INCIDENCE OF MYCOFLORA OF LOCALLY MALTED SORGHUM (*Sorghum bicolour* L. MOENCH) USED FOR LOCAL DRINK (KUNU) PRODUCTION FROM KEFFI NASARAWA STATE

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Abstract: Seed borne mycoflora of lo cally malted sorghum used for local drink (kunu) production from different locations in Keffi LGA was investigated. The survey was carried out in four locations in Keffi Local Government Area, namely; Tudun Wada, AngwanLambu, Keffi Market and Sabon Kasuwa. Out of 400 grain samples investigated, 280 grains had fungi isolates. The fungi isolates were *Aspergillusniger*, *Apergillusflavus*, *Rhizopusoryzae* and *Mucorracemosus*. Their frequencies of occurrence were 23.21%, 32.50%, 22.50% and 21.79%, respectively. Keffi market had the highest incidence of fungi (32.14%) while SabonKasuwa had the least incidence of fungi (21.42%). Pathogenicity test conducted showed that species of fungi isolated are associated with locally malted sorghum in Keffi, Nasarawa State. The presence of these fungi on the malted sorghum of public health was significant. Kunu producers are encouraged to sterilize the grains or boil the grains to kill mycoflora before usage.

Keywords: Mycoflora, Sorghum, Kunu and Keffi.

Introduction

Sorghum is a genus of plants in the grass family Poaceae. Sorghum (*Soghurm bicolour* L. Moench) is a native of Africa with many cultivated forms.It is an important crop worldwide used for animal fodder, the production of alcoholic beverages, and biofuels (ITIS, 2006). *Sorghum bicolour* is an important food crop in Africa, Central America and South Asia, and is the fifth-most important cereal crop grown in the world (FAO, 1995). Most varieties are drought and heat-tolerant, and are especially important in arid regions, where the grain is one of the staples for poor and rural people. These varieties form important components of pastures in many tropical regions. Some species of sorghum can contain levels of hydrogen cyanide, hordenine, and nitrates lethal to grazing animals in the early stages of the plants growth. When stressed by drought or heat, plants can also contain toxic levels of cyanide and nitrates at later stages in growth (Singh *et al*., 1997).

Sorghum is a powerhouse in terms of nutrients and can provide those individuals who add it to their diet with vitamins like niacin, riboflavin and thiamine, as well as high levels of magnesium, iron, copper, calcium, phosphorous and potassium, as well as nearly half the daily required intake of protein and a very significant amount of dietary fibre (48% of the recommended intake (Lau Luchsinger, 2000). The second main component of sorghum andmillet grains is protein. Both genetic and environmental factors affectthe protein content of sorghum and millets. In Sorghum the variability of species is large, probably because the crop is grown under diverse agro climatic conditions which affect the grain composition (Gerik *et al*., 2003).

Kunu (also known as kununzaki) is a cereal-based beverage in Nigeria. The cereals utilized in its production are wheat, millet, sorghum and maize in decreasing order of preference (Gaffa *et al*., 2002). Sometimes, the cereals could be used in composite for its production but this is more common with only millet and sorghum grains. The preferred ratio of mixing is 1:2 (w/w) sorghum/millet. The traditional production process involves steeping the grains in local household utensil such as buckets, drums, calabashes or earthen ware vessels (Adeyemi and Umar, 1994).

Sorghum has high phenol and tannin contents (US Grain Council, 2008) and these principles make it resistant to mould infestation, diseases and damage. Despite its inherent resistance to mould infestation, sorghum grain mould constitutes one of the most important biotic constraints to sorghum improvement and production worldwide. It is estimated that annual economic losses in Asia and Africa as a result of grain mould are in excess of US$ 130 million (Chandrashekar *et al*., 2000). The mycoflora and mycotoxins contaminating sorghum in Nigeria and many parts of the globe have been documented (Bandyopadhyay *et al*., 2000; Okoye, 1992). Since fungi and their toxins cause obvious reduction in crop and animal livestock production and diseases in human, the aim of this study was to isolate and identify fungi associated with seed borne mycoflora of locally malted sorghum used for local drink (kunu) production in Keffi Nasarawa State.

Materials and Methods

Study area

The laboratory experiments were carried out in the Plant Science and Biotechnology laboratory, Department of Biological Sciences, Nasarawa State University, Keffi. The survey was carried out within four (4) different locations in Keffi Local Government Area. These locations include; AngwanLambu, TudunWada, Keffi market and andSabonKasuwa.

Sample collection

Samples of sorghum grains were collected from four (4) locations in Keffi, Nasarawa State. These grains were obtained from regular sorghum grains retailers in these locations in twovisits. On the first visit which was in January 2015, fifty (50) grains where collected from each of the locations to make up to 200 seeds. On the second visit in February 2015, fifty (50) grains were collected from each location making it a total of 400 sorghum grains collected at random from these locations.

Preparation of sterile agar medium

Thirty-nine grams of Potato Dextrose Agar (PDA) powder was dissolved in 1 litre of warm distilled water as instructed by the manufacturers.The mixture was then heated gently over the Bunsen burner until the agar powder dissolves. This was plugged with cotton wool. The flask of agar was then placed in the autoclave with the required number of petri dishes. The Agar and dishes were sterilized by heating to about 121^{o}C for 15 min at 15 pound pressure. The flask of agar was then allowed to cool.

Pouring sterile Agar plates

Bunsen flame was ignited on the working benchand cotton wool was removed from the agar flask and the mouth of the flask was passed through the flame. The lid of the sterile petri dishes was lifted as little as necessary and about 0.5ml of agar was poured into the petri dishes. The lid was then replaced on the petri dishes by passing the mouth of the flask through the Bunsen flame and the cotton wool was thenreplaced. This was repeated for the number of dishes needed. The dishes were not disturbed until the Agar has set and this took about ten minutes.

Inoculating the sterile Agar plates

An inoculating loop was flamed and allowed to cool without touching any part of the loop. The inoculating loop was cooled after a few seconds, microorganisms from sorghum grains were inoculated unto the sterile plates using the loop. This process was repeated twice for each location (Cappucino and James, 2012), so that a total of 100 plates were used for each location. Each visit was a replicate. A total of 400 petri dishes were used for the (four locations) study.

Incubation of Agar plates

Plates were incubated in an incubator at 30^{o}C which was maintained for seven (7) days. The plates were placed upside down to prevent condensation. Fungi isolates were identified using Domsch*et al*.(1980) method, that is photomicrography of the isolates were obtained using research camera and then compared with a standard published by Domsch*et al*., (1980). Data obtained from the survey were subjected to chi-square for analysis where results obtained could lead to the H_o "Species of fungi isolated are not associated with seed borne mycoflora of locally malted sorghum for Kunu Production examined" could be rejected or accepted.

Pathogenicity test

To establish which of the microbial isolates caused the deterioration, 2cm long cylindrical covers was removed from the middle portion of healthy sterilized fruits. The fruits were first of all washed with 2% sodium hypochlorite and allowed to dry. Discs, 5mm in diameter of two-day old fungal cultures of each isolate were inoculated, fungi first, into the holes made on the fruits with cork borer. The covers of the fruits were replaced after 5mm pieces had been cut off to compensate for the thickness of the fungal culture.

Results and Discussion

The results from the survey have shown that locally malted sorghum in Keffi LGA, Nasarawa State, Nigeria are associated with seed borne mycoflora. These fungi are well known and have been reported in some countries of the world and states in Nigeria (Onyeka*et al*., 2003). From the laboratory results obtained, 400 sorghum grains were collected and sampled out of which 280 grains had fungi isolates while 120 grains samples had no fungal isolates (Table 1). The species of fungi isolated and identified from the locally malted sorghum grain include *Aspergillusniger*, *Aspergillusflavus*, *Rhizopusoryzae*and *Mucorracemosus.* The frequencies of occurrence were 23.21%, 32.50%, 22.50% and 21.79% respectively (Table 2). From this survey, Keffi market had more fungal attack (32.14%) than other locations while Sabon Kasuwa (21.42%) had the least mycoflora generally. SabonKasuwa which had the least mycoflora may be as a result of high level of hygiene and cleanliness exhibited by marketers over their products in that area as reported by (Valiki, 1968).

Table 1: Incidence of fungi species in different locations in Keffi Local Government Area of Nasarawa State

Location	Total number of seeds	No. with fungi spp	No. without fungi spp
Tudun Wada	100	68 (17.00)*	32 (8.00)*
Angwan Lambu	100	62 (15.50)*	38 (9.50)*
Keffi Market	100	90 (22.50)*	10 (2.50)*
Sabon Kasuwa	100	60 (15.00)*	40 (10.00)*
Total	**400**	**280 (70.00)***	**120 (30.00)***

*Numbers in parenthesis are percentages.

Table 2: Frequency of occurrence of fungal isolate from different locations in Nasarawa State

Location	*Aspergillusniger*	*Aspergillusflavus*	*Rhizopusoryzae*	*Mucorracemosus*	Frequency of Attack
Tudun Wada	28	18	12	10	68
AngwanLambu	12	20	14	16	62
Keffi Market	20	35	17	18	90
SabonKasuwa	5	18	20	17	60
Total	**65**	**91**	**63**	**63**	**280**
%Total	**23.21**	**32.50**	**22.50**	**21.79**	**–**

– = not applicable

Table 3: Incidence of occurrence of fungalisolates from different locations

Location	*Aspergillusniger*	*Aspergillusflavus*	*Rhizopusoryzae*	*Mucorracemosus*	Frequency of attack	Mean	% incidence
Tudun Wada	28	18	12	10	68	17.00	24.28
AangwanLambu	12	20	14	16	62	15.50	22.14
Keffi Market	20	35	17	18	90	22.50	32.14
SabonKasuwa	5	18	20	17	60	15.00	21.42
Total	**65**	**91**	**63**	**61**	**280**	**-**	**-**
Mean	16.25	22.75	15.75	S	-	-	-
%Total	**23.21**	**32.50**	**22.50**	**21.79**	-	-	-

The incidence of mycoflora species in different locations were 24.28%, 22.14%, 32.14% and 21.42%, respectively (Table 3). The frequency of occurrence of fungi in locally malted sorghum from different locations showed that *Aspergillusflavus* had the highest frequency of occurrence (32.50%) while *Mucorracemosus*had the least with (21.79%) frequency of occurrence. This could be due to production of wide range of enzymes by *Aspergillusflavus*more than the rest of the species. Such enzymes include cellulose, pectinase, suberinase, cutinase, liginase, etc.*Aspergillusflavus*is a major producer of carcinogenic aflatoxins on crops, worldwide. It is also an opportunistic human and animal pathogen, causing aspergillosis in immunocompromised individuals (O'German *et al*., 2009).

The frequencies of occurrence of fungi species are independent of different towns in Keffi Local Government Area. Species of fungi isolated are associated with seed borne mycoflora of locally malted sorghum production examined (Table 4). This could be due to the fact that each location is adjoined to each other and as such have little or no variation in climatic conditions.

Table 4: Chi-square on the relationship between fungi isolated at different locations in Keffi, Nasarawa State

Location	No. with fungi spp	No without fungi spp	Total
Tudun Wada	68 (70.00)*	32 (30.00)*	100
Angwan Lambu	62 (70.00)*	38 (30.00)*	100
Keffi Market	90 (70.00)*	10 (30.00)*	100
Sabon Kasuwa	60 (70.00)*	40 (30.00)*	100
Total	**280**	**120**	**400**

*Number in parentheses are expected frequencies

Table 5: Percentage infection of pineapple fruits artificially inoculated with fungi isolated from diseased fruits

Fungal isolate	No of grains inoculated	% infection after 5 days
Aspergillusniger	20	80
Aspergillusflavus	20	70
Rhizopusoryzae	20	60
Mucorracemosus	20	100

Rhizopusoryzae is a decomposer that breaks down bread and other foods, *Mucorracemosus* as fungi causes systemic infections that infect internal tissue and organs and may spread through many regions of the body (Solomon *et al.*, 1999).

Tabulated value 7.815 < Cal. 27.02; so H_o is rejected. Species of fungi isolated are not associated with seed borne mycoflora of locally malted sorghum for Kunu production examined.

Conclusion

During the period of the survey to determine fungi responsible for the seed borne mycoflora of locally malted sorghum used for local drink (kunu) production in Keffi Nasarawa State, *Aspergillusflavus* had the highest frequency of occurrence (32.50%) while *Mucorracemosus* had the least with (21.79%) frequency of occurrence. These fungi are injurious to health andthe public are advised to avoid consuming unsterilized sorghum. Efforts should be made for proper storage after harvesting so as to cub the conditions that enhance mycoflora growth in this region. This will also ensure that the taste of the 'kunu' is not distorted and its freshness is maintained.

Acknowledgement

We want to sincerely thank the Plant Science Unit, Department of Biological Sciences of Nasarawa State University, Keffi for the use of laboratory facilities.

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