

FUNGAL MICROBES ASSOCIATED WITH SPOILT PAWPAP FRUIT IN ISHIAGU AND IT'S HEALTH IMPLICATION

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ABSTRACT

A research survey was carried out in Ishiagu, Ivo Local Government Area of Ebonyi State, Nigeria, on mycofloral occurrence on spoilt pawpaw fruit (*Carica papaya*) and its health implication. Pawpaw fruit samples were collected from different dealers in Ishiagu market and taken to microbiology laboratory where three (3) samples in three (3) replicates were subjected to microbial analysis. to determine microbial occurrence. Based on the result of the laboratory analysis, a total of six species of fungal microbes were isolated. The isolates include; *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus nigricans*, *Penicillium sclerotigeruim*, *Fusarium oxysporum* and *Phoma sorghnia*. Among the isolates, *Aspergillus niger* had the highest occurrence of 36.67%, followed by *Aspergillus flavus* 15.00% and *Rhizopus nigricans* 11.76% while *Penicillium sclerotigeruim* and *Phoma spp.* had the lowest occurrence of 3.33% respectively. The presence of these fungi revealed that adequate nutrients are available for microbial growth and proliferation in pawpaw fruit. The observed fungi isolates is an indication that pawpaw fruits sold in Ishiahgu are contaminated with mycoflorals which could be detrimental to human life if consumed without caution. Urgent call for enlightenment and measures to reduce this and protect the consumers is required.

INTRODUCTION

Pawpaw (*Carica papaya*) fruit is usually grown in subsistence level, plantation and in the wild and is harvested by local farmers in Nigeria using long sticks into a large open basket or fiber bags. The basket/fiber bags may be contaminated with spoilage fungi and are often piled on top of one another during transportation and retail, resulting to bruising and squeezing of fruit. Bruised fruit become colonized by propagules of the pathogens associated with the fruit surface and those in the fluid lacking from already rotten fruits. *Aspergillsiger*, *Aspergillus flavus*, *Rhizopus nigrican*, *Curvularia lunata*. *Rhizopus Oryzae*, *Fusarium equiseti* and *Fusarium moniliforme* have been reported to be responsible for postharvest losses in pawpaw in southern Nigeria (Gupta and Pathak, 2004).

Invasion by pathogens either through natural openings or wounds is considered the most critical factor in fruit rot (Effiuvwevwere *et al*, 2000)

Besides the losses in income to the pawpaw fruit market the rot on fungal spoilt pawpaw could also cause a health hazard to the consumers (Wang *et al*. 2012; Holtmeyer and Wallin, 2002).

Most microbes that infect plant tissues often produce secondary metabolite known to be hazardous to animals including human (Baiyewu *et al*, 2007). Mycotoxins are naturally occurring toxic chemical (often of aromatic structures), compounds which are capable of inducing mycotoxicoses (toxic syndromes especially cancer) in humans following ingestion or

inhalation. However, they differ in their degree and manner of toxicity (Effiuvwevwere, 2000). Some of these metabolic include the ergot alkaloid by *Claviscp species*, Zearalenoe and vomitoxin by *Fusarium species* and aflatoxines, which are a group of highly toxic mutagenic and carcinogenic polyketode compounds have been detected in pawpaw in south western Nigeria (Baiyewu *et al* 2007). The pawpaw fruit is a fleshy, juicy fruit usually green but turning yellow when ripe. The fruit consist largely of water, sugar, vitamins A and C, protein and ash. It is one of the most nutritious and cheapest fruit grown and consumed in Nigeria (Effiuvwevwere, 2000). The fruit can be freshly eaten or cooked. In the southern part of Nigeria, pawpaw fruit production has improved the diet of the local people, whose diet generally consisted of starch staples lacking essential vitamin and minerals. These pawpaw fruit were usually displayed on benches and in basket for prospective customers in the open markets until sold, thereby exposing them to further microbial infection (Chuwuka *et a*, 2010). The fruit is however affected by a wide array of micro-organisms causing its decay.

Due to high consumption of pawpaw fruit, adequate attention should be directed towards harvesting, preserving, and marketing process of pawpaw in eastern part of Nigeria .Its major constraint, is the easy and regular spoilage leading to rot thereby reducing the quality. This leads to serious implications, as most of these microorganisms

produce mycotoxins that affect human (Holtmeyer and Wallin, 2002). This research will help to examine and determine the occurrence and microbial load on diseased/rotten pawpaw fruit in Ishiagu and provide possible remedies to avoid further deterioration to meet up with the quality required. This will help avert the health implications that could occur when spoilt ones are consumed. Based on the above, the objective of the study was to determine the microbial occurrence of spoilt pawpaw fruit in Ishiagu as well as to ascertain the health implication of spoilt pawpaw based on the specific microflorals identified.

MATERIALS AND METHODS.

The research project was carried out in Ishiagu, Ivo L.G.A. of Ebonyi state, Nigeria. It involved assessment of mycofloral occurrence on spoilt pawpaw fruit, in the study area. The laboratory assessment was carried out in pathology laboratory of Federal College of Agriculture, Ishaigu, Ebonyi state.

Ebonyi state is located within the South Eastern zone of Nigeria with annual mean temperature of 27°C (minimum) and maximum of 32°C and an average relative humidity of 80%mm. with annual rainfall of 1600mm to 2000mm.

Sample Collection

The pawpaw fruit (*Carica papaya*) for this experiment was purchased from three dealers in Ishiagu market. The pawpaw fruits were bagged with a black polythene bag. All the samples collected were taken to the plant pathology laboratory where the analysis was carried out.

Sterilization Of Materials

The petri-dishes, pipettes, measuring cylinder etc that were used for the analysis were sterilized in Hot air oven at a temperature of 160°C and holding time of 2hours while the medium to be used was sterilized in an autoclave at 121°C which is equivalent to 15lb for a holding time of 15minute.

Microbial Determination

The method used in the microbial determination was blotter technique where sterilized petridishes for the work were underlined with sterile cotton wool and white paper and moist with sterile distill water as an alternative to filter paper and covered with petri-dish lids.

The process was carried out separately with three pawpaw fruits which were purchased from the three different dealers in the Ishiagu market. All was incubated at room temperature for seven days in three replicates.

Pre-Culture Preparation

Medium used was Sabroaud Dextrose agar which was prepared as follows:

Mycological peptone	-	10.0g
Dextrose	-	40.0g
Agar	-	15.0g
Distilled water	-	1000ml

Any implicate fungi organisms was separated in a monoculture standard (pure culture) using Sabroaud Dextrose Agar medium, which was sterilized and allowed to gel in sterilize petri-dishes for proper identification of organisms.

Determination of percentage occurrence of fungi isolates

This was done to determine the level of occurrence of different fungi that were isolated. The total number of each isolate in the three samples which was screened was noted and expressed in percentage. Frequency of occurrence was also determined.

Identification of the Fungi Isolates

The fungal isolates were identified on the basis of their cultural behavior. Microscopic examination was carried out using lactophenol blue solution. A drop of it was introduced on a clean grease free slide and a fragment of the test fungus removed and introduced into the stain. The fungus was spread properly on the slide with the aid of a sterile spreader. A cover slip was gently placed on the slide avoiding damage to the conidia and examine under the microscope. The fungi were identified based on conidia colour and shape, mycelia display and other known characteristics peculiar to the organism in question.

Data Analyses:

Simple frequency percentage and standard error calculation were used to analyze the data and establish result.

RESULTS AND DISCUSSION

The result of this research revealed series of fungal microbes on spoilt pawpaw fruits assessed, as an indication of pawpaw fruits been contaminated in the study area. It equally shows that the pawpaw sold in Ishiagu market are of low quality. Based on the laboratory analysis on this research, the result obtained revealed hosts of storage microbes of fungi origin. The organisms isolated from pawpaw (*Carica papaya*) are as follows:

Aspergillus niger, *Aspergillus flavus*, *Penicillium sclerotigenium*, *Rhizopus nigricans*, *Fusarium oxysporum*, *Phoma sorghina*.

The result presented in Table 1 showed the occurrence (%) of fungi microbes on fresh pawpaw sold in Ishiagu market. The result revealed that *Aspergillus niger* and *Aspergillus flavus* had the highest occurrence of 30% and 16.79% respectively, followed by *Fusarium oxysporum* 10%, while *Rhizopus spp* and *Phoma sorghina* had 6.67% respectively. *Penicillium sclerotigenium* had the least occurrence of 3.33%. This is in conformity with Rico *et al* (2007) which stated that pathogenic micro-organisms can enter pawpaw fruits through damaged surfaces, harvesting materials and improper handling of the produce.

Alam *et al*, (2013) also stated that it could be due to harvest contamination from materials and equipment. These facilities/equipments could harbour these microbes, thereby infesting the harvested pawpaw fruits.

Table 4.2 showed the occurrence (%) of fungi microbes on spoilt pawpaw fruits obtained from the market in Ishiagu.

However, this result shows that *Aspergillus niger* had the highest occurrence 36.76%, followed by *Aspergillus flavus* 15%, *Rhizopus nigricans* 11.76% and *Fusarium oxysporum* 8.33%. while *Penicillium sclerotigerum* and *Phoma sorghina* had the least occurrence of 3.33% each. This could be due to transporting equipment after harvest such as sack, baskets which serve as sources of microbes and as such transfer microbes directly to the fruits, as the fruit has high nutritional constituents. They serve as fertile ground for microbes especially fungi according to Egwu *et al*, (2002) and Chukwuka *et al* (2010) who reported isolation of *Aspergillus niger*, *Fusarium avenaceum*, *Rhizopus stolonifer* and yeast in pawpaw fruit in Nigeria.

Table 4.3 showed the accumulative mean of microbial isolates from the fresh and spoilt sample (pawpaw fruits) sold in Ishiagu market.

The result revealed that *Aspergillus niger* had the highest accumulative mean of 66.76 followed by *Aspergillus flavus* 31.76 while *Rhizopus spp* and *Fusarium oxysporum* had the same mean of 18.33%. This result is in conformity with the previous works of Baiyewu *et al* (2007) and Chukwuka *et al* (2010) which reported the isolation of *Aspergillus niger*, *A. flavus*, *Rhizopus spp* and yeast from pawpaw in Nigeria. And this could equally result if the fruits are not properly handled, packaged and stored, which could predisposed them to fungal contamination and deterioration, causing a serious health hazard for humans and animals that consumes any of the contaminated fruits.

Table 4 showed the mean fungi colony forming unit (CFU) count on fresh pawpaw fruits which ranged from 5.0×10^{-3} to 8.0×10^{-3} . While that of spoilt pawpaw fruits in Table 5 ranged from 5.3×10^{-3} to 7.7×10^{-3} . This could be as a result of differences in spoilage and pathogenic micro-organisms present among the samples. This supported the work of Gultie and Sahile, (2013) and Egwu (2012) which reported that handling and transporting equipment after harvesting can damage fresh pawpaw fruits, rendering their products susceptible to diseases. Harvesting equipment such as sack, basins, basket, etc, can serve as major sources of microbial growth. Table 6 showed the characteristics of fungi species isolated from the pawpaw fruits during the laboratory work on the samples. The observation there showed that both perfect and imperfect fungi were in occurrence on the pawpaw fruits. The *Aspergillus niger* which appeared brownish black on medium

was in contrast with *A. flavus* which appeared greenish, showing that they are not of the same species, though the same genus. The various fungal characteristics observed on that Table equally revealed that the fungal isolates in attendance were of diverse genera and species.

This could equally be due to the fruits coming in contact with the disease causing organism through different sources such as field infection or during harvesting. The fruit could come in contact with the soil which is an important factor that aids spoilage of stored fruits. Air is another factor responsible for spoilage of fruits during their post harvest period. Fungal spores are always in the air; these spores and other particulate ones constitute the air spores (Holtmeyer and Wallin, 2002).

These identified fungal organisms associated with fresh and spoilt pawpaw fruit in the study area which included *Aspergillus niger*, *A. flavus*, *Rhizopus spp* and *Phoma sorghina*, suggested that these fungal organisms could be responsible for the rot and its severity on the fruits. This supported the findings of Baiyewu *et al* (2007) and Chukwuka *et al* (2010) which reported isolation of *Aspergillus niger*, *A. flavus*, *Rhizopus spp* and yeast from pawpaw in Nigeria.

Market conditions that favour contamination can be worsened by poor hygiene of the vendors, using unsafe container, poor handling practice and poor environmental conditions such as unsafe marketing environment. The consequences of the problems could lead to increased loss of pawpaw fruits due to microbial spoilage (Egwu, 2012).

In the study, isolation of fungal organisms from the fresh and spoilt pawpaw fruits was not accidental. *Aspergillus niger* and *A. flavus* were more widespread among all the fresh and spoilt pawpaw fruits examined. Similar findings on the isolation of fungal pathogens on fruits stored and sold in the market have been reported by earlier researchers (Baiyewu, and Amusa, 1999). Chukwuka *et al*, (2010) implicated *Rhizopus nigricans*, *A. flavus*, *A. niger*, *Fusarium spp*, and *Mucor spp*. in pawpaw fruit spoilage from a farm in Oyo state of Nigeria. *A. niger* was the cause of post harvest spoilage in sweet orange and acid lime in the field.

The recommended storage temperature for pawpaw is 10°C . Handling with care to reduce bruising is advised. Storage of unripe pawpaw below temperatures of 10°C will result in chilling injury and increased susceptibility to disease incidence.

Ripe pawpaw will store successfully at lower temperature, but transport of ripe fruit (more than 50% yellow colour) is not recommended due to the susceptibility to mechanical damage and bruising.

Most of the fungal organisms isolated in this study play a pivotal role in the deterioration of food and feed system and some of them are also able to

produce toxic compounds for serious health hazards for human and animals.

The mycotoxins produced by these fungi can cause serious health hazards including being carcinogenic, immunotoxic, teratogenic, neurotoxins, nephrotoxic and hepatotoxic effects and kashin-beck disease (Magnet *et al*, 2011).

Mycotoxins cannot be destroyed by either heating or processing of the infected fruits. Some fungal isolates in pawpaw which includes, *Aspergillus spp* and *Fusarium spp* that are secondary metabolites can cause deleterious effects when consumed by man and animals.

Besides the loses in income to the pawpaw fruit markets, the fungi contaminated rotten pawpaw could also cause a health hazard to the consumers. Perhaps resulting to losses in marketable quality and hygiene of pawpaw fruits leading to major economic problem in Nigeria and the world at large.

CONCLUSION

The study has established that *Aspergillus niger*, *Aspergillus flavus*, *Penicillium sclerotigerum*, *Rhizopus nigricans*, *Fusarium oxysporum* and *Phoma spp*. are microbes associated with the spoilage of pawpaw fruits (*Carica papaya*), and can cause health hazards in human and animals. The species of fungi present on the pawpaw fruits, show the lack of sanitary conditions of the environment, utensils and personal surfaces and general management practices. Since microbial contamination of these fruits occurs

primarily at the surface due to poor post harvest handling, attempts should be made to use antimicrobial techniques either by sprays or dips to reduce the problem. This study is suggesting or rather insisting the adoption of good handling and storage practices to avoid microbial contamination of any fruit or fruit products.

RECOMMENDATIONS

The present and subsequent deterioration that leads to spoilage due to microbial invasion of fruits (pawpaw), if not checked could lead to a serious health hazards when such pawpaw fruit is consumed. Losses due to post harvest spoilage or pathological decay are as a result of either latent infections in the field that becomes active following harvest or of cross-contamination during harvesting, storage and distribution.

It is therefore recommended that prevention begin in the field (farm) by using appropriate harvesting materials and an integrated strategy of good agricultural practice.

To reduce the rate of contaminations which in turn leads to the spoilage of fruits and also to the ingestion of contaminated pawpaw by consumers, it is recommended that the farmer, handlers, sellers and consumers should be properly educated and sensitized on the need to improve their personal hygiene which is one of the factors that affect post harvest pawpaw fruits thereby introducing contaminants and spoilage.

Table 1: The occurrence (%) of fungi microbes on fresh pawpaw fruits sold in Ishiagu market, Ivo LGA Ebonyi State.

Fungi isolates	Pawpaw dealers (Samples)			mean
	1	2	3	
<i>Aspergillus niger</i>	20	30	40	30.00
<i>Aspergillus flavus</i>	30	-	20	16.76
<i>Penicillium sclerotigerium</i>	-	-	10	3.33
<i>Rhizopus nigricans</i>	-	20	-	6.76
<i>Fusarium oxysporum</i>	20	-	10	10.00
<i>Phoma sorghina</i>	-	20	-	6.76
MEAN	11.76	11.76	1.33	73.61
SD	13.292	13.292	15.055	9.801
SE±	56.472	56.472	6.147	0.408

Table 2: Occurrence (%) of fungi microbes on spoilt pawpaw fruits obtained in Ishiagu market, Ivo LGA, Ebonyi State.

Fungi isolates	Pawpaw dealers (samples)			mean
	1	2	3	
<i>Aspergillus niger</i>	35	40	35	36.76
<i>Aspergillus flavus</i>	20	10	15	15.00
<i>Penicillium sclerotigerium</i>	15	10	10	11.76
<i>Rhizopus nigricans</i>	-	10	-	33.33
<i>Fusarium oxysporum</i>	10	10	5	8.33
<i>Phoma sorghina</i>	-	-	10	3.33

MEAN	13.33	13.33	12.500	18.10
SD	13.292	13.292	12.144	12.481
SE±	5.427	5.427	4.959	5.096

Table 3: Cumulative mean (%) of microbial isolates from fresh and spoilt pawpaw fruits sold in Ishiagu market.

Fungi Isolates	Pawpaw dealers (%) (Samples)			mean
	1	2	3	
<i>Aspergillus niger</i>	27.50	35.00	37.50	33.38
<i>Aspergillus flavus</i>	25.00	5.00	17.50	15.38
<i>Penicillium sclerotigerum</i>	0	5.00	5.00	3.38
<i>Rhizopus nigricans</i>	7.50	15.00	5.00	9.17
<i>Fusarium oxysporum</i>	15.00	5.00	7.50	9.17
<i>Phoma sorghnia</i>	0	10.00	5.00	5.00
Mean	12.50	12.50	12.916	12.66
SD	12.042	11.726	12.982	11.034
SE + _	4.916	5.301	5.301	4.506

Table 4: Mean fungi colony forming unit (CFU) on the fresh pawpaw fruits.

Parameter	S1	S2	S3
Medium °C	SDA	SDA	SDA
Sample 1 (CFU)	6	9	4
Sample 2 (CFU)	8	7	6
Sample 3 (CFU)	4	8	5
Mean	6.0	8.0	5.0
Dilution factor	10 ⁻³	10 ⁻³	10 ⁻³
Volume of Inoculum (ml)	1.0	1.0	1.0
MFC (CFU/ML)	6.0 x 10 ⁻³	8.0 x 10 ⁻³	5.0 x 10 ⁻³
SD	3.125	3.188	2.927
SE +	1.276	1.302	1.195

Table 5: Mean of fungi colony forming unit (CFU) on the spoilt pawpaw fruit.

Parameter	S1	S2	S3
Medium °C	SDA	SDA	SDA
Sample 1 (CFU)	8	6	6
Sample 2 (CFU)	6	9	4
Sample 3 (CFU)	5	8	6
Mean	6.3	7.7	5.3
Dilution factor	10 ⁻³	10 ⁻³	10 ⁻³
Volume of Inoculum (ml)	1.0	1.0	1.0
MFC (CFU/ML)	6.0 x 10 ⁻³	7.7 x 10 ⁻³	5.3 x 10 ⁻³
SD	3.035	3.208	2.939
SE +	1.239	1.310	1.200

Table 6: Characteristics of fungi species isolated

Macroscopic/Microscopy Characteristics	Isolated
Colony profusely branched which appear brownish black on medium, septet and rhizoids and conidia vesicles bearing chains, stolen and rhizoids absent, microscopic spores are brownish-black and conidia in chain.	<i>Aspergillus niger</i>
Greenish mycelia growth on medium septet, perpendicular and branches to the top. Conidia vesicles bearing chains. Stolon and rhizoids absent, conidia radiates from the vesicles.	<i>Aspergillus flavus</i>
Known as blue moulds, in the initial stage of growth, they have a colony appearance but later when the spores or conidia are formed, their appearance becomes powdery and the colour becomes blue brown or pink according to age.	<i>Penicillium spp</i>
White mycelia growth and branched in appearance profusely. Non-septet, upright sporangiospores with dark pear-shaped sporangium and presence off globular sporangiospores.	<i>Rhizopus spp</i>
Appearance of culture in medium is whitish, cream reverse, burgundy and macroconidia. Hyphae nature of conidiophores is septet, sexual spores borne or monophialides.	<i>Fusarium spp</i>
Colony profusely branched which appear orange, purple on medium septet and absence of stolen and rhizoids while conidia vesicles bearing chains. Microscopic spores are small, spherical and black in colour.	<i>Phoma spp</i>

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