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Incidence of *Aspergillus* Species on the Stored Millet (*Pennisetum Glaucum*) and Sorghum (*Sorghum Bicolor*) at Dawanau and Sharada Markets

Yahaya, S. M. Department of Biology, Kano University of Science and Technology, Wudil, Kano State

Hafsat, S. J. Department of Biology, Kano University of Science and Technology, Wudil, Kano State

Ali, M. U. Department of Biology, Kano University of Science and Technology, Wudil, Kano State

Amina, L. A. Department of Biochemistry, Kano University of Science and Technology, Wudil, Kano State

Tasiu, A. M. Department of Biochemistry, Kano University of Science and Technology, Wudil, Kano State

Ibrahim, A. M. Department of Biochemistry, Kano University of Science and Technology, Wudil, Kano State

Sadiya, A. B. Department of Biochemistry, Kano University of Science and Technology, Wudil, Kano State

Maimuna, M. D. Department of Biochemistry, Kano University of Science and Technology, Wudil, Kano State

Musa, M. J. Department of Biochemistry, Kano University of Science and Technology, Wudil, Kano State

Abstract

Experiment was carried out using plate culture method to isolate and identified the Aspergillus species responsible for losses of stored grains at Dawanau and Sharada markets. Six Aspergillus species were isolated and identified from the stored grains. These are A. candida, A. flavus, A. fumigatus, A. niger, A. oryzae, A. Candida and A. Parasiticus with A. flavus and A. niger as the most frequently encountered species having 47.0% and 46.0% frequency of occurrence respectively. The differences in fungal deterioration of the millet and sorghum between the two study areas was not statistically significant (P<0.05). The results of this study found that a post-harvest loss of the millet and sorghum in the two markets was due to attack by fungal species.

Keywords: Fungi, Millet, Sorghum, Dawanau, Sharada

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench), and pearl millet (*Pennisetum glaucum* L.) comprise the major components of human diet in Africa. Cereals like Sorghum, Millets, Wheat, Maize and Rice are major staple foods of the most population (Owolade *et al*., 2005). Sorghum is the second most important cereal after maize with 22% out of total cereal area cultivated (Owolade *et al*., 2005). Pearl millet is a climate hardy crop which is grown in harsh conditions, but as a subsistence crop. Harvested from an area of 20 m ha in the semi-arid regions of Africa, pearl millet contributes 19% area to cereal production (Anyanwu 2003; Anderson *et al*., 2004; Muhammad *et al*., 2014).

In West Africa crop production is mostly concentrated in the drier parts of the region owing to its drought tolerance. It is mainly grown in Northern areas of Nigeria and Republic of Niger (FAO 2002; Muhammad *et al*., 2014). The yields of millet range from two to three tons per hectare. In Nigeria the total production in the year 2013 was 80,000 metric tons from 115,000 hectares (FAO, 2001; Kavia 2007). It is an important component of the diets of pregnant and lactating women, infants and children (1993) as well as recuperating and sick patients (Muhammad *et al.*, 2003). Millet is traditionally utilized in different ways mainly to prepare thin porridge (Tuwo), stiff porridge (Akamu) and alcoholic drinks (Muhammad *et al.*, 2014).

Other products made from millet include; leavened breads and non-alcoholic beverages. In some cases flour from millet is blended with flours from sorghum, maize and cassava and used in food preparation (Kumasi *et al.,* 2009). The crop is also used for medicinal purposes such as treating measles, colds, anaemia and diarrhoea (Kumasi *et al.,* 2009). The straw may be used for thatching and weaving for example traditional baskets for serving ugali (Kumasi *et al.,* 2009).

In Nigeria sorghum is mostly grown in the drought-prone marginal agricultural areas of Northern and Eastern provinces (Muhammad *et al.*, 2014).The crop performs well in areas between 500 m and 1700 m altitude and rainfall of 420 to 630 mm (Kumasi *et al.,* 2009). Nutritionally sorghum contains higher protein levels than maize. Sorghum is grounded and used as porridge and the flour is mixed with wheat flour for bread, the flour is also mixed with maize or finger millet flour and used in preparation of many traditional dishes such as Tuwo (ICRISAT, 2014). According to Muhammad *et* *al.,* (2014), sorghum has been identified as a trap plant in push-pull strategy against stem borers and as reservoirs for their natural enemies.

However, both millet and sorghum are affected by many diseases resulting in high post-harvest losses in the field and after harvest. In addition to causing significant qualitative and quantitative yield reduction (Oerke 2005), these fungi are known to produce mycotoxins that pose serious health hazard to humans and animals that feed on contaminated grains (Samson 1991; Pestka and Smolinsky, 2005; Rooney, 2013).*Aspergillus* species are recognized as a major agricultural problem as the species are worldwide on a variety of plant hosts and primarily on cereal grains (Kumasi *et al.,* 2009;Leslie *et al.,* 2008). The presence of *Aspergillus* species in stored millet and sorghum may pose a threat to the health of both humans and livestock. However, in Nigeria there is no accurate data on the frequency and relative percentage of fungi on grains especially in millet and sorghum. Because of these reasons, it has not been possible to develop effective management strategies to prevent fungal infection and bio-deterioration of grains. This brought the need to provide baseline information that will assist in design of control strategies. Therefore, the main aim of the present study is to investigate the incidence of *Aspergillus* species on millet and sorghum on sale at Dawanau and Sharada markets of Kano State, Nigeria and also to determine between millet and sorghum which one is more prone to infection by *Aspergillus* species.

MATERIALS AND METHODS

STUDY AREA

Dawanau market is located at Bichi Local Government Area of Kano State, Nigeria, a distance of about 90 km away from Kano University of Science and Technology, Wudil. It is the largest cereals market in Kano State, with different varieties of cereals being available to customers from different parts of African countries. Despite being one of the largest cereals markets in Africa, there are no good storage facilities in the market. Some marketers store their grains on the floor of the stores while others kept their grains in sacs.

Sharada:**market** is located at Gwale Local Government Area of Kano State a distance of about 50 Km away from Kano University of Science and Technology, Wudil. It is one of the large vegetable markets in Kano State. There are no fruits and vegetables grown in Kano State that are not found at Sharada. Despite being one of the largest vegetable growing and selling markets at Kano metropolitant, there are no good storage facilities at Sharada market. Some marketers store their fruits and vegetables on the bare floor of the stores, while others kept theirs packed in baskets. Marketers hardly used chemicals on their fruits and vegetables. They however washed them either with water or detergents.

EXPERIMENTAL PROCEDURE

In this study an investigation was carried out to provide information on fungal colonies responsible for post-harvest losses of millet and sorghum. The investigation was carried out from September 2015 to February, 2016.

Isolation and Identification of Post-Harvest Fungi

This involved the isolation and identification of fungi associated with loses of quality and quantity of millet and sorghum respectively. The methodology that was used in this research was based on the works of Yahaya (2005) and Yahaya *et al*., (2016) and is described below:

Sample Collection and Handling

Sample of millet was obtained twice a week directly from the study area. Millet obtained were rinsed in three times differently in running tap water and allowed to dry and incubated at 25.7≠2°c for three days.

Colony Count and Subculture

Each week, growth of fungal colonies was monitored and the number of colonies that appeared was counted and recorded. Each distinct colony was sub cultured in to fresh Potato Dextrose Agar.

Pathogenicity Test

A pathogenicity test was carried out to prove Koch postulate. All fresh samples were separately washed in 10% (v/v) sodium hypochlorite solution and rinsed in three times in differently in running tap water and allowed to dry. A sterilized needle was used to streaked fungal hyphea from crushed and uncrushed grain sample and then placed on fresh samples of millet and sorghum. Controls were inoculated with sterile distil water. Materials were placed on the laboratory bench. Sterilized forceps was used to remove portions from the disease areas on the 4th day and placed on freshly prepared PDA plates and incubated at 25.7≠2° c for 3 days. Fungal growth that appeared was recorded.

STATISTICAL ANALYSIS

The data was analysed statistically using one way analysis of variance (ANOVA) and differences among the means was determine for significance at $P \leq 0.05$. This was achieving using computer program (SSPSS, 16.0).

RESULTS

A total of 181 fungal species were counted and recorded during the study at Dawanau and Sharada markets. The fungal colonies isolated have the following compositions *A. flavus* 47(25.9%), followed by *A. niger 46*(25.41%), *A. parasiticus 26* (14.36 %), *A. fumigatus* 23(12.70 %), *A. orzae* 19(10.49 %) and the least occurring colony of 20 (11.04 %) was recorded in *A. candida* (Table 1) Statistically the difference between the six colonies isolated in Millet and Sorghum in Dawanau and Sharada was not statistically significant at P = 0.336 (Table 1).

Incidence of *Aspergillus* species on Millet and Sorghum grains at Dawanau Market

Results showed that all the samples show infection of *Aspergillus species*. The highest occurring colony was *A. niger* 23 (23.71%) in both millet and sorghum this was followed by *A. flavus* with 21(11.60%), followed by *A. parasiticus* 15 (15.48%), *A. candida* 12(21.64%) *fumigatus* 11(11.34%). The least occurring colony was *A. Orzae* 10 (10.30 %). Variation in the colonies counted from millet and sorghum at Dawanau market shows that more colonies were counted on sorghum 53(54.63%) while 46 (47.42 %) was recorded on millet. The result showed that the differences between the *Aspergillus* species isolated in millet and sorghum at Dawanau market was not statistically significant at P = 0.323 (Table 2).

Incidence of *Aspergillus* species on Millet and Sorghum at Sharada Market

All samples collected from Sharada Market showed infection of *Aspergillus species*. The highest occurring colony was *A. flavus* in both millet and sorghum with 26(27%), followed by *A. niger* 23(24.4%), *A. fumigatus* 12(12.8%), *A. parasiticus* 14(7.73%), *A. orzae* 9(9.4%) and the least occurring colony was *A. Candida* 8(8.3%). Variation in the colonies counted from millet and sorghum at Sharada market showed that more colonies were counted on sorghum 48(53. 9%) while 41 (46.0%) was recorded on millet. The result showed that the differences between the *Aspergillus* species isolated in millet and sorghum at Sharada Market was not statistically significant P = 0.332 (Table 3).

Pathogenicity Test

The pathogenicity test confirmed Koch postulate for identification of the causative agent of a particular disease. The pathogens where present in all cases of the disease. The same pathogens was isolated from the diseased host and re-grown in pure culture when inoculated into a healthy grain sample of millet and sorghum the pathogen from the pure culture cause the same disease. The same pathogen was re-isolated from the new host and shown to be the same as the originally isolated pathogen (Table 4).

Table 1: ***Aspergillus*** **species counted from millet and sorghum sampled from both Dawanau and Sharada market**

Crops	*A. Candida*	*A. flavus*	*A. fumigatus*	*A. niger*	*A. orzae*	*A. parasiticus*	Total	%
Millet	12	22	11	23	8	8	84	46
Sorghum	8	25	12	23	11	18	97	54
Total	20	47	23	46	19	26	181	100
%	11.0	26.0	13.0	25.4	10.4	14.3		

Table 2: ***Aspergillus*** **species counted from millet and sorghum sampled from Dawanau market**

Crops	*A. Candida*	*A. flavus*	*A. fumigatus*	*A. niger*	*A. orzae*	*A. parasiticus*	Total	%
Millet	7	10	6	12	3	5	43	46
Sorghum	5	11	5	11	7	10	49	53
Total	12	21	11	23	10	15	92	100
%	13	22	11	25	10	16	97	

Table 3: ***Aspergillus*** **species counted from millet and sorghum sampled from Sharada market**

Crops	*A. Candida*	*A. flavus*	*A. fumigatus*	*A. niger*	*A. Orzae*	*A. parasiticus*	Total	%
Millet	5	12	5	11	5	3	41	46.0
Sorghum	3	14	7	12	4	8	48	53.9
Total	8	26	12	23	9	11	89	9.99
%	8.3	27.7	12.8	24.4	9.4	12.3		

TABLE 4: PATHOGENICITY TEST ON FRESH/APPARENTLY HEALTHY MILLET AND SORGHUM SAMPLED FROM SHARADA MARKETS

A. flavus	*A. fumigatus*	*A. niger*	*A. orzae*	*A. Candida*	*A. parasiticus*
+	+	+	+	+	+
+	+	+	+	+	+

Key: + = Isolates grow with a similar growth characteristic features to the original diseased samples

DISCUSSION

Incidence of *Aspergillus* species on millet and sorghum grains

The entire sample collected showed infection of *Aspergillus* species. A total of six *Aspergillus* species were isolated during the study from the stored grains collected from the two study areas. These include *A. candida, A. flavus, A. fumigatus, A. niger, A. oryzae* and *A. parasiticus* with *A. flavus and A. niger* as the most frequently occurring isolate with 26.0% and 25.0% frequency of occurrence respectively. Result of the pathogenicity test confirmed the pathogens as originally isolated pathogen of millet and sorghum sample from Dawanau and Sharada markets. More colonies where recorded at Dawanau then Sharada markets.

The result of the present study agreed with the results of Muhammad *et al*, (2014) who reported the incidence of *Aspergillus* species as *A. flavus* 25.5%, *A. niger* 20.3%, *A. fumigatus* 10.1%, *A. oryzae*5.0% and *A. parasiticus* 3.8% from the millet and sorghum sold in Tambuwal market of Sokoto State Nigeria. Also the result support the result of Francisca *et al.,* (2007) who observed an incidence of 30.53% on millet and 54.2% in sorghum among the grain sold at Nnewi market. The fungal organisms isolated from millet and sorghum grains in the present study are known to be spoilage organisms associated with many agricultural products including cereals, fruits and nuts (Labbe and Garcia, 2001; Francisca *et al*., 2007;Muhammad *et al*., 2014). These fungi might have colonized the grains during production in the field, transportation or storage. The variation in the frequency of their occurrence may have reflects differences in the inoculum density in the area or the prevailing environmental conditions favouring the growth of the fungi.

As reported by Klinch (2007) the result of the present study shows that the incidence of *Aspergillus* species on millet was higher in *A. flavus and A. niger*), this also agree with the result of Oladiran and Iwu (2013) in a study carried out at Wamakko grains market. They reported the incidence of *Aspergillus* species with high percentage of occurrence in *A. flavus, A. niger* and *A. fumigatus*. In (2001) this was similarly reported by Ehrlich (2007). Analysis by Food and Agricultural Organization (2002) shows that deterioration in grain quality due to the activity of *A. flavus* makes it unfit for marketing and consumption, they reported 25% of the world food crops are affected by mycotoxins each year resulting in monetary losses by the marketers, scarcity and high cost of the commodity. Aflatoxins contamination in grain poses a great threat to human and livestock health as well as international trade. Munkvold and Desjardins (1997) reported that crop loss due to aflatoxins contamination costs US producers more than $100 million per year on average including $ 26 million to peanuts

($69.34/ha). Munkvold and Desjardins (1997) estimates of the costs of control of mycotoxins in food products in the United State to be $0.5 to $1.5 billion/year and $5 billion/year for Canada.

In conclusion, the study has shown that *Aspergillus* species could account for the substantial losses of millet and sorghum stored and sold by grain sellers in the study area and the grains are susceptible to invasion and colonization by diverse moulds. In addition to causing significant qualitative and quantitative yield reduction (Parry 1990;Adamu 2002;Kimanya *et al*. 2008; Jimoh and Kolapo 2008), these fungi are known to produce mycotoxins that pose serious health risks to humans and animals that feed on contaminated grains (Pestka and Smolinsky, 2005). Therefore, efforts in good storage and processing practices should be intensified as preventive measures against toxigenic strain dispersal.

The presence of mycotoxins in sorghum and millets seems to depend on a number of factors including grain resistance, time of contamination and post-harvest handling and storage conditions. Given the growing contribution of sorghum and millets to diets, it is imperative that the incidence, extent and risk of mycotoxins in sorghum and millets be studied in order to design control strategies.

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