Fungi Associated with the Spoilage of Post-Harvest Pawpaw Fruits Sold in Ifite Awka, Anambra State

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Abstract: The fungi associated with the spoilage of pawpaw fruits in Ifite Awka, Anambra State were studied. Five spoilt and ten healthy fruits were used in the study with sabouraud dextrose broth and sabouraud dextrose agar as the growth media while the pour plate method was employed in their isolation. The fungal counts ranged between 3.8x10³ and 9.2x10³ Cfu/g. Sample 2 had the highest fungal count of 9.2x10³ Cfu/g while sample 3 had the least fungal count of 3.8x10³ Cfu/g. The fungi were isolated, and characterized on the basis of their cultural and microscopic features and identified as Penicillium sp, Candida sp, Aspergillus sp, Mucor sp and Fusarium sp. Penicillium sp and Aspergillus sp were isolated from all the samples examined while Candida sp had the least frequency of occurrence having been isolated from samples 2 and 3 only. The result of the pathogenicity test carried out on the healthy fruits showed that all the fungal species re-isolated from the healthy samples had the same cultural and microscopic features as the original fungal isolates, indicating that these isolates were responsible for the spoilage of the pawpaw fruits examined. These organisms may have gained entrance into the produce from the air, during transportation, processing in an unhygienic environment and through improper handling. The organisms are known to produce potent toxins which have deleterious effects on humans and animals, therefore care must be taken during their handling, transportation and processing of pawpaw fruits to minimize the entry and proliferation of these organisms in the produce, as well as the health risk their infestation pose to the consumers of the fruits.

Keywords: Fungi, Spoilage, Post-harvest, Pawpaw Fruits, Ifite Awka, Anambra State

INTRODUCTION

Pawpaw (Carcia papaya) is a popular fruit plant grown all over parts of the tropical and subtropical regions of the world. It is a fast growing but short-lived herbaceous plant with latex vessels in all its fruit. It has an upright, branched or unbranched stem covered all over with lead scars.

Pawpaw fruit is fleshy, juicy and usually green but becoming yellow when ripe. The edible portion is composed of mostly Water, Sugars, Vitamin A, Vitamin C, Proteins and Ash. In Nigeria, Pawpaw is one of the popular and cheapest fruits grown and consumed for its nutritional content.

There has been a great increase in the demand for pawpaw fruits over the
years and this may be due to their increased consumption patterns in the tropics. Pawpaw, part from being taken as food has some medical values. The latex from the trunk of the tree is applied externally to speed the healing of wounds, ulcers, boils and warts. The seed is also used to expel worms and the flower may be taken as an infusion to induce menstruation. Pawpaw contains high level of sugars and nutrients and its low pH values make it particularly desirable to fungal decay.

Spoilage in pawpaw can also be referred to as rot or decay. Spoilt pawpaw fruit is characterized by excess softening, mycelia growth, loss of moisture, unpleasant odour, shrinkage and total drying up of water in the fruit. The spoilage may be caused by microorganisms, insects, rodents, physical injury such as bruising and freezing as well as chemical breakdown of the fruit.

In Nigeria, pawpaw fruit is usually grown in the wild and harvested by local farmers using long sticks into large open baskets or fibre bags. The basket or fibre bags may be contaminated with spoilage fungi and are often piled on top of one another during transportation and retailing resulting to bruising and squeezing of the fruit. Bruised fruit readily becomes colonized by the propagules of the pathogens associated with the fruit surfaces and those in the fluid leaking from already rotten fruits. These fruits are usually displayed on benches and baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial contamination beside those associated with the fruit surface and those from other infected fruits.

Aspergillus Niger, Aspergillus flavus, Rhizopus nigricans, Curvularia lunata and Fusarium equiseti have been reported to be responsible for the post-harvest losses in pawpaw in southern Nigeria. Besides the losses in income to the pawpaw fruit markets, the fungi-spoilt fruits could also cause health hazards to the consumers due to their production of mycotoxins which are capable of inducing mycotoxicoses in man.

Generally, spoilage fungi can be toxigenic or pathogenic. Toxigenic fungi have been reported in pawpaw. Pathogenic fungi on the other hand could cause infections or allergies. Aspergillus species produce ochratoxins that are hazardous to humans and animals.

Though the consumption of pawpaw is increasing due to its nutritional value, their spoilage by fungi needs attention, therefore in this study, the fungi associated with the spoilage of post-harvest pawpaw fruits sold in Ifite Awka, Anambra State were isolated, characterized and identified.

MATERIALS AND METHODS

Sample Collection
Fifteen pawpaw fruits (five spoilt and ten healthy) were purchased from different locations in Ifite Awka, Anambra State and taken to the Microbiology Laboratory of Nnamdi Azikwe University, Awka in sterile polythene bags for the fungal isolation.
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Sample Processing
One gram of each of the spoilt pawpaw fruits was carefully cut with a sterile scalpel and enriched in a sterile sabouraud dextrose broth for twenty four hours. Tenfold serial dilution was prepared after the enrichment.

Fungal Isolation
Aliquots of the serially-diluted samples (10^4) were introduced into sterile culture plates containing chloramphenicol at a concentration of 0.05mg/ml to inhibit bacterial growth and sterile sabouraud dextrose agar (SDA) added. Incubation was carried out in an inverted position at room temperature for five days after which the colonies that developed were counted, subculture on sterile SDA plates and later stored on sterile SDA slants for identification.

Identification of the Fungal Isolates
The fungal isolates were identified on the basis of their cultural and microscopic features. 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 bubbles. The slide was thereafter mounted and examined under the microscope. The fungi were identified with the descriptions given by Oyeleke and Manga 14.

Pathogenicity Test
The method of Chukwuka et al. 15 was used. Five healthy pawpaw fruits were properly washed with tap water, rinsed with distilled water and surface-sterilized with ethanol (75%). A sterile 4mm corn borer was used to make holes in each of the fruits which were individually inoculated with each of the fungi. Petroleum jelly was used to seal the holes to prevent contamination. Five fruits wounded with the cork borer but not inoculated were used as controls. The inoculated fruits and controls were placed in clean polythene bags (One fruit per bag). Each of the fruits were moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at 280C for five days after which the fungi were re-isolated and compared with the original isolates.

RESULTS AND DISCUSSION
The fungal counts of the spoilt pawpaw fruits are shown in Table 1.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Fungal Counts (Cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.4x10^3</td>
</tr>
<tr>
<td>2.</td>
<td>9.2x10^3</td>
</tr>
<tr>
<td>3.</td>
<td>3.8x10^3</td>
</tr>
<tr>
<td>4.</td>
<td>7.7x10^3</td>
</tr>
</tbody>
</table>
They ranged between 3.8 and 9.2x10³ CFU/g. The cultural and microscopic features of the fungal isolates are presented in Table 2.

### Table 2. Cultural and Microscopic Features of the Fungal Isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Cultural Features</th>
<th>Microscopic Features</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colonies were green and velvety</td>
<td>Conidia were greenish and globose. Conidiophores were simple, long and erect. Hyphae were septate and branched. Spore heads were brush-like, with chains of Conidia</td>
<td>Penicillium sp</td>
</tr>
<tr>
<td>2</td>
<td>Colonies were creamy, smooth, convex and opaque with yeasty odour</td>
<td>Budding, spherical to elongated cells with pseudomycelia were seen</td>
<td>Candida sp</td>
</tr>
<tr>
<td>3</td>
<td>Colonies with black with white edges</td>
<td>Conidia heads were large, globose, dark-brown and biseriate. Conidia were globose and rough-walled. Conidiophores were smooth-walled</td>
<td>Aspergillus sp</td>
</tr>
<tr>
<td>4</td>
<td>Colonies were grey</td>
<td>Sporangia were black with zygospores. Sporangioles were long and branched. Hyphae were non-septate and branched</td>
<td>Mucor sp</td>
</tr>
<tr>
<td>5</td>
<td>Colonies were pink and cottony</td>
<td>Microconidia and macroconidia were seen. Microconidia were ovoid. Macroconidia were borne on phialides</td>
<td></td>
</tr>
</tbody>
</table>

They were identified as Penicillium sp, Candida sp, Aspergillus sp, Mucor sp and Fusarium sp. The occurrence of the fungi in the spoilt pawpaw fruits is presented in Table 3.

### Table 3. Occurrence of the Fungal Isolates in the Spoilt Pawpaw Fruits

<table>
<thead>
<tr>
<th>Samples</th>
<th>Penicillium sp</th>
<th>Candida sp</th>
<th>Aspergillus sp</th>
<th>Mucor sp</th>
<th>Fusarium sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = detected  
- = undetected

Penicillium sp and Aspergillus sp occurred in all the samples examined. The results of the pathogenicity test carried out on the healthy pawpaw fruits are shown in Table 4.
Table 4. Result of the Pathogenicity Test on the Healthy Pawpaw Fruits

<table>
<thead>
<tr>
<th>Samples</th>
<th>Penicillium sp</th>
<th>Candida sp</th>
<th>Aspergillus sp</th>
<th>Mucor sp</th>
<th>Fusarium sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = detected
- = undetected

The fungi had the same cultural and microscopic features as the ones inoculated into the healthy pawpaw fruits.

Duckwort 16 reported Penicillium italicum and Aspergillus flavus as the most common spoilage fungi involved in the blue mold and black mold diseases of pawpaw fruits respectively. Baiyewu and Amusa 4 implicated Aspergillus flavus and Penicillium italicum as pathogenic organisms of pawpaw fruits. Akinmusire 13 reported that Aspergillus flavus was responsible for sunken spots on pawpaw fruits. He also isolated Aspergillus flavus, Candida tropicalis and Mucor sp from pawpaw fruits in Maiduguri, Northern Nigeria, with Aspergillus flavus having the highest percentage occurrence. Similar report was also made by Effiuvwevwere 10.

Chukwuka et al. 15 reported that Penicillium sp, Aspergillus sp and Fusarium sp were responsible for pawpaw fruit decay in a farm in Oyo State, South Western Nigeria. Awoite et al. 17 isolated Penicillium sp, Candida sp, Aspergillus sp, Mucor sp and Fusarium sp from pawpaw during their post-harvest period. Sankat and Maharaj 18 implicated fungi as contaminants of pawpaw fruits since the fruits are high in moisture and nutrients. Gupta and Pathak 9 also isolated Aspergillus sp and Fusarium sp from post-harvest pawpaw fruits in Southern Nigeria.

Aspergillus sp and Penicillium sp occurred in all samples examined while Candida sp occurred least frequently. The result is at variance with the report of Chukwuka et al. 15 who reported that Rhizopus nigricans was the most predominant fungi encountered in pawpaw fruits while Aspergillus sp was the least fungus encountered.

The pathogenicity test result showed that the fungal species re-isolated from the healthy pawpaw fruits had the same cultural and microscopic features as the fungal species isolated from the spoilt fruits, indicating that the organisms were responsible for the spoilage of the fruits.

The presence of these fungi in the pawpaw fruits is a public health concern because of the health risk they pose to the consuming public. The organisms are the source of highly potent mycotoxins which are known to have consequences for man and animals. The presence of these organisms may be as a result of improper handling and processing, use of contaminated water for washing, cross contamination from other fruits, the use of dirty processing knives and trays, damage on the fruits and processing in an unhygienic environment. The spoilage
of the fruits by these organisms may be influenced by the moisture content, PH value, temperature and nutrient composition of the produce, thus the low PH and moisture content of pawpaw fruits make them susceptible to fungal spoilage.

These organisms colonize the pawpaw fruits after they degrade certain specific cell wall polymers such as the Protopectin which is the cementing substance of the produce. The organisms are known to be pathogenic and produce diseases of man such as Aspergillosis, which may be fatal if untreated. The control of spoilage of pawpaw fruits is therefore imperative. Care must be taken during the harvesting, cleaning, sorting, packaging, transport and storage of the fruits to reduce the incidence of these fungi on the fruits as well as the health risk posed to the consuming public.

CONCLUSION
The high occurrence of fungi in the pawpaw fruits examined demands that appropriate control measures should be employed during its handling to minimize loss due to spoilage and unacceptability. Adequate mycological knowledge of the produce is crucial. In addition, an appropriate sanitation programme must be put in place to prevent the entry and proliferation of these organisms, thereby drastically minimizing the health risk posed to the consumers. Routine inspection of post-harvest pawpaw fruits by food regulatory agencies is recommended.

REFERENCES