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In Vitro Assessment Of Microbial Post-Harvest Spoilage Of Locust Beans

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Abstract: ***Post- harvest microbial spoilage of locust beans (Parkia biglobosa) was investigated. Both pour plate and streak plate methods were used to isolate both the bacteria and fungi associated with the spoilage. Sub culturing was carried out until pure cultures were obtained. Morphological characteristics, Gram staining and biochemical tests were carried out for identification. The predominant bacterial isolates were Staphylococcus aureus, Streptococcus latics, Bacillus subtilis while the fungi were Aspergillus niger, Aspergillus fumigatum. Based on the results of this research, bacteria and fungi are responsible for the post-harvest spoilage of locust beans. Fermentation of locust beans should be monitored and good manufacturing practices should be done. The result of the investigation shows that Staphylococcus is coagulase positive and this as a result of its pathogenic ability. In order to slow down post harvest spoilage and minimize the associated adverse health effects, great caution should be taken to follow strict hygiene, good agriculture practice, cultivation, harvest, storage, transportation and marketing.***

Keywords: Microorganisms, Locust beans, Spoilage, Post-harvest

I. INTRODUCTION

The Africa locust bean tree, *Parkia biglobosa* are perennial trees legumes which belongs to the sub-family mimosoideae and family leguminosae (now family Fabaceae). They grow in the savanna region of West Africa up to the southern edge of the Sahel zone 13^{o}C. A mature locust bean tree (20-30 years) can bear about a tone and above of harvested fruits. From experience, the tree can start to bear fruit from five to seven years after planting (Gernah *et al.*, 2007).

The most important use of African locust bean is found in its seed, which is legume, although it has other food and non–food uses, especially the seed which serves as a source of useful ingredients for consumption (Gernah *et al.*, 2007).

Locust bean, commonly referred to as "iru" by Yoruba, "dawa dawa" by Hausa and "ogiri", by Igbo, is a local seasoning or condiment used in soups and stews. A very popular soup ingredient, globally, it is referred to as African locust bean with the botanical name as *Parkia biglobosa* (Makale, 2016).

The roots are used as a lotion for sore eyes (Farombi, 2003). Although microorganisms of all groups including bacteria, protozoa, algae, viruses, fungi, together with insects and rodents play significant roles in food deterioration; the most active and more versatile organisms that affect locust bean seeds and its products causing spoilage when stored are several species of bacteria and fungi (Omafuvbe *et al.*,2000).

II. MATERIALS AND METHODS

Sample collection: The locust beans was bought from Owode market in Offa, Kwara State in a polythene bag and transported to the laboratory for microbiological analysis

Inoculation method: Inoculation method employed was pour plate method. It was done by introducing 0.1ml from each dilution aseptically into sterile Petri dishes and cooled molten agar was poured into each of these plates aseptically

and was then incubated. For bacteria incubation was done at 37^0C for 24 hours and for fungi at 25^0C for 3-5days.

Sub-culturing: Discrete colonies on solid media were transferred by streak plate method to fresh agar plates followed by incubation. This was repeated several times until axenic (pure) cultures were obtained

Staining of fungal isolates and microscopic observation: A drop of lactophenol cotton blue was dropped on clean grease-free slide. Amycological pin was used to pick a small portion of the fungal culture and transferred into the drop of lactophenol blue. The slide was gently covered with cover slip and examined under low power objective (×10) and high power objective (×40).

Staining of bacterial isolates and microscopic observation:

Gram staining materials: Microscope, crystal violet, Lugol's iodine solution, carbol fuchsin, water, 95% alcohol, slide and wire loop.

Catalase test: 2ml of hydrogen peroxide (H202) solution was poured into a sterile test tube. A sterilized wire loop was used to aseptically pick a good growth of the test organism into the test tube containing the hydrogen peroxide solution. Effervescence of oxygen bubbles indicates a positive catalase reaction.

Coagulase test: This test specifies the ability of the test organism to cause clotting or agglutination of blood plasma. The fresh blood was spun after it was collected with sterile needle and syringe and centrifuged at about 200 revolution per minute to obtain the blood plasma. Wire loop was sterilized in a blue flame into red hot and allowed to cool. Colony of bacterial was picked with the aid of sterile loop and it was dropped on a slide. The inoculum was emulsified on the slide and a loop full of freshly prepared plasma was added and emulsified together. Agglutination shows coagulase positive.

Indole test: Two (2) ml of peptone water was introduced into sterile test tube and was inoculated with inoculums picked from 24 hours old culture; it was then incubated at 370C for 48 hours. After incubation 0.5mls of Kovac's reagent was added and shaken gently. Yellow colour shows negative, red colour shows positive indole test.

Motility test: A tube of nutrient broth was inoculated with a colony of the organism and incubated at 37°C for 24 hours. A drop of the broth culture was placed on the center of a clean cover slip. A ring of Vaseline was made on a clean slide. The two were affixed and inverted on the microscope using ×40 objective lens.

III. RESULTS AND DISCUSSION

The results of the experiments conducted on the microbial spoilage of locust beans, based on the microscopic observation, morphological characterization and biochemical tests revealed the predominant bacteria to be Staphylococcus aureus, Streptococcus lactis and Bacillus subtilis as shown in Table 3. The predominant fungi as shown in table 4 were Aspergillus niger, Aspergillus fumigatum and Fusarium oxysporum.

The table 1 below shows the total viable bacterial counts on nutrient agar plate. At the end of the count, the average count from sample A1 count was 9.2×10^{-8} cfu/g, A2 was 7.2×10^{-8} cfu/g and A3 was 6.0×10^{-8} cfu/g.

Sample	cfu/g
A1	9.2×10^{-8}
A2	7.2×10^{-8}
A3	6.0×10^{-8}

Table 1: Total viable count of bacterial isolates on Nutrient agar

Sample	cfu/g
F1	4.0×10^{-4}
F2	3.6×10^{-4}
F3	3.1×10^{-4}

Table 2: Total viable count of fungal isolates on Potato dextrose agar

The table above shows the total viable fungi counts on potato dextrose agar plate. At the end of the count, the average count from sample F1 count was 4.0×10^{-4} cfu/g , F2 was 3.6×10^{-4} cfu/g and F3 was 3.1×10^{-4} cfu/g.

Test	Isolate A1	Isolate A2	Isolate A3
Catalase	+ve	+ve	+ve
Coagulase	+ve	-ve	-ve
Methyl red	-ve	-ve	-ve
Motility	-ve	+ve	+ve
Indole	-ve	-ve	-ve
Probable organism	*Staphylococus aureus*	*Streptococus lactis*	*Bacillus subtilis*

Table 3: Results of biochemical tests on bacterial isolates

The table above shows the results of biochemical tests on the isolates. Isolate A1 was found to be catalase +ve, coagulase +ve, methyl red -ve, motility -ve, indole –ve, Isolate A2 was found to be catalase +ve, coagulase -ve, methyl red -ve, motility +ve, indole –ve, Isolate A3 was found to be catalase +ve, coagulase -ve, methyl red –ve, motilily +ve, indole –ve

The table above shows the identities of the bacterial isolates. Isolate A1 was identified as *Staphylococcus aureus* , A2 was identified as *Streptococcus lactis* , A3 was identified as *Bacillus subtilis.*

Isolate	Colony character	Probable organism
F1	Black velvely to cotton surface profused growth. Conidiosphore arising from long, branched, smooth-watted, conidia are large mostly globose, hypae appeared septate.	*Aspergillus niger*
F2	Yellow-green, conidiophores upright, bearing phialides at the apex, conidia globose, hyphae appeared septate	*Aspergillus flavus*
F3	White- pink, cotton aerial mycelium conidiophores rickly branched philalide	*Fusarium oxysporum*

Table 4: Morphological characterization and identification of fungal isolates

The table above shows morphological characteristics and identities of the fungal isolates. Isolate F1 was identified as

Aspergillus niger, isolate F2 was identified as Aspergillus flavus while isolate F3 was identified as Fusarium oxysporum.

From Table 2, fungi have the highest counts of 4.0x10-4 and the lowest count of 3.1x10-4.The highest count was as a result of increase in microbial load. The dynamics of fermentation in any food matrix is a complex microbiological process involving interactions between different microorganisms (Omafuvbe et al., 2003).

The result of this study agreed with the findings of Victor (2009) who reported that the organisms isolated from fermented Iru were Bacillus, Staphylococcus specie and Micrococcus which were all bacterial isolates.

Aspergillus fumigatum produces a toxin called aflatoxin which can contaminate food leading to allergic diseases. Aspergillus species cause a disease called Aspergillosis which brings about allergic broncho pulmonary, asthma cystic fibtosis and sinusitis infection (Benett, 2010).

Based on the results of this research, bacteria and fungi are responsible for the post-harvest spoilage of locust beans. Fermentation of locust beans should be monitored and good manufacturing practices should be done. The result of the investigation shows that Staphylococcus is coagulase positive and this as a result of its pathogenic ability.

In order to slow down post- harvest spoilage and minimize the associated adverse health effects, great caution should be taken to follow strict hygiene, good agriculture practice: cultivation, harvest, storage, transportation and marketing.

This study helped to determine the bacteria and fungi that cause post- harvest spoilage of locust beans which reduce the shelf life of locust beans. The growth and some subsequent consequences of the occurrence of bacteria and fungi can be controlled through proper monitoring and selection of sound seed and initial selection of sound and healthy locust seed before planting and storage should be ensured.

Spoilage can be controlled through the planting of healthy ones and proper handling before, during and after harvest. The locust beans should be well protected from insect infestation which serves as vector to agricultural products.

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