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Microbial Ecology of Organisms Causing Pawpaw (*Carica papaya* L.) Fruit Decay in Oyo State, Nigeria

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Abstract: This study isolated, identified and characterized the microorganisms associated with the pawpaw fruit decay and examined the influence of certain growth parameters or environmental factors on the growth and development of microbes. Of all the samples studied (ripe and unripe pawpaw fruits), five species of fungi were found to be associated with the fruit decay. The most common fungi found were *Aspergillus flavus*, *A. niger*, *Fusarium sp*., *Mucor sp*. and *Rhizopus nigricans*. These fungi species were found in varying degrees. *R. nigricans* was the most predominant, followed by *Mucor sp.* and *Fusarium sp*. while *Aspergillus* species was the least encountered. *R. nigricans* was the only organisms found to be associated with the unripe fruits. The effect of temperature on growth and development of the organisms showed that mycelial growth and spatial distribution was high between 30 - 35°C. Water content value of 25-40%/g of the samples caused rapid microbial growth and subsequent maceration and liquefaction of the fruits. The effect of the pH showed that fungi growth is enhanced predominantly in acidic environment. Their optimum growth was recorded at pH 4.5-6.0. Pathogenicity tests revealed that of all the isolated fungi, *R. nigricans* was highly pathogenic leading to rapid disintegration of treated fruits in 3-5 days. This was followed by *Mucor* sp. and *Fusarium* sp. while *Aspergillus species* were least pathogenic and caused the least amount of rot on pawpaw fruits.This study showed that pawpaw fruit decay and microbial growth depends on various factors such as temperature, pH and moisture. Since pawpaw fruits were usually infected by pathogenic organisms, to be effective, production, preparation and preservation of food such as fruit salads made with pawpaw must be carried out as rapidly and hygienically as possible using good quality equipment, produce and materials.

Key words: Carica papaya · *A. flavus* · *A. niger* · *Fusarium sp* · Moisture · *Mucor sp* · Pawpaw · pH · Post-harvest diseases · *R. nigricans* · Temperature

INTRODUCTION

The *Carica papaya* (pawpaw) is a member of the small family "Caricaceae" allied to the "Passifloraceae". This family comprises of Jamilla, Jacarata, Cyclicmorpha and Carica genera [1]. The only genus having species cultivated for their fruits is Carica. Until recently, the Caricaceae was thought to comprise 31 species in three genera (namely Carica, Jacaratia and Jarilla) from tropical America and one genus, Cylicomorpha, from equatorial Africa [2]. However, a recent taxonomic revision proposed that some species formerly assigned to Carica were more appropriately classified in the genus Vasconcella [3]. Accordingly, the family's classification has been revised to comprise Cylicomorpha and five South and Central American genera (Carica, Jacaratia, Jarilla, Horovitzia and Vasconcella), with *Carica papaya* the only species within the genus Carica [3].

Although opinions differ on the origin of *C. papaya* in tropical America [4], it is likely that *C. papaya* originates from the lowlands of eastern Central America, from Mexico to Panama [2]. Its seeds were distributed to the Carribean and south-east Asia during Spanish exploration in the 16th Century, from where it spread rapidly to India, the Pacific and Africa [5]. Carica papaya is now grown in all tropical countries and many sub-tropical regions of the world. It is one of the tropical plants grown all over West Africa mainly for local consumption. *Carica papaya* is a soft-wooded perennial plant that lives for about 5-10 years, although commercial

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plantations are usually replanted sooner [6]. Fruit are ready to harvest five to six months after flowering, which occurs five to eight months after seed germination [6]. The fruits range in size from 7- 30 cm long and vary in mass from about 250 to 3000g [7].

It is an interesting tree in that the male and female parts exist in different trees. Pawpaw fruits, apart from being taken as food also have some medicinal importance. The fruits, leaves, seeds and latex are used medicinally [8]. Its main medicinal use is as a digestive agent; it is prescribed for people who have difficulty digesting protein and is used to break up blood clots after surgery, which is due to the presence of enzyme papain in the plant's latex. The latex from the trunk of the tree is also applied externally to speed the healing of wounds, ulcers, boils and warts. The seed is used to expel worm and the flower may be taken in an infusion to induce menstruation [9-10]. It has also been that reported that annonaceous acetogenins derived from the extracts of the twigs of the pawpaw tree may be good chemotherapeutic agents for cancer as these compounds inhibit enzymes necessary for metabolic processes in tumour cells [10, 11-16]. Increasing interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decisions about their use [10, 17].

Economically, *Carica papaya* is the most important species within the Caricaceae, being cultivated widely for consumption as a fresh fruit and for use in drinks, jams, candies and as dried and crystallised fruit [5]. Green fruit and the leaves and flowers may also be used as a cooked vegetable [18]. Nutritionally, papaya is a good source of calcium and an excellent source of vitamins A and C [2]. Papaya also has several industrial uses. Biochemically, its leaves and fruit are complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications [19]. Of these, however, papain, is a particularly important proteolytic enzyme that is produced in the milky latex of green, unripe papaya fruits (note that ripe papaya fruit contain no latex or papain). The latex is harvested by scarifying the green skin to induce latex flow, which is allowed to dry before collection for processing [2]. Evolutionarily, papain may be associated with protection from frugivorous predators and herbivores [19]. Commercially, however, papain has varied industrial uses in the beverage, food and pharmaceutical industries including in the production of chewing gums, in chill-proofing beer, tenderising meat, drug preparations for various digestive ailments and the treatment of gangrenous wounds. Papain has also been used in the textiles industry, for degumming silk and for softening wool [5] and in the cosmetics industry, in soaps and shampoo.

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 [20]. It is one of the most nutritious and cheapest fruits grown and consumed in Nigeria [20]. The fruit can be freshly eaten or cooked [20]. 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 [20]. 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 [21], beside those associated with the fruit surface and those from adjacent infected fruits [20, 22].

The fruit is however affected by a wide array of microorganisms causing its decay. These microorganisms, under the influence of environmental factors, pose a serious threat to pawpaw fruits production. Although available literatures revealed that the importance of pawpaw fruit is increasing daily, the incidence of microbial attack on this fruit demands attention. *Nysius vinitor* is presumed to vector a seed-rotting disease caused by a yeast with distinctive ascospores closely resembling those of *Nematospora sinecauda* [23], but this association has not been tested [24]. Although, *N. vinitor* has been ruled out as a vector of phytoplasmas that cause economically significant diseases of pawpaw fruit [24]. White *et al.* [25] suggest it should be considered in future studies in the vector of a pawpaw dieback, mosaic and yellow crinkle viruses. The utilization of pre-harvest management practices is important to reduce direct losses and to increase efficacy of post-harvest quarantine treatments. Since the discovery of the melon fly in Hawaii, a number of methods have been employed in attempts to reduce or prevent damage by this pest. They include mechanical control, cultural control, biological control and chemical control.

Researches have shown that microbial activities are greatly influenced by a number of parameters at different rates. This however does not mean the ability of an organism to survive or endure a given low or extreme conditions of environmental factors, but its ability to carry out its complete life cycle. The environmental factors affecting the life of microorganism as observed by various workers include temperature, hydrogen ion concentration (pH), oxygen, moisture content etc. Considering the importance of pawpaw, the need to boost its production and increase its production areas arises. This could be done, among other things, by preventing or

controlling the attack of microbes on pawpaw via studying the ecology of these microbes. Therefore, this study was carried out to isolate, identify and characterize the microorganisms associated with the fruit decay and to examine the influence of certain growth parameters or environmental factors on the growth and development of microbes associated with pawpaw fruit decay.

MATERIALS AND METHODS

Study Area: Pawpaw fruits were obtained from a farm at Abaso village near Olorunda-Abaa in Lagelu local government area of Oyo State, Nigeria. The reason for being selective in the area of collection of samples is due predominantly to the abundance of pawpaw trees in the area. This however afforded us the opportunity of selecting the fruits that has not been affected by microbes.

Specimen Collection and Preparation: Careful harvesting of pawpaw fruits was made. This prevented the pre-exposure of the fruits to the attacks of microbes and other decaying agents since pawpaw fruits could bruise easily. Six pawpaw fruits were collected from each farm; 3 ripe and 3 unripe pawpaws. These fruits were immediately taken to the laboratory for investigation of microbial infection and physiochemical analysis.

Physiochemical Analysis: The following parameters were determined: sample weights, temperature, water content and pH. The weights of the samples were determined using weighing balance. The experiment was set up using large desiccators for each of the samples. Temperature was determined using digitron thermometer (model 275-K) as described by the methods of FAO [26] and standardized mercury in glass centigrade thermometer as described by Ademoroti [27] and the readings were taken three times (morning, afternoon and evening) daily and the average was taken as the temperature reading of such fruit. The water in samples was analyzed for physiochemical and bacteriological quality and the chemical characteristics were determined by the methods of FAO [26] and Ademoroti [27]. The pH values of the pawpaw fruit samples were determined by a combined glass calomel electrode and a pH meter (Jenway, UK) as also contained in Ademoroti [27]. The pH meter was standardized with two different buffer solutions of different pH values (4 and 7) immediately before sample measurement. The electrodes were thoroughly rinsed with buffer solutions between samples and after all the measurements. The water content of the pawpaw fruits was also determined as described by Ademoroti [27].

Microbiological Analysis: Nutrient agar and potato dextrose agar (PDA) were used for the isolation and enumeration of microorganisms associated with decay of pawpaw fruits. All media were prepared, mixed thoroughly and sterilized by autoclaving at 121°C for 15 min according to the manufacturer's specification. The fruits were washed free of soil in sterile distilled water. By means of a sterile scalpel and needle, slices of tissue from advancing region of decay were cut, dipped in 70% alcohol for 1 min and washed with distilled water three times. 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 ± 1°C for 5 days under 12 h photoperiod. The fungal colonies that appeared were primarily identified using cultural and morphological features [28]. The isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt et al. [29] and Oyeleke and Manga [30].

Pathogenicity Analysis of the Isolated Fungi: Uninfected pawpaw fruits were washed with sterile water and their surfaces sterilized with 70% alcohol and then allowed to dry. 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. Six pawpaw fruits were inoculated each with each of the isolates and this experiment was replicated three times. Controls consisted of six 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 ± 1°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.

RESULTS

This study shows different microorganisms associated with the fruit decay and the influence of certain growth parameters or environmental factors on the growth and development of microbes associated with pawpaw fruit decay. Figure 1 shows the temperature (°C) of the pawpaw fruits samples used in this study. The minimum and maximum temperatures recorded from the various samples of pawpaw fruits analyzed were 30°C and 35°C respectively (Figure 1).

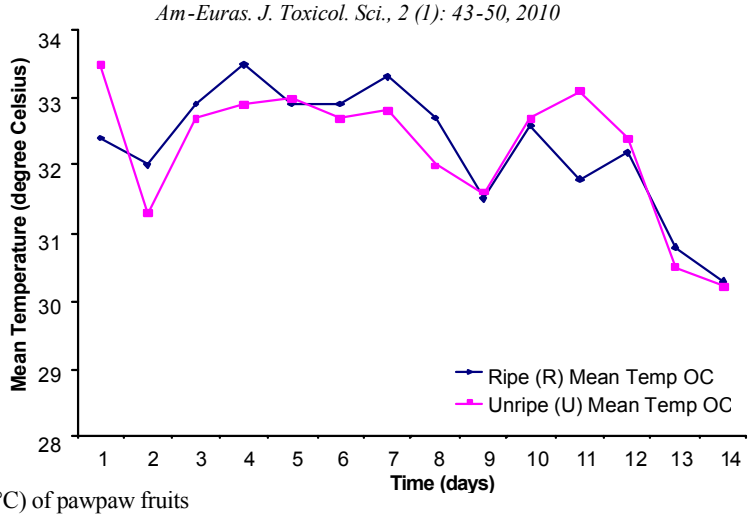

Fig. 1: Temperature (°C) of pawpaw fruits

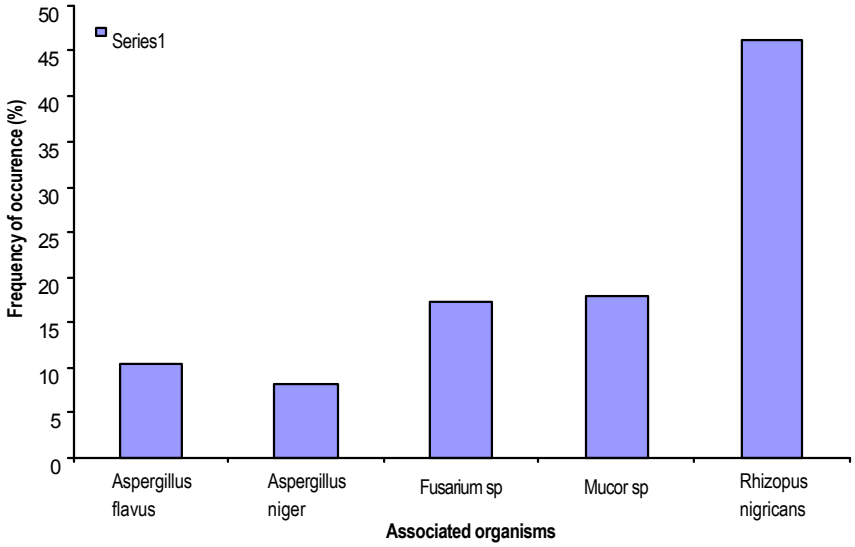

Fig. 2: Frequency of occurrence of fungi associated with pawpaw fruit in Oyo State, south western Nigeria

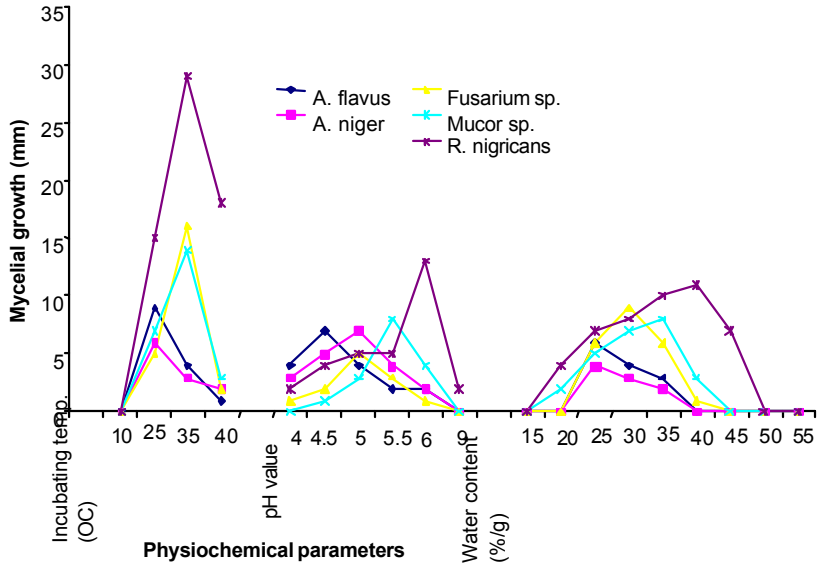

Fig. 3: Pathogenicity of fungal Isolates from pawpaw fruit and the effect of physiochemical parameters on the microbial load (number of colony)

In this study, five organisms were found to be associated with pawpaw fruits spoilage. Figure 2 shows the frequency of occurrence of fungi associated with pawpaw fruit in Oyo State, south western Nigeria. Of these organisms, *R. nigricans* was the most predominant. This was followed by *Mucor* sp. and *Fusarium* sp. *A. flavus* and *A. niger* was less predominant as shown in Figure 2.

Figure 3 shows the pathogenicity of fungal Isolates from pawpaw fruit and the effect of physiochemical parameters on the microbial load (number of colony). The growth and development in terms of numbers of colony produced by each organism at various temperatures was monitored for a period of two weeks (Figure 3). The growth rate increased with temperature up to the maximum, which is varying from species during which growth rate start decreasing. All the associated organisms performed well between the temperatures above 34°C. The only exception was *Rhizopus nigricans* which flourished up to 35°C. This led to further incubation at varying temperatures (Figure 3) which shows that its growth started to decline at temperatures above 35°C and below 40°C. However, no growth was observed in any of the species at 10°C.

The pH values of the ripe and uninfected pawpaw fruits were found as 7.5. As these fruits become infected and populated with microbes, the pH started decreasing, tending towards acidity. The microbial growth was recorded at pH values ranging between pH 4.0 and 6.0. *R. nigricans* and *Mucor sp*. grows well at pH 5.5 – pH 6.0 while *Fusarium* sp. and *A. niger* show rapid growth at pH 5.0 - pH 5.5. *A. flavus*, however tends to be more acidophilic as rapid growth was recorded at pH 4.5 – pH 5.0. However, *R. nigricans* was found to be more adapted than any other species by growing even at pH 9.0; although its growth at this pH value was severely retarded. The highest microbial load was recorded at pH 5.0 (Figure 3).

The water content of the various samples of pawpaw fruits used was determined right from the time they were fresh to the point of decay. The highest number of microbial load was observed at water content value of 30.0% per gram of the samples. *R. nigricans* showed rapid growth at water content value of 35.0% and 40.0% followed by *Mucor* sp. which thrives well at water content value of 30.0% and 35.0%. *Fusarium* sp., *A. niger* and *A. flavus* follow in that order having highest value at 30.0%, 25.0% and 25.0% respectively. However, none of the organisms was found growing at water content value 15.0% and 55.0% per gram of the samples respectively (Figure 3).

DISCUSSION

The findings of this study showed that *R. nigricans*, *A. flavus, A. niger, Fusarium sp.* and *Mucor sp.* were found with pawpaw fruits from a farm in Oyo State, Southwestern Nigeria. The incidence of occurrence of these pathogens in these farms in the State was not significantly different from each order. All the five organisms isolated were confirmed to be pathogenic on the pawpaw fruits but in varying degrees. *R. nigricans* was the most pathogenic, followed by *Mucor* sp. and *Fusarium* sp. in this order whereas *A. flavus* and *A. niger* were less pathogenic. When inoculated into healthy susceptible pawpaw fruits, the characteristic symptoms originally observed was also noticed again. All the five organisms were successfully taking part in the decay and are thus confirmed as the causal organism of pawpaw fruit decay. These pathogens have been reportedly isolated from pawpaw fruits in Nigeria [22].

Gupta and Pathak [31] had earlier reported that *A. niger, A. flavus, R. nigrican, C. lunata*, *R. oryzae, F. equiseti* and *F. moniliforme* were responsible for post harvest losses in pawpaw in South Western Nigeria. In consonant with Oyeniyi [32] who identified microflora isolated from rhizosphere of pawpaw tree as bacteria and fungi (*A. niger*). Besides the losses in income to the pawpaw fruit marketers, the rotten fruits could also cause a health hazard to consumers. Krogh [33] 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. Fungi associated with pawpaw roots were studied by Oluma [1] while Oyeniyi [32] carried out a survey of microflora in the rhizosphere of pawpaw. It is however note-worthy to intensify efforts in combating the production constraints associated with pawpaw fruits spoilage caused by microbes. Researches have shown that the recent disruption of the global food supplies is predominantly due to post-harvest losses associated with microbes.

This study has also shown that fruit decay and microbial growth depends on various factors such as temperature, pH and water content. The optimum temperature range for the growth of these microbes was 30.0° to 35.0°C. This however agreed with the work of Domsch and Gams [34] who reported optimum temperature for growth of *Fusarium* sp. as 31.0°C. The temperature for the ripe and unripe fruits was almost always the same; and yet the microbial growth was restricted to the unripe fruits but flourished in the ripe ones. It can therefore be said that temperature alone does not bring about microbial growth; other

requirements have to be met. It also connotes that temperature is not solely responsible for the ripening of fruits. This is in line with Jawetz *et al*. [35] who reported that the internal temperature of the fruits has not been lesser than the ambient temperature. Growth increases with temperature up to a maximum which vary from species to species after which growth starts declining.

Temperature fluctuations recorded in the various samples used were also considered. This could be largely due to different rate of metabolic activities of the associated organisms. *R. nigricans* was found dominating while others were in minority. This was however in consonant with the work of Sussman and Ainsworth [36] which attributed this to biotic interactions resulting from competition for water, space and nutrients among the various organisms present. This study showed that *R. nigricans* was the best adapted in the spoilt fruits. The invasion of pawpaw fruits by microbes followed a displacement pattern from *R. nigricans*, *Mucor* sp., *Fusarium* sp., *A. flavus* and *A. niger* in that order.

The effect of moisture on growth and development of the associated organisms showed that water was essential for normal body functioning. This was so because none of the organisms was able to grow at too low moisture content. The moisture content value of 25.0 - 40.0% per gram of the sample was found to contribute immensely to the growth and development of these organisms; though each has its own optimum value. However, no growth was recorded at water content value of 15.0% and 55.0% per gram of the samples respectively in any of the associated organisms. The highest number of microbial load was however recorded when the water content was 30.0% per gram of the various pawpaw fruit samples. *R. nigricans* showed rapid growth at water content value of 30.0% - 40.0% while *Mucor* sp. thrive well at water content value of 30.0% – 35.0%. *Fusarium* sp. on the other hand did well at water content value of 25.0 - 30.0%. This agreed with Jawetz *et al*. [35] who stated that molds are capable of active growth at water content value of 20.0% and above but not exceeding 50.0%.

A pH of 7.5 was reported for the ripe and uninfected pawpaw fruits. As these fruits becomes infected and populated with microbial growth, the pH start decreasing tending towards acidity. The pH at which microbial growth was enhanced ranged from pH 4.0 - pH 6.0. *R. nigricans* and *Mucor* sp. grew well at pH 5.5 – pH 6.0 while *Fusarium* sp. showed rapid growth at pH 5.0 - pH 5.5. *A. flavus* and *A. niger*, however tend to be more acidophilic as their rapid growth was recorded at pH 4.5 - pH5.0. This agreed with Domsch and Gams [34] who indicated the optimum pH range for molds to be 4.2 - 7.0. However, *R. nigricans* was found to be adapted than any other species by growing even at pH 9.0; although its growth at this pH value was severely retarded.

The isolation of these pathogens confirmed the studies of Baiyewu [20], Baiyewu and Amusa [21], Baiyewu *et al*. [22], Gupta and Pathak [31] and Kuthe and Spoerhase [37] that *R. nigricans, A. niger* and *A. flavus* found associated with rotten pawpaw are highly pathogenic causing appreciable losses in pawpaw fruits at post harvest. Baiyewu [20] also isolated *Fusarium* spp., *A. flavus* and *Rhizopus* spp. among other pathogens from pawpaw fruit. In our studies, the pathogenicity analysis revealed that all isolated fungi *R. nigrican* proved highly pathogenic causing a rapid disintegration of inoculated fruits in three to five days. *Mucor* sp. and *Fusarium* sp. was moderately pathogenic while the least pathogenic was *A. flavus* and *A. niger*. However, from the result of this study, *A. flavus* are not likely to be pathogens of pawpaw fruit but rather contaminants. Hence necessary precaution in preventing contamination of this produce by these bacteria and fungi will enhance the microbial quality of the produce [22]. Also, the presence of these fungi pathogens in theses pawpaw fruits could pose a serious threat to the health of its consumers. These fungi have been discovered to produce secondary metabolites in plants tissues potentially harmful to humans and animals [38]. Aflatoxin has been associated in cancer of the liver (heptatoma), aflatoxicosis and also with acute hepatitis in humans, especially in the developing world [22, 33, 38-40].

From this study, it can be concluded that pawpaw fruits from a farm in Oyo State, Southwestern Nigeria were grossly contaminated by pathogenic microorganisms as *R. nigricans*, *Mucor sp.*, *Fusarium sp.*, *A. flavus* and *A. niger* were found in varying degree. Microorganisms are naturally present on all foodstuffs and can also be brought in by outside elements (wind, soil, water, insects, animals, human handling [41]. They can become contaminated during growing, harvesting and transport of the raw materials and/or processing into finished products [42]. According to Baiyewu et al. [22], it is therefore necessary and important that both the farmer who harvests the fruits into bags for transportation, the marketers and consumers take necessary and appropriate precautions in preventing contamination and eating of contaminated fruits. This will however reduce the risk of mycotoxins associated with fungi contamination which are deleterious to human health. In line with the assertions of Krige *et al*. [43], since pawpaw fruits were usually infected by pathogenic organisms, to be effective,

production, preparation and preservation of food such as fruit salads made with pawpaw must be carried out as rapidly and hygienically as possible using good quality equipment, produce and materials.

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