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# Fungi associated with deterioration of sour-sop (*Anona muricata*. Linn) fruits in Abia State, Nigeria

**Okwulehie, Ikechukwuka Cyriacus* and Alfred, Nnamdi Kingsley**

Department of Biological Sciences, Michael Okpara, University of Agriculture Umudike, P. M. B. 7267, Umuahia, Abia State, Nigeria.

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***Anona muricata*** **commonly known as sour-sop, belong to the family Annonaceae. It is a small slender tropical tree usually grown for its large fleshy and juicy fruits. The fruit of** ***A. muricata*** **plays an important role in the diet of many in many parts of the tropics including Nigerian. Unfortunately, the usefulness of the fruits of** ***Anona*** **is decimated by many fungi species. Investigation of the fungi that cause the deterioration of sour-sop** ***(Anona muricata*****) was carried out in order to recommend the appropriate control measures. Mature fruits of** ***A. muricata*** **were collected from different locations in Abia State. Isolation, characterization and identification of fungi were made by plating washings from skin surface and extracted juice from pulp of the fruits in test tubes containing potato dextrose agar (PDA) and yeast malt extract agar (Difco) into which streptomycin sulphate was incorporated to inhibit bacterial growth. Inoculated tubes were incubated at 28 ± 2°C for 48 h. Control experiment was carried out with sterile peptone water instead of using the washings. The following filamentous and yeast fungi were isolated from the skin surface and pulp of the fruits:** ***Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Colletotrichum*** **sp.,** ***Fusarium solani, Mucor*** **sp.,** ***Penicillium chrysogenium, Penicillium*** **sp.,** ***Rhizopus stolonifer*** **and** ***Rigidoporus*** **sp.,** ***Candida albicans, Saccharomyces cerevisiae, Saccharomyces rouxii*** **and** ***Torulopsis*** **spp. The pathogenicity tests of the filamentous fungi isolated were carried out on mature green Anona fruits and were found to be pathogenic. The filamentous fungi were mainly responsible for the deterioration of the fruits of** ***A. muricata*** **in Abia state while the yeasts were fermentative.**

**Key words:** Abia state, *Anona muricata* (Sour-sop) fruits, fungi, deterioration.

## INTRODUCTION

*Anona muricata* Linn, commonly known as sour-sop in Nigeria, belonging to the family Annonaceae, is a small slender tropical tree usually grown for its large fleshy and juicy fruits. According to Keay et al. (1964), *A. muricata* is the most widely grown of the *Anona* species in the tropics. In Nigeria, the plant is restricted to the rainforest Zones of Nigeria and cultivated mainly in home gardens in Abia, Imo, Rivers, Ebonyi and Delta States.

The fruit of *A. muricata* plays an important role in the diet of many in many parts of the tropics including Nigerian. According to Hugues and Phyippe (1989), the fruit is most widely used for the preparation of refreshing (canned) juice, dessert ice-cream, flavoring pure ingredient in fruit salad and in jelly making. Judging from the size of the fruit, the quantity and quality of the juice from the fruit, it could be said that it has high potential as a raw material for the production of good quality table wine The fruit fresh pulp of *A. muricata* contains 1.0% protein, 1.0% oil, 1.0% fibre and about 18% carbohydrates. According to Tice et al. (1991), there is also about 26 mg ascorbic acid, 5 mg vitamin A, 0.07 mg of thiamin, 8.0 mg riboflavin and 9 mg of niacin per 100 g.

*A. muricata* alkaloids have been reported to have anti-inflammatory and anti-microbial properties (Kumar and Tandon, 1979) According to Mahta and Pandsksena (1976), A. muricata is considered ripe for consumption only when the mature healthy fruit becomes soft and the skin has begun to darken.

*Corresponding author. phylyke@yahoo.com

**Table 1**. Yeast and filamentous fungi isolated from the skin surface and pulp of ripe *A. muricata* fruits harvested from tree top.

| Parts of fruits | Yeast fungi | | Filamentous fungi | |
|---|---|---|---|---|
| | **20 - 100% incidence** | **<20% incidence** | **20 - 100% incidence** | **< 20% incidence** |
| Skin surface | *Saccharomyces cerevisiae, Candida albicans* | *Torulopsis sp* | *Penicilium chrysogenum Aspergillus flavus* | *Rhizopus stolonifer* |
| Fleshy pulp | *S. cerevisie, C.albicans* | 0 | *Aspergillius niger* | *Penicilium chrysogenum* |
| | *Torulopsis* | 0 | | *Aspergillius flavius* |

0 = indicates the non-detection of fungi.

The numerous uses and the potentialities of the fruit of *A. muricata* are however reduced by the high rate of its deterioration both on the tree top and in storage. Such information aroused the interest of the researchers in investigating the deteriorative agents involved in the deterioration of the fruits. To the best of knowledge of the authors, the fungi responsible for the deterioration of the fruits have not been documented. Therefore it is the aim of this study to identify and isolate the fungi associated the fruit deterioration.

## MATERIALS AND METHODS

### Sample collection and storage

The ripe and unripe mature fruits of sour-sop used in the investigation were collected in July, 2004, from different locations in Umudike, Amawom and Umuahia municipality of Abia State of Nigeria. The fruits from Umudike and Amawom were collected from home gardens, by harvesting from tree-tops using sickle and by picking ripe fruits that fell onto the ground under the parent tree. The fruits from Umuahia municipality were purchased from Umuahia main market. The fruits were placed in sterile polythene bags and stored in the refrigerator at about 5°C for one week to arrest further deterioration, before being used for the study. The fruits were not pre-treated with any chemical before storage.

### Isolation, characterization and identification of fungi

The investigation of the fungi associated with the fruit entailed observations made on five fruits from each of five tree tops in Umudike and Amawom and five locations in Umuahia main market. Isolation, characterization and identification of the fungi were carried out following the method of Okigbo (2001) and Okwulehie (2004). The fungi were isolated from washings from the skin and extracted juice from the pulp of the fruits obtained from (i) tree-top (ii) ground under the parent tree and (iii) bought from the market. Five serial dilutions of the extracted juice and skin washing were prepared using sterile peptone water and plated on potato dextrose agar and yeast malt extract agar (Difco) in 30 cm Petri dishes and incubated at 28 ± 2°C for 48 h (Okigbo, 2001; Okwulehie, 2004). The identification of the fungi associated with the juice and skin-washing was carried out at the National Root Crop Research Institute (NRCRI) Umudike, following standard procedures by Dade and Gunnel (1969), Arx (1974) and Okigbo (2001). Control-plating was carried out using sterile peptone water and in both the test and control plates, 0.5 g.per litre of streptomycin sulphate powder was incorporated as antibiotics to prevent bacterial growth (Okwulehie, 2004).

To determine the mycoflora on the skin of the fruits, five fruits from each location were placed in sterile 1 litre flat -bottomed glass container, each containing 500 ml of distilled water. The skins of the fruits were carefully washed with sterile water to avoid damaging them since they had become soft. The washings were transferred into sterile 1-litre flasks. Serial dilutions (up to $10^{-5}$ dilution), were prepared and 0.1 ml was spread-plated on potato dextrose agar (PDA) plates using a sterile dropper, in four replicates.

The mycoflora from the extracted juice of *A. muricata* fruit was determined following the methods used by Okigbo (2001) and Okwulehie (2004) . The fruit juice was expressed from the pulp of the washed fruits. Before the juice expression, the outer skin of the fruit was surface-sterilized with 70% ethanol and washed with sterile distilled water, then the juice was expressed through a tiny opening punctured by using a sterile needle and directed into a sterile test tube. Serial dilutions of the juice were made using 1 ml of the juice. Aliquots (0.1 ml) of the dilution were spread-plated on the medium in each Petri-dish using a sterile pipette. Control-plating was done using only sterile peptone water. The Petri dishes were incubated at 28 ± 2°C in the incubator. To purify the culture, distinct colonies of isolated fungi were sub-cultured onto potato dextrose agar in slants.

Pathogenicity tests were performed with all fungal isolates from the skin washings and juice of the fruits following the method of Okigbo (2001). Intact fruits of *A. muricata* which had been surface-sterilized using 70% ethanol and cleaned with sterile distilled water were used. Fungal spore suspensions (about 20 ml) were prepared by centrifuging and re-suspended in three changes of sterile distilled water. Holes (1 cm) in diameter were bored into the intact fresh but unripe fruits of *A. muricata* using size 3 flamed cork-borers, and inoculated by dropping 5 ml spore suspension into the holes using sterile pipette. The holes were sealed with sterile PDA plugs. Healthy ripe and unripe fruits were inoculated with sterile distilled water after surface- sterilizing with 70% ethanol. These served as control. Fruits were placed in sterile cellophane bags and placed in the incubator at 28 ± 2°C and left for two days to allow the spore to germinate and establish. The fruits were then exposed to natural condition in the laboratory and inspected daily to check for symptoms. Portions of the inoculated and infected tissues were plated on PDA for re-isolation and identification of the pathogenic fungi following the method described by Raper and Fannel, (1973).

## RESULTS

Various filamentous and yeast fungi were isolated from the skin surface and pulp of the fruits of *A. muricata* collected from the various locations in Abia State (Tables 1 - 3). They were categorized as major and minor groups depending on the incidence. The incidence was expressed

**Table 2.** Yeast and filamentous fungi, isolated from the fruits of *A. muricata* picked from the ground under the tree.

| Parts of fruit | Yeast fungi | | Filamentous fungi | |
|---|---|---|---|---|
| | 20 - 100% incidence | < 20% incidence | 20 - 100% incidence | < 20% incidence |
| Skin surface | *C. albicans*<br>*S. cerevisae* | *S. rouxii*<br>*0* | *Colletotrictium* sp, *Aspergillus* sp, *Aspergillus niger, Mucor* sp, *Botryodiplodia theobromae, Penicilluim chrysogerium, Penicillium* sp *and Rhizopus stolonifer.* | *Rigidosporus lignosus*<br>*Fusarium soloni*<br>*Penicillum sp.* |
| Fleshy Pulp | *S. cerevisiae*<br>*C. albicans* | *0*<br>*0* | *P. chrysogenuim*<br>*A. niger*<br>*R. stolonifer* | *B. theobromae* |

0 = Indicates non-detection of fungi.

**Table 3.** Yeast and filamentous fungi isolated from the fruits of *A. muricata*, purchased from Umuahia main market.

| Parts of fruit | Yeast fungi | | Filamentous fungi | |
|---|---|---|---|---|
| | 20 - 100% incidence | < 20% incidence | 20 - 100% incidence | < 20% incidence |
| Skin surface | *C. albicans*<br>*S. cerevisial* | *0*<br>*0* | *P. chrysogencium*<br>*R. stolonifer*<br>*A. flavus*<br>*A. niger*<br>*Mucor sp* | *0*<br>*0*<br>*0*<br>*0*<br>*0* |
| Fleshy pulp | *C. albians*<br>*S. cerevisiaa*<br>*Torulpsis sp* | *0*<br>*0*<br>*0* | *P. chrysogenium*<br>*A. niger* | *0*<br>*0* |

0 = Indicates non-detection of fungi.

as percentage of replicate portions or parts of the treatment group from which a given fungus was isolated. The filamentous and yeast fungi included in the major categories were those with percentage incidence of 20% and above, while fungi within the minor group had less than 20% incidence.

Results of the study are presented in Table 1. Two yeast fungi *Saccharomyces cerevisiae and Candida albicans* and three filamentous fungi *Penicillium chrysogenum, Colletotrichum* sp *and Aspegillus* sp. were isolated from the skin of *A. muricata*. The yeast and filamentous fungi with less than 20% were *Torulopsis* sp and *Rhizopus stolonifer*, *respectively.* The yeast components of the pulp of the fruits collected from the tree top with 20% incidence and above were *S. cerevisiae, C. albicans* and *Torulopsis* sp, while the only mould of the same incidence was *Aspergillus niger. Penicillium chrysogenium and A. flavus* have less than 20% incidence.

The percentage incidence of the yeast and filamentous fungi isolated from the skin surface and pulps of the fruits of *A. muricata* picked from under the tree are presented in Table 2. The yeast fungi from the skin of the fruits more than 20% incidence and above were *C. albicans and S. cerevisiae*, while the yeast component with less than 20% incidence was *Torulopsis.* Similarly the filamentous fungi with 20% and above incidence were *Colletotrichum* sp. *Fusarium* sp, *Aspergillus niger* , *Mucor* species, *Botryodiplodia theobromae, Penicilluim* sp*., P. chrysogenium* and *Rhizopus stolonifer* while those with less than 20% incidence were *Rigidoporus lignosus* and *Fusarium solani.*

The yeast fungi of the pulp of *A. muricata* fruits picked from the ground under the tree with 20% incidence and above were the same as those found on the skin, while the filamentous fungi were *P. hrysogenum. A. niger and R. stolonifer*, The only filamentous fungus of the pulp with less than 20% incidence was *B. theobromae*.

The fruits purchased from Umuahia market contained *C. albicans* and *S. cerevisiae* with *Torulopsis sp.* less than 20% incidence. The filamentous fungi isolated from the skin of the fruits of *A. muricata from the market* were *P. chrysogenuim, R. stolonifer, A. flavus, A. niger and Mucor* sp. The incidence of *Torulopsis* sp. *o*n the skin of the fruits purchased from the market was low. Similarly, the pulp of the fruits from the market contained *C.*

*albicans* and *S. cerevisiae* and the mould, *P. chrysogenium* and *A. niger* at 20% and above incidence.

## DISCUSSION

Micro- organisms especially fungi have been reported to cause extensive deterioration of fruits and vegetables (Fajola, 1979; Erinle, 1982; Amadioha and Uchendu, 2003). Some of these micro-organisms cause rotting, discoloration or fermentation of the fruits which affect their preservation. This study showed that the fruits of *A. muricata are* attacked by various fungi which cause deterioration. In the present study, many filamentous and yeast fungi were isolated from the fruits of *A. muricata* collected from various locations in Abia State. A close observation of the mycoflora showed that many of them were the same as those isolated from black plum (Okigbo, 2001). Filamentous and yeast fungi were isolated from the fruits collected from the tree-top, from the ground under the parent plants and from Umuahia main market. This is an indication that the isolated mycoflora were responsible for the deterioration of the fruits of *A. muricata* in Abia State.

Fruits picked under the *A. muricata* trees showed more species of filamentous fungi (Table 2) followed by those from Umuahia main market (Table 3), possibly because of their contact with the soil and handling by the traders, respectively. Moreover, most of the fungal species were soil-borne. For example, *Fusarium* and *Botryodiplodia* sp. Similarly, the load of pathogenic fungi which have colonized the rottened fruits under the trees, have resulted in increased inoculum levels around and about the tree.

The filamentous fungi isolated from the deteriorated fruits of *A. muricata* were the pathogens and were responsible for the deterioration of the inoculated fruits. These fungi could affect the market value and shelf life as well as nutrient levels of the fruits of *A. muricata* in Abia State. Similar findings of fungal deterioration of fruits were reported for paw-paw in Abia State (Echerenwa and Umechuruba, 2004). The yeast fungi may also hasten the deterioration by their fermentative activities which soften the fruits and predispose them to attack by pathogens. Prompt removal of over-ripe fruits under the *A. muricata* trees is recommended to reduce inoculums levels of the pathogenic fungi. Careful handling is also recommended to ensure minimal damage of the fruit.

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