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MICROORGANISMS ASSOCIATED WITH THE SPOILAGE OF CUCUMBER, GARDEN EGG AND PAWPAW IN MAKURDI METROPOLIS, BENUE NIGERIA

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Abstract: **A total of nine cucumbers, each of Garden egg and pawpaw samples were collected from Wurukum, High level and Wadata markets and cultured on appriopate agar, for colony count and isolation of bacteria according to their cultural and biochemical characteristics. The results revealed that garden egg from High Level Market had the highest bacterial count ($1.9x10^5$cfu/g) and the least was pawpaw from High Level Market ($1.1 x 10^5$ cfu/g). However, it was not statistically significant. The bacteria isolated were; *Propianol bacteria* (23.3%), *Escherichia coli* (16.6%), *Staphylococcus aureus* (36.7%), *Bacillus spp* (10.0%) and *Corynebacteria* (13.3%). The fungal isolates were *Aspergillus flavus* (10%), Aspergillus *fumigatus* (20%), *Aspergillus nidulus* (10%), *Aspergillus terreus* (20%) and *mucor* (40%). The result of this study shows fruits sold in Wurukum, High Level Market and Wadata Market are contaminated and may cause harm to consumers, so measures such as proper handling should be taken to control the contamination of these fruits.**

Keywords: **Wurukum, High Level Marke, *Propianol bacteria, Aspergillus fumigatus, Aspergillus flavus*.**

1. INTRODUCTION

Fruits and vegetables are generally very vital to the human system due to their various minerals and vitamin contents. Some of them are more nutritious than others and are in higher demands. Some fruits and vegetables are native to some areas and this is due to climatic and soil requirements, these play's important roles in fruits germination, growth and maturation because without appropriate requirements, the yield may be very low and some of them may not even survive in such environments (Samson, 1996).

In the field, the physical environment of leaf surfaces is considered to be inhospitable for the growth and survival of bacterial, for example, lack of nutrients and free moisture, temperature and humidity fluctuations, and ultraviolet light (Dickinson, 1986). Environmental conditions, however, can greatly influence bacterial populations; the presence of free moisture on leaves from precipitation, dew, or irrigation may promote survival and growth of bacterial populations (Blakeman, 1981, Andrews, 1992, Beattie and Lindow, 1999).

Cucumber, Garden egg and pawpaw are botanically called *Cucumis salivus*, *Solanum melongena* L and *Carica papaya.* They are tropical plants because they are grown in the tropical regions. Their spoilage is due to biotic factors and being that they are perishable fruits, some microorganisms attack most especially the ripe fruits causing spoilage in Cucumber, Garden egg and pawpaw is not in exception. Spoilage is most often due to the metabolic activity of microorganisms as they grow and utilize the nutrients in the food. Naturally, fruits and vegetable will have their qualities deteriorated by

many intrinsic and extrinsic factors and primarily by the growth and action of different categories of microorganisms. Many factors lead to the effect of these organisms on fruits and vegetables (Cucumber, Garden egg and pawpaw) thereby causing spoilage of the fruits which are mainly of different species and varieties and they are mainly fungi and bacteria. The microorganisms are differentiated by the characterization and color changes (Yeh *et al.,* 1998).

Also, environmental factors might initiate action of microorganisms and subsequently lead to spoilage; for instance, temperature effect on Cucumber, Garden egg and pawpaw may initiate spoilage and cause decay to set in.The effect of pH may be felt thereby promoting the requirement for the microorganisms involved either by increasing or decreasing the acidity or alkalinity of the soil which has direct influence on the fruits and vegetables (Cucumber, Garden egg and pawpaw) and initiate sequential break in normal nutrient or mineral content of the plant. Fruits and vegetables such as Cucumber, Garden egg and pawpaw may be contaminated with microorganisms from sources such as soil, water, animals, birds and insects. Production processes of harvesting, washing; slicing and packaging can create additional conditions where contamination can occur. Also the contamination of fruits and vegetables by bacteria could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Effiuv, 2000).

Because of extremely large number of variables that might influence contamination of raw fruits or vegetables, it is difficult to design well-controlled experiments that would address risks factors for contamination.

Since most fruits and vegetables contain high level of water and nutrients, they serve as good substrates that support the growth of spoilage and pathogenic microorganisms (Anan and Okaka, 1998). Contamination of raw fruits and vegetables with pathogenic organisms of human health significance can occur directly or indirectly via animals, or insects, soil, water, dirty equipment and human handling. For example, fruit flies have been shown to transfer *Escherichia coli* H7 to damaged apples under laboratory condition (Janisiewicz *et al.,* 1999). This may have implications during harvesting and in packing shed or processing facilities, where damaged produce is inevitable and flies may be difficult to control. Pathogens that can be found in fruits and vegetables such as Cucumber, Garden egg and pawpaw includes the following: *Esterobacter spp*, *Salmonella spp*, *Bacillus spp*, *E. coli*, *Listeria monocytogenes* etc.

In developing countries like Nigeria, spoilage of fruits can occur directing or indirectly via animals, insects, soil and human activities such as improper preservation which causes damage in fruits and lead to difficulties in flies control and its pathogenic organism to human.

Fruits are hawked and consumed almost everywhere in Makurdi and yet there is inadequate information regarding its microbial load and health implications.

It has been reported that various source of fruits serve as mineral vitamins in order to nitrify the human body.

The result of this study may provide information which will help reduce consumption of contaminated fruits and consequently reduce infections associated it.

Objectives of the Study

i. To determine the total bacterial counts in garden egg, pawpaw and cucumber sold in Makurdi metropolis.

ii. To isolate, characterize and identify fungal and bacterial isolates obtained from these fruits within Makurdi metropolis.

2. MATERIALS AND METHODS

Area of Study:

The Makurdi town area of Benue State lies within the middle belt zone of Nigeria, its bearing is 300km southeast of Abuja, the federal capital territory and 887km north east of Lagos, (Egozo, 2000).

The area lies within the host humid zone with little seasonal temperature variation through the year and experiences two major seasons (that is) between November to March and between April to October respectively. Makurdi has an average annual temperature of about 31.5^0C with the relative humidity between 65 and 97% rainfall in Makurdi one varies between 100 and 25cm, (Egozo, 2000)

Sample Collection:

Nine (9) samples each of Cucumber, Garden egg and pawpaw fruits were collected from three different major markets (Wurukum, High level and Wadata) in Makurdi. Each sample was collected in isolation in a sterile polyethene bags and conveyed to the Microbiology laboratory in Food Science and Technology Department in the Federal University of Agriculture, Makurdi, for analysis within 24 hours of collection.

Sterilization of Glass Wares:

Petri-dishes, conical flasks, plastic containers, slides and cover slips were thoroughly washed with detergent and rinsed with distilled water, allowed to dry in air then placed in hot air oven at a temperature of 100^0C for one hour.

Sterilization of Media:

The media used for the work: - Nutrient agar, sabourand dextrose agar, simen's citrate agar, lactophenol cotton blue were all sterilized in the autoclave at 121^0C for 15 minutes.

Sample Preparation:

The fresh fruits collected were labeled according to the locations (markets) from which they were collected. The fruits were swabbed using sterilized swab sticks and inserted into a test tube containing 10ml of distilled water and shaken vigorously to a homogenous suspension to form a stock solution. 1ml of the suspension was aseptically transferred using a sterile pipette into 9ml of sterile distilled water in another test tube to give 10^{-1} dilution. The serial dilution was up to 10^{-4}. 0.1ml of each dilution was inoculated on nutrient agar and spread by a glass spreader. The culture plates were incubated at 37^0C for 24 hours to allow colony formation. After which total microbial count was carried out to estimate the total number of colonies per ml. Plate contain 30-300 colonies were counted and CFU calculated using the number of colonies multiplied by the dilution factor.

Isolation of Organisms:

Colonies from nutrient agar were subcultured on saborand agar and incubated at 37^0Cfor 24 hours to obtain a pure culture after which Gram stain and biochemical tests were carried out.

Gram's Staining:

Each organism in the stock culture bottle was picked with an inoculating loop and used to make a smear on a clean grease free glass slide with a drop of distilled water.

The slide was placed on a staining well and floated with crystal violet forabout 30 seconds after heating the smear through a Bunsen burner flame for a few seconds. The wire loop was heated to red hot to ensure sterilization and cooled at 45 ^{0}C. The colony of the test organisms was picked with the loop and emulsify on the microscopic slide that contains the drop of distilled water. After emulsification, the slide was swirled in the air three times and then passed through the flame 5 – 7 times to ensure that the smear is heat fixed.

The crystal violet was washed with distilled water and iodine and allowed to stand for 30 seconds. 70% alcohol was used to rinse off the iodine like distilled water. The slide was finally flooded with safranin for 30 seconds, rinsed with distilled water and allowed to dry off before viewing under the microscope using the oil immersion objective (X1000) (Bergey *et al.,* 1994).

Catalase Test:

This test was carried out according to the methods by Cheesbrough, (1984). 2 to 3drops of hydrogen peroxide solution was put in test tube, with a sterile wire loop a colony of the test organism was removed and immersed in the hydrogen peroxide solution (H_2O_2). Emergence of bubbles in a few seconds shows a positive result and absence of bubbles indicate a negative result.

Citrate Test:

This test will be done by preparing Simon's citrate agar was prepared as recommended by the manufacturers. Making of streak of the isolates were streaked on it using a wire loop and incubating at 35^{o}C for 48 hours. A bright blue colour in the medium indicates a positive test while no colour change indicates a negative result (Ochei, 2007).

Coagulase Test:

The slide method was used. A small colony of bacteria was emulsified with human plasma on a slide and the slide was rocked for thirty seconds. A coagulase positive result was e slide will be inverted to enable the jelly contact the edges to the cover slip. The indicated by clumping (Singleton, 1997).

Mortility Test:

A source of ridges was made on a clean glass slide using petroleum jelly (vaseline). A drop of both of the test organism was placed on a clean cover slip.

Thslide will be inverted and viewed in the microscope under X 40 objective motile organisms were observe moving across the microscopic field.

Preservation of the Fruits:

The swabbed fresh fruits were preserved in nine different transparent containers. The containers were purchased from the market and sterilized using ethanol and each fruit was kept in one of the sterilized containers. The containers were tightly sealed and the lids wrapped with masking tape in order to preserve it. The containers were labeled based on the fruits in each container and monitored daily for changes in appearance that may culminate into spoilage.

Identification of Fungal Isolates:

After preservation, different types of fungi species and mucor growths were formed on the surface of the decayed (spoilt) fruits.

Two Methods of Identifying Fungal isolates was used, The direct observation of the isolate with the unaided eyes and the use of the microscope for observation of microscopic features.

Direct Observation:

Colonies were observed directly from the containers and compared with a pictorial chart for proper identification.

Microscopic Identification:

The containers with different types of species were selected for microscopic examination. Part of the colonies were collected with the aid of a sterile wire loop and rubbed thoroughly on a glass slide after which lactophenol cotton blue was added and viewed under the microscope using X40 objective lens and compared with a pictorial chart for proper identification (Smith and Moses, 1985).

3. RESULTS

Three different types of fruits Cucumber (*Cucumis salivus*), Garden egg (*Solanum melongena* L) and Pawpaw (*Carica papaya*) were purchased from three different markets in Makurdi namely Wurukum, Wadata and High level. A total of nine (9) fruits were examined for the presence of bacteria and fungi.

The bacteria associated with the spoilage of this fruits were *Propianol bacteria, Escherichia coli, Staphylococcus aureus, Bacillus species, Corynebacteria*. While the fungi included *Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus, Aspergillus nidulus and mucor spp.*

Table 1 shows the total viable count of bacterial isolates associated with the spoilage of garden egg, cucumber and pawpaw with garden egg from High Level having the highest count ($1.9x10^5$) and pawpaw from High level also had the lowest number of count ($1.1 x10^5$).

Table 2 shows frequency of occurrence of bacterial isolates from selected fresh fruits with *Staphylococcus aureus* having the highest frequency of occurrence (36.7%) and the least organism was *Bacillus species* (10%).

Table 3 shows frequency of occurrence of fungal isolates from spoilt fruits with mucor having the highest frequency (40%) *Aspergillus flavus* and *Aspergillus terreus* having the least frequency (10%) each.

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Table 1: Total viable count of Bacterial isolates associated with the spoilage of cucumber, Garden egg and pawpaw in CFU/g

S/N	Samples	Locations		
		Wurukum Market	High level Market	Wadata Market
		CFU/g		
1	Pawpaw	1.3×10^5	1.1×10^5	1.2×10^5
2	Cucumber	1.2×10^4	1.3×10^5	1.6×10^5
3	Garden egg	9.03×10^3	1.9×10^5	1.2×10^5

$\chi^2 = 2.6, \quad df = 2, \quad \text{P} - \text{value} = 0.896$

Table 2: Frequency of occurrence of Bacterial isolates from selected fresh fruits

Isolated Organisms	Wurukum Market	Highlevel Market	Wadata Market	Total Number (%)
Propianol bacteria	3(2.33)	2(2.8)	2(1.87)	7(23.3)
Escherichia coli	1(1.67)	3(2.0)	1(1.33)	5(16.6)
Staphylococcus aureus	3(2.00)	5(2.40)	3(1.60)	11(36.7)
Bacillus Spcies	1(1.00)	1(1.2)	1(0.80)	3(10.0)
Corynebacteria	2(1.33)	1(1.6)	1(1.07)	4(13.3)
Total	10	12	8	30

$\chi^2 = 0, \quad df = 2, \quad \text{P} - \text{value} = 1.000$

Table 3: Frequency of occurrence of fungal isolates in spoilt fruits

S/N	Species of fungi	Number (%)
1	*Aspergills flavus*	1(10)
2	*Aspergillus fumigatus*	2(20)
3	*Aspergillus terreus*	1(10)
4	*Aspergillus nidulus*	2(20)
5	*Mucor*	4(40)
Total		**10(100%)**

4. DISCUSSION

Out of the three different types of fruits used, the bacteria isolates were *propianol bacteria* (2.33%), *Escherichia coli* (16.6%) *Staphylococcus aureus* (36.7%), *Bacillus species* (10.0%) and *Corynebacteria* (13.3%). Gram positive organisms had the highest effect in the spoilage of these three fruits. While Gram negative organisms had the lower effect. This observation agree with the findings of Anna (2000) who indicated that Gram positive organisms may be responsible for initiating and causing spoilage in cucumber, garden egg and pawpaw fruits.

The presence of these bacteria present a health risk factor to people who consume fruits without proper washing. For example, the presence of *Staphylococcus aureus* in fruits could cause gastroenteritis in the individual who consume fruits without proper washing. Its isolation (*Staphylococcus aureus*) could be an indication of unhygienic handling of the fruits by the sellers (Ehiri, 2001).

The presence of *Escherichia coli* indicates the use of water with feacal contamination which agrees with Odunfa (1985) and Washima (2008).They stated that *Escherichia coli* isolated speaks of poor sanitary conditions of sources of water used during washing of the fruits.

Bacillus spp are pathogenic and can cause food poisoning endocarditis and bacteremia. Their presence in some of the fruits samples may be because, some of the fruits are openly sold in the market exposed to the spores of the organisms which are dormant to the lethal effects of heat drying and ultraviolet radiation (Doyle *et al.*, 1997).

The result of the analysis showed that there was no statistically significant difference in the total viable count of bacterial isolates associated with the spoilage of Cucumber, Garden egg and Pawpaw in CFU/g.

The frequency of occurrence of bacterial isolates from selected fresh fruits in the different markets showed that *staphylococcus aureus* occurred more frequently (36.7%) followed by *Propianol bacteria* (23.3%), *Escherichia coli* (16.6%), *Corgnebacteria* (13.3%) and *Bacillus species* (10.0%) which have the least occurrence.

The fungal isolates were *Aspergillus flavus, Aspergillus fumigatus, Aspergellus nidulus, Aspergillus terreus* and *mucor*. Mucor had the highest effect (40%) on spoilage of the fruits in Makurdi and *Aspergillus favus* and *Aspergillus terreus* had the least effect (10% each) on fruit spoilage in Makurdi metropolis.

5. CONCLUSION

Microbial spoilage of Cucumber, Garden egg and Pawpaw is a gradual process. Most factors like temperature, pH, water content and certain organism which are always associated with the fruits can also create a favorable environment for the growth and spread of these organisms. Microorganisms may enter fruits through damage of the natural structures such as punctures, wounds, splits and cuts and this may lead to decay.

6. RECOMMENDATIONS

It is therefore recommended that:

1. Fruits should be washed properly to reduce the number of microorganism.
2. Consumers should avoid consumption of spoilt fruits to avoid health implications that may lead to death.
3. Both sellers and consumers should protect fruits from flies.
4. Government should create avenues to educate the masses on proper handling, storage and distribution of fruits to avoid contamination.

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