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ISOLATION AND IDENTIFICATION OF FUNGI INVOLVED IN THE POST-HARVEST SPOILAGE OF GUAVA (*Psidium guajava*) IN AWKA METROPOLIS

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Abstract

The postharvest spoilage of Guava fruit (Psidium guajava Linn.) from four selected markets in Awka, Anambra State, Nigeria was investigated. Isolation of associated fungi from guava fruits was carried out on Sabouraud dextrose agar (SDA). A total of seven (7) fungi were isolated namely Colletotrichum gloeosporioides, Fusarium oxysporum, Mucor sp., Rhizopus stolonifer, Aspergillus niger, A. fumigatus and A. parasiticus. F. oxysporum was the most prevalent of the seven fungi isolated and appeared in all the four locations. Guava fruit spoilage was most severe under humid environment and was enhanced by wounds on fruit surfaces. Pathogenicity test carried out revealed that of the seven isolates four proved pathogenic when artificially inoculated into healthy guava fruits. These were F. oxysporum, A. niger, A. fumigatus and A. parasiticus. These organisms are, therefore, the causal agents of guava fruit rot under the conditions of this study. The rest isolates did not reproduce fruit rot following artificial inoculation.

Keywords: Psidium guajava, postharvest, pathogenic, isolation, spoilage.

INTRODUCTION

Guava (*Psidium guajava* Linn.), family Myrtaceae is a large dicotyledonous shrub, or small evergreen tree native to Mexico, the Caribbean and Central and South America. The genus *Psidium* comprises approximately 150 species of small trees and shrubs but only a few produce edible fruits while the rest are wild with inferior quality fruits (Mani *et al*., 2011). About 20 species have edible fruits of which the most commonly cultivated is the common guava (*Psidium guajava* L.). Guava is hardiest among tropical fruit trees and excels most other fruit crops in productivity and adaptability being easily pollinated by insects. Various cultivars have white, pink, or red ovoid or pear-shaped berry fruits enveloping numerous, cream to brown, kidney-shaped or flattened seeds (Orwa *et al*., 2009). Guava fruits have a pronounced and typical fragrance, similar to lemon rind but less sharp. The pulp inside may be sweet or sour, and off-white ("white" guavas) to deep pink ("red" guavas). The seeds in the central pulp vary in number and hardness, depending on species.

P. guajava (apple guava, common guava) is rich in vitamin C and is grown abundantly throughout western Nigeria (Adel, 1999). It is one of the leading fruits of Mexico and contains moisture, proteins and carbohydrate. A lot of research has been carried out on guava, especially on the leaves regarding their constituents, pharmacological properties and history in folk medicine (Gutiérrez *et al.*, 2008). Most research, however, has been conducted on apple guava (*P. guajava*), with other species remaining unstudied. Extracts from apple guava leaves or bark are implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain (Ojewole, 2006, Chen *et al.*, 2007, Mahfuzul *et al*., 2007) while essential oils from the leaves display anti-cancer activity *in vitro* Manosroi *et al*., 2006).

Guava (*P. guajava* Linn.) is affected by about 177 pathogens of which, 167 are fungal, 3 bacterial, 3

algal, 3 nematodes and one epiphyte (Misra, 2004). Wilt is the most important disease of guava. Besides this, fruit rot and postharvest diseases are also important and cause serious losses. Guava fruit diseases are of two types, field and postharvest diseases. Postharvest diseases develop during transit and storage. Postharvest fungal diseases of fruits represent one of the most severe causes of loss in production. Fruits constitute a vital part of human diet. Postharvest diseases of fruits are the most severe causes of losses in production and are responsible for bio-deterioration of tropical fruit pulp. The principle of spread of fungal infection in fruits supports that a single infected guava fruit can be the source of infection to other guava fruits during storage and in transit (Jay, 2003). Microorganisms are associated, in a variety of ways with all the foods we eat, guava fruits inclusive. This research was, therefore, embarked upon to identify the postharvest fungi associated with guava fruits sold in Awka metropolis of Anambra State.

MATERIALS AND METHODS

Sample Collection

Samples of guava fruits were collected from four markets in Awka metropolis namely Eke-Awka, Amaenyi, Amawbia and Okpuno markets. These are the major markets and depots for fruits, vegetables and food items in genereal in Awka, Anambra State. Forty (40) guava fruit samples showing rot symptoms were randomly collected from the four markets at the rate of 10 fruits from each market. The samples from different markets were held in separate sterile polyethylene bags.

Inoculum Preparation and Isolation

The method of Balali *et al*., (1995) was adopted. The different samples were surface-sterilized separately with absolute ethanol and rinsed in two changes of sterile distilled water. The portions on the samples showing symptoms were cut off with sterilized scalpel. The cut portions were homogenized into a pulp using a sterilized laboratory blender. The homogenized pulp was immediately transferred into a sterile beaker, wrapped tightly with aluminum and then stored under aseptic condition for subsequent use.

The medium used for fungal isolation in this study was Sabouraud Dextrose Agar (SDA) prepared routinely. Chloramphenicol (30mg/l) was added to the medium to discourage bacterial contamination (Adamu *et al.*, 2009). Sterile molten medium in Petri dishes were subsequently inoculated with 1ml of different dilutions of the homogenized guava pulp. With the aid of a flamed glass rod, the inoculum was spread evenly over the surface of the medium (Pelczar, 1993 and Chan 1997, Cheesbrough, 2000). Inoculated plates were incubated at room temperature (28 ± 2^{o}C) for 5 - 7 days and observed daily. Observed colony development was sub-cultured promptly and bottle slants prepared for storing.

Identification of Isolates and Pathogenicity Test:

Slides of pure cultures of the guava isolates were prepared for microscopic observation and identification. The cultural and morphological characteristics of the isolates were observed and noted and formed part of the criteria used for identification (Barnett and Hunter, 1987; Domsch *et al.*, 1993). Detailed morphological characteristics of the fungi such as hyphae (septation), reproductive structure (sporangia/conidia) in chain or single; the type of spore, etc were observed and recorded.

Pathogenicity test was carried out with the seven guava isolates in this study. Fresh, healthy guava fruits were collected and taken to the laboratory. The fruits were surface-sterilized with 80% absolute ethanol and then inoculated with the isolates after wounding. Inoculation was made by using sterile 5mm-diameter cork borers to create holes on the test guava fruits. Mycelial plugs were cut from pure culture plates of the isolates and plugged into the holes on the fruits. The control fruits were inoculated with sterile agar plugs. All the inoculated fruits were incubated at room temperature (28 ± 2^{o}C) and observed daily for symptom development. Re-isolations were made from fruits showing rot symptoms and identifications carried out.

RESULTS

Seven different fungi comprising five genera were isolated from guava fruits collected from four markets in Awka metropolis, Anambra State. The fungi were identified as *C. gloeosporioides*, *F. oxysporum*, *Mucor* sp., *R. stolonifer*, *Aspergillus niger*, *A. fumigatus and A. parasiticus* **(Plate 1)** These fungi were isolated from all samples collected from the four markets in Awka metropolis.

Aspergillus species were more prevalent than any other species isolated in this study. The samples from Eke-Awka were the most infested of all the samples because in addition to the fact that all the fungi appeared in the samples, the frequency of occurrence of each fungus was very high (**Table 1**). *F. oxysporum* was the single most frequent species in all the locations put together. The fungal load of guava fruits in Awka meteropolis ranged between mean values of 8.33 to 14.67 colony forming units per gramme (cfu/g) of guava fruits expressed as the total viable count (TVC). However, washing before sale was found to reduce the microbial load of guava in this study.

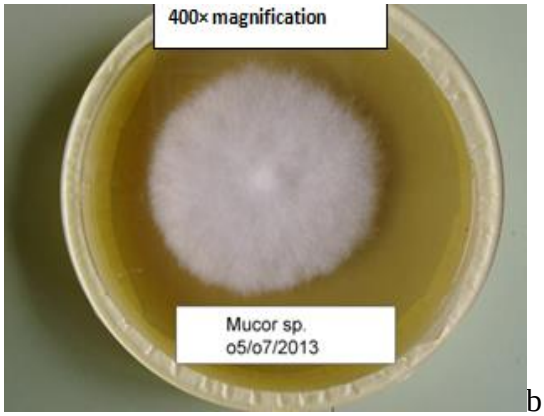
Results of the pathogenicity test showed that of the seven organisms isolated only four proved to be pathogenic on guava fruits. The four pathogens were *F. oxysporum, A. niger, A. fumigatus and A. parasiticus. F. oxysporum* was the most pathogenic, inducing a mean infection diameter of 7.30±0.3mm followed by *A. parasiticus* (6.47±0.3), *A. niger* (5.80±0.4) and *A. fumigatus* (5.56±0.3), in that order. Analysis of variance showed that there was significant difference (P<0.05) in the virulence of the pathogens under the conditions of this study. There was no disease development in guava fruits inoculated with sterile agar plugs.

TABLE 1 : FREQUENCY OF OCCURRENCE OF FUNGAL ISOLATES IN GUAVA FRUITS FROM FOUR MARKETS IN AWKA METROPOLIS

	Locations				
Fungal isolates	**A**	**B**	**C**	**D**	**Frequency(%)**
F. oxysporum	+	+	+	+	100
Mucor sp.	-	-	+	+	50
Aspergillus niger	+	-	+	-	75
A . parasiticus	+	+	+	-	50
A. fumigates	+	+	+	-	50
R. stolonifer	+	+	+	-	75
C.gloeosporioides	-	-	+	+	50

(+) presence, (-) Absence, A = Amawbia market, B = Amaenyi market, C= Eke-Awka market, D = Okpuno market.

a 



b

Rhizopus stolonifer ***Mucor* sp.**

c 

d

Fusarium oxysporum *Aspergillus niger*

e 

Colletotrichum gloeosporioides

PLATE 1 (A-E) Showing Pure Culture Plates of Guava (*Psidium guajava*) Isolates

DISCUSSION

The fungi associated with postharvest spoilage of guava (*P. guajava* Linn.) were identified in this study as *C. gloesporioides*, *F. oxysporum*, *Mucor* sp., *R. stolonifer*, *A. niger*, *A. fumigatus and A. parasiticus*. Guava was also shown to have a wide range of fungal load per gramme of the fruits in Awka meteropolis. These organisms are commonly implicated in the postharvest deterioration of many fruits and vegetables and have been reported severally (Booth, 1976, Amadi and Oso, 1996, Amadi, 2005 and Oyetunji *et al*., 2012). Generally, contamination of agricultural produce is a function of many factors including infestation in the field prior to harvest, handling during harvesting and methods of packaging and distribution of produce to the market. The variation in fungal loads of guava observed in this study can be attributed to the differences in the level of sanitation in handling and of the market environments. Wounds are known to be the major pre-disposing factor of fruits and vegetables to microbial attack both in transit and in storage.

Proof of pathogenicity is the single most reliable criterion used in implicating associated microorganisms in the causal processes of disease development. Four isolates namely *F. oxysporum*, *A. niger*, *A. fumigatus and A. parasiticus* proved pathogenic on inoculated guava fruits in this study. This finding is consistent with the reports of Peter *et al.*, (2002), Amadi *et al*., (2009) and Renu and Lal, (2009) that *Fusarium* and *Aspergillus* species are notorious causal agents of rot in many fruits and vegetables including watermelon, carrot and guava. As a further proof of its virulence, among the seven fungi isolated in this study, only *F. oxysporum* was found in all the locations thereby showing a hundred percent (100%) prevalence. Mathew *et al.,* (2010) had reported *A. niger* and *R. stolonifer* in postharvest diseases of guava.

In conclusion, it is important to point out that guava fruits are sensitive to physical damage. Efforts should be made, therefore, during harvesting and handling to minimize wounding. This way, the shelf life of guava fruits can be prolonged a little more and so, make them more available at least during the season. In addition, field sanitation should be maintained to minimize the load of inoculum that may be carried from the field into storage. This will reduce the incidence of postharvest spoilage of the fruit.

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