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ISOLATION OF MICROORGANISMS ASSOCIATED WITH DETERIORATION OF BANANA FRUITS CONSUMED IN DAMATURU, YOBE STATE.

***IBRAHIM ADAMU; **SUNDAY DAVID NAJOJI; & *IBRAHIM BABALE GASHUA**

*Department of Science Laboratory Technology, School of Science & Technology, the Federal Polytechnic Damaturu, Yobe State, Nigeria.
**Department of Basic Science, School of General & Remedial Studies, the Federal Polytechnic Damaturu, Yobe State, Nigeria.

ABSTRACT

A Banana is a long curved fruit that grows in clusters and has soft pulpy flesh and yellow skin when ripe. It is an edible fruit and is an herbaceous flowering plant belonging to the genus *Musa* and the family *Musaceae*. The massive production of bananas and the result of poor storage facilities leads to a large waste of

Introduction

A Banana is a long curved fruit that grows in clusters and has soft pulpy flesh and yellow skin when ripe. It is an edible fruit and herbaceous flowering plant belonging to the genus *Musa* and the family *Musaceae*. Banana is also eaten as a cooked vegetable (and is then called plantains). All the edible banana fruits are seedless (parthenocarpic) and belong to two main species, *Musa acuminata* Colla and *Musa balbisiana* Colla. The hybrid from these two species *Musa* x *paradisiaca* Linnaeus is also available nowadays (Sidhu and Zafar, 2018; Moreno et al., 2021). It is known in English as banana and in Hausa as *Ayaba*. The fruit is variable in

bananas due to microbial deterioration. Microbes associated with spoilage are mostly due to contamination from external sources during post-harvest practices as a result of transportation to the market or during storage of the fruit. The study aims to isolate microorganisms associated with the deterioration of banana fruits and to assess common ways that have been used to improve the storage life of the fruit sold in Damaturu Yobe State. The standard pour plate and streak plate methods were used in the identification and isolation of microorganisms from deteriorated Banana fruits. Yeast cells were the most isolated fungal isolates in spoilt Bananas. While *Penicillium* and *Aspergillus niger* had the least fungal isolate rate. *Streptococcus pyogenes* were the most isolated Bacteria species in fresh Bananas, their occurrence was 28% while *Staphylococcus aureus* shows a percentage occurrence of 24%. Coccobacilli species were strictly isolated from spoilt Bananas and show a percentage occurrence of 48%. This study shows that microorganism especially fungi, causes deterioration and spoilage of banana fruits and consumption of some of the infected bananas fruit causes disease in man as a result of toxics secreted by fungi.

Keywords: banana, microorganism, deterioration, isolation, identification.

size, colour, and firmness, but is usually elongated and curved, with soft flesh-rich starch covered with a rind that may be green, yellow, red, purple, or brown when ripe, (Mwang et al., 2007).

In the developing world, banana is a major staple crop of considerable importance. The world-wide massive production of bananas and as result of poor storage facilities leads to a large waste of bananas due to microbial deterioration. Microbes associated with spoilage are mostly due to

contamination from external sources during post-harvest-practices as a result of transport to the market or during storage of the fruit.

The colour of the bananas could suggest the level of deterioration or infection. This type of food infection or contamination is usually observed, that causes health hazards. These organisms invade bananas by riddling through natural openings or damaged sites. The damaged fruit because of microbial action reduces its market value and nutritional importance (Ramavat and Ahuja, 2020).

The global fruit processing industry has experienced consistently increased demand over the last five years, for processed fruit and fruit products and consumer spending has increased. Banana is a very popular fruit in the world market and is consumed as a staple food in many countries. It is grown worldwide and constitutes the fifth most important agricultural food crop in terms of world trade. It is either eaten raw or processed and also as a functional ingredient in various food products (Singh et al., 2016).

Microorganisms, natural disasters such as earthquakes, etc. have caused a substantial deterioration in banana fruit production and have naturally affected Global fruit production. Microscopic organisms are the major and significant factor for banana fruit spoilage (Oyewole, 2012; Ramavat and Ahuja, 2020). The quantity of banana products in Nigeria has not been estimated. However, it is known to be produced in varying amounts within the country. Massive production of banana fruit and poor storage and handling process always lead to their spoilage.

All parts of the banana plant have pharmaceutical applications: the flowers in bronchitis and dysentery and on ulcers; cooked flowers are given to diabetics; the astringent plant sap in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery, and diarrhea, and it is applied on hemorrhoids, insect and other stings and bites; young leaves are placed as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and the leaves are taken in dysentery, and diarrhea and used for treating malignant ulcers; the roots are administered in digestive disorders,

dysentery and other ailments; banana seed mucilage is given in cases of diarrhea in India (Kumar, 2012; Hassan et al., 2018).

The Study Area

Damaturu is the capital of Yobe State which is one of the 36 states of the Federal Republic of Nigeria. Yobe is a state located in northeastern Nigeria. A mainly agricultural state, it was created on 27 August 1991. The State was carved out of Borno State. Yobe State shares borders with Borno State to the east, Gombe State to the south, Bauchi and Jigawa States to the west and Niger Republic to the north. It borders to the north with the Diffa and Zinder Regions of Niger. Because the state lies mainly in the dry savanna belt, conditions are hot and dry for most of the year, except in the southern part of the state which has more annual rainfall. The state is located within latitude 11 North and longitude 13.5 East. The State experiences a typical tropical climate with distinct seasons, the rainy season and the dry season. The rainy season last from April to October with annual rainfall in the range of 150 -180 mm while the dry season begins in November and ends in March. The mean monthly temperatures fluctuate between 25 and 47°C in the year. The state is an agricultural area producing mainly maize, rice, millet, groundnut, gum Arabic, and cotton. Its people also engage in livestock rearing and fishing. It has one of the largest cattle markets in West Africa, which is located in Potiskum.

Materials and Methods

Sample collection: Fifty (N=50) samples of fresh and spoilt bananas were collected from banana vendors from the Damaturu metropolis. Twenty-five samples (25 fresh and 25 spoilt) were aseptically collected from the Damaturu market and the banana plantation in clean polythene bags separately in a cool box and taken to the Biology Laboratory, Department of Science Laboratory Technology (SLT), for identification and analysis.

Sample treatment/preparation: The Banana fruit samples were subjected to treatment using mortar and pestle to produce a paste.

Media Preparation: The media used for the experiment were nutrient agar and sabroud dextrox agar for the isolation of bacteria and fungi respectively. The media was prepared using the manufacturer's instruction and sterilized using an autoclave at 121°C for 15 minutes.

Total Bacteria Count: Total bacteria count was determined using the standard pour plate technique method described by Agbabiaka *et al.,* (2015), with slight modifications, One gram (1g) of banana pulp was mashed in a plastic mortar and homogenized in 9ml sterile distilled water. A10^{-6} serial dilution was prepared and 1ml was inoculated into nutrient agar (NA) and incubated at 37 °C in an incubator plate was examined for bacterial growth after 24 hours or 48 hours and colonies were counted using a colony counter and enumerated as cfu/g. The media was also melted in a water bath and then poured into various Petric dishes and allowed to solidify. The wire loop was dipped into the sample and streak on the media and was incubated at the same temperature for the same period.

Total fungus count: Total fungal count was determined using the standard pour plate technique method described by Agbabiaka *et al* (2015) with sight modification. A10^{-6} serial dilution was prepared and 1ml was inoculated onto Saboround Dextrose Agar (SDA) plate and was incubated at room temperature for 72 hrs and the colonies were counted using a colony counter. The streak plate technique was also employed at the same temperature and period.

Isolation and Identification of Bacteria Isolates from fresh and spoilt samples:

The pour plate method of Harrigam and Mc cane (1990) described by Mbajiuka et al. (2014) and the streak plate method of Koch (1881) as described by Sue-Katz (2008) were used. After each banana sample was meshed under running tap water for 60 sec and the surface was sterilized

with aspirin with cotton wool soaked in 70% ethanol. A tenfold dilution of the banana pulp was weighed and macerated in a plastic mortar and homogenized in 9 ml sterile distilled water. A10^{-4} serial dilution NA plates were incubated at 37 ^{o}C for 24 hrs while the media was melted in the water bath and then poured into various Petric dishes and allowed to solidify. The wire loop was dipped into the sample and streak on the media and was incubated at the same temperature and period.

All bacteria isolates were characterized and identified considering their cultural, morphological, and biochemical characteristics following the methods described by (Ayitso and Onyango, 2016). Methyl red test, citrate test. Coagulase test and sugar fermentation. An unincubated plate (containing only the medium) served as the control.

Isolation and Identification of fungal isolates:

Fresh samples: The pour plate method of Harrigan and Mccane (1990) described by Mbajuika *et al.*, (2014), and the streak plate method of Koch (1881) as described by Sue-Katz (2008) were used after each banana sample was washed under rung tap water for 60 sec and then surface sterilized by coping with cotton wool soaked in 70% ethanol. A tenfold dilution of the banana pulp was prepared using sterile distilled water. A10^{-4} serial dilution was prepared and 1ml was aseptically inoculated on SDA plates and incubated plates containing only the medium served as the control fungal isolate was characterized based on their macroscopic appearance on culture medium, microscopic morphology, and types of asexual spores produced (Fawole and Oso, 2004).

Spoilt samples: A sterile swat was used to take a sample from the damaged part of the banana and then incubated onto SDA plates and incubated at room temperature at (28 ± 2) ^{o}C for 24 hrs and inoculated plates (containing only the medium) served as the control (Amusa *et al,* 2013).

Identification Test: The identification test was carried out according to the method of Cowan and Steel (1993). These tests include gram stain, coagulase test, catalase test, and identification agent.

Gram Reaction: A sterile wire loop was used to obtain a colony of the isolate on the plate and emulsified on a clean glass slide, it was smeared and air dried completely. The fixed smear was covered with crystal violet stains for 30 seconds. The stain was washed with clean water, covered again with lugos iodine for 30 seconds, and rinsed with clean water. The smear was decolorized with acetone and flushed with clean water; the smear was then covered with a natural red stain for 2 minutes and rinsed with clean water. The back of the slide was wiped clean and allowed to air dry after which the smear was examined microscopically. First, the x 40 objective was to check the stain to see the distribution of the staining reagent and then with the oil immersion objective to examine the bacteria.

Catalase test: This test was used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci from non-catalase-producing bacteria such as streptococci. A smear of the bacterium was made on a slide using a sterilized wire loop. About 2 drops of 3 hydrogen peroxide were added to the slide, the presence of bubbles signifies a positive reaction.

Coagulase Test: Coagulase test was used to differentiate staphylococcus aureus (positive) which produces the enzyme coagulase from *S. epidemis* and *S. saprophyticus* (negative). A drop of physiological saline was placed on each end of the slide, with a wire loop to emulsify a portion of the isolated colony in each drop to make two thick suspensions. A drop of human, rabbit, or cow plasma was added to one of the suspensions and mixed gently. A clumping of the plasma was looked for within 10 seconds if no plasma was added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping. Clumping indicates a positive reaction.

Results and discussion

Five different types of fungi and three bacteria isolates were obtained in this study. Fungi isolated from these samples examined include; *Aspergillus niger, Rhizopus spp,* and *Penicillium*. The yeast isolated was *Candida spp,* Mucor was also isolated and three strains of bacteria identified by their biochemical characteristics were *Staphylococcus aureus* and *Streptococcus species* isolated from the fresh banana while *Cocobacilli species* were strictly isolated from the spoilt bananas. Yeast cells were the most isolated fungal isolates in spoilt Banana. While *Penicillium* and *Aspergillus niger* had the least fungal isolate rate.

Streptococcus pyogenes were the most isolated Bacteria species in fresh Banana, its occurrence was 7(28%) while *Staphylococcus aureus* shows a percentage occurrence of 6(24%), Coccobacilli species were strictly isolated from spoilt Banana and shows a percentage occurrence of 12(48%).

This study shows that the fungi *Aspergillus niger, Rhizopus spp*, *Penicillum spp,* Mucor, and yeast strains identified as *Candida species* are important agents of spoilage of banana fruit. The bacteria identified in this study are *Staphylococcus aureus, Streptococcus,* and *Coccobacillii species*. The pathogenicity test carried out suggests that the role of bacteria as banana rot-causing organisms is minimal since the result shows not much different from the test control.

This disparity in the result seems to suggest that although a lot of banana spoilage organisms start from the farm and *Candida spp* could be post-harvest contaminants acquired probably during transportation from the farm to the market.

Conclusion

Banana production and storage are faced with a lot of challenges, especially in prolonging the shelf life. This study shows that microorganism especially fungi, causes deterioration and spoilage of banana fruits and consumption of some of the infected banana fruit causes disease in man as a result of

toxins secreted by fungi, e.g. Aflatoxin. Some of these microorganisms are fungi while others are bacteria. Bacteria spoilage of banana fruit is less compared to the fungi spoilage of bananas. Since banana has a slightly acidic pH, it is most likely to be spoilt by fungi.

Some of the common contaminating microorganisms which were isolated from the banana fruit already spoilt could come from the farm while some of the microorganisms were acquired as post-harvest contaminants probably in temporary storage and in transit which finally manifest their spoilage activities in the market.

The careless handling and storage of banana fruit which could bruise or cut the banana peel causes spoilage of the fruit as a result of penetration and activities of microorganisms. Storing the banana in a dirty environment or the use of dirty tables and rage used in covering tables increases the incidence of contamination.

Various spoilage conditions and environmental situations also increase the rate of spoilage of the fruit e.g. the fruit should be stored at room temperature and in the refrigerator should be stored at a temperature of 14 ^{o}C. The handlers should prevent bruises and injuries on the banana fruit.

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