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Isolation and Identification of Rot Fungi Associated with Fruits Sold in Some Markets within Kano Metropolis, Nigeria

I. Sani[*], A. S. Kutama[**] and A. A. Nuhu[***]

[*]*Department of Plant Biology, Bayero University, Kano, NIGERIA*
[**]*Department of Plant Biology, Bayero University, Kano, NIGERIA*
[***]*Department of Biological Sciences, North West University, Kano, NIGERIA*

Email ID: saibmuk@gmail.com

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ABSTRACT: The study was carried out between July and August, 2009. Totally ten commonly sold spoilt fruits (orange, banana, pineapple, date palm, tomato, garden egg, water melon, guava, Shear butter, and coconut), were collected from retailers at Rimi, Kurmi and Kofar Wambai markets in Kano Municipal local government. The samples outer surfaces were disinfected with 0.1g/lit sodium hypochlorite solution and then homogenated using distilled water in a blender. The homogenates were cultured on potato dextrose agar (PDA) plates and incubated aerobically at room temperature for 7 days. Pure cultures of the resulting fungal colonies were obtained from primary plates and identified by using morphological and microscopical characterizations. All the samples were infected with one or more fungal species such as *Aspergillus, Penicilium, Rhizopus, Mucor, Alternaria Cladosporium, Neurospora* and *Fusarium*. Among this, the most predominant pathogenic fungus was *Aspergillus.* Pathogenicity test with isolated fungi on fresh fruits showed that some were found to grow and cause spoilage. Proper handling from the farm and storage and as well as avoiding the mixing of spoilt and good fruits were identified to be important factors that could be helped to prevent contamination and loss. Fumigation with chemicals like sulphur dioxide, washing with fungicides or hot water and use of standard storage facilities were also recommended to prevent economic loss due to fungal pathogens. Analysis of variance at (P<0.05) showed no significant difference between the frequency of occurrence of the fungal isolates. This indicates that any of the isolates has the potential to cause infection on any fruits

Keywords: Contamination; Disinfection; Fruits; Fungi; Storage.

INTRODUCTION

Contamination of fruits and other defects of fresh fruits are termed “market disease”. Losses due to market diseases amount to millions of naira in Nigeria, and molds are responsible. Although the surface of fresh fruits harbors large number of yeasts and molds, yeasts generally lack means to invade and infect plant tissue and therefore, secondary rather than primary agent of spoilage[1]. Infection of fruits and subsequent rotting can occur in orchard and vineyard as well as during different marketing steps which include the harvest, grading, packaging, transport, storage and various manipulations in the wholesale and retail markets[2]. Contamination of fruits during harvest as well as growth of fungi in lesions and other damaged areas is largely responsible for the large populations that are often observed. For example, commercially harvested concord grapes yield an average count of 68,000/g (mostly yeast) compare to the population of only 38,000/g in fruits that have been collected and transported under relatively specific condition[3]. Fruits play an important role in the diet of people by providing essential vitamins and minerals that aid in the smooth of body metabolic activities[4]. However, most fruits are seasonal, the product is easily attacked by microorganisms under common storage conditions; and hence the huge waste especially where storage facilities are poor or lacking. Lack of sound method of processing and poor harvesting techniques have resulted in the non availability of both fruits and associated products. Thus, deterioration of stored fruits is a major contributor to economic problems on an individual and to a larger

extent, on a Nation[5]. The recent scientific manipulation of factors necessary for delaying growing of microorganisms for preservation of fruits and fruit products has created a great impact in improving the standard of people[6]. The goal of food microbiology is to develop ways of improving the quality of foods which basically means the control of chemical, physical and microbiological changes during storage and processing[7]. Microbial spoilage of fruits and vegetables have been attributed to plant pathogens acting on roots, stems leaves, flower, fruits and other parts used as food and to saprophytic organisms which may be secondary invaders after the action of pant pathogens, or which may enter healthy fruits or vegetables or grow on its surface[8].

Over 55% of the edible fruits sold in the fresh fruits market of Northern Nigeria are not cultivated in that region. They are continuously being imported from the Southern part of the Country where the climate and other environmental variables are conducive for their growth and cultivation. These fruits suffer from a lot of post – harvest handling damages. Erinle[9] reported that depending on the mode of transportation, the fruits are packed in baskets, cardboards, tins, boxes, wooden crafts and trays. In large area of production, motorized handling is usually needed. Harvey[10] classified post-harvest losses based on the casual agent as mechanical, physiological, pathogenic and chemical. Reuben[11] observed that slight bruising creates favorable conditions for decay organisms to get established.

Therefore, this study aims to isolate and identify the common fungal fruits sold in Kano metropolitan markets, to suggest some possible ways of reducing spoilage of fruits in Nigeria and also find out the most predominant fungal pathogens.

MATERIAL AND METHODS

Study Site: Three major market sites were selected in the Kano Municipal; Rimi, Kofar Wambai and Kurmi markets. The sites were selected because they serve as the main deports of Kano Municipal markets where the main fruits dealers and suppliers sell to petty traders who in turn circulate the product. Kofar Wambai is situated along the IBB road, Rimi market along Emir Palace road, opposite Murtala Muhammad General Hospital, while Kurmi market is situated near Jakara Hospital within the State central zone.

Sample collected: A total of 10 different diseased fruits were selected from each market. The sampling was done randomly selecting five retail points[12]. Diseased fruits were identified by physical examination following the method of [13]. Various types of spoiled fruits were selected; those that look sunken and shriveled those with different coloured lesions with water soaked appearance around the wound were collected. Similarly, healthy ones were collected for use as control.

Sample Preparation: The method of [14] was adopted for the preparation of the samples, isolation and identification of fungi. Diseased fruits were cut from the lesion edge with a disinfected knife. The cut portions of the lesion were disinfected with 0.1g/lit sodium hypochlorite solution containing 2% chlorine for 5 minutes. They were rinsed in three changes of distilled water and homogenized using blender. The homogenate was serially diluted with normal saline solution (8.5% Nacl in water) using sterile pipettes.

Media Preparation: Potato Dextrose Agar (PDA) was prepared and sterilized by autoclaving at 121^0C for 15 minutes. Then it was transferred in to sterile Petri plates (20ml each) and allowed for solidification.

Isolation of the Fungi: 0.1 ml of serially diluted aliquots of each samples were spreader over PDA plates containing 0.3g/lit chloramphenicol and cyclohexamide for the prevention of bacterial contaminations using sterile disposable microsyringe (SAM). All the plates were incubated at 30^0C for 6-7 days. The fungal colonies developed over the medium were purified and maintained in PDA medium

Identification of the Isolated Fungi: The cultural characteristics of the isolate was determined after incubation at 30°C for 6-7 days by using culture techniques of [14], identification was carried out, thus; small blocks (about 1.0cm^2) of sterile SDA were aseptically cut and transferred in to the centre of sterile slide. The edge of the block was inoculated with the scrapings of the fungal isolate and covered. The set up was incubated at 30^0C for 5-7 days. When a typical structure developed, small portion of it was placed on a slide using wire loop and stained with drops of 0.5% lactophenol blue solution and viewed under microscope.

Pathogenicity Test: Healthy fruits were surface disinfected using 70% ethanol and three holes approximately 0.5mm were made with a flamed cooled wire loop. Scrapings of the isolated colonies were inoculated into the holes. Both infected and uninfected fruits were kept separately for 7-14 days at 30^0C[15]. After incubation, the results were observed.

RESULTS AND DISCUSSION

A total of 486 fungal isolates were obtained from the ten different spoilt fruits from all the three markets screened. The isolates were classified by grouping into eight taxonomic genera namely; *Aspergillus, Rhizopus, Mucor, Alternaria, Neurospora, Penicillium, Cladospirium and Fusarium.*

The diseased fruits were found to be heavily infected with eight genera of fungi. The study has also attempted to examine the influence of these pathogens on healthy fruits. It indicated that there are a number of fungal pathogens which are responsible for various post-harvest disease i.e. storage disease pathogens in fruits. This is in accordance with the report of [16] that different genera of fungi which are pathogenic to the fruits live in the necrotic areas and cause disease in human if consumed directly. Of the eight identified genera in this study, the most frequently occurring genus was *Aspergillus* found in all the ten fruits obtained from each market. This result is in agreement with the report of [17] on fruit rot caused by fungal pathogens and the work of [18] on isolation and identification of pathogenic fungi associated with fresh edible fruit. Correspondingly,[19] reported that *Aspergillus, Penicillium* and *Alternaria spp* at various degree of frequency when determining pathogens found in pawpaw and orange. Further, [20] reported that high temperature favors the growth of these pathogenic fungi while hot and humid weather confer the conducive atmosphere. He also reported that the cool temperature and dry or low humidity reduces the rate of the multiplication of the fungal propagules. This might be the reason why fruits when kept in refrigerator last longer despite their being perishable items. This is true going by an earlier report of [21], reported that metabolic activities generally decrease with decline in temperature.

Table 1: Fungal isolates obtained from Kurmi, Rimi and Kofar Wambai markets and their relative abundance (%)

Market	Kurmi		Rimi		Kofar Wambai	
Isolate	**No. of isolates**	**Relative abundance (%)**	**No. of isolates**	**Relative abundance (%)**	**No. of isolates**	**Relative abundance (%)**
***Alternaria* spp**	15	8.7	15	13.0	11	5.8
***Pencillium* spp**	14	8.1	1	0.9	22	11.6
***Fusarium* spp**	6	3.5	11	9.6	5	2.7
***Aspergillus* spp**	29	16.9	36	31..3	55	29.1
***Mucor* spp**	44	25.6	16	13.9	36	19.0
***Rhizopus* spp**	39	22.7	27	23.5	37	19.6
***Neurospora* spp**	15	8.7	4	3.5	17	9.0
***Cladosporium* spp**	10	5.8	5	4.3	6	3.2
Total no. of isolates	172	100	115	100	189	100

Table 2: Pathogenicity test on different plants

Sample	Isolates							
	***Mucor* spp**	***Rhizopus* spp**	***Aspergillus* spp**	***Penicillium* spp**	***Fusarium* spp**	***Alternaria* spp**	***Cladosporium* spp**	***Neurospora* spp**
Banana	+	+	+	+	+	+	+	+
Coconut	+	+	+	+	+	+	+	+
Orange	+	+	+	+	-	+	+	+
Garden egg	+	-	+	-	-	-	-	+
Tomato	+	+	+	+	+	+	+	+
Date palm	-	+	-	-	-	-	-	-
Water melon	+	+	+	+	+	+	+	+
Shear butter	+	+	+	+	+	+	+	+
Guava	+	+	+	+	+	-	+	-
Pineapple	+	+	+	+	+	-	-	-
Control								

+ = isolate able to cause infection (virulent), - = isolate unable to cause infection (inanimate).

The results have also indicated that pathogens responsible for post–harvest disease in all the fruits are the same irrespective of the sampling sites; this is contrary to the work of [17] in which the organisms responsible for the post harvest disease in orange and banana are entirely different from those in pawpaw and pineapple.

The test of Pathogenicity on healthy fruits of different pathogenic isolates has shown that there were considerable variations in the diameter of the lesions produced by each pathogen on each fruits. Also some were able to cause damage on re-infection (virulent) while some were not. According to report of [22], this could be due to the inability of independent growth and survival on the fruits and some organisms might be dependent on the break down products of other spoilage–causing organisms on condition for survival.

It must be realized that good storage merely limits storage losses of good product over relatively long period of storage. The sellers were observed to practice different method of storage. However, in spite of the method adopted, spoilage was still recorded. Statistical analysis (ANOVA) had shown that the effect of pathogens is virtually the same on all fruits, hence, no significant difference ($P<0.05$) was observed between the frequency of the occurrence of the fungal isolates on the different fruits.

CONCLUSION

Conclusively, the study revealed that different fungi from different taxonomic genera were responsible for post-harvest storage disease in different fruits. Therefore, the present of pathogenic fungi on fruits remains significant to public health concern and also these might be vehicle for transmitting food-borne diseases.

Recommendations:

- An improved or modern means of transportation should be facilitated in order to avoid delay during transportation from farm to the selling points.

- Modern storage facilities should be provided to prevent horizontal contamination by the already spoilt fruits.
- Containers for carrying the fruits are advised to be disinfected.
- Harvest at right stages of maturity would also help to avoid excessive mold spoilage.
- In case of tomatoes, people should stop using the so-called "Gwale Gwaje". Because they are highly contaminated, and can cause a serious health implication which may even lead to death.

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