UJMR, Volume 3 Number 1 June, 2018 *ISSN: 2616 - 0668*
https://doi.org/10.47430/ujmr.1831.012
Received: 26th June, 2018 **Accepted: 11th July, 2018**

ISOLATION AND IDENTIFICATION OF FUNGI FROM SOME SELECTED VEGETABLES IN KANKARA LOCAL GOVERNMENT AREA, KATSINA STATE

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Abstract
Samples of vegetables (infected and healthy) were investigated in this study to identify and isolate fungal species growing present. The vegetables are Tomato (*Lycopersicum esculentum*), Pepper (*Capsicum annum*), Cucumber (*Cucumis sativus*) and Onion (*Allium cepa*) which were collected from local farms around Kankara local government area. Potato dextrose agar (PDA) was used for isolation of the fungi and morphological features and fungal identification key were used to identify the isolated organisms. All the vegetables collected showed the presence of fungi. The fungi isolated are *Aspergillus flavus, Rhizopus stolonifer, Aspergillus niger, Aspergillus parasiticus, Penicillium digitatum*., *Penicillium citrinum* and *Mucor* spp. among all, *Penicillium citrinum, Aspergillus niger*., and *Aspergillus. parasiticus* had the highest rate of occurrence followed by *Penicillium digitatum* and *Aspergillus flavus* while *Mucor* spp. and *Rhizopus stolonifer* were the least.
Key words: Fungal species, Identification, Isolation, Kankara, Vegetables

INTRODUCTION

Generally, vegetables are considered as the leafy outgrowth of plants or plants shoot used for 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 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 the therapeutic agents and are valued mainly for their high vitamin and mineral content. Studies have evaluated the association of fruit and vegetables consumption with the reduction of risk of many diseases (Hung *et al*., 2004).

The incidence of microorganisms in vegetables maybe expected to reflect the sanitary quality of the processing steps and the microbiological condition of the raw product at the time of processing (Nguyen-the and Carlin, 1994). However, pathogenic microorganism may 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 transporting equipment. Certain fungi such as *Aspergillus, Fusarium,* and *Penicillium* spp. grow on vegetables and their growth may result in production of toxins known as mycotoxins (such as alkaloids and polyketones that are toxic to animals including humans), which can cause a variety of ill effect in human from allergic responses to immune suppression and cancer (Pitt and Hocking, 1997).

Microorganisms have 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 fruit and vegetables limits the efficacy of this treatment (Burnett and Beuchat, 2001).

Vegetables are frequently consumed raw without being exposed to the processes that reliable eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce fungal levels but cannot be relied upon to eliminate pathogens. Eating or drinking contaminated foods or drinks can cause food borne disease (Adebayo *et al*., 2012). Many different types of bacteria, viruses and parasites can contaminate food, so there is numerous different food borne infections. The consumption of carrot, cucumber, onion and cabbage in Nigeria has increased tremendously in recent years due 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. The primary causative agents of microbial spoilage are bacteria, yeasts and mold (Jay *et al.*, 2005). Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and post-harvest handling or during storage and distribution (Barth *et al.*, 2009). The same types of soil borne spoilage microbes that occur on produce are the same spoilage microorganisms that are present on harvesting equipment, handling equipment, in the packing house, in the storage facility and on food contact surfaces throughout the distribution chain (Barth *et al*, 2009).

Fungi cause spoilage of many vegetables and crops. They lead to the destruction of plants quality and reduce the yield of crops. Several times, our vegetables are being spoiled by fungi which lead to decreases in the productivity of the vegetables. This research work is initiated to isolate and identify fungi that spoils these vegetables in order to find a necessary means to prevent and control the spoilage of these vegetables and which will exert a tremendous effect in the socio-economic status of the people.

MATERIALS AND METHODS

Sample collection

Samples of vegetables (healthy and infected) each of pepper, tomato, cucumber and onion were collected from local farms in and around Kankara L.G.A of Katsina State. The samples were transported to the laboratory of Biology Department, Umaru Musa Yaradua University in separate sterile plastic bags for fungal analysis. Vegetables were surface sterilized by exposing them in 90% ethyl alcohol for 1 minute (BDH chemicals Ltd poole England) and then into 1% sodium hypochlorite for 3 minutes and rinsed three times in sterile distilled water. segments (3-5cm) of tissues from the samples were cut out with a sterile scalpel and placed on previously prepared media in petri-dishes and incubated at appropriate temperature.

Sample Processing

One gram (1g) of each sample was dispensed into a prepared 9ml of distilled water contained in the McCartney bottles. The content was shaken for homogenous mixture. One in ten serial dilutions (10^{-1} – 10^{-5}) of the samples were prepared.

Isolation and identification of fungi

From each of serially diluted tubes 1ml was inoculated onto plates of Potato Dextrose Agar (PDA, Difco) which also contains Chloramphenicol to avoid growth of unwanted bacteria. The plates were allowed to solidify and incubated at room temperature for 2-5 days. The plates were incubated at 28 ±1°C for five days.

Following incubation, plates containing 30-300 colonies were selected and counted using digital colony counter. The counts were expressed as Colony Forming Unit per gram (cfu/g). The fungal colonies that appeared were primarily identified using morphological features (Barnett and Hunter, 1972). The fungal isolates were purified by sub-cultured transplanting to new set using potato dextrose agar (PDA, Difco). The pure strains of isolated fungi were identified using fungal identification keys (Domsch *et al*, 1993; Klich, 2002; Samsin and Varga, 2007).

Microscopic Examination of fungal isolates

Lacto phenol cotton blue was dropped on a glass slide and small portion of fungal colony from the sub-structure plates was taken, using a sterile inoculating needle and transferred to a glass slide, it was then emulsified with a sterile needle and then covered with a cover slip gently, to avoid air bubbles. Observation under low and high power objective lens was carried out, the observation include, searching for different features of fungi including, the hyphae, conidia, sporangiophore (reproductive structure), and identification was carried out microscopically by examining the colony.

RESULTS

The table above shows the actual fungal colony that grows as colony forming units per gram (cfu/g)) of the spoiled and healthy vegetable samples, where it ranges from $1x\ 10^1$ to $3.\ 8 \times 10^1$. Spoiled onion has the highest number of colony 3.8×10^1 and healthy cucumber has the lowest with 1.0×10^1

Seven (7) fungi were isolated from spoiled vegetables sample; *Rhizopus stolonifer*, *Aspergillus flavus* were isolated from tomato and *Penicillium citrinum* was isolated from onion, tomato and pepper while *Mucor* spp. and *Penicillium digitatum* were isolated from cucumber and *Aspergillus parasiticus* was isolated from onion and cucumber while *Aspergillus niger* was isolated from pepper and onion samples. While five (5) isolates were obtained from the healthy vegetables; *Aspergillus parasiticus, Aspergillus flavus* were isolated from tomato and *Penicillium citrinum* was isolated from cucumber and pepper while *Aspergillus niger* was isolated from onion and *Penicillium digitatum* was isolated from pepper samples (Table 2).

Table 1: Fungal Counts (cfu/g) of Spoiled and Healthy Vegetables

VEGETABLE SAMPLES	SPOILED VEGETABLES	HEALTHY VEGETABLES
Tomato	2.6×10^1	2.0×10^1
Cucumber	1.8×10^1	1.0×10^1
Pepper	3.0×10^1	2.0×10^1
Onion	3.8×10^1	3.0×10^1

Table 2: Fungi isolated from Spoiled and Healthy Vegetables

Fungal isolates	Spoiled Vegetables affected	Healthy Vegetables affected
Penicillium citrinum	Tomato, Pepper and Onion	Pepper, Cucumber
Rhizopus stolonifer	Tomato	-
Aspergillus flavus	Tomato	Tomato
Mucor spp.	Cucumber	-
Aspergillus parasiticus	Cucumber, Onion	Tomato
Penicillium digitatum	Cucumber	Pepper
Aspergillus niger	Onion, Pepper	Onion

Table 3 shows the morphological characteristics of different fungal isolates identified apparently from healthy and the spoiled vegetables.

Table 3: Morphological characteristics of the fungal species isolated

Fungal species	Morphological characteristics
Aspergillus niger	- Non-branched conidiophores with bulb end carries conidia or conidia like sun rays. -Pink-like black growth
Aspergillus flavus	-Pink-like green growth. -Non-branched conidiophores with bulb end Carries conidia.
Penicillium citrinum	-Blue green colony. -Reverse side is yellow to orange
Mucor spp.	-Simple conidiophores, conidia are produce in chain.
Aspergillus parasiticus	-They have roughned conidiophores. -Blue green colony and reverse dull yellow to dull green -They are vesicle spherical.
Penicillium digitatum	-Brush-like conidiophores carries conidia. -Green or green-greyish color colonies grows over fruits especially citrus.
Rhizopus stolonifer	-Sporangia contain spores, have rhizoids. -Cotton like white growth spotted with black color.

DISCUSSION

The findings of this study showed that *Aspergillus flavus*, *A. parasiticus*, *Aspergillus* spp., *Penicillium* spp., *P. citrinum*, *Rhizopus* spp., and *Mucor* spp. were found in vegetables grown in rural area around Kankara Local Government Area, Katsina State, Nigeria. *Penicillium digitatum*, *Rhizopus niger*, and *Aspergillus flavus* were found to be associated with spoilage or deterioration of vegetable. These fungal species have been reportedly isolated from Pawpaw fruits in Nigeria (Baiyewu *et al*., 2007; Chukwuka *et al*., 2010). The result obtained showed that of all the isolated fungi *Aspergilus niger*, *Aspergillus parasiticus* and *Penicillium citrinum* were highly prevalence among all the vegetables and *Aspergillus flavus* and *Penicillium digitatum* have moderate occurrence while *Rhizopus stolonifer* and *Mucor* spp. have the least prevalence among the

vegetables. All the seven species were found to cause spoilage of the vegetables; this is congruent with findings of (Baiyewu *et al*., 2007, Chukwuka *et al*., 2010). Generally, fungi that caused food spoilage are considered toxigenic or pathogenic (Al-Hindi *et al*., 2011).

Toxigenic fungi have been isolated from spoiling fruits and vegetables (Al-Hindi *et al*., 2011). During refrigeration some moulds may produce mycotoxins (Tournas and Stack, 2001). The fungi isolated in this study have been reported to produce secondary metabolites in plants tissues. These secondary metabolites are potentially harmful to humans and animals (Eaton and Groopman, 1994; Baiyewu *et al*., 2007). A good example is Aflatoxin which has been associated in cancer of the liver (heptatoma), aflatoxicosis and also with acute hepatitis in humans, especially in the developing countries (Krogh, 1992; Prasad, 1992; Eaton and Groopman, 1994; Muhammad *et al*., 2004; Baiyewu *et al*., 2007). Pathogenic fungi, on the other hand, could cause infections or allergies (Monso, 2004). *Aspergillus* spp. are known to produce several toxic metabolites, such as malformins, naphthopyrones (Pitt and Hocking, 1997) 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.

Aspergillus spp. and *Rhizopus* spp. isolated from infected pepper, onion and tomato in the study responsible for the soft rots of these vegetables. This is also reported by Baiyewu *et al*., (2007). This is in correlation with the work of Nijis *et al*., (1997) who reported that *Aspergillus* spp. is the predominant organism associated with the spoilage of orange. The isolation of *Aspergillus flavus*, *Penicilliuum citrinum* and Aspergillus parasiticus from Tomato and Cucumber (healthy and infected) is also congruent with findings of Efiuvwevwere, (2000) who reported that *Aspergillus* spp. are responsible for the rotting of pineapple. The isolation of *Rhizopus* spp. and *Mucor* spp. from Tomato and Cucumber (infected) agreed with work of Efiuvwevwere, (2000); Nijis *et al*., (1997); Purseglove, (1977), who reported that *Fusarium* spp. and *Rhizopus* spp. are responsible for the soft rot of tomato.

Colonization of fruits and vegetable by the invading microorganism is a critical phase in the microbial spoilage of produce. Also, the prevalence of fungi as the spoilage organisms of fruits and vegetables is due to a wide range of factors which are encountered at each stage of handling from pre-harvest to consumption and is related to the physiological and physical condition of the produce as well as the extrinsic parameters to which they are exposed (Efiuvwevwere, 2000). Efiuvevevwere, (2000) also reported that high moisture and relative humidity led to greater fungal growth in farm produce which tends to lower the storability of fruits and vegetables.

CONCLUSION

This research reveals the presence of fungal species associated with spoilage of vegetables. Hygienic practices should be encouraged during harvest, marketing and processing such as adequate washing with water and salt before consumption.

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