A STUDY OF PATHOGENIC FUNGI CAUSING POST HARVEST LOSSES OF PINEAPPLE SOLD AT WUDIL AND YAN LEMO MARKETS OF KANO STATE

Abstract

Pineapple (Ananas comosus) sold in the market is lost due to post-harvest diseases. This research was conducted to determine the fungal pathogens responsible for post- harvest losses of pineapple sold at Wudil and Yen lemo markets. Two samples of pineapples were purchase twice a week from both Wudil and Yanlemo markets for a period of four month. The samples and were investigated for the presence of fungal pathogen using standard microbiological methods. The methods involves mounting small portion of pine apple in plate containing Potato dextrose agar to isolate the fungi. Three fungal pathogens belonging to *Aspergillus* species were isolated. and *Aspergillus*. *niger* had the highest frequency of occurrence of (*50%*). Followed by *A. flavus* was the second with (*27%*). And The *A. fumigatus* had the least lowest frequency of occurrence of (*23%*). The differences between the fungal isolates recorded was statistically significant different (P>< 0.05) between the two markets, where higher fungal isolates were recorded at Yanlemo market 159 (40.6%) than and Wudil 38 (9.71%). The result of the study found showed that the post- harvest losses of pine apple in the two markets are attributed to fungal infection. Therefore, safe guarding the two markets from debris and dumps of rotten fruits and vegetable may assist in reducing fungal inoculums in the two markets.

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Keywords: Fungi, Post- harvest, Incidence, Wudil, Yan lemo

INTRODUCTION

The pineapple (*Ananas comosus*) is a tropical plant with edible multiple fruit consisting of coalesced berries. also called pineapples, and It is the most economically significant plant in the Bromeliaceae family (Tournas, 2005; Yahaya, 2005; Ikhiwili, 2012). Pine apples

may be

cultivated from a crown cutting of the fruit, possibly flowering in 20–24 months and fruiting in the following six months (Coppens and Leal, 2003).

Pineapples can be consumed fresh, cooked, juiced, or preserved. They are found in a wide array of cuisines. In addition to consumption, the pineapple leaves are used to produce the textile fiber *pina* in the Philippines, commonly used as the material for the men's *barong Tagalog* and women's barot saya formal wear in the country ( ). The fiber is also used as a component for wallpaper and other furnishings (Jones and Wilson, 2006). The fruit is a good source of vitamin A, B, C and also calcium, magnesium, potassium and iron. It is also a good source of bromelin, a digestive enzyme. It is consumed fresh or in the form of juice, jam, squash and syrup (Jones and Wilson, 2006).

Pineapple is mainly essential for its dietary needs and can be consume as an ingredient in fruit juices and can be eaten raw (Masefield *et al.*, 2002). In West Africa pine apple is grown in gardens and irrigation schemes. In Nigeria, most pine apple is grown in the southern parts of the country (Durgesh *et al*, 2008) and there is no record of any systematic or organized traditional storage method for the fruits. They are usually sold immediately after harvesting. They are packed in baskets, cardboard boxes, or wooden crates ready for transportation to the markets(Durgesh *et al*, 2008).

Like many other fruits pine apple are highly perished product and the quality is affected by post harvest handling, transportation, storage and marketing. This may result in decay and production of microorganism which become activated because of the changing physiological state of the fruits (Hayatu, 2000). It ~~has also been~~is estimated that 20% of all fruits harvested for human consumption are lost through microbial spoilage ~~causing one or more of 250~~

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~~market diseases.~~ Spoilage of fruit usually occurs during storage and transit and also while waiting to be processed. (Durgesh *et al.*, 2008; Al-Hindi *et al.*, 2011).

Pineapples are subject to a variety of diseases, ~~the most serious of which is~~which includes wilt disease, ~~vectored by mealy bugs typically found on the surface of pineapples, but possibly in the closed blossom cups (Durgesh *et al* 2008). Other diseases include~~ pink disease, bacterial heart rot, anthracnose, fungal heart rot, root rot, black rot, butt rot, fruit let core rot, and yellow spot virus (Durgesh *et al* 2008; Marin-Cevada *et al.*, 2010). Contamination of pine apples may take place at all stages during pre and post-harvest stages. Cultivation and operation or preparation of fruit is responsible for this contamination (Johannessen, *et al.*, 2002).

The ripened pine apple fruits are easily affected while the green ones show resistance to infection because they don't meet the nutritional requirements of the fungi. The enzymes potential necessary for invading green fruits is greater for ripe ones and it is temporarily beyond the capability of the fungi (Alao, 2000).

Ayanda *et al.*, (2013) identified fungi and bacteria as the major organisms causing deterioration of pineapple and other fruits in Nigeria and this occur by the secretion of extra cellular cell wall degrading enzymes, this factor influences virulence of pathogens. ~~(Ayanda *et al.*, 2013).~~ In another related study NARI, (2004) shows that in Nigeria, fungi constitute the major limiting factor to the production of perishable fruit. Losses caused by fungal attack vary from 20-30% (Park *et al.*, 2008). The consequences to man and environment with respect to Phytotoxicity on the use of fungicides for control of fungal diseases worldwide, there is therefore, an urgent need for alternative method of plant disease control. This scenario necessitated the search for and the development of ecologically sustainable fungal control methods which are effective against the target species but cause minimal adversity for

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non-target species (Suleiman and Emua, 2009). However, for a successful control of losses due to pathogenic microorganism baseline information is required to know precisely the types of pathogens that are involved which will prepare ground for the control strategies. Therefore, this study ~~is~~ aimed at a isolating and ~~identification~~ identifying ~~of~~ fungal pathogens responsible for post- harvest losses of pineapple on sale at Wudil and Yen lemo market which will provide baseline information for the development of control strategy.

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MATERIALS AND METHOD

Study site

Wudil Market: Is located at Wudil Local Government Area of Kano state. It is one of the largest markets in Kano state. ~~Despite being one of the largest markets in Kano state, there~~It has ~~were~~ no good storage facilities ~~in the market~~for fruits including pineapples. Some marketers store their grains on rusted basins and ~~. Also~~ hardily use chemicals on their grains.

Yan lemo Market: Is located at Tarauni local government area of Kano state and is similar in form and structure to Wudil market above~~. It is one of the largest markets in Kano state. Despite being one of the good grain markets in Kano state, there were no good storage fertilities in the market. Some marketers stored their grains in rusted basins or on the floor of the stores. Also hardly used chemical on their grains..~~

Experimental procedure

~~In this study a~~A survey was carried out to provide information on the incidence of fungal species responsible for the ~~looses~~loses of pineapple on sale at Wudil and Yen lemo markets~~. The investigation period was from~~within February ~~to~~ and March, 2018.

Sample collection and collection site

Two samples of pineapple were purchased twice a week directly from vegetable sellers each at Wudil and Yen lemo Markets and transported to laboratory at Kano university of science and Technology in polyethylene bags for plating. The methodology used in this research follows the one used by Yahaya *et al* (2016).

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Sample Handling

Pine apple obtained from Wudil and Yen lemo markets were surface sterilized~~satirized~~ by immersion in 3% (v/v) sodium hypochlorite solution for three minutes. Then ~~were~~ rinsed three times in ~~three changes of~~ running tap water and allowed to dry. Portion (2mm) was cut with sterilized scapel and placed on Potato Dextrose Aga (PDA) plate and incubated at 25-27^{0}c for three days.

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Isolate count and subculture

Each week growth of fungal isolate was monitored and the number of isolates that appeared was counted and recorded. Each distinct isolates was sub cultured into fresh PDA.

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Pathogenecity test

Pathogenicity test were conducted to ~~prove Koch~~prove Koch's postulate. Diseases free pineapples were surface sterilized with 10% (v/v) sodium hypochlorite solution and rinsed three times in ~~three changes of~~ running tap water and ~~allowed to~~wiped dry using sterile cloth. A 2mm diameter cycle was made on the samples then samples were streak with fungal hyphae on the circular portion. Controls were inoculated with sterile distilled water. Materials were placed on the laboratory bench, sterilized forceps were used to removed portion from disease areas on the 4th day after inoculation and placed on freshly prepared PDA plates and incubated at 25-27^{0}c for three days. Fungal growth that appeared was recorded.

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Microscopic examination

~~For each examination a streak of f~~Fungal mycelium was placed on a clean glass slide and examined under a microscope. One drop of cotton blue lactophenol was added and the cover slip placed. The slide was mounted on the microscope and observed at magnification of x10 and x40. Morphological characteristics of fungal isolated were determined and identified using method described by Dorothea, *et al., (*1976*)*. Lengths of hyphae were determined with eyepieces reticule by using colonial and morphological characteristics. Photographs of *Aspergillus species* were taken from mounted slide using camera Lucida at biology laboratory Kano University of science and technology, Wudil.

Statistical Analysis

The data were analyzed statistically using one way analysis of variance (ANOVA) and difference among the means were determined for significance at P< 0.05. This was achieved using computer program (SPSS, 16.0).

RESULT

A total of Three hundred and ninety one fungal isolates were counted during the study at both Wudil and Yan lemo market. *A. niger* was the highest occurring species with 197 isolates accounting to ~~(~~50.31%~~). This was~~ followed by *Aspergillus fumigatus* 89 isolates at ~~(~~*22.25%*~~)~~. ~~The third occurring colonies was A~~*spergillus*. *flavus was third with* 65 isolates at ~~(~~16.62%~~)~~. While the ~~least~~ lowest occurring ~~isolate 40 (10.23%)~~ was *R. stolonifer spp* with 40 isolates at 10.23% (Table 1).

Variation of the colony counted in Wudil and Yan lemo market

Higher numbers of fungal species were isolated at Yan lemo market with 159 (40.66%). While Wudil market recorded 38 (9.71%) species (Table 1). The differences of the colonies counted at Wudil and Yan lemo markets were statistically significant (P< 0.005). During the

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study, high numbers of fungal species were recorded in the second week 106 (27.10%). While the least number of fungal species 86 (21.99%) was recorded in the first week (Table 2).

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Pathogenecity Test

The results of the pathogenicity test confirmed all the four criteria outline in Koch postulates for identification of the causative agent of a particular disease. The pathogen where present in all cases of the disease. The same pathogens was isolated from the diseased host and grown in pure culture. When inoculated into a healthy sample of banana fruit the pathogen from the pure culture causes the same disease. The same pathogen was re isolated from the new host and shown to be the same as the originally isolated pathogen (Table 3).

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Table **1: Number of fungal colonies isolated at Wudil and Yan lemo Markets**

Identified fungi	Wudil Market	Yan lemo market	Total	Mean	%
A. niger	12	185	197	98.5	50.31
A. fumigatus	10	79	89	44.5	22.25
A. flavus	10	55	65	32.5	16.62
R. stolonifer	6	34	40	20	10.23
Total	**38**	**159**	**391**	**195.5**	**99.99**

Table 2: Total Number of Fungal colonies Isolated on weekly basis at Wudil and Yan lemo Markets

Colonies	Weeks 1	2	3	4	Total	Mean	%
A. niger	44	53	44	56	197	98.5	50.31
A. fumigatus	18	29	24	18	89	44.5	22.25
A. flavus	12	19	18	16	65	32.5	16.62
R. Stolonifer	12	5	10	13	40	20	10.23
Total	86	106	96	103	**391**	**195.5**	**99.99**

Table 3: Pathogenicity test after inoculation for 4days on fresh watermelon.

Fungi	*A. niger*	*A. fumigatus*	*A. flavus*	*R. stolonifer*
pathogenicity	+	+	+	+
test	+	+	+	+

Key: + = Isolates grow with a similar growth characteristic features to the original diseased samples

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DISCUSSION

A total of 391 isolates were counted during the study. Out of the isolated fungi *A. niger* was the most frequently occurring isolate with percentage occurrence of 53.31%. This was followed by *A. fumigatus with 22.25% and A. flavus 16.62.* The list occurring isolate was *R. stolonifer* 10.23. Pathogenicity test confirmed the pathogens as originally isolated pathogen

of pine apple on sale at Wudil and Yanlemo markets. More colonies where recorded at Yanlemo then Wudil markets.

The quality of perishables is affected by post - harvest handling, transportation, storage and marketing. This may result in decay and provide suitable condition for invasion by microorganism which may become activated because of the changing physiological state of the fruits (Durgesh *et al.*, 2008).

The findings of this ~~research~~ study ~~support~~ were similar to the report of Baiyewu, *et al.*, (2010) and Chukwuka, *et al.*, (2007) who studied fungal deterioration of some vegetables in Nigeria and found that losses is attributed to the activities of *A. niger*, *A. fumigatus* , *Mucor, and Rhizopus stolonifer*. The finding also agrees with the work of (Hayatu 2000) who isolated, *A. niger, A. flavus, Rhizopus, and Mucor* from samples of vegetables grown at Nassarawa local government area of Kano state.

In the present study *A. flavus* and *R. stolonifer* were the third and fourth occurring colonies with percentage occurrence of 16.62% and 10.23% respectively. The occurrence of these pathogens might be related to their ability to produce resistant spores, as reported by Jay (Droby, 2006; Yahaya *et al* 2016) that spores of *Aspergillus* are more resistant to high temperature. And in conjunction to this, *Aspergillus* species have been implicated in the spoilage of fruits and vegetables in Nigeria (Hayatu, 2000; Yahaya *et al*., 2016). From the isolates counted it was clear that the fungi isolated from pine apple were observed to have been contaminated with multiple pathogen. Bukar *et al*., (2009) reported that temperature and relative humidity of the fungi have a significant effect on the growth of the pathogens and their subsequent relevance to spoilage Yahaya *et al*., 2016; Pawlowska, *et al*., 2012; Tafinta *et al*., 2013).

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The high colony counts obtained at Yanlemo market could be attributed to the damping of waste and spoiled fruits and vegetables ~~in many points~~ within the markets acting as source of contaminants. ~~Therefore, the rotten and spoiled fruits dump around the market might have encouraged the growth of microorganism~~ in addition to discharge of effluent which might have assists in infecting these fields. Such discharge from rotten and spoiled fruits could contain some nutrients that might favour the growth of the fungi as against the lower number of colonies isolated at Wudil market where the area is free from household and industrial effluents (Lelieveld, *et al.*, 2003; Yahaya *et al*., 2016).

Therefore, It can be concluded that the four fungal species namely *Candida albican*, *A. niger*, *A. fumigatus*, and *A. flavus*, are the common post -harvest fungi associated with pine apple on sale at the studied markets. The results obtained in this study indicate that Wudil, area is the most suitable for marketing of fresh and healthy fruits. This is because in Wudil area there is total absence of household and industrial effluents in the area surrounding the market this might have accounted for the least isolate count. Yanlemo site is the least suited for marketing of fruits because; effluents from rotten and spoiled fruits within the market area were the source of infection. The nutrient discharge might contain toxic chemicals that on long time exposure could pose serious health hazards to the consumers of these fruits. Therefore, to safe guard the consumers from buying produce which may be of health hazard effort should be made ensure that all rotten and spoiled fruits are not dump within and surrounding area of the markets. Rather rotten and spoiled fruits should be disposed properly away from the market. Likewise marketing of fruits should be prohibited in any area close to the refused dump. Otherwise the presence and subsequent spoilage due to these fungi, if not checked could lead to serious economic loss and possible health hazards when these fruits are consumed.

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