Quest Journals
Journal of Research in Pharmaceutical Science
Volume 2 ~ Issue 7 (2015) pp: 07-10
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Isolation and Identification of Fungal Species Associated With the Spoilage of Some Selected Edible Fruits in Iree Town Of Boripe Local Government, Osun State, Nigeria

[1]Akinro, E.B. [2]Adetuberu, I.A., [1]Efunwole, O.O. and [2]Olakunle, T. P.
[1]*Department of Science Laboratory Technology, Osun State, Polytechnic, Iree.*
[2]*Department of Applied Sciences, Osun State Polytechnic, Iree.*

Received 28 January, 2015; **A**ccepted 05 March, 2015 © The author(s) 2015. Published with open access at **www.questjournals.org**

ABSTRACT:- Four Spoilage fruits were investigated in this study. They were pawpaw *(Carica papaya),* Pineapple *(Ananas comosus),* Orange *(Citrus sinensis)* and Tomato *(Lyeoptersieon esculentum),* which were collected from a market at Iree town. All the fruits collected showed signs of spoilage by fungal species. The fungal species isolated were *Aspergillus flavus, Rhizopus stolonifer, Aspergillus niger, Candida tropicalis, Phytophthora* sp., *Fusarium oxysporum* and *Mucus* sp. Among all, *Aspergillus* species had the highest rate of occurrence followed by *Rhizopus* and *Candida* species while *Phytophthora* sp., *Mucus* sp. and *Fusarium* sp. were the least. Finally, the pathogenicity tests showed that all the fungi isolated were pathogenic to all the fruits.

Keywords:- *Spoilage, fruits, fungal species, pathogenicity.*

I. INTRODUCTION

Fruits and vegetables generally give the body every necessary vitamins, fats, minerals and oil in the right proportion to maintain growth and development on humans, (1).

Fruits and vegetable however, have serious challenges to their existence and these include changes in climatic condition, pests, inadequate rainfall and fungal attack (1). Over the years, there has been an increase in the need to identify and isolate the fungal associated with their spoilage.

Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic, these changes may be accompanied by alteration in taste, smell, appearance or texture (7). Numerous microbial defects of agricultural crops are characterized by the types of microorganism responsible for the deterioration, the process of infection in the case at fungal invasion follows the development of fungal penetrating structure (appresorium) (7). Colonization of fungi is a critical phase in the microbial spoilage of post harvested fruits. The colonization process involves the ability of the microorganism (fungi) to establish itself without the produce (host). This is initiated when fungi depolymerises certain specific cell wall polymers (such as protopectin, the cementing substance) of the produce (10).

The contamination of fruit and vegetables by fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (4).

Fungi affecting Tomatoes *(Lycopersicum esculentum)* includes *Fusarium oxyspanium, Fusarium stolonifer, Aspergillus niger* and *Rhizopus stolonifer.* They are responsible for tomato soft rot, as was isolated by (6). Result on the percentage incidence and rot shows that *Rhizopus stolonifer* caused the greatest rot on tomato fruit. A lot of breeding works have been carried out on tomato up to the point where we now have tomato hybrids that could withstands adverse environmental condition and resistant to diseases and pests (3).

The preponderance of the isolated moulds from orange (*Citrus sinesis*) belongs to *Aspergillus* sp., and this confirms their prevalence in foods and fruits exposed to tropical humid climate thus constituting potential health risks to consumers of this fruit and its by-products (5). The aim of this study is to isolate

and identify those fungi associated with source fruits which eventually caused their spoilage and result to economy loss.

II. MATERIALS AND METHOD

Pawpaw, pineapple, orange and tomato fruits were purchased from a market from Iree town, in Boripe Local Government of Osun State. A total of twenty four fruits comprising of six fruits from each fruit were purchased. Among each of the six fruits, there were three affected, and three healthy looking fruits. Thus, a total of twelve rotten fruits are involved. The fruits were put in 90% ethanol sterile polythene bags and transported to the laboratory for analysis.

Isolation of fungi from each of the blemished fruits was carried out using the method of (1);(2). 3-5cm of the tissues from the margins of the rotten fruits were cut with a sterile; scalped and placed on the prepared Sabouraud Dextrose Agar in petridishcs and incubated at $28+1^0$C for 5 days. The detected fungi were carefully subcultured on Sabouraud Dextrose Agar in plates and slants. The plates and slants were grown for seven days in an incubator at 28^0C.

Fungal isolates from plates were prepared into mounts on microscopic slides and examined under the microscope for comparison of fungal morphology with description given by Samson and Reenen-Hoekstra (9).

III. PATHOGENICITY OF ISOLATED FUNGI

Three healthy fruits from each sample were surface sterilized with 90% ethanol, and incisions were made on them using a sterile 4mm cork borer, similar sterile cork borer was used to cut pellets of Agar containing the cultures of fungal mycelia of the isolates. The fungi were inoculated onto the hole created on the healthy fruits in a laminar flour chamber.

The inoculated wound was sealed with petroleum jelly. Two controls with incision but not inoculated were established, were placed in a clean polythene bag (one fruit per bag) each moistened with-wet balls of absorbent cotton wool to create a humid environment and incubated at 28^0C for 5 days. After 72 hours, the incubated fruits were observed for symptom development. The causal agents were pre-isolated from the infected fruits and compared with the original isolates.

IV. RESULTS

Seven fungi species, *Aspergillus* sp., *Rhizopus* sp., *Candida* sp., *Fusarium* sp., *Phytophthora* sp. and *Mucor* sp. were isolated from a total of twelve fruits. *Aspergillus flavus* was isolated from pawpaw *(Carica papaya)* and pineapple *(Ananas comosus)*, while *Aspergillus niger* was isolated from orange *(Citrus sinensis)*, *Rhizopus* sp. was obtained from pawpaw *(Carica papaya)* and Tomato *(Lycopersicon esculentum) Candida sp* was isolated from orange *(Citrus sinensis)* and tomato *(Lycopersicon esculentum)* while *Fusarium* sp. and *Mucor* sp. were isolated from tomato *(Lycopersicon esculentum)* and *Phytophthora* sp. from pineapple *(Ananas comosus* (table 1).

The frequency of occurrence shows that *Aspergillus* sp. had the highest frequency in which it occurred in five out of the twelve fruits tested (Table 2). Pathogenicity tests showed that the isolated organisms were pathogenic, because they were able to produce the same spoilage signs in the healthy fruits into which they were re-inoculated (Table 3).

TABLE 1: Identification of fungal isolates from fruit samples with different spoilage patterns.

Fruit Sample	Spoilage	Macroscopic Examination	Microscopic Examination	Organisms
Pawpaw (*Carica papaya*)	Sunken large depression	White colony with light yellow green, become dark yellow-green	Radiate conidial head, coarsely roughened vesicle conidia and flask shaped pholides chains of rough	*Aspergillus flavus*
Pawpaw (*Carica papaya*)	Wrinkled appearance with depression soaked with water	Whitish colony later becoming brown-black	Non-septate mycelia, with sporangiousphore, ovaid in shape with sub-globose columella	*Rhizopus stolonifer*
Pineapple (*Ananas comosus*)	Sunken large depression	White colony with light yellow-green later becoming dark-yellow-green	Radiate conidial later split to loose columps. Rough conidia bone on vesicle	*Aspergillus flavus*

Pineapple (*Ananas comosus*)	Production of gas due to fruit becoming spongy with white deposit	Greenish white colonies, opaque, smooth and convex	Budding, spherical to elongate cells, forming pseudomycelia	*Candida tropicalis*
Pineapple (*Ananas comosus*)	White-foaming-like grown with sunken depression	Bluish-brown colour appearance thread like filament (hyphae)	Possession of 300 spared with flagella	*Phytphthora* sp.
Orange (*Citrus comosus*)	Dark brown discolouration, gas production due to spongy	Black colour with white edges	Large conidial heads, dark brown becoming radiate and split to columns	*Aspergillus niger*
Orange (*Citrus comosus*)	Fruit becoming spongy with gas production	Greening white colourise, orange smooth, convex	Budding spherical and forming pseudomycelium	*Candida tropicalis*
Tomato (*Lycopersican esculentum*)	Wrinkled appearance and depression	Whitish becoming brown-black	Non-septate mycelia with branching sporangiosphores	*Rhizopus stolonifer*
Tomato (*Lycopersican esculentum*)	Wrinkled appearance and depression	Grey colony becoming brownish	Non-septate mycelia with branches sporangiosphores	*Mucor* sp.
Tomato (*Lycopersican esculentum*)	Whitish-pink mycelia growth	Colonises are whitish-pink with a purple mycelium	Mucor conidial, ovoid to ellipsoidal in shape slightly cured and pointed at both ends	*Fusarium oxyspernums*

TABLE 2: Frequency of occurrence of each isolate in fruit samples

Fruit Sample	Total Number of Samples	Fungal Species	Percentage Frequency
Pawpaw (*Carica papaya*)	2 1	*A. flavus* *A. stolonifer*	66.7% 33.3%
Pineapple (*Ananas comosus*)	1 1 1	*A. flavus* *Candida tropicalis* *Phytophthora* sp.	33.3% 33.3% 33.3%
Orange (*Citrus sinensis*)	1 2	*C. tropicalis* *A. niger*	33.3% 66.7%
Tomato (*Lycopersicon esculentum*)	1 1 1	*Rhizopus stolonifer* *Mucor* sp. *Fusarium oxysporium*	33.3% 33.3% 33.3%

TABLE 3: Pathogenicity Tests

Fungal Species	Fruit Inoculums	Spoilage Pattern
Aspergillus flavus	Pawpaw Pineapple	Sunken spots Sunken depression
Aspergillus niger	Orange	Dark brown discoloration with sunken spots
Rhizopus stolonifer	Tomato Pawpaw	Water soaked wrinkled appearance
***Mucor* sp.**	Tomato	Water soaked. Appearance with whitish depression
Candida tropicalis	Orange Pineapple	Fruit become spongy with prominent of gas and white deposits appearance
***Fusarium oxysporium Phytopthora* sp.**	Tomato Pineapple	Whitish-pink mycelia growth. Whitish foamy-like growth with sunken depression

V. DISCUSSION

Aspergillus sp. and *Rhizopus sp.* isolated from pawpaw in the study responsible for the soft rots of pawpaw.
This is also reported by (2).

Aspergillus niger and *Candida tropicalis* were found responsible for the deterioration of orange, this is in correlation with the work of (5) who reported that *Aspergillus* sp. is the predominant organism associated with the spoilage of orange.

The isolation of *Phytophthora sp.*, *Aspergillus flavus* and *Candida tropicalis* from pineapple is in correlation with the work of (4) who reported that *Aspergillus* sp. and *Candida* sp. are responsible for the rotting of pineapple.

The isolation of *Fusarium* organism, *Rhizopus stolonifer* and *Mucor* sp. from tomato revealed and supported the work of (4);(3);(8), who reported that *Fusarium* sp. and *Rhizopus stolonifer* 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 (4).

Efiuvevevwere,(4) 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.

VI. CONCLUSION

Since fruits and vegetables have been very important in human nutritional qualities, this then calls for proper handling of the produce from the pre-harvest to consumption.

All the fruits are advised to be eaten fresh or cooked either to avoid long term exposure to spoilage. Also, the high moisture content of fruits and vegetables will be a serious limiting factor in their preservation. Finally, the farmers who harvest the fruits into bags for transportation, the marketers and consumers should take necessary precautions in preventing contamination and also try to create an environment that would discourage the growth or multiplication of microorganisms.

All the above will help to reduce the risk to poisoning by aflatoxin and other mycotoxins which are normally produced by those fungi isolated in this study. Hence, this is a great concern to all.

REFERENCES

[1]. Amusa, N.A., Kehinde, I.A. and Ashaye, O.A. (2002). Biodeterioration of breadfruit in storage and its effects on the nutrient composition. *African journal of biotechnology*, 1(2): 57-60.

[2]. Baiyewu, R.A., Amusa, N.A., Ayoola, O.A. and Babalola, O.O. (2007). Survey of the post-harvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya*) in South Western Nigeria. *African journal of agricultural research*, 2(4): 178-181.

[3]. Chuku, E.C., Ogbonna, D.N., Onuegbu, B.A. and Adeleke, M.T.V. (2008). Comparative studies on the fungi and biochemical characteristics of snake grand (*Trichosanches curcumerina* Lim) and 'tomato (*Lycopersican esculentus* mill) in Rivers State, *Nigeria. Journal of applied sciences*, 8(1): 168-172.

[4]. Efiuvwevwere, B.J.O. (2000). Microbial spoilage agents of tomato and assorted fruits and vegetable (An illustrated references book). Paragraphics of lishing company, Port Harcourt, Pp: 1-39.

[5]. Nijis, Doe Van H.P. Egmerd, Rombouts, F.M. and Notermans, S.H.W. (1997). Identification of hazardous *Fusarium* secondary metabolites occurring in Food Raw Materials. *Journal of food safety*; 17: 16-191.

[6]. Onnegbu, B.A. (2002). Fundamentals of crop protection, ASCEN, RSUST, Port Harcourt, Pp: 204-208.

[7]. Paul, S. and Sainsbuy, D. (1981). Dictionary of microbiology. John Wiley and Son's publication, New York.

[8]. Purseglove, W. (1977). Tropical crops dicotyledons vol. 1 and 2 ELBS, Pp: 137-533.

[9]. Robert, A., Samson and S. Ellen., Van Reenen-Hoekstra.(1988). Introduction to food borne fungi 3rd edition. CBS.

[10]. Snowdon, A.L. (1988). A review of the nature and causes of post harvest deterioration in fruits and vegetables, with special references to those in International Trade. Biodeterioration, 7: 585-602.