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International Digital Organization for Scientific Research **ISSN: 2550-7931**
IDOSR JOURNAL OF APPLIED SCIENCES 8(3) 140-146, 2023.
https://doi.org/10.59298/IDOSR/2023/10.2.1420

Isolation of Seed-Borne Mycoflora from Cashew Nuts Vended in Selected Markets in Awka

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ABSTRACT
This study investigated the mycoflora associated with Cashew nuts sold in Awka markets. Samples of Cashew nuts were purchased from local markets (Eke-Awka, Amaenyi and Good-Will). Samples were analysed for the moisture contents and the presence of fungi by adopting dilution plating method. Data were analysed using Analysis of Variance (ANOVA) via Statistical Analysis System (SAS) of Version 9.1. Means of treatment were compared using Duncan's Multiple Range Test (DMRT) at $p<0.05$. The results of the study revealed thatSix fungal species were isolated which included *Fusarium oxysporum, Aspergillus niger, A. flavus, A. fumigatus, Penicillium oxalicum* and *Mucor* species. The results showed that Eke-Awka Market recorded the highest percentage moisture content of 6.8%, which was followed by Amaenyi Market with a percentage moisture content of 6.6%, while least was Goodwill Market with moisture content of 4.1%. The presence of fungi in stored food commodity deteriorates the nutritive value and secrete toxins injurious to human health. The occurrence of pathogenic fungi on cashew nuts could be avoided or diminished if proper farming, harvesting and storage methods were adopted.
Keywords: Isolation, Mycoflora, Cashew, Nut, Market, Awka

INTRODUCTION

The cashew plant, *Anacardium occidentale* L., is a tropical evergreen, perennial tree with a darkish-green leathery foliage, crooked branches and very irregular crown, a small to medium sized tree belonging to the family Anacardiaceae [1]. For over 400 years after introduction, cashew trees were exploited mainly for apple and no commercial value was attached to the nuts [2]. The first commercial cashew planting in Nigeria was in the mid 1950 at Ogbe, Oji, Udi and Mbala by the defunct Eastern Nigeria Development Corporation (ENDC) and Iwo, Eruwa and Upper Ogun by the defunct Western Nigeria Development Corporation (WNDC) [3]. The cashew nut liquid as a by-product of processing cashew is mostly composed of anacardic acids [4] which have been used effectively against tooth abscesses due to their lethality to a wide range of gram-positive bacteria [5]. The nut oil from cashew seeds is used topically as an anti-fungal agent, also for healing cracked heels as opined by [5]. [6], reported that consuming nuts at least four times a week resulted in a 37% reduced risk of coronary heart disease compared to those who never or seldom ate nuts. The cashew nut is a popular snack often eaten on its own and approximately 75% of their fat is unsaturated fatty acids which includes health [7]. In relation to the seed-borne mycoflora of cashew nut, fungi play a significant role in deteriorating the nutritive value of the nut by utilizing the nutrient for their growth. Plant based edible stuffs are very susceptible to fungal contamination [8]. Fungi are found in different food commodities including nuts and other parts of the plants and these results to economic losses on cashew nuts [9]. Several environmental factors like humidity and temperature during storage of these nuts influence the infestation by fungi and

aflatoxin production [10].Contamination of foodstuffs by aflatoxins has created serious concerns on food safety in Africa and especially Nigeria [11]. Mycotoxins are diverse group of secondary metabolites produced by molds which contaminate foods and have toxic effects on the health of humans and animals. *Aspergillus* spp, *Penicillium* spp, *Rhizopus* spp and *Mucor* spp are the most frequent species recovered from non-disinfected cashew nuts [12]. Fungi grow to produce secondary metabolites under favorable chemical, physiological and environmental conditions, especially when temperature and moisture are suitable [13]; [14]; [15]. Mycotoxicoses are becoming increasingly implicated in human and animal pathology [16]. However, the mycotoxigenic *Aspergillus, Penicillium* and *Fusarium* are responsible for secretion of different metabolic toxic compounds [17]; [18] and could be considered the most serious fungal genera that contaminate cashew nuts [19]. [20], summarily stated that a proper study of mycoflora of cashew nuts may improve and establish a new variety of seeds as a necessary immediate measure to increase the average cashew nut yield in Nigeria. Considering the significance of cashew in the livelihood, the effect of mycoflora will cause a significant yield reduction of cashew in Nigeria, hence this study therefore, investigated the mycoflora associated with cashew nuts (*Anacardium occidentale* L.) vended in selected Markets in Awka.

MATERIALS AND METHODS

Sample Collection

Fresh Cashew fruits (*Anacardium occidentale* L.*)* samples were purchased from three different local markets in Awka axis namely; Eke-Awka Market, Amaenyi Market and Goodwill Market. The nuts were detached from the fruits and put in nylon bags separately and brought into the laboratory of Department of Botany, Nnamdi Azikiwe University, Awka, where the nut shells were further opened to get the freshly enclosed edible cashew nuts for isolation of fungi.

Preparation of Sample for Analysis

The fresh cashew nuts were washed with tap water and oven dried at a temperature of 60^0C for 72 hours. The samples were ground into fine powder using grinding machine after which the ground samples were sieved to obtain powdered processed sample used for the analysis.

Determination of Moisture Content

The moisture content was determined according to AOAC (2000) method. An empty petri-dish was dried in an oven for 10 minutes and allowed to cool in a desiccator and then weighed (W_1). Approximately 2g of the sample was weighed into the petri-dish (W_2) and placed in an oven at 105°*C* for 5hours. It was then brought out, cooled in a desiccator continuously until a constant weighed is expressed (W_3). The procedure was repeated for the different sample location of cashew nuts.

$$\% \text{ Moisture} = \frac{W_2 - W_3 \times 100}{W_2 - W_1 1}$$

Where;
W_1 = weight of empty Petri-dish
W_2 = weight of sample + Petri-dish before drying
W_3 = weight of sample + Petri-dish after drying

Mycological Analysis

Sample suspension was prepared by adding twenty grams of ground sample in 100 ml of sterile distilled water for two hours. Then, it was shaken for ten minutes using a mechanical shaker. Serial dilutions were prepared from 10^{-1} to 10^{-6}ml for each sample location under aseptic condition as fungal spores sediment more quickly. One ml of appropriate dilution was transferred into petri dishes containing PDA medium using sterile pipette. Six replicates were produced. Then the plates were incubated at 27°Cfor 72 hours and then examined daily for the development of fungi growth.

Sub-culturing/Identification of Test Fungi Pathogens

When growth has established, subcultures were prepared using inocula from different organisms in the mixed cultures to obtained a pure culture. This was done by transferring hyphal tips from the colony edge of the mixed cultures to fresh plates of PDA using flame sterilized blades. After sub-culturing, the plates were incubated at 27°C until pure cultures were obtained. The Petri dishes of pure cultures of the test fungi were then sealed with paraffin to prevent contamination. The resulting pure cultures were used for characterization and subsequent identification of the fungi isolates with the aid of a compound microscope and identification guides [21]; [22].

Fungal Count and Mean

The results of the fungal colonies were carefully counted and their means calculated. The number of colonies was multiplied by the dilution factor, divided by the aliquot plated as follows:

$$\text{CFU/ML} = \frac{\text{Number of colonies} \times \text{the dilution factor}}{\text{Plating volume}}$$

Percentage Occurrence

Percentage occurrence was calculated using the following formula:

$$\% \text{ occurrence} = \frac{\text{No. of colonies of a particular fungi species in all plates} \times 100}{\text{Total no. of colonies of all the fungal colonies in all the plates}}$$

Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA) via Statistical Analysis System (SAS) of Version 9.1. Means of treatment were compared using Duncan's Multiple Range Test (DMRT) at $p<0.05$ [23].

RESULTS

Isolation of Fungal Pathogens from Cashew Samples

The incidence of occurrence of fungi isolates associated with cashew nuts in Awka markets indicated that six fungi were isolated and they included *Fusarium oxysporum*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Penicillium oxalicum* and *Mucor* species (Table 1).

Table 1: Fungi isolated from cashew nuts from different locations in Awka

S/N	Location	Fungal isolates
1	Eke-Awka Market	*Fusarium oxysporium* *A. niger* *A. flavus*
2	Amaenyi Market	*A. fumigatus* *A. niger* *A. flavus*
3	Goodwill Market	*A. niger* *Penicillium oxalicum* *Mucor* species

Fungal Count and Mean of the Cashew Nut Samples

The fungal colonies of the three different locations in Awka were observed and counted carefully. The data showed 133 fungal colonies from Eke-Awka, 90 fungal colonies from Amaenyi and 65 fungal colonies in Good-will market (Table 2).

Table 2: Fungal colonies and total mean

S/N	Location	Number of colonies	Mean colonies
1	Eke-Awka	133	1.33
2	Amaenyi	90	9.0
3	Goodwill	65	6.5

Percentage Occurrence

The results of the percentage occurrence of fungi isolates on cashew nuts obtained from Eke-Awka Market revealed that *A.niger* showed highest occurrence of

45.1% while the least occurrence of 10.5% was recorded against *F. oxysporum* (Table 3). The results of the percentage occurrence of fungi isolates on cashew nuts obtained from Amaenyi Market showed that *A. niger* had highest occurrence of 43.3% while *A. fumigatus* showed the least occurrence of 16.7% (Table 4). The results of the percentage occurrence of fungi isolates on cashew nuts obtained from Goodwill Market depicted that *Mucor* species showed highest occurrence of 53.8% while the least occurrence of 9.2% was recorded against *P. oxalicum* (Table 5). The results of the moisture contents of the cashew nuts obtained from three different locations showed that Eke-Awka Market recorded highest moisture content of 6.8% while the least moisture content of 4.1% was noticed against Goodwill Market (Table 6).

Table 3: Percentage occurrence of fungi isolates on cashew nuts from Eke-Awka Market

S/N	Fungi isolates	Individual occurrence	% occurrence
1	*Fusarium oxysporium*	14	10.5
2	*A. niger*	60	45.1
3	*A. flavus*	59	44.4
	Total colonies	133	

Table 4: Percentage occurrence of fungi isolates on cashew nuts from Amaenyi Market

S/N	Fungal isolates	Individual occurrence	% occurrence
1	*A. fumigatus*	15	16.7
2	*A. niger*	39	43.3
3	*A. flavus*	36	40.0
	Total colonies	90	

Table 5: Percentage occurrence of fungi isolates on cashew nuts from Goodwill Market

S/N	Fungal isolates	Individual occurrence	% occurrence
1	*A. niger*	24	37.0
2	*Penicillium oxalicum*	6	9.2
3	*Mucor* species	35	53.8
	Total colonies	65	

Moisture Content

Table 6: Moisture contents of cashew nuts obtained from different locations

S/N	Location	W_1(g)	W_2	W_3	Moisture content %
1	Eke-Awka Market	6.192	7.786	7.676	6.8
2	Amaenyi Market	6.200	7.715	7.615	6.6
3	Goodwill Market	6.197	7.253	7.210	4.1

DISCUSSION

Cashew nut infection by pathogenic fungi has been reported in a number of studies and revealed a high risk due to contamination with mycotoxins [24]. It is a fact that plant based edible stuffs are very susceptible to fungal contamination [8]. From the results of this study, it was shown that six fungi were isolated which included *F. oxysporium, A. niger, A. flavus, A. fumigatus, P. oxysporium and Mucor* species. This finding is in line with reports of [25]. Most of the fungi were previously reported from cashew nuts in many parts of the world [26]; [24][27]; [25]. Incidence of these isolates depends on a number of factors including moisture content and storage time [26]. Low moisture levels limit mould growth after harvesting and during storage. The percentage moisture contents for the three sample locations revealed that Eke-Awka Market recorded highest moisture content of 6.8%, which was closely followed by Amaenyi Market with moisture content of 6.6%, while the least moisture content of 4.1% was noticed against Goodwill Market. This agrees with the preliminary study conducted by

some authors that moisture content below 5.8% is approximately equivalent to 70% relative humidity. This is generally considered the maximum level for safe storage [28]. However, samples from Eke-Awka and Amaenyi had moisture contents above the limit and consequently were predisposed to more fungal growth and mycotoxin contamination (Table 6). Cashew nut is one of the few commodities that travel a long distance between times of harvest and when consumed [29] and these can lead to the initiation of these fungal activities thereby causing loses of commercial and nutritional values in the nuts and most importantly endanger the life of consumers by exposure to mycotoxins infestation. *Aspergillus* was represented by three species and showed the widest diversity among all isolated fungi which are *A. niger, A. flavus, A. fumigatus*, and followed by *Mucor* species, *Fusarium* and *Penicillium*. [30] stated that these fungal species are found common to the soil and different agricultural food commodities.

The contamination of toxigenic fungi and aflatoxins may occur from the soil during growth or harvesting or from the environment during storage and sale which are conductive for fungal growth proliferation and aflatoxin contamination [31].

CONCLUSION AND RECOMMENDATION

This study demonstrated that cashew nuts vended in Awka are contaminated with several mycotoxigenic fungi which included *F. oxysporum*, *A. niger*, *A. flavus*, *A. fumigatus*, *P. oxalicum* and *Mucor* species. It is therefore, recommended that strict mycological hygiene measures should be established in cashew farming, harvesting and storage to minimize mycotoxin contamination and to discourage high humidity that favours the growth of these fungi.

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