MYCOLOGICAL STUDIES ON POST HARVEST DETERIORATION OF PINEAPPLE (*Ananas comosus* L. Merr.) FRUITS IN KEFFI, NASARAWA STATE

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Abtract: Post harvest deterioration of Pineapple (*Ananas comosus*) fruits in Keffi was studied. Eighty decayed fruit samples were collected from four (4) different locations in Keffi Local Government Area. The locations were Keffi Market, Angwan Lambu, Angwan Kaje and Angwan Fulani. Out of 80 decayed fruit samples collected 51 fruits had isolates. The fungal isolates were *Rhizopus stolonifer, Aspergillus niger, Penicillium sp, Rhizopus nigricans.* The frequencies of occurrence of these isolates are 47.06% 33.33%, 11.76% and 7.84%, respectively. Keffi market had the highest incidence of pathogens (29.41%) while Angwan Kaje had the least incidence of pathogens (17.65%). There was no significance difference (P>0.05) on the frequency of occurrence of the isolates in relation to locations in Keffi Local Government Area. Pathogenicity test carried out showed that *Rhizopus stolonifer* and *Aspergillus niger* were causal organisms while *Rhizopus nigricans* may have been a mere contaminate. Factors that enhance the spread of these organisms in these areas should be controlled.

Keywords: Fungi, deterioration, pineapple fruits, Keffi

Introduction

Pineapple (*Ananas comosus*) one of the genus *Ananas* in the family of Bromeliaceae and are known to have commercial and nutritional importance. They play a vital role in human nutrition by supplying some necessary substances such as vitamins and essential mineral in human daily diet that can help to keep a good and normal health. Pineapples are widely consumed. One of the factors that impact negatively on the economic value of pineapple is that they have a short shelf-life. This is as a result of many factors prominent among which is the activity of pathogens. It has been reported by Zhu (2006) that 20 – 25% of harvested pineapple fruits are lost via the activities of pathogens during harvested chain (Amao *et al*., 2011). Pineapple is a perennial shrub, which grows to 1.0 to 1.5 meters (3.3 4.9 ft) tall, although sometimes it can be taller. In appearance, the plant itself has a short, stocky stem with tough, waxy leaves. When developing its fruit, it usually produces up to 200 flowers, although some large-fruited cultivars can exceed this. Once it flowers, the individual fruit of the flowers join together to create what is commonly referred to as a pineapple.

After the first fruit is produced, side shoots (called 'suckers' by commercial growers) are produced in the leaf axils of the main stem. These may be removed for propagation, or left to produce additional fruits on the original plant (Davidson, 2008). Pineapple (*Ananas comosus*) is ranked the third most important tropical fruit in the world. Nigeria is the sixth in the list of world pineapple producers with about 800,000 tonnes per annum (Amao *et al*., 2011). Pineapple fruits are exposed to contamination by microbes through contact with soil, dust and water by handling at harvest or during postharvest processing. This makes them harbour wide range of microorganisms including plant and human pathogens (Eni *et al*., 2010). Therefore this study is aimed at isolating and identifying fungi associated with deterioration of pineapple (*Ananas comosus*) fruits in Keffi, Nasarawa State-Nigeria.

Materials and Methods

Study area

The laboratory experiments were carried out at Biotechnology Laboratory, Plant Science Unit of Biological Sciences Department, Nasarawa State University, Keffi. The survey was carried out within four Locations in Keffi Local Government Area. These locations are; Angwan Lambu, Angwan Kaje, Angwan Fulani and Keffi Market.

Sample collection (pineapple; Ananas comosus)

Samples of decayed Pineapple fruits were collected from four locations in Keffi Local Government Area. These pineapple fruits were obtained from regular pineapple retailers in these locations. A total of 80 pineapples were collected at random from these locations, which lasted for four months. During the first visit in the month of February five decayed pineapple fruits were collected from each location, making a total of twenty pineapple fruits for that month. Hence by the month of May total of 80 decayed pineapple fruits were obtained. Each visit constitutes a replicate. Hence, there were four replicates.

Preparation of sterile Agar medium

39 g of Potato Dextrose Agar (PDA) powder obtained from Sigma-Aldrich USA as dissolve in 1 litre of warm distilled water as instructed by the manufacturer. The sample was then heated gently over the Bunsen burner until the agar starts to boil. Stirring was done through out to dissolve all agar powder. This was ploughed with cotton wool. The flask of agar was then placed together with clean petri dishes in the autoclave (YX-280B, U Clear England). The agar and the dishes were sterilized by heating to about 120^{o}C for 15 min at 15 ponds pressure. The flask of agar was then allowed to cool (Ogaraku *et al*., 2012).

Pouring sterile Agar plates

The sterile petri dishes were placed on a flat surface. Cotton wool was removed from the agar and the flask was passed through flame. The lid of the sterile petri dishes was shifted as little as possible and about 0.5 m of agar was poured in to the petri dish. The lid was then replaced on the petri dish by passing the mouth of the flask through the Bunsen flame and the cotton wool is then replaced.

This was repeated for the number of dishes needed. The dishes were not distorted until the agar was set and this took about (10) min. It was allowed to cool and solidify (Ogaraku *et al*., 2012).

Inoculating the sterile Agar plates

An inoculating loop was passed through a Bunsen burner (Spiritual flame) and cooled for a few seconds. Microorganisms from the deteriorating pineapple fruits were sampled in the sterile plates using the loop. Each plate was sealed with adhesive tape and labelled. This process was repeated twice for each of the locations (Haq and Dania, 1995).

Incubation of Agar sterile plates

Plates were incubated in an incubator at 30^{o}C which was maintained for seven days. The plates were placed upside down to prevent condensation. Fungi isolation was done using Dongmo and Oyeyiola (2006) method and fungi identification was done using Domch *et al*. (1980) method. Here photomicrography of each isolate was obtained and compared with a standard published by Domsch *et al*. (1980).

Pathogenicity test

To establish which of the microbial isolates caused the deterioration, 2 cm long cylindrical covers were removed from the middle portion of healthy sterilized fruits. The fruits were first of all washed with 2% sodium hypochlorite and allowed to dry. Discs, 5 mm in diameter of two-day old fungal cultures of each isolate were inoculated, fungi first, into the holes made on the fruits with cork borer. The covers of the fruits were replaced after 5 mm pieces had been cut off to compensate for the thickness of the fungal culture (Ogaraku *et al*., 2012).

Results and Discussion

From the laboratory results obtained, 80 pineapples fruits were collected and sampled, out of which 51 pineapple fruits had fungal isolates while 29 pineapples fruits had no fungi species (Table 1). Some of the fungi species isolated and identified from the deteriorated pineapple (*Ananas comosus*) fruits include; *Rhizopus stolonifer, Aspergillus niger, Penicilium spp, and Rhizopus nigrican*. The result showed that pineapple fruits in Keffi, Nasarawa State, Nigeria suffer from different infection by fungi. The fungi that cause the infections are well known and have been reported in some countries and states in Nigeria (Makun *et al.*, 2009). Reports have showed that fruits are rotten by organisms that either infect the produce while still immature or attached to the plant or during harvesting and subsequently handling and marketing.

Table 1: Incidence of fungi species in locations of Keffi Local Government Area

Location	Total No. of spp	No. with Fungi spp	No. without Fungi spp
Keffi Market	20	15 (29.41)*	5 (17.24)*
Angwan Lambu	20	13 (25.49)*	7 (24.13)*
Angwan Kaje	20	14 (27.45)*	6 (20.68)*
Angwan Fulani	20	9 (17.64)*	11(37.93)*
Total	**80**	**51**	**29**

(Number in parenthesis are expected frequencies and "No" stands for number)

According to this report, rotting is greatly aided by mechanical injuries on the surface of produce such as finger nail scratches, abrasions, insect punches, abscission layers and cut stems. The frequencies of occurrence were 47.06 % 33.33%, 11.76% and 7.84%, respectively (Table 2). Keffi market had more fungal attack (29.41%) than other locations while Angwan Kaje had the least (17.65%) fungal diseases generally (Table 3). The high incidence of these diseases in Keffi can be attributed to high incidence of economic activities in this area. The population of residences is more compared with other locations resulting in the handling and marketing of the pineapple fruits. It may also be attributed to variation in geographical terrain and local micro climate condition (Wallbrige, 1981). The distance, means of transport and length of time the fruits were kept in the market condition after harvest may also be responsible for these variation in incidence of occurrence (Onyeka *et al*., 2003). Genetic composition of the pineapple fruits may also have played a prominent role in its low and high incidence (Onyeka *et al*., 2003), this is because the genetic composition would have being altered by environmental factors, such as high relative humidity, which predisposed the fruits to the attack of the fungal species isolated in this work.

Table 2: Frequency of occurrence of fungi isolates from different locations

Location	*Rhizopus stolonifer*	*Asergillus niger*	*Penecillum* sp	*Rhizopus nigrican*	Frequency of attack
Keffi market	7	4	3	1	15
Angwan Lambu	5	6	1	1	13
Angwan Kaje	9	4	0	1	14
Angwan Fulani	3	3	2	1	9
Total	**24**	**17**	**6**	**4**	**51**
% Total	**47.06**	**33.33**	**11.76**	**7.84**	

Table 3: Incidence of occurrence of fungi isolate from different locations in Keffi

Location	*Rhizopus Stolonifer*	*Aspergillus niger*	*Penecillium* spp	*Rhizopus nigrican*	Frequency	Mean	% incidence
Keffi Market	7	4	3	1	15	3.75	29.41
Angwan Lambu	5	6	1	1	13	3.25	25.49
Angwan Kaje	9	4	0	1	14	3.50	27.45
Angwan Fulani	3	3	2	1	9	2.25	17.65
TOTAL	24	17	6	4	51		
Mean	6.00	4.25	1.50	1.00			
%Total	**47.06**	**33.33**	**11.76**	**7.84**			

Also, the frequencies of occurrence of fungi deterioration of pineapple fruit from different locations showed that *Rhizopus stolonifer* had the highest frequency of occurrence (47.06%), while *Rhizopus nigricans* had the least with (7.84%) frequency of occurrence. This could be due to the production of wide range of enzymes by *R. stolonifer* more than the rest species. The presence of the above mentioned fungi are of public health and economic importance. Some of fungal pathogens produce Mycotoxins which are hazardous to human and animal health (WHO, 1979). *Aspergillus* species are known to cause infection of the respiratory track, *Aspergillus* species are also used to produce soya sauce by fermentation of soya beans, also used to produce citric and galli acids which serve as additive during the manufacture of different products from ink to chewing gum (WHO, 1983).

Table 4: Chi-square on the relationship between fungi isolates and different locations in Keffi Local Government Area

Location	No. with fungi spp	No. without Fungi Spp	Total
Keffi Market	13 (12.75)*	7 (7.25)*	20
Angwan Lambu	15 (12.75)*	5 (7.25)*	20
Angwan Kaje	14 (12.75)*	6 (7.25)*	20
Angwan Fulani	9 (12.75)*	11 (7.25)*	20
TOTAL	**51**	**29**	**80**

*(Numbers in parenthesis are Expected Frequencies and "No" stands for Number)

Table 5: Percentage infection of pineapple fruits artificially inoculated with fungi isolated from diseased fruits

Fungal isolate	No of fruits inoculated	% infection after 5 days
Aspergillus niger	5	80
Rhizopus stolonifer	5	100
Penicillium sp	5	60
Rhizopus nigricans	5	00

Pathogenicity test carried out indicated that *Rhizopus stolonifer* had the highest infection (100%) after five days, followed by *Aspergillus niger* which had (80%) while *Rhizopus nigrican* had no infection (0%) (Table 5) Pathogenicity test carried out showed that *Rhizopus stolonifer* and *Aspergillus niger* were causal organisms while *Rhizopus nigricans* may have been a mere contaminate. Economically, the retailers of pineapple fruits in Keffi Local Government Area are deprived of a reasonable percentage of their produce as some fruits decay before reaching the consumers.

Ho: The frequency of occurrence of fungi species is independent of different locations in Keffi Local Government Area.

Since the tabulated value is greater than the calculated value there we accept the hypothesis i.e X^2 tabulated $7.81 > X^2$ calculated 4.49.

Conclusion

During the period of the survey to determine fungi responsible for the decayed pineapple fruits (*Ananas comosus*) from four different locations of Keffi, Nasarawa State, the highest fungal decay came from Keffi Market (29.41%) of fungi decay while Angwan Kaje had the least (17.65%) of the fungi decay. From the frequency of occurrence of fungi decay of pineapple fruits of various locations *Rhizopus stolinifer* had the highest (47.06%) frequency of occurrence. The most common fungi species affecting pineapple fruits are *Rhizopus stolonifer* and *Aspergillus niger*, these two species are prevalent in this area. Measures should be carried out through genetic engineering to improve on the storage capacity of pineapple in this area.

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