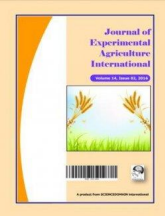

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A Study of Pathogenic Fungi Causing Post Harvest Losses of Pineapple Sold at Wudil and Yan Lemo Markets of Kano State

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SMY, ABK and MUA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ML, YSA, MH, LWH, HA, IS, JA, AYM, SBS, MIH, AIS, AB, MU and TA managed the analyses of the study. Also managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

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 purchased twice a week from both Wudil and Yanlemo markets for four months. The samples were investigated for the presence of fungal pathogen using standard microbiological methods. The methods involve mounting small portion of pineapple in the 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* with (*27%*). The *A. fumigatus* had the lowest frequency of occurrence of (*23%*). The differences between the fungal isolates recorded were significantly different (P<0.05) between the two markets, where higher fungal isolates

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were recorded at Yanlemo market 159 (40.6%) and Wudil 38 (9.71%). The study 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.

Keywords: Fungi; post harvest; incidence; Wudil; Yan lemo.

1. INTRODUCTION

The pineapple (*Ananas comosus*) is a tropical plant with edible multiple fruit consisting of coalesced berries it is the most economically significant plant in the Bromeliaceae family [1,2,3]. 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 [4].

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 fibre *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 [5]. The fibre is also used as a component for wallpaper and other furnishings [5]. The fruit is a good source of vitamin A, B, C and also calcium, magnesium, potassium and iron [5]. 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 [5].

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 [6,7] 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 [7].

Like many other fruits pine apple highly perish product, and the quality is affected by post harvest handling, transportation, storage and marketing [8]. This may result in decay and production of microorganism which become activated because of the changing physiological state of the fruits [9]. It is estimated that 20% of all fruits harvested for human consumption are lost through microbial spoilage [8]. Spoilage of fruit usually occurs during storage and transit and also while waiting to be processed [7,10].

Pineapples are subject to a variety of diseases, which include wilt disease, pink disease, bacterial heart rot, anthracnose, fungal heart rot, root rot, black rot, butt rot, fruit let core rot, and yellow spot virus [7,11]. 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 [12].

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 [8]. The enzymes potential necessary for invading green fruits is greater for ripe ones and it is temporarily beyond the capability of the fungi [8].

Ayanda et al. [13] identified fungi and bacteria as the major organisms causing deterioration of pineapple and other fruits in Nigeria and this occurs by the secretion of extra cellular cell wall degrading enzymes, this factor influences virulence of pathogens. In another related study [14] 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% [15,16]. 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 identifying 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.

2. MATERIALS AND METHODS

2.1 Study Site

2.1.1 Wudil market

Is located at Wudil Local Government Area of Kano state. It is one of the largest markets in Kano state. It has no good storage facilities for fruits including pineapples Some marketers store their grains on rusted basins and hardily use chemicals on their grains.

2.1.2 Yan Lemo market

Is located at Tarauni local government area of Kano state and is similar in form and structure to Wudil market above.

2.2 Experimental Procedure

A survey was carried out to provide information on the incidence of fungal species responsible for the loses of pineapple on sale at Wudil and Yen lemo markets within February and March, 2018.

2.3 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 methods used in sampling and plating follows the one used by [17].

2.4 Sample Handling

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

2.5 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.

2.6 Pathogenecity Test

Pathogenicity test was conducted to prove Koch's postulate. Diseases free pineapple were surface sterilized with 10% (v/v) sodium hypochlorite solution and rinsed three times in running tap water and wiped dry using sterile cloth allowed to dry. A 2 mm diameter cycle was measured on the samples and then 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 the removed portion from disease areas on the 4^{th} day after inoculation and placed on freshly prepared PDA plates and incubated at 25-27°C for three days. Fungal growth that appeared was examined.

2.7 Microscopic Examination

Fungal mycelium was placed on a clean glass slide. 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 x 10 and x 40. Morphological characteristics of fungal isolated were determined and identified using method described by Dorothea, et al. (1976). Photographs of *Aspergillus species* were taken from mounted slide using camera Lucida at biology laboratory Kano University of science and technology, Wudil.

2.8 Statistical Analysis

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

3. RESULTS

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% followed by *Aspergillus fumigatus* 89 isolates at *22.25%). Aspergillus. flavus* was third with 65 isolates 16.62%. While the lowest occurring was *Rhizopu. stolonifer* 40 isolates at 10.23% *spp* (Table 1).

3.1 Variation of the Colony Counted in Wudil and Yan Lemo Market

Higher numbers of fungal species were isolated at Yan lemo market with 159 at 40.66%. While Wudil market recorded 38 at 9.71% species (Table 1). The differences between the colonies counted at Wudil and Yan lemo markets were statistically significant (P< 0.005). During the study period high numbers of fungal species were recorded in the second week 106 at 27.10%. While the least number of fungal species 86 at 21.99% was recorded in the first week (Table 2).

3.2 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 [18]. The pathogen where

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				Total	Mean	%
	1	2	3	4			
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 4 days on fresh pineapple

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 A

present in all cases of the disease. The same pathogens were isolated from the diseased host and grown in pure culture. When inoculated into a healthy sample of pineapple fruit the pathogen from the pure culture causes the same disease [19]. The same pathogen was re isolated from the new host and shown to be the same as the originally isolated pathogen (Table 3).

4. DISCUSSION

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 [7].

In the present study *A. niger* was the most frequently occurring isolate followed by *A. fumigatus,* this findings were similar to the report of [20] and [21] 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 [9] 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 isolates, the occurrence of these pathogens might be related to their ability to produce resistant spores, as reported by [22] and [17] that spores of *Aspergillus* are more resistant to high temperature. And in conjunction with this, *Aspergillus* species have been implicated in the spoilage of fruits and vegetables in Nigeria [21,17]. The result obtained in the present study shows that the pineapple fruits were contaminated by multiple pathogen [23,24,25]. [17] 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.

The high colony counts obtained at Yanlemo market could be attributed to the damping of waste and spoiled fruits and vegetables within the markets acting as source of contaminants in addition to discharge of effluent which might have assisted in infecting the two markets. 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 [17].

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 from rotten and soiled fruits 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 of properly away from the market. Likewise marketing of fruits should be prohibited in any area close to the refused dump of rotten and spoiled fruits. Otherwise, the presence and subsequent spoilage due to these fungi could lead to serious economic loss and possible health hazards during consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tournas VH. Moulds and yeasts in fresh and minimally processed vegetables and sprouts. International Journal of Food Microbiology. 2005;99:71–77.
2. Yahaya SM. Contribution of harvest to pathogenic and non pathogenic losses of vegetables in some selected irrigated areas of Kano State, Msc. Thesis, Bayero University Kano; 2005.
3. Ikhiwili OM. Isolation and characterisation of microorganisms associated with rot diseases of fruit, stem and leaf of *Carica papaya* L. A Project Report Submitted to the Department of Biological Sciences, College of Science and Technology, Covenant University, Canaanland, Ota, Ogun State, Nigeria. 2012;5–6.
4. Coppens d'Eeckenbrugge G, Leal F. Chapter 2: Morphology, anatomy, and taxonomy. In Bartholomew DP, Paull RE, Rohrbach KG. The Pineapple: Botany, Production, and Uses. Wallingford, UK: CABI Publishing. 2003;21.
   [ISBN 0-85199-503-9]
5. Jones J, Wilson W. Chapter 11: Science. An incomplete education. Ballantine. 2006; 544.
   [ISBN 978-0-7394-7582-9]
6. Masefield. Essential of fruits as dietary needs and ingredient in fruit juices and can be eaten raw. International Journal of Biology; 2002.
7. Durgesh PM, Ranjana GK, Varsha KV. Microbiological analysis of street vendect fruits juices from Mumbai City, India. Journal of Food Safety. 2008;10:31-34.
8. Alao SEL. The importance of post harvest loss prevention. Paper presented at graduation ceremony of school of food storage technology. Nigerian Stored Products Research Institute, Kano. 2000; 1-10.
9. Hayatu M. Post harvest physiological studies of some selected members of family solanaceae. Unpublished M. Sc thesis. Bayero University, Kano. 2000;2–25.
10. Al-Hindi RR, Al-Najada AR, Mohamed SA. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. Afr. J. Microbiol. Res. 2011;5(4):443–448.
11. Marin-Cevada Vianey, Caballero-Mellado Jesús, Bustillos-Cristales Rocão, MuñOz-Rojas Jesús, Mascarúa-Esparza Miguel A, Castañ Eda-Lucio Miguel, LóPez-Reyes Lucãa, MartãNez-Aguilar Lourdes, Fuentes-RamãRez Luis E. Tatumella ptyseos, an unrevealed causative agent of pink disease in pineapple. Journal of Phytopathology. 2010;158(2):93–99.
    DOI: 10.1111/j.1439-0434.2009.01575.x
12. Johannessen GS, Loncarevic S, Kruse H. Bacteriological analysis of fresh produce in Norway. International Journal of Food Microbiology. 2002;77:199-204.
13. Ayanda O, Ajayi AA, Olasehinde GI. Isolation characteristic and extracellular enymes detection of microbial isolates. International Journal of Biology, Chemistry Science. 2013;7(2):641-648.
14. NARI. Post-harvest handling technical series. Technical Bulletin, No. 28, Cucumber Post Harvest Care and Market Preparation, National Agricultural Research Institute. 2004;13.

15. Park IK, Kim J, Lee YS, Shin SC. *In vivo* fungicidal activity of medicinal plant extracts against six phytopathogenic fungi. International Journal of Pest Management. 2008;54:63-68.
16. Suleiman MN, Emua SA. Efficacy of four plant extracts in the control of root rot disease of cowpea (*Vigna unguiculata* [L.] Walp). African Journal of Biotechnology. 2009;8:3806-3808.
17. Yahaya SM, Fagwalawa LD, Ali MU, Lawan M, Mahmud S. Isolation and identification of pathogenic fungi causing deterioration of lettuce plant (*Lactuca sativa*). A case study of yankaba and sharada vegetables markets. Journal of Plant Science and Research. 2016; 3(1).
18. Yahaya SM, Abubakar Y, Ali MU, Lawan M, Ajingi YS, Haruna M, Mardiyya AY. Fungal infection of banana (*Musa sapientum)* sold at wudil and yanlemo markets of Kano state. Dutse Journal of Pure and Applied Sciences. 2018;4(1):254-262.
19. Linling L, Zheng L, Juan H, Hua C, Zhiqin L, Shuiyuan C, Qing T. Isolation and identification of pathogenic fungi causing decay in luotian chestnut during late storage period and research on pathogenicity. Plant Diseases & Pests. 2013;4(6).
20. Baiyewu RA, Amusa NA, Ayoola OA, Babalola OO. Survey of the postharvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya* L.) in South Western Nigeria. Afr. J. Agric. Res. 2007;2(4):178–181.
21. Chukwuka KS, Okonko IO, Adekunle AA. Microbial ecology of organisms causing pawpaw (*Carica papaya* L.) fruit decay in Oyo State, Nigeria. Am. Eurasian J. Toxicol. Sci. 2010;2(1):43–50.
22. Droby S. Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Hortic. 2006;709:45–51.
23. Bukar A, Mukhtar MD, Adamu S. Isolation and identification of postharvest spoilage fungi associated with sweet oranges (*Citrus sinensis*) traded in Kano metropolis. BAJOPAS. 2009;2(1):122-124.
24. Pawlowska AM, Zannini E, Coffey A, Arendt EK. Green preservatives: Combating fungi in the food and feed industry by applying antifungal lactic acid bacteria. Advance. Food Nttural Resource. 2012;66:217–238.
25. Tafinta IY, Shehu K, Abdulganiyyu H, Rabe AM, Usman A. Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) fruits in Sokoto State. Niger. J. Basic. Sci. 2013;21(3):193–196.

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