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POINT OF MARKETING SURVEY FOR FUNGI ASSOCIATED WITH SPOILAGE OF ORANGES DISTRIBUTED AT SOME DEPOTS IN KANO NIGERIA

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ABSTRACT
The recommendation of Food and Agricultural Organization (FAO) and the National Agency for Food, Drug Administration and Control (NAFDAC) prompts the current survey. This was with the aim of appraising health risk of oranges (Citrus sinensis) contaminated with fungi as well as generating a clue towards identifying the species involved. Accordingly, four markets, Naibawa, Jakara, Rimi and Brigade considered as Orange marketing depots in Kano Metropolis, Nigeria were screened. Five orange samples showing evidence of blemish such as moldy, decay and sunken rind were collected from each market. Samples were processed through decontamination of the outer rind with 75% ethanol, Fungal enumeration was carried out by plating on the potato dextrose agar (with chloramphenicol antibacterial) in duplicates and incubated aerobically at room temperature. Evidence of fungal growth was monitored for 1-7 days. Pure isolates of the fungi with the help of macroscopy and wet mount of mycelial specimen at x 40 objective power were conducted. Species were confirmed with the help of Atlas. Pathogenicity test was undertaken through Kotch's postulates for symptoms of contamination and spoilage within 3-7 days. The findings confirmed involvement of Penicillium spp in 50%, Aspergillus spp in 25%, Fusarium spp at 17.5% frequency. Rhizopus spp showed 7.5% proportion. It was concluded that, oranges dispensed have shown a preponderance of spoilage fungi indicating health hazard, poor quality and can lead to economic losses. Better post-harvest and storage handling, Quarantine by Consumer Protection Agencies are recommended.
Keywords: Citru sinensis, Fungi, Spoilage, Oranges, Market Survey

INTRODUCTION

Fruits are very important for their high dietary and nutritional qualities. Fruits play a vital role in human nutrition by supplying the essential growth factors such as vitamins and minerals to the regular diet, which is necessary for the good and normal health consumption of fruit and products has dramatically increased by more than 30% during the past few decades (Barth *et al.,* 2009). Fresh fruit and consumption increased by 25.8 and 32.6% respectively and far exceeded the increases observed for processed fruit and products. Fruits and vegetables are the major source of macronutrients such as a fiber and micronutrients such a minerals, vitamin C, thiamin, riboflavin, B-6 niacin, folate A and E (Rickman *et al.,* 2007).

Traditional varieties of fruits like avocado, banana, orange and papaya are affected by a wide array of microorganisms. 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 (Akinmusire, 2011). One of the limiting factors that influence health and economic values of the fruit is the relatively short lifespan. Fruits deteriorate rapidly after harvest and in some cases do not reach consumers at optimal quality after transport and marketing. The main causes of fruit deterioration are dehydration, weight loss, color change and microbial spoilage. However, deterioration rate is affected by different factors like temperature, relative Humidity and storage atmosphere condition (Sherratt *et al.,* 2006).

Available literatures revealed that the importance of fruit is increasing daily, and the incidence of microbial attack on this fruit demands attention (Janisiewicz and Korsten, 2002).

Fungal contamination of fruits and or infection may occur during the growing season, harvesting, transportion post-harvest, storage and marketing conditions, or after purchased by the consumer. The important post-harvest disease causing fungi include *Penicillium* spp*, Aspergillus* spp, and *Rhizopus stolonifer* spp (Ogawa *et al.,* 1995). Fruits particularly orange are eaten raw. Spoilage fungi that typically produce more diverse and greater amounts of extracellular depolymerases successfully attack fruits (Barth *et al.,* 2009). More so, an abundance of extracellular pectinases and hemicelluloses produced by the fruits are important factors for fungal spoilage (Miedes and Lorences, 2004).

The recommendation of Food and Agricultural Organization (FAO) and the National Agency for Food, Drug Administration and Control (NAFDAC) prompts the current survey. This was with the aim of appraising health risk of oranges (*Citrus sinensis)* contaminated with fungi as well as generating a clue towards identifying the species involved even at the point of market.

MATERIAL AND METHODS

Survey of Orange Sales Depots

The investigation was conducted in Kano city, Nigeria within the period of June to July, 2013. It is one of the largest fruit marketing depots servicing especially North-western Nigeria. Popular foci include Naibawa –Yan'Lemo, Brigade, Jakara, Rimi market, Yankaba and Sabon gari markets. Observation on storing facilities, personal hygiene of sellers and environmental sanitation in the sites were monitored according to methods adopted by Sherratt *et al.,* 2006. Specific points for collection of samples were selected based on the approved consent of the sellers in the markets.

Samples collection

Four markets, Naibawa, Jakara, Rimi and Brigade considered as Orange marketing depots in Kano Metropolis, Nigeria were screened. Type of orange with the common post-harvest disease systems were collected by purchasing as any other buyer and packaged in sterile polyethylene bag. Five orange samples showing evidence of blemish such as moldy, decay and sunken rind were collected from each market. Samples were processed through decontamination of the outer rind with 75% ethanol.

Isolation of fungi

Five orange samples showing evidence of blemish such as moldy, decay and sunken rind were collected from each market (Bukar *et al.,* 2009). Samples were processed through decontamination of the outer rind with 75% - 90% ethanol. Fungal enumeration was carried out by plating on the potato dextrose agar (with chloramphenicol antibacterial) in duplicates and incubated aerobically at room temperature. Evidence of fungal growth was monitored for 1-7 days. Isolation for pure cultures of the fungi with the help of macroscopy and wet mount of mycelial specimen at x 40 objective power were conducted. Species were confirmed with the help of Atlas. Pathogenicity test was undertaken through Kotch's postulates for symptoms of contamination and spoilage within 3-7 days (Oviasogie *et al.,* 2015).

Fruit of orange was surfaced sterilized by exposing them in 90% ethanol for 1 min and 1% sodium hypochloride and then it was rinsed three times in sterile distilled water, segment (3-5cm) was cut with a sterilized scalpel was placed in previously prepared potato dextrose agar (PDA) in Petri dish and the plate were all incubated at ambient temperature for 5 to 7 days (Effiuvwevwere, 2000).

To get a pure culture, each of the emerging mycelium was transferred to fresh solid medium. Pure cultures of each colony on different plate were obtained and they were prepared for characterization (Alhindi *et al.,* 2011).

Characterization of the isolate

The pure isolated fungi were identified using cultured and morphological features according to the most documented key in fungal (Klich, 2002); Samsun and varga, 2007). The identification of fungal isolates was based on colony characteristic and conidial features of the isolate within genus level.

The isolate were also identified by comparing their characteristic with those of known taxa as described by Jolt *et al.,* (1994) and Oyeleke and Mange (2008). Accordingly, slide cultures were prepared and for each of the isolate the identified fungi were smoothly caught (2-3cm) with sterilized scalpel and placed on slide. The technique adopted by Bukar *et,*al (2009) was applied for identification of the unknown isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on a clean slide with the aid of mounting needle where a small portion of mycelium from the fungal culture was removed and place on drop of lactophenol cotton blue stain.

The mycelium spread very well on the slide with the aid of the needle. A cover slip was gently applied with little pressure to eliminate bubbles. The slides was mounted and observed under compound light microscope at x10 and x40 power of objectives respectively.

RESULTS

During market survey, poor handling practices in supply chain, storage conditions, distribution, marketing practices and transportation were observed. From this personal observation the poor hygienic conditions of the vending house/store, the venders and the utensils used for keeping and weighing the fruits were in deplorable conditions, unacceptable to the standard earmarked by the Food and Agricultural Organizations. Factors of mixing the deteriorating fruits with the healthier one and exposure to flies and dumping for many days in polythene bags at higher temperatures before sales were very common in the market. This could have aggravated prevalence of fungi as the spoilage organism of oranges in the four markets studied.

The main suppliers of fresh oranges were from Benue, Rivers, Cross rivers, Enugu, Imo, Kogi, Ekiti, Ondo, Ogun and part of Kaduna and Plateau states among others in Nigeria. This means the spoilage could be along transit or haulage period from farm to market.

The findings confirmed involvement of *Penicillium* spp in 50%, *Aspergillus* spp in 25%, *Fusarium* spp at 17.5% frequency. *Rhizopus* spp showed 7.5% proportion. It was observed that, oranges dispensed have shown a preponderance of spoilage fungi indicating health hazard and poor quality (Table 1).

The physical observation of the diseased fruit revealed brownish, necrotic patches on the skin of the oranges. From this outcome, *Penicllium* and *Aspergillus* were the dominant spoilage species of oranges whereas, *Rhizopus* spp, *Fusarium* spp were found in lower frequencies (Table 2 & 3).

Table1: Fungal Contamination of Oranges Sold in some markets in Kano Nigeria (June, 2013)

s/n	Sampling site	Number of sample collected	Contamination Rate (%)
1.	Naibawa market	5	100
2.	Rimi market	5	100
3.	Jakara market	5	100
4.	Brigade	5	100
	Total		

Table 2: Physical and morphological characteristic of fungi associated with orange (fruit) in some markets in Kano (June, 2013)

S/N	Physical appearance	Microscopic morphology	Identified genus
1.	Greenish colonies	Conidia in long chain on repeatedly branched conidiophores resembling brush like head.	*Penicillium spp*
2.	colonies with loose white to yellow mycelium rapidly becoming dark brown	Black, brownish black, purple brown conidiophores and yellow to green conidia with dark sclerotia.	*Aspergillus spp*
3.	Orange pink	Septate sickle cell like conidiosphore	*Fusarium spp*
4.	White to dark grey colonies, fast growing and filling the prtri dish with dense cottony mycelium, producing mass of sporangia.	Noseptate mycelium with root like rhizoids: black columellate, sporangiasphore in clusters and dark sporangia containing dark to pale spores.	*Rhizopus spp*

Table 3: Percentage proportions of the predominant fungal isolated in the orange fruits examined

S/N	Species name	Percentage proportion
1	*Penicillium spp*	50%
2	*Aspergillus spp*	25%
3	*Rhizopus spp*	7.5%
4	*Fusarium spp*	17.5%

DISCUSSION

This investigation reaffirms the earlier works such as those of Bukar *et al.*(2009) that reported occurrence of oranges contaminated with spoilage fungi being sold in Kano orange depot at Naibawa market. Findings of this study also showed that *Aspergillus spp, Fusarium spp, Penicillium spp, Rhizopus* spp were still isolated from fruits sold in main markets of Naibawa, Rimi, Bridage and Jakara Market of Kano metropolis. All the post-harvest spoilage moulds were found to be associated with spoilage or deterioration of orange (*Citrus sinensis*). The work of Al-Hindi *et al.,* (2011) in other part of the globe have reported that *A. niger, P. digitatum and R. stolonifer*, *Byssochlamy sp*, *Cladosporium* spp. and *Mucor* were implicated in spoilage of *Citrus sinensis*. Tournas and Katsoudas (2005) also reported that *Fusarium spp*. were the most common fungi in citrus fruits. Bukar *et al.,* (2009) also revealed that, the most predominant fungus isolated from the orange (*Citrus sinensis*) were, *Aspergillus spp*; others include *Mucor spp*, Penicillium *spp*, *Rhizopus spp Fusarium spp,* and *Alternaria spp*. A study conducted by Oviasogie *et al.,* (2015) also confirmed that the fungal pathogens associated with the spoilage of orange (*Citrus sinensis*) were *Aspergillus spp. Penicillium spp. Mucor spp*. *Rhizopus spp. Candida tropicalis Saccharomyces cerevisiae and Alternaria spp* in which *Aspergillus spp*. was the predominant fungal pathogen.

In the current study *Penicillium spp*. was the most incriminated spoilage fungi of orange than other fungal species and this result is in agreement with the study conducted by Mbajiuka and Enya (2004) in which abundant presence of *Penicillium nalgiovense*, *Penicillium notatum* and *Penicillim expansium* were found among other fungi species involved in deterioration of Orange fruit.

The fruits sampled from Jakara, Rimi and Bridgade markets were also found to be massively infected with four genera of fungi namely *Aspergillus, Fusarium, Penicillium* and *Rhizopus*. The prevalence of fungi as the spoilage organism of fruits 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 conditions of the produce as well as the extrinsic parameters to which they are subjected (Effiuvwevwere, 2000). Damage inflicted on produce at the time of harvest is a major cause of infection since most of the spoilage microorganisms invade the produce through such damage tissues; similarly, the extent of deterioration is influenced by the depth of the wound. Furthermore, the incidence of infection is worsened by poor sanitary practices such as cross-contamination, contact infection during the transportation of fruits (Effiuvwevwere, 2000).

Akinmusire (2011) also remarked that the contamination of fruits by fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation. From personal observation the poor hygienic conditions of the vending house/store, the venders and the utensils used for keeping and weighing the fruits were responsible factors for mixing the deteriorating fruits with the healthier one and many other factors aggravate prevalence of fungi as the spoilage organism of fruits. In addition, the overall weather conditions of Jimma town, especially the high moisture content favours the growth of spoilage moulds on fruits and vegetables vended at Agip, Kochi and Bishishe. According to Alemu *et al.,* (2011) from a climatic point of view, abundant rainfall makes this region one of the best watered of Ethiopian highland areas, conducive for the growth of post-harvest fruit spoilage moulds.

The overall analysis of the isolation and identification process implied various causes for the growth of the moulds on fruits. As clearly described by Korsten (2006), postharvest losses of fruits is a serious problem, because the values of fresh product significantly increase while passing from the farm to the consumers table and due to overpopulation the demand for fruits increases in the world. Fungal pathogens are mainly responsible for postharvest losses of fruits (Korsten 2006).

This study detected the profile of spoilage fungi involved in the deterioration of orange sold in four main markets of Naibawa, Rimi, Bridage and Jakara. It showed that, fungi were involved in the spoilage of fruits. Fungal flora of orange samples found from April to May 2013 were dominated by moulds of *Penicillium, Aspergillus, Fusarium, Rhizopus*. Generally, of all isolated moulds *Penicillium* spp. was the dominant isolate 50% followed by *Aspergillus* spp.25%, *Fusarium* spp 17.5%, *Rhizopus* spp 7.5% respectively.

CONCLUSION

The results of this study indicate that fruits sold at Naibawa, Rimi, Bridage and Jakara were massively infected with spoilage fungi due to several factors as poor hygienic conditions of the vending site/store, venders and vending utensils. In addition, the high humid content of the vending site also contributes for the spoilage. Mechanical injuries of fruits such as bruises or cuts was occur during harvesting or

post harvesting, provide infection site for spoilage of fruits. The high moisture content of fruits and vending/storing site will be a serious limiting factor in their preservation. Another bedeviling factor could be that, the fruits used in this study are produced in the neighboring states, thus, they are transported to the city in locally woven baskets and sacks under weather conditions that encourage the incubation of these contaminating fungi.

RECOMMENDATIONS

By way of recommendation, the high prevalence of the spoilage fungi demand that appropriate control measures against infection, should be employed if farmers expect good performance of their produce. Adequate mycological knowledge and handling practices of these produce would therefore help minimize wastes due to deterioration and unacceptability. It is therefore important that both the farmer who harvests the fruits into bags for transportation, the marketers and consumers take necessary precaution in preventing contamination and also try to create an environment that will not encourage the growth or multiplication of spoilage fungi. In addition, the hygienic conditions of vending/storing site, the venders and vending utensils should have to be improved in order to provide fresh and quality fruits for the consumers. This will help in providing fresh and quality fruits for the consumers as well as in preventing the consumption of contaminated fruits thereby reducing the risk of health problems which are produced by these fungi that have been isolated in this study.

This research pointed out the common fungi which spoil fruits of orange, which are sold in Kano City Market. Thus proper handling of these fruits should be practiced to reduce the fungal contaminants. Since the majority of analyzed samples showed the presence of different fungal genera, appropriate measurement must be taken by responsible bodies to reduce the fungal load and to enhance the quality of fruits sold in Kano City.

In most unorganized markets, fruits were available in local retain shops without appropriate temperature control and unsuitable storage. This is purchased by households; this leads to the disturbance of the health of the people. So, inhibition of fungal growth by lowering storage temperature through storage under refrigeration and use of fungicides must be applied.

The hygienic and sanitary conditions observed in fruit sellers were not very satisfactory. Thus, they should improve the sanitary condition of fruit markets. In order to improve the quality of fruits, it is better to encourage creation of awareness for consumers as well as sellers and to all others about observing critical control points.

Molecular characterization of the isolates reveals the true diversity of spoilage fungi associated with fruits are highly encouraged for future investigations of this kind coupled with the traditional morphological characterization.

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