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Microbial Organisms Associated With The Post-Harvest Rot of Bell Pepper (*Capsicum annuum* L.) Fruit In Mubi, Nigeria

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Abstract

The study was aimed at assessing the microbial organisms associated with the post-harvest rot of bell pepper (*Capsicum annuum*) fruits in Mubi, Nigeria. A total of sixty samples of both the healthy and spoilt pepper fruit were collected from three different markets (Mubi main market, kasuwan dole and kasuwa kuturu) within Mubi. After proper washing the fruit samples using running tap water and dipping in 1% hypochlorite for one minute and rinsing in three changes of distilled water, the fungi and bacteria were isolated by direct plating and streaking methods using potato dextrose and nutrient agar media respectively. Three fungal species which included: *Aspergillus niger, Aspergillus flavus* and *Rhizopus stolonifer* all found in samples obtained from the three different markets; and bacteria from two different genera, *Clostridium* and *Bacillus* were isolated and confirmed through pathogenicity test *A. niger* of fruit sample from kasuwan dole had the highest percentage frequency of occurrence with 50 % and *A. flavus* in samples from Mubi main market was the lowest with 21.42 %. *Bacillus* sp. in samples from Mubi main market and Kasuwan kuturu had the highest and lowest percentage frequency of 100 % and 44.44 % respectively. The fungal and Bacterial species which involved *A. niger*, *A. flavus* and *R. stolonifer* and *Clostridium* sp. and *Bacillus* sp. respectively caused post-harvest fruit-rot of *C. annuum.* Therefore, *C. annuum* spoilt fruit should be discarded and never consumed to avoid diseases associated with these microbes.

Keywords: Isolation; Identification; Microorganisms; Fruit-rot; Pathogenicity

Introduction

Bell pepper (*Capsicum annuum* L.), is a cultivar group of annual or perennial plants in the family Solanaceae grown for its edible fruits. The plant has woody stems that grow brightly colored fruits. The alternating leaves are elliptical and smooth edged, that come to a distinct point. The plant produces white or purple bell-shaped flowers which are 2.5 cm in diameter. The red, yellow, purple, or brown fruits of the plant are produced each season about 3-6 weeks after flowering (Anon, 2011). Bell pepper is one of the most commercially important crops grown in Nigeria. Currently, much attention is given to it due to its possible links to the prevention of a certain type of cardiovascular diseases, atherosclerosis, hemorrhage, delaying of the ageing process, improving physical resistance and increasing appetite (Marin *et al.*, 2008). They are remarkable vegetables as a result of their significant pro-vitamin A concentration, through their concentration of carotenoids such as beta- carotene (Duthie, 2000). They also contain a high level of vitamins C and E in addition to carotenoids and xanthophylls. Besides being rich in phytochemicals, peppers provide a good amount of fiber. Both hot and bell pepper fruits contain substances that have been shown to increase the body's heat production and oxygen consumption for about 20 minutes after eating (Materska *et al.*, 2003).

Peppers are mostly prone to attack caused by pest and microbial organisms such as fungi, bacteria and nematodes that are pathogenic (Ademoh *et al.*, 2017). Due to attack by pathogenic microbial organisms, pepper fruit usually get rot before they get to consumers. As a result, millions of naira is being lost by both the farmers and sellers of this farm produce. These fruit rot microbial organisms could be introduced into the crop through the seed itself, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution (Barth *et al.*, 2009). Most bell pepper

fruits are in association with a variety of bacteria and fungi but due to a particular environmental condition, only a small proportion of the kind of microorganisms present would be able to grow rapidly and cause its deterioration. This study was, therefore, intended in identifying the pathogenic bacterial and fungal organisms that are responsible for the fruit-rot of bell pepper fruits in Mubi.

Materials and Methods

Study Area

This study was carried out in Mubi, Adamawa State, Nigeria. Mubi lies between latitude 10^0 14'48"N and 10^0 17'34"N of the equator and between longitude 13°14'46"E and $13^0$17'39"E of the prime meridian. The area has a tropical climate with average temperature of about 32-35° C and relative humidity ranging from 28-45 % and the annual rainfall is about 1056 mm (Adebayo, 2004). Most of the inhabitants of Mubi are Farmers, with significant number of them as business men and women and very few as civil servants.

Sample Collection

A total of sixty (twenty from each market) sample of bell pepper fruits (both rotten and healthy) were collected from three different markets within Mubi metropolis. These markets include: Mubi Main Market (MMM), Kasuwan Dole (KD) and Kasuwan Kuturu (KK). The collections were made into a sterilized polythene bags.

Media for Isolation

The media that were used for this study include Potato Dextrose Agar (PDA), for fungal isolation and Nutrient Agar (NA), for bacterial isolation.

Preparation of Potato Dextrose Agar (PDA)

About 30 g of powdered PDA was dissolved in 1000 ml of sterile distilled water and sterilized by autoclaving at 121^0C for 15 minutes. After heating, the media was allowed to cool before pouring into sterile petri dishes and left to solidify under aseptic conditions.

Preparation of Nutrient Agar (NA)

About 38 g of NA was weighted into a conical flask and made up to 1000 ml with distilled water. The conical flask was then sterilized by autoclaving at a temperature of 121^0 C for 15 minutes. After heating, the media was allowed to cool before pouring into a sterilized petri dishes and to solidify under aseptic conditions.

Isolation of the Fruit-rot Pathogens of C. annuum

Fungal isolation

Isolation of fungi from infected pepper fruit carried out according to the method described by Mailafia *et al*. (2017).

Bacterial isolation

The infected pepper fruit were first washed under a running water tap, then dipped into 1 % Sodium Hypochlorite to surface sterilize for one minutes and rinsed in three changes of sterile distilled water. They were then blotted dry by using sterile blotting paper. A sterile wire loop was used to get some cells of the fruit tissue and streaked on NA petri dishes and incubated for 23-24 hours at a temperature of 35-37°C.. After incubation, bacterial colonies which were of different colors were observed on the plates and re-isolated and sub-cultured on separate sterile NA media.

Identification of Fungal and Bacterial Isolates

Identification of the fungal isolates

The fungal isolates obtained were identified the methods described by Barnet and Hunter (1999) and Aziagba *et al.* (2015).

Identification of bacterial isolates

The bacteria isolates were only identified to genus level. The identification was based on the morphological and microscopical characteristics. Gram staining was conducted to study the cellular morphology of the isolated bacteria.

Determination of Percentage Frequency of the Isolated Organisms

The percentage frequency of the bacteria and fungi isolated from the *C. annuum* fruit were determined using the formula below:

$$\% \text{ frequency} = \frac{\text{Number of times an organism was encountered}}{\text{Total organisms isolated}} \times 100$$

Pathogenicity Test

Pathogenicity test was carried out using the method described by Okigbo and Emoghene (2009) to determine the pathogenicity of the isolates obtained from the infected pepper fruits.

Results

Fungal Pathogens Isolated From Infected C. annuum Fruit and their Percentage Frequency of Occurence in the Market

The assessment of *C. annuum* infected fruit obtained from three different markets within Mubi metropolis for the presence of fungi indicated the presence of three different fungal species on the fruits from these three markets. These include: *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* with 42.86 , 21.42 and 35.72 %; 50, 25 and 25 % and 25, 37.5 and 37.5 % of the frequency of occurence in MMM, KD and KK markets respectively. *A. niger* isolated from pepper fruit obtained from KD market had the highest percentage frequency of occurence and that of KK the lowest. *A. flavus* and *R. stolonifer* of fruits from KK had equal and the highest percentage frequency of occurence with 37.5 % than those of other markets (Table 1).

Bacteria Pathogens Isolated From Infected C. annuum Fruit and their Percentage Frequency of Occurence in the Market

Determination of the presence of bacteria on *C.annuum* fruits obtained from three different markets (MMM, KD and KK) within Mubi indicated the presence of two main genera of bacteria, namely: *Bacillus* sp. and *Clostridium* sp. *Bacillus* sp. was found present in pepper fruit samples obtained from all the three markets while *Clostridium* sp. in fruit samples from KD and KK (Table 2).

Pathogenicity Test of the Fungal and Bacterial Isolates Obtained From Infected C. annuum Fruit

Confirmation of the pathogenicity of the three fungal species (*A. niger*, *A. flavus* and *R. stolonifer*) and the two bacterial genera (*Bacillus* sp. and *Clostridium* sp.) isolated from *C. annuum* fruit showed positive as all the fungi and bacteria isolated from the infected fruits initiated diseases symptoms similar to those seen on the bell pepper fruit from which the fungi and bacteria were isolated.

Table 1: Fungal Pathogens Isolated From Infected *C. annuum* Fruit and their Percentage Frequency of Occurence in the Market

MARKET	FUNGAL SPECIES	NO. OF OCCURRENCE	% FREQUENCY
MMM	*Aspergillus niger*	6	42.86
	Aspergillus flavus	3	21.42
	Rhizopus stolonifer	5	35.72
KD	*A. niger*	8	50.00
	A. flavus	4	25.00
	R. stolonifer	4	25.00
KK	*A. niger*	4	25.00
	A. flavus	6	37.5
	R. stolonifer	6	37.5

Key: MMM= Mubi Main Market; **KD =** Kasuwan Dole; **KK=** Kasuwan Kuturu

Table 2: Bacteria Pathogens Isolated From Infected *C. annuum* Fruit and their Percentage Frequency of occurrence in the Market

MARKET	BACTERIA GENERA	NO. OF OCCURRENCE	% FREQUENCY
MMM	*Bacillus* sp.	5	100
KD	*Bacillus* sp.	6	54.55
	Clostridium sp.	5	45.45
KK	*Bacillus* sp.	4	44.44
	Clostridium sp.	5	55.56

Key: MMM= Mubi Main Market; **KD =** Kasuwan Dole; **KK=** Kasuwan Kuturu

Discussion

The presence of the three fungal species: *A. niger, A. flavus* and *R. stolonifer* on the bell pepper fruit samples obtained from the three different markets within Mubi was an indication of the high health risk associated with the consumption of uncooked or partially cooked vegetables. The three fungal species are pathogenic (Udoh *et al*., 2015), causing

diseases in plants, animals and even humans. *A. flavus* cause aspergillosis and produce aflatoxin, a carcinogenic mycotoxin (Jariwal *et al.*, 2018); *A. niger* cause otomycosis, cutaneous infections and pulmonary diseases (Loudon *et al*., 1996; Araiza *et al.*, 2006; and Person *et al*., 2010); and *R. stolonifer* cause zygomycosis in persons with weak immune system (Ribes *et al*., 2000). Several studies associated *A. niger*, *A. flavus* and *R. stolonifer* with the post-harvest rot of vegetables and fruits such as tomatoes, watermelon, pawpaw, orange, lettuce and red pepper (Udoh *et al*., 2015; Yahaya *et al.*, 2016; Mailafia *et al.*, 2017). Pathogenic fungi, similar to the ones isolated in this study were also reported by Yaradua *et al*. (2018) when they assessed infected pepper, cucumber, onion and tomato fruit samples. Moss (2002) reiterated that 60 % of fruits spoilage was due to the activities of *A. niger* and *R. stolonifer* and accounted for about 10 % of mould deterioration of vegetables.

Rod-shaped Gram positive bacteria species from the genera *Bacillus* and *Clostridium* were found responsible for the fruit rot of the bell pepper (*C. annuum*) obtained from the three different markets in the study area. Bacterial species from the genus *Bacillus* were the common, found in all the samples from the three markets. Bacterial species from the genus *Clostridium* cause infections through the production of powerful toxins which are responsible for diarrhoea and cramping (Chukwu *et al*., 2015); and bacteria from the genus *Bacillus* cause diseases such as food poisoning, ocular infection, pneumonia, meningitis, endocarditis and musculoskeletal infections in humans (Drobniewski, 1993). Many researchers reported the presence of *Clostridium* (Chukwu *et al.*, 2015 and Klapec *et al.*, 2016) and *Bacillus* (Beuchat, 1995 and Obeng *et al*., 2018) species on some vegetables. Vegetables and fruits contamination with pathogenic microbes could be linked to the processes of storing, harvesting, transporting and handling methods (Mailafia *et al.*, 2017).

Conclusion

The fungal and Bacterial species which involved *A. niger*, *A. flavus* and *R. stolonifer* and *Clostridium* sp. and *Bacillus* sp. respectively caused post-harvest fruit-rot of *C. annuum.* Therefore, *C. annuum* fruit must be properly washed before consumption to avoid diseases associated with these microbes.

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