i> spp	48mm
4	<i>Alternaria</i> spp	52mm

Table 6 Decay rate of fungi isolated from *Musa spp* fruits after 7days of incubation

	Fungal isolates	Length of rot (mm)
1	<i>Fusarium</i> spp	84mm
2	<i>Mucor</i> spp	75mm
3	<i>Rhizopus</i> spp	54mm

Discussion

This study shows that *Fusarium* spp, *Cladosporium* spp, *Chrysonilia* spp and *Alternaria* spp were found in the spoilt sweet orange while *Fusarium* spp, *Mucor* spp and *Rhizopus* spp were found in the spoilt banana. Out of the fungi isolated from sweet orange, *Cladosporium* spp and *Fusarium* spp have the highest frequency of 40% and 30% followed by *Chrysonilia* spp and *Alternaria* spp with 10% and 20% frequency of occurrence. Furthermore, out of the fungi isolated from banana, *Fusarium* spp has the highest frequency of 50%, followed by *Mucor* spp, 30% and then *Rhizopus* spp with 20%, which is the least frequency of occurrence. This is in agreement with Ifeanyi¹⁰ and Bello¹¹ whom both isolated about seven different genera from different fruits, and when these isolates were aseptically inoculated into healthy susceptible fruits, the characteristics symptoms originally observed were also noticed. All the organisms were successfully taking part in the decay and are thus confirmed as the causal organism of fruit decay.^{8,12} Thus, these fungi were also found to be associated with the deterioration of sweet orange and banana fruits. All the organisms isolated were confirmed to cause spoilage on the sweet orange and banana fruits but in varying degrees. Of all the isolated fungi from sweet orange, *Cladosporium* spp and *Fusarium* spp were the most pathogenic (virulent) with rapid disintegration of the fruits in 7days having a rot length of 74mm and 70mm respectively while the least pathogenic fungi was *Alternaria* spp and *Chrysonilia* spp having rots length of 52mm and 48mm respectively. The most pathogenic fungi for the banana fruit was *Fusarium* spp and *Mucor* spp having a rot length of 84mm and 75 mm respectively while the least pathogenic fungus was *Rhizopus* spp with rot length of 54mm. Generally, fungi that causes spoilage are considered toxigenic or pathogenic (Al-Hindi et al., 2011). Some fungi may produce mycotoxins.¹³ The fungi isolated in this study have been reported to produce secondary metabolites in plant tissues. These secondary metabolites are potentially harmful to humans and animals.^{7,14} A good example is aflatoxin which has been implicated in cancer of the liver (hepatoma), aflatoxicosis and also acute hepatitis in humans, especially in the developing world.^{8,14-17} Pathogenic fungi, on the other hand, could also cause infections or allergies.¹⁸

Summary

Citrus sinensis and *Musa sapientum* are consumed all over the world as an excellent source of vitamins, phytochemicals and other powerful natural antioxidant that builds the body's immune system. These biologically active compounds prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood which promote human health. However, the impact of diverse diseases, in this case, fungi (sweet orange scab, citrus black spot, powdery mildew for orange and panama and leaf spot disease for banana) limits their production, nutritional value and market qualities. These diseases can be controlled through chemical treatment of fruits, use of biological control agents, proper packaging and storage facilities and other disease management practices to reduce postharvest damages. Considering its health benefits, there is need for public awareness on the importance of sweet orange and banana, especially in the rural areas as the fruit is relatively cheap and common almost all year round.

Conclusion

In this study, *Cladosporium* spp, *Fusarium* spp, *Chrysonilia* spp and *Alternaria* spp were detected in spoilt sweet orange, while *Fusarium* spp, *Mucor* spp and *Rhizopus* spp were found banana. The presence of these fungi on sweet orange and banana fruits poses a serious threat to the health of consumers as the organism could produce mycotoxins, which are harmful when consumed. Most of the fungi isolated were observed to be able to infect healthy orange and banana fruits within a short time, which poses a serious economic threat to sellers of these fruits in Sokoto metropolis.

Recommendation

Fruits and vegetables in general are known to be perishable, and have a relatively short shelf-life causing them to deteriorate rapidly. Therefore, sweet orange and banana fruits should be consumed shortly after harvesting or properly refrigerated (or stored using other means) and should be discarded if any alteration in color or taste of the fruit is noticed as this can be hazardous to human health.

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None.

Conflict of interest

The author declares that there in none of the conflict.

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