EFFECT OF FUNGAL ROTS ON NUTRITIONAL QUALITY OF *Anona senegalensis* L. AND *Anona squamosa* L. FRUITS SOLD IN YOLA, ADAMAWA STATE, NIGERIA

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B. G. Zakari[1]*, R. Z. Suleiman[2] and F. K. Channya[1]
[1]Department of Plant Science, Modibbo Adama University of Technology, P.M.B. 2076, Yola, Adamawa State, Nigeria
[2]Department of Forestry, Ministry of Environment, PMB 2170, Yola, Adamawa State, Nigeria
*Corresponding author: bgzakari@yahoo.com

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Abstract: The aim of this work was to analyze the effect of fungal rot on nutritional quality of Annona fruits sold in Yola, Adamawa state, Nigeria. Rotted annona fruits were randomly sampled from five markets in Yola. Fungi associated with the annona rots were isolated using Potato Dextrose Agar (PDA) culture medium and were identified as *Aspergillus niger*, *Poma herbarum* and *Rhizopus stolonifer*. Results from proximate analysis of the infected fruits and healthy fruits of *Annona senegalensis* and *Annona squamosa* indicated that there were increase in the moisture (15.4%) and ash (14.6%) contents of *A. senegalensis* and 14.8% moisture and 14.6% ash contents of *A. squamosa* in the freshly harvested fungal infected fruits relative to the apparently healthy ones, while there were decrease in the protein (11.4%), fat (8.3%), fibre (30.2%) and carbohydrate (20.1%) content of the fungal infected fruits relative to the apparently healthy ones. Correlation test carried out show that the nutrient composition of the fungal infected and the apparently healthy fruits are significant at the 0.01 level (2-tailed). The results are clear indications that fungi not only cause rotten on fruits but also reduce the nutritional values of fruits as well.

Keywords: *Annona senegalensis*, *Annona squamosa*, fruits, fungi, *Rhizopus stolonifer*

Introduction

Annona (*Annona senegalensis*, *Annona squamosa*) belong to the family Annonaceae, and are wide spread in the tropics and subtropics of the world (Samson, 1980). AgroForestry (2013) reported the name Annona was derives from the Latin "annual harvest"; it is quite a prolific bearer, and may produce fruit within two to three years. The tree yields up to 10 tons/ha and each fruit weighs 0.5 to 2 kg. Annona has nutritional and medicinal values; it was reported by Southampton Centre for Underutilised Crops (SCUC) (SCUC, 2006) as an important fruit food globally and one of the most highly price tropical fruits.

The fruit makes an excellent drink. Its white edible pulp contains 80% water, 1% protein, 18% carbohydrate and fair amount of vitamins B_1, B_2, and C. Annona are also economically important crop in many countries of Africa and Asia as well as in South, North and Central America (Pinto *et al*., 2005). The seeds have been used as pesticide against moths and cockroaches (Jaramillo *et al*., 2000) and leaves when chewed and applied on incisions after surgery will help fasten the healing process (Padma *et al*., 2001). Annona production has been bedeviled by a lot of constraint globally. The most serious one is postharvest disease due to fungal pathogens (Padma *et al*., 2001; Dholvitayakhun *et al*., 2013).

Despite the numerous economic importance of Annona, especially, its fruits, visits made to some Annona markets within Yola metropolis revealed that several fruits were found deteriorating in the markets. The study was conducted to investigate the fungal pathogens associated with the deterioration of Annona fruits in Yola, Adamawa State and the effect of the fungal pathogens on the nutrient composition of the fruits.

Materials and Methods

The study area

The study was conducted in Yola, Yola North Local Government Area of Adamawa State. Yola lies between latitudes 9°11′N to 9°19′N and longitudes 12°20′E to 12°30′E (Adebayo, 1999). Yola has a tropical climate marked by dry and rainy seasons. The rainy season starts around May and ends in late October while the mean total rainfall is 1,113.3 mm (Adebayo, 1999). Maximum temperature in Yola is about 40°C in April, while minimum temperature could be as low as 18.3°C between December and early January while relative humidity is lowest (26%) in month of January and February and increases to 58, 69, and 79% in May to July, respectively (Adebayo, 1999).

Sample collection and preparation

A total of one hundred and twenty Wild soursop (*Anona senegalensis* L.) and Sugar apple (*Anona squamosa* L.) samples were collected randomly from Jimeta modern, Jimeta old, Jambutu, Saminaka and Pallujah markets all in Yola, Nigeria. Both infected and healthy fruits were collected and incubated in sterilized desiccators for the development of rots.

Isolation of the pathogens

Fungi associated with diseased Annona fruits were isolated using the blotter method as recommended by the International Seed Health Testing Association (2005). Portion of the diseased fruits were cut into small pieces, washed with clean water, surface sterilized with 0.01% mercuric chloride for 30 seconds. These were then rinsed in sterile distilled water and inoculated on solidified potato dextrose agar (PDA). The plates were incubated at room temperature (25±2°C) for 24 h. Fungal that grew from the incubated plates were sub-cultured repeatedly until pure cultures were obtained.

Identification of the pathogens

Fungi isolated from the infected fruits were identified by examine their characteristics on the culture media. Their morphological structures were also observed under the light microscope. These structures were then compared with the structures in the fungi identification manual (Watanabe, 2010).

Pathogenicity test

In ascertaining the pathogenicity of the fungi isolated, the approach of (SPT-TCA, 1999) was employed. Healthy *Anona senegalensis* and *Anona squamosa* fruits were washed with tap water and then surface sterilized with 0.01% mercuric chloride. A 2 mm diameter cork borer was used to bore holes into the healthy fruits. 2 mm disc of the pure culture of each isolate was cut and placed in the hole. Tissues were replaced and wounds were sealed with sterile vesper prepared from wax and Vaseline. The controls were set up in the same manner except that sterile agar was used instead of the isolate. The experiments were replicated five times. The plates were incubated at room temperature (25±2°C) for 24 h. Disease

symptoms were examined and compared with the original disease symptom observed earlier bon the infected fruits.#

Analysis of the nutrient composition

Ten healthy and diseased annona fruits were used for this analysis. Fiber and pulp were excised from the fruits, weighed, cut into pieces and dried in a hot air oven at 60^{o}C. The dried pieces of the samples were grounded into fine powder and analyzed for moisture, carbohydrates, ash, crude fibre, protein and fat following the (AOAC, 1980).

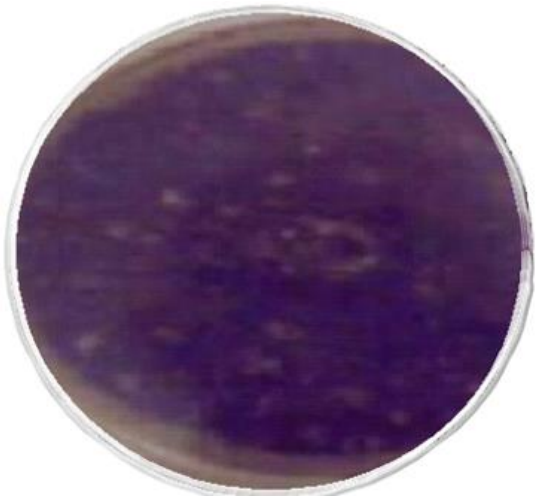
Statistical analysis

All experiments were conducted in the laboratory using Completely Randomized Design (CRD) as described by (Gomez and Gomez, 1984). All the experiments were replicated four times. Data collected were analyzed using analysis of variance(ANOVA), while the means that were significant were separated by least significant difference (LSD) at 1% probability level (P<0.01).

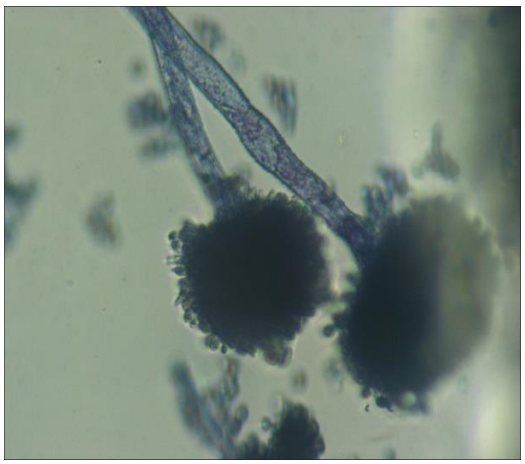
Results and Discussions

Isolation and identification of fungal isolates

The isolated fungi from the *Annona* fruits were identified as *Aspergillus niger*, *Poma herbarum* and *Rhizopus stolonifer* (Plates 1 – 6). The result of the pathogenicity test carried out showed that the fungal organisms re-isolated had the same characteristics with those isolated originally from the ripe fruits; hence they are the causal agent of the fruits rot observed. This agrees with the report of (Zakari et al., 2015; Nava-Diaz, 2000) that *Aspergillus niger*, *Poma herbarum* and *Rhizopus stolonifer* were among the fungi associated with the fruits of *Annona* species. These fungal pathogens might have followed the fruits from the field to the storage room. (Olunloyo, 1986) reported that fungal pathogens might be resident on the leaves and stems on plants from where they were dispersed into the fruits to initiate infection spore during rainfall.



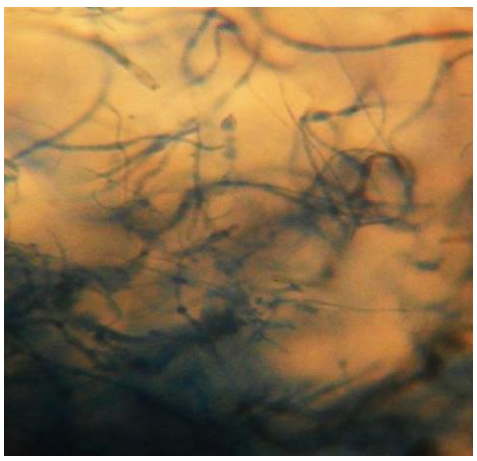
A



B

Plate 1 and 2: Seven- day- old culture (A) and micrograph (B)of *Aspergillus niger*

A



B

Plate 3 and 4: Seven-day-old culture (A) and micrograph (B) of *Poma herbarum*

A

B

Plate 5 and 6: Seven-day-old culture (A) and micrograph of *Rhizopus stolonifer*

Analysis of the nutrient composition

Table 1 and 2 show the results of the proximate composition of the fungal-infected and apparently healthy fruits of *A. senegalensis* and *A. squamosa*. Results show that in both fruits there were increase in moisture and ash content of the fungal infected fruits relative to the apparently healthy ones, while the protein, fat, fibre and carbohydrates content of the apparently healthy fruits of both plants were relatively higher. It could therefore be deduced that the relative increase of moisture in the infected fruits may be caused by the digestion, degradation and dissolution of the fruit tissue into a mush by the pathogens. These degradative activities might have also resulted to the relative reduction in the protein, fat, fibre and carbohydrate contents of the infected ones. These might have been broken down by the fungi into smaller molecules which they absorbed. (Bonner, 1997) reported that complex molecules as polysaccharides and protein are required by fungi for building the hyphal wall and for respiration to obtain energy. This suggests that these pathogens might have denied man of these essential nutrients upon consumption through their degradative activities, thereby causing some great damaging effect to human health. Van Duyn *et al*. (2000) stated that the deficiency of fibre in our diet leads to artherosclerosis, diverticular diseases and intestinal cancer. It was observed that there was a decrease in the vitamins A and C composition of the fungal infected fruits relative to the apparently healthy ones. The relative reduction in the vitamins A and C content of the infected fruits may be attributed to these degradative activities. Vining (1990) observed that vitamins act as co-enzymes in carboxylation, fatty acid metabolism, pyruvic carboxylases and also as important growth factors in all fungi. This means that the consumption of the fungal-infected fruits by man could lead to such vitamin deficiency diseases as scurvy, dry skin and dermatitis type effect, since the pathogen has utilized the vitamin content of the fruits for their growth.

Table 1: Proximate composition of infected and healthy fruits of *A. senegalensis*

Nutrients	AHFAS conc. (%)	FIFAS conc. (%)
Moisture	9.6	15.4
Ash	13.1	14.6
Crude protein	13.7	11.4
Fat	9.3	8.3
Carbohydrate	33.7	30.2
Crude fibre	20.6	20.1
Vitamins	**AHFAS conc. (mg/100g)**	**FIFAS conc. (mg/100g)**
A	0.7	0.4
C	0.3	0.1

AHFAS= Apparently healthy fruits of *A. senegalensis;* FIFAS= Fungal-Infected fruits of *A. senegalensis*
**Correlation is significant at the 0.01 level (2-tailed).

Table 2: Proximate composition of infected and apparently healthy fruits of *A. squamosa*

Nutrients	AHFASq conc. (%)	FIFAsq conc. (%)
Moisture	11.3	14.8
Ash	8.7	11.3
Crude protein	11.9	10.6
Fat	12.6	11.7
Carbohydrate	38.1	35.4
Crude fibre	17.4	16.2
Vitamins	**AHFASq conc. (mg/100g)**	**FIFASq conc. (mg/100g)**
A	1.1	0.8
C	0.6	0.3

AHFASq= Apparently healthy fruits of *A. squamosa;* FIFASq= Fungal-Infected fruits of *A. squamosa.*
**Correlation is significant at the 0.01 level (2-tailed).

A correlation test carried out show that the nutrient composition of the fungal infected and the apparently healthy fruits of *A.senegalensis* and *A. squamosa* have significant at the 0.01 level (2-tailed).Although the results show that the degradative activities of the pathogen actually affected the nutrient composition of the diseased fruit, a correlation test

carried out show that the nutrient composition of the fungal infected and the apparently healthy fruits of *A. senegalensis* and *A. squamosa* have significant at the 0.01 level (2-tailed).

Conclusion

It may be concluded from this study that *Aspergillus niger*, *Poma herbarum* and *Rhizopus stolonifer* are common pathogenic fungi which cause *Annona* fruit rots in the study area. The result from the pathogenicity test indicated that all the isolated fungi are pathogenic and attributed to the cause of *Annona* rots for the first time in Yola. It is also clear from the result that fungi not only causes rotten on fruits but also reduces the nutritional values of fruits as well. Fungal infection may leads to a reduction in carbohydrate and protein contents of the annona pulp which might have a remarkable effect on the value of the fruit, especially in the food industry. We therefore recommended timely spraying of the fruits with fungicides to reduce the damaging activities of the fungal pathogen and contamination with mycotoxins and other related fungal metabolites that might be harzadous to human health. Since some of these pathogens gain access via wounds created by insect pests, there is also the need for further investigations of the pests causing injuries on annona fruits, with the aims of reducing their activities.

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Conflict of Interest

Authors declare that there are no conflicts of interest.

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