Microorganisms Associated With Spoilage Of Stored Vegetables In Uyo Metropolis, Akwa Ibom State, Nigeria

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Abstract: Microorganisms associated with spoilage of stored vegetables were studied using standard microbiological methods. The analysis was done on carrots, cucumber, cabbage and onions with soft rot symptoms using various media. It showed that *Escherichia coli* (28.6%) were the most predominant bacterial isolates associated with vegetable spoilage in Uyo metropolis. This was followed by *Enterobacter* spp. (21.4%), *Staphylococcus aureus* (14.3%), *Erwinia* spp. (14.3%) *and Pseudomonas* spp. (14.3%) while *Salmonella* spp. (7.1%) was least predominant. The results obtained from the microorganism s associated with the spoilage of stored vegetables showed that the total aerobic counts for cucumber ranged from 1.28 x 10^6 to 3.20 x 10^6 cfu/g and the total coliform count ranged from 2.35 x 10^6 to 3.28 x 10^6 cfu/g. The total aerobic count for carrots ranged from 2.07 x 10^6 to 2.20 x 10^6 cfu/g while the total coliform count ranged from 2.80 x 10^6 to 3.00 x 10^6 cfu/g. The total aerobic count for cabbage ranged from 1.43 x 10^6 to 2.10 x 10^6 cfu/g while the total coliform count ranged from 3.10 x 10^6 to 4.20 x 10^6cfu/g. Generally, the total Salmonella-Shigella counts ranged from 0.0 x 10^6 to 1.80 x 10^6 cfu/g. Only cabbage had the highest count for total Salmonella-Shigella. The aerobic count for onions ranged from 1.8 x 10^6 to 2.0 x 10^6 cfu/g and the total coliform count ranged from 2.00 x 10^6 to 3.10 x 10^6 cfu/g. Fungi isolated from these vegetables were *Aspergillus niger*, *Rhizopus stolonifer* and *Aspergillus fumigatus* with fungal count ranging from 1.80 x 10^6 to 3.0 x 10^6 cfu/g. The frequency of occurrences showed that bacterial isolates were most predominant (63.6%) compared to the fungi isolates (36.4%). No protozoa or viruses were found to be associated with vegetable spoilage in Uyo metropolis. The frequency of occurrences of bacteria isolates showed that *Escherichia coli* (28.6%) were most. This was followed by *Enterobacter* spp. (21.4%), *Staphylococcus aureus* (14.3%), *Erwinia* spp. (14.3%) *and Pseudomonas* spp. (14.3%). *Salmonella* spp. (7.1%) was least predominant. It also showed that of the three fungi species isolated from vegetables, *Rhizopus stolonifer* (37.5%) *and Aspergillus fumigatus* (37.5%) were most predominant while *Aspergillus niger* (25.0%) was least predominant. Pathogencity tests revealed that all the isolates were pathogenic to the different vegetables examined leading to rapid disintegration of treated fruits in 3-5 days. It showed that each infected vegetable gave the initial organism that caused the spoilage of the fruit. The rot symptoms obtained were similar to those observed previously on the fruits when subjected to identification procedures. The presence of indicator and other organisms examined in this study is of special concern and perhaps the greatest danger associated with food for human consumption is contamination by human excrement. The need for microbial assessment of vegetables for production of salads and other use is also emphasized to reduce possible contamination.
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1. Introduction

Generally, vegetables are considered as the leafy outgrowth of plants or plants shoot used food. These include those plants or plant part used in making soup or served as an integral part of main meal (Yusuf et al 2004). Vegetables can also be regarded as the edible component of plants, such components includes leaves, stalk, roots, tubers, bulbs, flowers and seed (ICMSF 1998). Vegetables are important protective food and highly beneficial for the maintenance of health and prevention of diseases. They contain valuable food ingredients which are essential for the proper function of the body. Vegetable contain various medicinal and therapeutic agent and are valued mainly for their high vitamin and mineral content. Studies have evaluated the association of fruit and vegetable s consumption with the reduction of risk of specific diseases (Hung et al., 2004).

The incidence of microorganisms in vegetables may be expected to reflect the sanitary quality of the processing steps and the microbiological condition of the raw product at the time of processing (Ngugen and Carlin, 1994). For almost 100 years, vegetables contaminated in the field have been recognized as a source of human infection and unless. Many of the viruses (Rosenblum et al., 1990), bacteria

(Ho et al., 1986) and protozoan on vegetables which have caused food poisoning are derived from human faeces. However, pathogenic microorganism of human origin may also be present in minimally processed vegetables as the minimal technological processing may be unable to remove the original contamination resulting from air, soil, water, insects, animals, workers, harvesting and transportation equipment. Certain fungi such as Aspergillus, Fusarium, and Penicillium spp. as commonly occurring filamentous fungi grow in vegetable and their growth may result in production of toxins known as mycotoxins, which can cause a variety of ill effect in human from allergic responses to immunosuppression and cancer (Pitt et al., 1996).

The ability of public health agencies to identify through enhanced epidemiological and surveillance techniques, raw vegetables as probable sources of infectious microorganisms has undoubtedly resulted in increased numbers of documented outbreaks. The risk of illness associated with raw vegetable products can be reduced by removing or killing pathogenic microorganisms by washing or treating them with sanitizers. However, the hydrophobic cutin, diverse surface morphologies and abrasions in the epidermis of fruits and vegetables limits the efficacy of this treatment (Burnett and Beuchat, 2001).

Vegetables are frequently consumed raw without being exposed to the processes that reliably eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce bacterial levels but cannot be relied upon to eliminate pathogens. Eating or drinking contaminated foods or drinks can cause food-borne disease. Many different types of bacteria, viruses and parasites can contaminate food, so there is numerous different food borne infections. The consumption of carrot, cucumber, onions and cabbage in Nigeria has increased tremendously in the recent years properly due to increased awareness on their health important. Carrot is known to contain an important biologically active compound, carotenoid (Asagbra and Oyewole, 2002). It has been estimated that 20% of vegetables harvested for human consumption are lost through microbial spoilage by one or more of 250 market diseases, the primary causative agents of microbial spoilage are the bacteria, yeasts and mold (Jay, 2005).

Storage fungi can cause decrease of germination capability, loss in weight, discoloration of kernels, heating and mustiness, chemical and nutritional changes, and mycotoxin contamination (Sauer *et al.* 1992; Bulaong and Dharmaputra, 2002). They can change fat quality of peanuts by hydrolytic enzymes producing free fatty acids and glycerol (Pomeranz 1992; Bulaong and Dharmaputra, 2002). Altogether, these changes lead to a lower quality or rejection of commodity as foodstuff (Bulaong and Dharmaputra, 2002). Three fungal species, namely *Aspergillus flavus, A. parasiticus,* and *A. nomius* produce aflatoxins as secondary metabolites (Pitt and Hocking 1997). The toxins are known to be carcinogenic, hepatotoxic and teratogenic in test animals (Bulaong and Dharmaputra, 2002). Moisture content is the most important factor affecting fungal growth in stored products (Bulaong and Dharmaputra, 2002). According to Sauer *et al.* (1992) some fungal species were observed to become dominant with as little as 0.2% change in moisture content.

Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution (Barth et al., 2009). Those same types of soil-borne spoilage microbes that occur on produce are the same spoilage microorganisms that are present on harvesting equipment, on handling equipment in the packinghouse, in the storage facility, and on food contact surfaces throughout the distribution chain (Barth et al., 2009). Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices (GAP) will provide dramatic reductions in yield loss due to spoilage at all subsequent steps in the food-to-fork continuum (Eckert and Ogawa, 1988; Barth et al., 2009).

The problem of fungal growth and aflatoxin contamination of foodstuffs remains, especially in developing countries where handling and storage technologies are still being developed (Bulaong and Dharmaputra, 2002). Pitt and Hocking (1996) reported that aflatoxin exceeding 50 ppb contaminated 45, 22 and 25% of 215, 81 and 94 peanut samples collected from retailers in 1990/1991 in Indonesia, Philippines and Thailand, respectively. In line with this study Lubulwa and Davis (1994) reported that the total annual cost of aflatoxin contamination in Indonesia, Philippines and Thailand was about $A 158 million. Indonesia incurred 84% (= $A 132 million) of this cost, Thailand incurred 13% (= $A 21 million) and Philippines 3% (= $A 5 million) of the cost. Dharmaputra and Putri (1997) found that *P. funiculosum* was a dominant species in peanuts with different percentages of splitted kernels (0, 25, 50, 75 and 100%) stored for 2 months under laboratory conditions. Its population was between 0.7x10 - 1.2xl0^3 cfu/g (wb). Pitt *et al.* (1998) reported that *P. funiculosum* were found in 4% of 256 retail samples examined and 9% of all kernels examined (50 kernels/sample) in Bogor and Yogyakarta, Indonesia. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006; Al-Hindi et al., 2011).

However, in Nigeria, local utilization of carrots, cabbage, onions, and cucumbers is limited to direct unprocessed eating either wholly or salads. This situation has lead to a growing awareness on the need to evaluate microorganism associated with spoilage of these vegetables sold in Uyo metropolis. The aim of this wok was to determine the microbial load of stored vegetables sold at direction locations in Uyo, and to isolate, characterized and identify the specific organisms in different stored vegetables in Uyo metropolis, Akwa Ibom State, Nigeria.

2. Materials And Methods

2.1. Collection of samples

Twenty four (12 healthy and 12 infected) samples each of carrot, cabbage, cucumber and onions were purchased from the market place and storage room all around Uyo metropolis, Akwa Ibom State, Nigeria. These samples were placed in separate sterile plastic bags and transported to the laboratory for microbial analysis. These vegetables were not locally cultivated in the state. A total of 12 randomly selected infected vegetables and 12 unblemished, healthy and clean looking vegetables were purchased. Vegetables 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 media in Petri dishes and incubated at appropriate temperatures.

2.2. Enumeration, Isolation and Identification of Microorganisms

Samples were stored and allowed to ferment. The spoiled parts of each samples from different location was weighed and grinded using mortar and pestle. One gram of each sample was dispensed into a prepared 9 ml of distilled water contained in the McCartney bottles. The content was shaken for homogenous mixture. Ten fold serial dilutions were made and 10^{-5} dilution of the samples from different location were plated out using Nutrient agar, MacConkey and Salmonella-Shigella agar for total aerobic and coliform counts. These were incubated for 24 hours at 37^0c in the incubator. Sabourand dextrose agar and potato dextrose agar (PDA, Difco) were used for the total fungal counts and incubated at 28 ± 1oC for 5 days under 12 h photoperiod. The fungal colonies that appeared were primarily identified using cultural and morphological features (Barnett and Hunter, 1972). For bacterial colony purification, the streak techniques in the Nutrient agar was employed, while the fungal isolates were transplanted to new set Sabourand agar by picking. The bacterial plates were incubated at 37^{0C} for 24 h. The fungal plates were left at room temperature for 5 days. The discrete colonies from these subcultured plates and series of biochemical tests were done on the bacterial isolates for proper characterization and identification. The bacterial isolates were also identified by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994) and Oyeleke and Manga (2008). The pure isolated fungi were identified using cultural and morphological features according to the most documented keys in fungal identification (Domsch et al., 1993; Klich, 2002; Samson and Varga, 2007).

2.3. Pathogenicity of isolated organisms

Twelve (12) healthy vegetables collected from the each study sites were surfaced sterilized in ethyl alcohol as described above. Vegetables were wounded with a sterile 5 mm cork borer, and inoculated with bacterial colony and 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. Twelve vegetables were inoculated each with each of the isolates and this experiment was replicated. Controls consisted of twelve vegetables 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^OC and 37^OC for 5 days. After 72 h, the inoculated vegetables were observed for symptom development. The causal agents were re-isolated from the infected vegetables and compared with the original isolates.

3. RESULTS ANALYSIS

3.1. Total Bacteria and Fungi Counts Obtained from Vegetables

The results obtained from the microorganism s associated with the spoilage of stored vegetables showed that the total aerobic counts for cucumber ranged from 1.28 x 10^6 to 3.20 x 10^6 cfu/g and the total coliform count ranged from 2.35 x 10^6 to 3.28 x 10^6 cfu/g. The total aerobic count for carrots ranged from 2.07 x 10^6 to 2.20 x 10^6 cfu/g while the total coliform count ranged from 2.80 x 10^6 to 3.00 x 10^6 cfu/g. The total aerobic count for cabbage ranged from 1.43 x 10^6 to 2.10 x 10^6 cfu/g while the total coliform count ranged from 3.10 x 10^6 to 4.20 x 10^6cfu/g. Generally, the total Salmonella-Shigella counts ranged from 0.0 x 10^6 to 1.80 x 10^6 cfu/g. Only cabbage had the highest count for total Salmonella-Shigella. The aerobic count for onions ranged from 1.8 x 10^6 to 2.0 x 10^6 cfu/g and the total coliform count ranged from 2.00 x 10^6 to 3.10 x 10^6 cfu/g. The total fungal count ranged from 1.80 x 10^6 to 3.00 x 10^6 cfu/g (Table 1).

Table 1: Total Bacteria and Fungi Counts Obtained from Vegetables

Samples	Location	Total Aerobic Counts (CFU/g)	Total Coliform Counts (CFU/g)	Total Salmonella-Shigella Counts (CFU/g)	Total Fungal Counts (CFU/g)
Cucumber	Storage room	1.28 x 10^6	2.35 x 10^6	0.0 x10^6	1.80 x 10^6
	Market place	3.20 x 10^6	3.48 x 10^6	0.0 x10^6	2.00 x 10^6
Carrot	Storage room	2.07 x 10^6	2.10 x 10^6	0.0 x10^6	2.10 x 10^6
	Market place	2.20 x 10^6	3.0 x 10^6	0.0 x10^6	2.50 x 10^6
Cabbage	Storage room	1.43 x 10^6	3.10x 10^6	1.30 x10^6	2.70 x 10^6
	Market place	2.10 x 10^6	4.20 x 10^6	1.80 x10^6	2.07 x 10^6
Onions	Storage room	1.80 x 10^6	2.20 x 10^6	0.0 x10^6	2.80 x 10^6
	Market place	2.80 x 10^6	3.00 x 10^6	0.0 x10^6	3.00 x 10^6

3.2. Frequency of occurrences of Microorganisms Associated with Vegetables spoilage in Uyo Metropolis

Table 2 shows the frequency of occurrences of microorganisms associated with vegetables spoilage in Uyo metropolis. It showed that bacterial isolates were most predominant (63.6%) compared to the fungi isolates (36.4%). No protozoa or viruses were found to be associated with vegetable spoilage in Uyo metropolis (Table 2).

Table 2: Frequency of occurrences of Microorganisms Associated with Vegetables spoilage in Uyo Metropolis.

Isolates	No. (%)	Cucumber	Carrot	Cabbage	Onions
Bacteria	14(63.6)	3(21.4)	4(28.6)	4(28.6)	3(21.4)
Fungi	8(36.4)	3(37.5)	2(25.0)	2(25.0)	1(12.5)
Protozoa	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Viruses	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	**22(100.0)**	**6(27.3)**	**6(27.3)**	**6(27.3)**	**4(18.2)**

3.3. Frequency of occurrences of Bacteria Associated with Vegetables spoilage in Uyo Metropolis

Table 3 shows the frequency of occurrences of bacteria associated with vegetables spoilage in Uyo metropolis. It showed that *Escherichia coli* (28.6%) were most predominant bacterial isolates associated with vegetable spoilage in Uyo metropolis. This was followed by *Enterobacter* spp. (21.4%), *Staphylococcus aureus* (14.3%), *Erwinia* spp. (14.3%) *and Pseudomonas* spp. (14.3%). *Salmonella* spp. (7.1%) was least predominant (Table 3). *Escherichia coli* were present in all the vegetables examined. *Enterobacter* spp. was present in all the vegetables examined except for cabbage, in which it was not found. *Salmonella* spp. was only present in cabbage. *Staphylococcus aureus* was only found in cucumber and carrot. *Erwinia* spp. was present in carrot and cabbage only. *Pseudomonas* spp. was present in cabbage and onions only (Table 3).

Table 3: Frequency of occurrences of Bacteria Associated with Vegetables spoilage in Uyo Metropolis.

Bacteria	No. (%)	Cucumber (%)	Carrot (%)	Cabbage (%)	Onions (%)
Escherichia coli	4(28.6)	1(25.0)	1(25.0)	1(25.0)	1(25.0)
Pseudomonas spp.	2(14.3)	0(0.0)	0(0.0)	1(50.0)	1(50.0)
Erwinia spp.	2(14.3)	0(0.0)	1(50.0)	1(50.0)	0(0.0)
Staphy. aureus	2(14.3)	1(50.0)	1(50.0)	0(0.0)	0(0.0)
Enterobacter spp.	3(21.4)	1(33.3)	1(33.3)	0(0.0)	1(33.4)
Salmonella spp.	1(7.1)	0(0.0)	0(0.0)	1(100.0)	0(0.0)
Total	**14(100.0)**	**3(21.4)**	**4(28.6)**	**4(28.6)**	**3(21.4)**

3.4. Frequency of occurrences of Fungi Associated with Vegetables spoilage in Uyo Metropolis

Table 4 shows the frequency of occurrences of fungi associated with vegetables spoilage in Uyo metropolis. It showed that of the three fungi species isolated from vegetables, *Rhizopus stolonifer* (37.5%) *and Aspergillus fumigatus* (37.5%) were most predominant while *Aspergillus niger* (25.0%) was the least predominant (Table 4). *Aspergillus niger* was present in both cucumber and onions only. *Rhizopus stolonifer and Aspergillus fumigatus* were present in all vegetables except for onions (Table 4).

Table 4: Frequency of occurrences of Fungi Associated with Vegetables spoilage in Uyo Metropolis.

Fungi	No. (%)	Cucumber (%)	Carrot (%)	Cabbage (%)	Onions (%)
A. niger	2(25.0)	1(50.0)	0(0.0)	0(0.0)	1(50.0)
A.fumigatus	3(37.5)	1(33.3)	1(33.3)	1(33.4)	0(0.0)
R. stolonifer	3(37.5)	1(33.3)	1(33.3)	1(33.4)	0(0.0)
Total	**8(100.0)**	**3(37.5)**	**2(25.0)**	**2(25.0)**	**1(12.5)**

4. DISCUSSION

The total aerobic counts obtained in this study is lower compared to that reported by Kaneko et al. (2003), however, the total coliform counts and the total fungal counts were slightly higher than that reported by Kaneko et al. (2003). The bacteria isolates obtained in this study were also slightly different from those identified by Kaneko et al. (2003) in a similar study. The initial bacteria of stored produce may have been derived from contamination of air, soil, water, insects, animals, workers and harvesting and transportation equipments (FDA, 2002). . Also, the densities of Lactic acid bacteria (LAB) in fruit and vegetable products usually range from 10^2 to 10^6 CFU/wound (Trias et al., 2008). In the study by Trias et al. (2008), the highest concentrations of microorganisms were in ready-to-eat vegetables. This was due to the presence of cut surfaces, which allow higher nutrient availability (Ongeng et al., 2006) and affects not only LAB but all the microbiota related to the fresh product (Badosa et al., 2008; Trias et al., 2008). The microbial population levels found in this study were in agreement with data reported for ready-to-eat salads in other studies (Carlin et al., 1989; Trias et al., 2008).

The bacterial isolates identified in this study include *Escherichia coli*, *Enterobacter* spp., *Staphylococcus aureus, Erwinia* spp., *Pseudomonas* spp., and *Salmonella* spp. This is consistent with the findings of previous studies. Microorganisms most commonly found in vegetables generally involve Pseudomonas and Erwinia as coliforms and Micrococcus (ICSMF, 1998). Sufficient moisture, abusive temperature and adequate time will ensure a continuing increase in the bacteria population. Pathogens such as *Bacillus cereus, Salmonella* and *Escherichia coli* are naturally present in some soil, and their presence on fresh produce is not rare. The genus *Erwinia* belongs to the family Enterobacteriacease. They are all associated with plants where they are known to cause plant diseases of the rot and wilt type. These Gram negative rods that are related to the genera *Proteus, Serratia, Escherichia, Salmonella* and others. *E. coli and Salmonella* spp. are indicators of feacally contaminated products or vegetables.

Food processors may be sources of microbial chance inoculation, microbial food poison, food intoxication and food spoilage hence, food processors may be counter productive by being responsible for public health hazard and loss of revenue (Bankole *et al*., 2005). In this present study, all the vegetables examined haboured *Escherichia coli,* while prominent microorganisms variously haboured include *Enterobacter* spp., *Staphylococcus aureus, Erwinia* spp., *Pseudomonas* spp., *Salmonella* spp., *Aspergillus niger*, *Rhizopus stolonifer and Aspergillus fumigatus*. The detection of *E. coli* in this study showed poor hygienic standard in the handling of these salad vegetables or it could be also be from contamination during harvest. Presence of *E. coli* indicates recent contamination by faecal matter and possible presence of other enteric pathogens known to be causative agents of food borne gastroenteritis and bacterial diarrhea disease (Jiwa et al., 1981).

Most strains of *Staphylococcus aureus* are known to be pathogenic due mostly to the heat stable enterotoxin they produce in direct relationship to their inoculum level. Considering the notoriety of the resistance of *S. aureus* to methicillin, other penicillin and celphalosporins (Davis, 1997; Adeleke and Odelola, 1997), its detection in cucumber and carrot samples poses a lot of health risk to nourishment seeking consumers. The presence of *Staphylococcus auerus*, a pathogenic organism of public health concern and significance in these vegetables might have contaminated the stored vegetables from source as a result of handling by farmers or retailers. Improper handling and improper hygiene might lead to the contamination of food and this might eventually affects the health of the consumers (Dunn *et al*., 1995; Omemu and Bankole, 2005; Okonko *et al*., 2008 a,b,c,d, 2009a,b; Mgbakor et al., 2011).

Most of the organisms found in this study are those commonly found in soil and water. But the presence of other indicator organisms like *E. coli* and *Enterobacter aerogenes* in those vegetable samples might be the result of possible contamination during sales or unhygienic handling of stored vegetables. The presence of the most frequently isolated index of food quality and indicators of faecal contamination such as *Escherichia coli Enterobacter aerogenes* and *Salmonella* spp., reported in this study is an indication of faecal contamination of the food as a result of possible unhygienic handling (Okonko *et al*., 2008 a,b,c,d 2009a,b) or contamination of the vegetable itself during processing or directly from source and this might have adverse effect on the health of the consumers (Okonko *et al*., 2008a,b,c,d, 2009a,b).

In this study, the fungi isolated were *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Aspergillus niger*. These pathogens have been reportedly isolated from Pawpaw fruits in Nigeria (Baiyewu et al., 2007; Chukwuka et al., 2010; Akintobi et al., 2011). Several fruit spoilage fungi from different region has been isolated and identified (Al-Hindi et al., 2011). The most common fungi found in a study by Akintobi et al. (2011) were *Aspergillus flavus*, *A. niger*, *Fusarium solani*, *Penicillium digitatum*, *Rhizopus stolonifer* and yeasts. Baiyewu et al. (2007) had also reported that *Rhizopus nigricans*, *Aspergillus flavus*, *Aspergillus niger* and *Fusarium moniliforme* among others, were responsible for post harvest losses in Pawpaw in South Western Nigeria. Chukwuka et al.

(2010) recently reported that *R. nigricans*, *A. flavus, A. niger, Fusarium sp.,* and *Mucor sp.* were responsible for with Pawpaw fruits decay from a farm in Oyo State, South Western Nigeria. The isolation of *Rhizopus stolonifer* from vegetables further confirmed the studies of Efiuvwerwere (2000), Chuku *et al*. (2008), Akinmusire (2011), and Akintobi et al. (2011) who reported that *Fusarium* sp, and *Rhizopus stolonifer* were responsible for the soft rot of tomato. Onyia *et al.* (2005) also reported that *Fusarium monilifome*, *Aspergillus niger* and *Rhizopus stolonifer* were isolated from rotten tomato fruits.

Aspergillus spp. were widespread among all examined vegetables spoilage. *A. niger* is a fungus commonly found on grapes (Chulze, 2006), apples (Oelofse, 2006) and tomatoes (Yildz and Baysal, 2006). Bali et al. (2008) reported that black mold *A. niger* were caused post harvest spoilage in sweet orange and acid lime at field. The group of diseases caused by Aspergillus are called aspergillosis, the symptoms include fever, cough, chest pain or breastlessness. Usually, only patients with already weakened immume systems or who suffer other lung condition are susceptible. Gupta and Pathak (1986) had earlier reported that *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus nigrican*, *Curvularia lunata*, *Rhizopus oryzae*, *Fusarium equiseti* and *Fusarium moniliforme* were responsible for post harvest losses in pawpaw in South Western Nigeria. 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). Aflatoxin production depends among others on the strain of *Aspergillus sp*. (Kozakiewicz 1996; Pitt and Hocking 1997; Bulaong and Dharmaputra, 2002).

Generally, spoiling fungi are considered toxigenic or pathogenic (Al-Hindi et al., 2011). Toxigenic fungi have been isolated from spoiling fruits (Stinson et al., 1981; Al-Hindi et al., 2011). During refrigeration some moulds may produce mycotoxins (Tournas and Stack, 2001; Al-Hindi et al., 2011). Pathogenic fungi, on the other hand, could cause infections or allergies (Monso, 2004; Al-Hindi et al., 2011). *Aspergillus* spp. are known to produce several toxic metabolites, such as malformins, naphthopyrones (Frisvad and Samson, 1991; Pitt and Hocking, 1997; Al-Hindi et al., 2011) and they can produce Ochratoxins (OTA), a mycotoxin which is a very important toxin worldwide because of the hazard it poses to human and animal health (Peraica et al., 1999; Petzinger and Weidenbach, 2002) thus extra care should be taken during personnel handling of these fruits; such as harvesting, cleaning, sorting, packaging, transport and storage (Al-Hindi et al., 2011).

All the three fungi organisms isolated were confirmed to be pathogenic on vegetables but in varying degrees. It showed that all the isolated fungi were highly pathogenic leading to rapid disintegration of treated vegetables in 3-5 days. These three fungi isolates (*Rhizopus stolonifer*, *Aspergillus fumigatus* and *Aspergillus niger*) were successfully taking part in the vegetable decay and are thus confirmed as the causal organisms of decay (Baiyewu et al., 2007; Chukwuka et al., 2010; Akintobi et al., 2011). Okereke et al. (2010) indicated that the fungi species isolated from the infected mangoes included *A. niger, Fusarium* sp and *A. Flavus* and that *Fusarium* sp and *A. Flavus* could not prove pathogenicity when inoculated into healthy mango fruits.

The contamination of vegetables by pathogenic bacteria and fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Effiuvwevwere, 2000; Okonko et al., 2008a,b,c,d, 2009a,b; Akinmusire, 2011; Akintobi et al., 2011). Post harvest handling and transport of fruit is inadequate (Baiyewu et al., 2007). Therefore most of the fruits and vegetables harvested do not usually get to the major cities in time due to the nature of transport systems existing in the rural areas (Baiyewu et al., 2007; Akintobi et al., 2011). While fruit with bruises are not isolated from the unbruised ones and thereby causing cross-infections, consumers are supplied mostly with partly rotten fruits (Baiyewu et al., 2007). This portends a great risk of aflatoxin and other mycotoxins to the consumers (Baiyewu et al., 2007; Akintobi et al., 2011).

5. CONCLUSION

Microorganisms are naturally present on all foodstuffs and can also be brought in by outside elements (wind, soil, water, insects, animals, human handling. They can become contaminated during growing, harvesting and transport of the raw materials, and/or processing into finished products (Lelieveld *et al.*, 2003). 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 (Baiyewu et al., 2007). This will however reduce the risk of mycotoxins associated with fungi contamination which are deleterious to human health (Baiyewu et al., 2007; Chukwuka et al., 2010).

From this study, some of the isolated organisms have serious public health risk while others fasten spoilage of the vegetables. High numbers of these microorganisms in raw consumed and salad produce would lead to the consumer's illness with attendant symptoms and consequences of the particular or combined microbial presence. Reduction of risk for human illness associated with raw product can be better achieved through controlling points of potential contamination in the field during harvesting, during processing or shipment, storage or distribution in the retail markets food services facilities or home (Beuchat and Ryn, 1997)

The presence of indicator and other organisms examined in this study is of special concern and perhaps the greatest danger associated with food for human consumption is contamination by human excrement (Okonko *et al*., 2008a,b,c, 2009a,b). The need for microbial assessment of vegetables for production of food salads and for other use should also be emphasized to reduce possible contamination (Fagade *et al*., 2005). The isolation of these fungi pathogens confirmed the studies of Gupta and Pathak (1986), Baiyewu (1994), Baiyewu and Amusa (1999), Effiuvwevwere (2000), Chulze (2006), Oelofse (2006), Yildz and Baysal (2006), Baiyewu et al. (2007), Chuku *et al*. (2008), Bali et al. (2008), Okereke et al. (2010), Chukwuka et al. (2010), Al-Hindi et al. (2011), Akinmusire (2011) and Akintobi et al. (2011) that *Rhizopus spp.,* and *A. niger* found associated with rotten Pawpaw are highly pathogenic causing appreciable losses in Pawpaw fruits at post harvest.

Although salad vegetables are commonly associated with food poisoning, they harbour disease causing organisms. The growth of these in the environment such oil which is a rich source of microbes. Poor agricultural practices such as irrigation with contaminated water also may introduce microorganisms. Poor storage and transportation practices can result in contamination as well as poor handling by dealers, processing and consumer in the home. Side by side is the huge nutritional benefit derivable from consumption of these vegetable especially that are therapeutic, curative and preventive. Just as with other foods consumer have some responsibilities to carry when handling these vegetables, wash hand with warm water and soap before and after handling the vegetables. Rinse raw product in clean water, brush off debris. For cutting, plastic board should be used instead of wooden ones.

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