

Full Length Research Paper

Survey of the post harvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya* L) in South Western Nigeria

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Survey and collection of the marketed pawpaw fruit with rot symptoms were conducted in the South Western Nigeria in 2000 and 2001 respectively. Pawpaw fruits showing rot symptoms that are displayed for sale in three different market places in three major cities in south western Nigeria namely, Ibadan, Abeokuta and Akure were collected and examined for the presence of the inducing pathogens and for aflatoxin contamination. The most commonly fungi found in rotten pawpaw fruits were: *Rhizopus nigricans*, *Curvularia lunata*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme*, *Colletotrichum capsici* and *Trichoderma viride*. *Rhizopus nigricans*, *F. moniliforme* *A. flavus* and *A. niger* had the highest rate of occurrence among the isolated fungi while *C. lunata* was the least encountered. Pathogenicity tests revealed that of all the isolated fungi, *R. nigricans*, *C. lunata* and *F. moniliforme* were highly pathogenic with the first two leading to rapid disintegration of treated fruits in 3-5 days. *A. niger* was moderately pathogenic, while *A. flavus* *T. viride* caused the least amount of rot on pawpaw fruits. Aflatoxins were detected from infected pawpaw fruits, both before and after autoclaving fruit for 15 min at 121°C.

Key words: *Carica papaya*, aflatoxins, post-harvest diseases, survey.

INTRODUCTION

Pawpaw (*Carica papaya* L.) is a popular fruit plant grown all over wetter parts of West Africa, tropical and sub-tropical regions of the world usually as compound fruit crop or semi-wild from discarded seeds (Kuthe and Spoerhase, 1974). Pawpaw fruits are also produced in many countries on a small scale for local consumption only.

Pawpaw 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 leaf scars (Agnew, 1968). 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 (Baiyewu, 1994). It is one of the most nutritious and cheapest fruits grown and consumed in Nigeria (Baiyewu, 1994). The fruit can be freshly eaten or cooked. It can also be used in the preparation of jellies, juice and jams. It has a pleasant sweet taste and flavour and has a great application in the preparation of fruit salad and deserts. Pawpaw has a mild laxative action and the seeds are used medicinally against worms and ulcer (Baiyewu, 1994). 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 (Baiyewu 1994).

There has been a great increase in the demand for pawpaw fruits over the years and this may be due to their

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increased consumption pattern in the tropic (Adisa, 1983). Pawpaw fruit is usually grown in the wild and is harvested by local farmers using long sticks into a large open baskets or fibre bags (Adisa, 1983). The baskets / fibre bags may be contaminated with spoilage fungi and are often piled on top of one another during transportation, resulting to bruising and squeezing of fruit. Bruised fruits readily become colonized by propagules of the pathogens associated with the fruit surfaces and those in the fluids leaking from already rotten fruit (Baiyewu, 1994). These pawpaw fruits were usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infection (Baiyewu and Amusa, 1999) beside those associated with the fruit surface and those from adjacent infected fruits (Baiyewu, 1994).

A visit to most of these markets revealed that between 15 and 40 % of fruit displayed for sale showed symptoms microbial infection. These fungal infected pawpaw fruit were sold at lower prices and were usually preferred by the low-income earners. Gupta and Pathak (1986) had earlier reported that *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus nigricans*, *Curvularia lunata*, *Rhizopus oryzae*, *Fusarium equiseti* and *Fusarium moniliforme* were responsible for post harvest losses in pawpaw in South Western Nigeria. Besides the losses in income to the pawpaw fruit marketers, the rotten fruits could also cause a health hazard to consumers. Krogh (1992) has earlier reported that most microbes infecting plant tissues often produced secondary metabolites in their hosts, which are known to be hazardous to animals including man. Some of these metabolites include the ergot alkaloids on cereals by *Clavisep* sp, fumonisin on maize by *Fusarium* sp, aflatoxins and ochratoxins on several plants produced by *Aspergillus* sp. (Prasad, 1992). Aflatoxins, which are a group of highly toxic, mutagenic and carcinogenic polyketide compounds, were first reported in groundnut feed that poisoned thousands of poultry and pigs (Goldblatt and Stoloff, 1983).

This research was embarked upon to survey the post harvest deterioration and aflatoxin contamination of pawpaw fruit sold in popular markets in South Western Nigeria.

MATERIALS AND METHODS

Fruit source and isolation of fungi

Pawpaw fruits both fresh and those found with symptoms of fungal infection were purchased from three different markets located in three different states; Oyo, Ogun and Ondo States of Nigeria. These states are located in low humid part of South Western Nigeria. The vegetation is typically tropic. The climate is characterized by dry November to April and wet May to October seasons. The mean annual rainfall of 1150 - 1500 mm occurs mainly between April and October with the major peak in June. Higher relative humidity (rH) values (rH 80 - 95%) are recorded during the rainy season than the dry season (rH 20 - 50%). A total

of 90 randomly selected fungal infected fruits and 90 unblemished, healthy and clean looking fruits were purchased (10 each from each market). Fruits were surface sterilized by exposing them in 1 min 90% ethyl alcohol (BDH chemicals Ltd Poole England) and then 3 min to 1% sodium hypochlorite and then rinsed three times in sterile distilled water. Segments (3 - 5 cm) of tissues from the margins of the rotted areas were cut out with a sterile scalpel and placed on previously prepared potato dextrose agar (PDA, Difco) in Petri dishes and incubated at $28 \pm 1^\circ\text{C}$ for 5 days under 12 h photoperiod. The fungal colonies that appeared were primarily identified using cultural and morphological features (Barnett and Hunter, 1972). This experiment was conducted in year 2000 and 2001 respectively.

Pathogenicity of isolated fungi

Thirty (30) healthy pawpaw fruits collected from the each study sites were surfaced sterilized in ethyl alcohol as described above. Fruits were wounded with a sterile 5 mm cork borer, and inoculated with mycelia disc (3 mm in diameter) of the fungal test isolate. The inoculated wound was sealed with Vaseline petroleum jelly. The inoculation was done in a laminar flow chamber. Five pawpaw fruits were inoculated each with each of the isolates and this experiment was replicated three times. Controls consisted of five fruits wounded with the sterilized cork borer but not inoculated. The inoculated fruits and the controls were placed in clean polyethylene bag (one fruit per bag) each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at $30 \pm 1^\circ\text{C}$ for 5 days. After 72 h, the inoculated fruits were observed for symptom development. The causal agents were re-isolated from the infected pawpaw fruit and compared with the original isolates.

Aflatoxin detection using thin layer chromatography

Ten grammes (10 g) each of the rotten sample pawpaw fruits obtained from each of the markets, as well as from clean (non infected fruits) inoculated with the fungal isolates respectively were extracted with chloroform (May and Baker (Ltd) England) and concentrated (Muhammad et al., 2004). Of the extracted samples, 5, 10 and 15 μL were spotted on three different points on a ruled base line of the thin layer chromatography (TLC) coated with plates silica gel (Merck[®] TLC grade7749) (Muhammad et al., 2004). Also 5, 10 and 15 μL of the aflatoxin standard were spotted on another three points near the previous sample extract spotted points (Muhammad et al., 2004). These were then developed in TLC tanks containing the solvents (toluene, isoamylalcohol and methanol) at a ratio of 3: 3: 2. When the solvent emigrated to about 2/3 of the plates, the plates was removed, air dried and examined under UV light at a distance of 365 mm (Fennell et al., 1973). The aflatoxin levels were semi-quantified based on comparisons with control levels, while the presence of aflatoxin in pawpaw samples from various sampling centers were determined. The data obtained were subjected to statistical analysis and the means were separated using Duncan's multiple range test (DMRT).

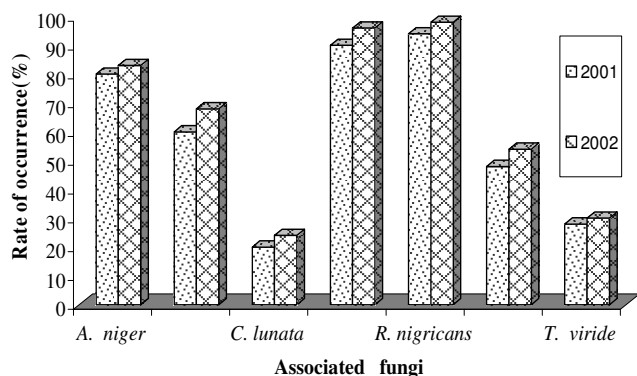
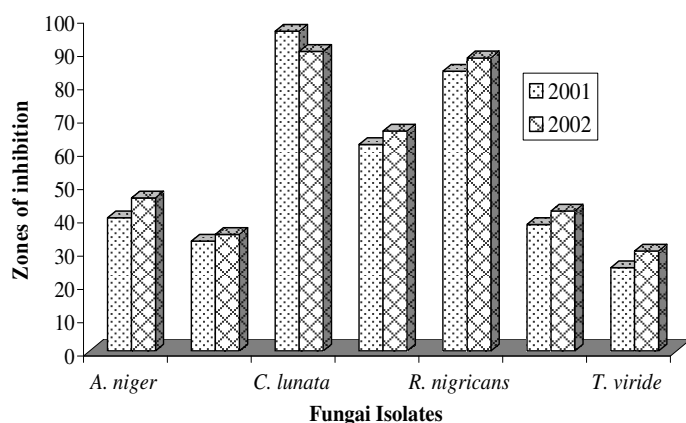
RESULTS AND DISCUSSION

Results of this study showed that *R. nigricans*, *C.lunata*, *A. flavus*, *A. niger*, *Fusarium moniliforme*, *Colletotrichum capsici* and *Trichoderma viride* were found with marketed pawpaw fruits in Southwestern Nigeria (Figure 1). The incidence of occurrence of these pathogens in the three markets in each of the States was not significantly differ-

Table 1. Detection of aflatoxin in rotten pawpaw fruit obtained from the market places.

Markets	2000		2001	
	Rotten pawpaw fruits	Fresh pawpaw fruits	Rotten pawpaw fruits	Fresh pawpaw fruits
Oje market	+	-	+	-
Fiditi	-	-	-	-
Oja'ba	+	-	+	-
Adatan market	+	-	+	-
Ibara market	+	-	+	-
Omidia market	+	-	+	-
Oba Adesida market	-	-	-	-
Morkin market	+	-	+	-
Araromi market	+	-	+	-

Note: + =Aflatoxin positive
- =Aflatoxin negative

**Figure 1.** Rate of occurrence of fungi associated with marketed pawpaw fruit in south western Nigeria.**Figure 2.** Pathogenicity of fungal Isolates from rotten pawpaw fruit

ent from each order. In the humid forest of South Western Nigeria, the high rainfall pattern, high humidity and the temperature of between 19 and 31°C prevailing in the

agro ecology favors the development of fungal diseases both in the field and the market. The isolation of these pathogens confirmed the studies of Gupta and Pathak (1986) and Kuthe and Spoerhase (1974) that *R. nigricans*, *A. niger*, *C. lunata*, *A. flavus*, *F. moniliforme*, and *C. capsici* found associated with rotten pawpaw are highly pathogenic causing appreciable losses in pawpaw fruits at post harvest. Baiyewu, (1994) also isolated *Fusarium* spp., *A. flavus*, *Rhizopus* spp., *Botryodiplodia* and *Cuvularia* spp. from pawpaw fruit. In our studies, the pathogenicity test revealed that all isolated fungi *R. nigricans*, *F. moniliforme*, *C. capsici* and *C. lunata* proved highly pathogenic causing a rapid disintegration of inoculated fruits in three to five days. *A. niger* was moderately pathogenic while the least pathogenic was *T. viride* and *A. flavus* (Figure 2). However, from the result of this study, *A. flavus* and *T. viride* are not likely to be pathogens of pawpaw fruit but rather contaminants. Hence necessary precaution in preventing contamination of this produce by these bacteria will enhance the microbial quality of the produce.

The TLC spot extracted from most of the infected pawpaw fruit, *A. flavus* inoculated fruits and the standard aflatoxin fluoresces produced bluish spots of equal intensities. Fungi have been discovered to produce secondary metabolites in plants tissues potentially harmful to humans and animals (Eaton and Groopman, 1994). Aflatoxin has been associated in cancer of the liver (hepatoma) and also with acute hepatitis in humans, especially in the developing world (Eaton and Groopman, 1994; Krogh, 1992; Prasad, 1992; Funnell et al., 1973). Aflatoxin was detected in 80% of the sampled pawpaw fruits (Table 1).

The detection of aflatoxins in the market pawpaw fruits and the high incidence of *A. flavus* isolated from infected fruit (Figure 1) strongly suggest that *A. flavus* might be the main producer of these toxins. Other fungal isolates found associated with these pawpaw fruits have never

been reportedly associated with aflatoxin production. *A. flavus* has been reportedly isolated from pawpaw fruits in Nigeria (Baiyewu, 2001). Aflatoxins have been reportedly detected in grapes, tomatoes and oranges in France (Sage et al., 2002; Muhammad et al., 2004). Singh (1983) reported that out of 342 samples of different fruits and spices obtained from the stores of commercial centers screened for aflatoxin, 95 of them were positive.

Post harvest handling and transport of pawpaw fruit is inadequate. Therefore most of the pawpaw fruits harvested do not usually get to the major cities in time due to the nature of transport systems existing in the rural areas. While fruit with bruises are not isolated from the unbruised ones and thereby causing cross-infections, consumers are supplied mostly with partly rotten fruits. Low-income earners who cannot afford the price of the non-infected or clean pawpaws mostly consume the partly rotten fruits. The fact that most people have not been diagnosed as having hepatoma or aflatoxicosis does not mean that the toxic metabolite does not exist in their body system (Muhammad et al., 2004). To date, no tests have been conducted if aflatoxins are in the urine and blood to determine the presence and risk of such metabolites in most working class people in this region. Aflatoxin M1, for example, has been reportedly detected in the urine the Philippine women that consumed peanut butter containing aflatoxin (Sage et al., 2002). It is therefore important that both the farmer who harvests the fruits into bags for transportation, the marketers and consumers take necessary precaution in preventing contamination and eating of contaminated fruits thereby reducing the risk of aflatoxin and other mycotoxins that are deleterious to human health.

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