

AME

**BIOLOGICAL AND ENVIRONMENTAL
SCIENCES JOURNAL FOR
THE TROPICS**

ISSN 0794 - 9057

Volume 5 Number 2 June, 2008



AN INTERNATIONAL JOURNAL



BEST JOURNAL 5(2):62 - 66
Date received: November, 2007
Date accepted: January, 2008
Printed in Nigeria.



SCREENING OF SOME CEREALS USED IN INDUSTRIAL PRODUCTIONS FOR *FUSARIUM* SPECIES

Ameh*, J.B; Abdullahi, I.O and Mustapha, A.K

Department of Microbiology, Ahmadu Bello University, Zaria

*Corresponding author

ABSTRACT

A study was carried out to evaluate the frequency of contamination of stored maize and Sorghum used in industrial productions with members of the genus *Fusarium*. Ninety one samples comprising white maize, yellow maize, white sorghum, and yellow sorghum were obtained from 18 storage facilities located in both Kaduna and Zaria cities of Kaduna State. They were plated out using the direct plating method using potato dextrose agar (PDA) and malt extract agar (MEA) as the growth media. The plates were incubated at room temperature ($21^{\circ}\text{C} \pm 1^{\circ}\text{C}$), for 48-96 hours. The resulting growth was screened both visually and microscopically for *Fusarium* species. Twenty nine samples yielded at least one species of *Fusarium* giving a frequency of 31.86% infection. The temperatures of the storage facilities were determined and they ranged from $29-39^{\circ}\text{C}$ with an average of 33°C . The moisture content values for the grains were determined using the oven method and they ranged from 5.3 13.0%. The results from this study can be used to guide against the use of *Fusarium*-infected cereals in industrial productions.

Key words: Maize, Sorghum, *Fusarium*, Contamination

INTRODUCTION

Maize (*Zea mays*) and sorghum (*Sorghum bicolor*) are important raw materials in various food and beverage industries, and in animal feed production among other uses. They are also the staple food of a large number of people especially in Northern Nigeria, of which Zaria is a part. For these purposes, these grains are usually purchased in large quantities and stored in bags or silos, pending processing. The major sources of deterioration of stored grains are insects and fungi (Ominski et al., 1994). Along with *Fusarium*, the major genera commonly encountered on maize and to a lesser extent, sorghum, in tropical regions, are *Aspergillus* and *Penicillium* (Samson, 1991; Orsi et al., 2000). Many members of the genus are known to be toxigenic, producing mycotoxins such as fumonisins, deoxynivalenol and other trichothecenes, zearalenone, and moniliformin, resulting in many human and animal diseases (Calvin and Harrison, 1992). These toxins have not been successfully removed from the grains after contamination, in spite of various attempts reported by Voss et al. (1996), and Marin et al., (1998). It is thus safer to keep the grains free of *Fusarium* infection and subsequent toxin production.

Popular locally brewed drinks such as *kunu*, *burukutu*, and *koko* among others, and industrially brewed non-alcoholic and alcoholic drinks are produced from these two grains. In

the light of this, it is important from both industrial and health standpoints to evaluate their contamination and infection frequencies with *Fusarium* species.

MATERIALS/METHODS

Collection of Samples

Samples of the grains were collected from 100kg-sacks of grains using a grain probe and stored in paper bags to minimize moisture build-up.

Determination of Storage Temperature

The temperatures of the stores, at the point of sample collection were recorded using $0-100^{\circ}\text{C}$ thermometers. In all, 19 stores in Kaduna and Zaria were visited and 91 samples were collected; 32 samples of white corn (WC), 21 for yellow corn (YC), 14 for white sorghum (WS) and 24 for yellow sorghum. The samples weighed between 500-900g. The samples were then conveyed to the laboratory, placed in waterproof bags and stored at 4°C until required.

Determination of Moisture Content

The moisture content (MC) value of the samples was determined using the high constant temperature oven method. Portions of the samples were taken and slowly ground in a porcelain mortar to avoid heat build-up and minimize moisture loss. Two-10g portions were then weighed in crucibles and dehydrated in a



pre-heated oven set at 130°C for 30 minutes. The crucibles were then left to cool and re-weighed. The average weight loss was then recorded and expressed as a percentage of the initial weight. This is the MC value.

Scoring of the Grains

The grains were scored, a process essentially used as a means of assessing physical damage visible to the naked eye. The proportion of visibly mouldy, rotted, or discoloured seeds in each of the 91 samples was determined by scoring all the seeds in a representative sample of 100 seeds. The samples were then classified as good-quality maize or sorghum, i.e. having less-than 34% incidence of visibly diseased seeds; or poor-quality maize or sorghum i.e. having more than 34% incidence of visibly diseased seeds (Kedera et al., 1999).

Isolation of *Fusarium* Species from the Grains

Five grains were randomly selected per sample and directly plated on malt extract agar, another five grains were selected and surface sterilized by immersion in 1% sodium hypochlorite (NaOCl) solution for 30 seconds. The grains were rinsed thrice in sterile distilled water and transferred to MEA. The plates were then incubated at ambient temperatures for 7 days after which they were viewed directly at x100 magnification for gross colony observations and observed features recorded. The *Fusarium* species present were microscopically identified according to the method of Nelson et al. (1983).

RESULTS AND DISCUSSION

Moisture Content (MC) Determination

The moisture content values of the grains ranged from 5.3–13.0% (Table 1) with an average MC value of 7.43% and this is well below the threshold required to prevent fungal growth on cereal grains as according to Gupta (1996). *Fusarium* species can only grow on stored grains with moisture contents of at least 18%, while other fungi grow on starchy cereals at MC levels lower than 13.5%. This however, is in contrast to the results obtained in this study, as 29 out of 91 samples were positive for *Fusarium* contamination. Of these, none had a moisture content up to the threshold value of 18%. This anomaly may be explained by the fact that moisture content values do not necessarily reflect the average, thus the values for some grains may be above the average, and since storage fungi will grow where the moisture

conditions are favourable and not necessarily according to the average moisture content of the grains (Sweets et al., 2004). It is likely that these grains could be responsible for the observed fungal growth. "Hot spots" and fluctuating store temperatures could encourage localized fungal growth in spite of low overall MC values.

Scoring of the Grains

Forty nine out of the 53 maize samples, equivalent to 92.45% of the maize samples were of good quality. All the yellow maize samples were of good quality while four white maize samples were of poor quality and yielded *Fusarium* species from both the surface-sterilized and non-surface sterilized grains. This is to be expected because damaged grains are more susceptible to *Fusarium* (and other fungal) infections (FAO, 2002). On the other hand, all 38 samples amounting to 100% of the sorghum samples were of good quality.

Fusarium species isolated from the grains

Nine samples of sorghum, equivalent to 23.68% had *Fusarium* species, while 20 out of 53 maize samples, equivalent to a frequency of 37.73% were infected giving a combined frequency of *Fusarium* contamination of stored maize and sorghum grains sampled to 31.86% (Table 2).

Only 12 of the surface-sterilized samples had *Fusarium* while 28 unsterilized grains yielded *Fusarium*. Only YC 16 yielded *Fusarium* in the surface-sterilized grains and not in the raw sample, while two samples, YC8 and YC16 yielded more than one species of *Fusarium* after sterilization as compared to 13 non-sterilized samples that had multiple infections.

The most frequently isolated species was *Fusarium verticilloides* with nine isolates on maize and two on sorghum (Table 3) and this is consistent with the report of Chen et. Al. (1992); Doko et. al. (1996); Allah Fadi (1998) and Baba-moussa (19998) who all reported that *F. verticilloides* was the most frequently isolated species (68%) on maize grains. The recovery of isolates of *F. sporotrichioides* from a sample of sorghum and maize was unexpected since this is usually found in temperate to cold regions (Onyike and Nelson, 1992). The least frequently isolated species was *F. poae* which was isolated once on each grain type. On the sorghum samples, the most frequently encountered species were *F. verticilloides*, *F. moniliforme* and *F. andiyazi* which occurred twice each. This is also consistent with available literature that reported that *F. andiyazi* is frequently



associated with sorghum (Leslie, 2002).

The length of storage is also an important factor as by April when most of the samples were taken, the grains had been in storage for almost 6 months. This length of time

, coupled with the hot temperatures and the onset of the rainy season can be used to explain the high prevalence of *Fusarium* and its toxin in the grains.

Table 1: Storage temperatures and moisture content values for samples that showed *Fusarium* growth.

Number	Sample	Moisture content (%)	Storage temperature (°C)
1	WC1	6.1	38
2	WC4	8.6	36
3	WC7	5.3	32
4	WC8	6.0	36
5	WC10	9.6	35
6	WC13	7.4	38
7	WC20	9.5	35
8	WC25	9.6	34
9	WC29	8.4	30
10	WC30	8.3	35
11	YC1	8.2	32
12	YC2	6.9	31
13	YC3	10.5	33
14	YC6	8.9	32
15	YC8	6.9	31
26	YC9	11.5	30
17	YC11	10.4	29
18	YC16	10.9	32
19	YC20	5.6	34
20	YC21	5.6	33
21	WS6	7.0	30
22	WS9	7.0	33
23	WS11	6.0	35
24	WS13	9.0	30
25	WS14	10.5	34
26	YS3	12.2	33
27	YS8	6.0	36
28	YS13	9.5	34
29	Ys16	13.0	31

Key: WC = white corn; YC = yellow corn; WS = white sorghum; YS = yellow sorghum

Table 2: occurrence of *Fusarium* species on the grain samples

Sample No	Serial No	Sterile Grain	Unsterile Grain	Multiple <i>Fusarium</i> Infection Sterile Grain	Unsterile Grain	Other fungi isolated
Wc1	1		+		+	Pe, Cu
WC4	2		+			Rh
WC7	3		+		+	Rh, As
WC8	4	+	+			Cy
WC10	5		+			Pe, Cy
WC13	6	+				As, Pe, Rh
WC20	7		+			Rh
WC25	8		+		+	Rh, Mu
WC29	9	+	+			Mu, Cy
WC30	10	+	+		+	Mu, Fe
YC1	11	+	+		+	CU, As
YC2	12		+			Pe
YC3	13	+	+		+	Rh, As
YC6	14		+		+	As
YC8	15	+	+	+		Mu, Cy
YC9	16		+			Mu, Rh
YC11	17	+	+		+	Cu
YC16	18	+		+		As, Pe, Tr
YC30	19	+				Cy, Mu
YC21	20	+	+			Tr, As
WS6	21		+			Pe
WS9	22		+			Rh, Pe
WS11	23		+		+	Mu, As
WS13	24		+			As
WS14	25		+			As, Rh
YS3	26		+			Pe
YS8	27	+	+		+	Rh, Cu
YS13	28		+		+	Pe, As
Ys16	29		+		+	Cu

Key: + = *Fusarium* infection; Pe = *Penicillium*; Tr = *Ttrichoderma*; Cu = *Curvularia*; Cy = *Cylindrophora*; As = *Aspergillus*; Rh = *Rhizopus*; Mu = *Mucor*

Table 3 frequency of occurrence of the various species of *Fusarium*

<i>Fusarium</i> species	Maize	Sorghum
<i>Fusarium graminearum</i>	5 isolates (14.21%)	1 isolate (2.86%)
<i>F. Verticilloides</i>	9 " (25.71%)	2 " (5.71%)
<i>F. Poae</i>	-	1 " (2.86%)
<i>F. Versicolor</i>	2 " (5.71%)	1 " (2.86%)
<i>F. Acuminatum</i>	2 " (5.71%)	-
<i>F. Proliferatum</i>	3 " (8.57%)	2 " (5.71%)
<i>F. andiyazi</i>	-	2 " (5.71%)
<i>F. Sporotrichoides</i>	1 " (2.86%)	1 " (2.86%)
<i>F. Culmorum</i>	2 " (5.71%)	-
<i>F. Oxysporum</i>	1 " (2.86%)	-

CONCLUSION

In general, these grains that are used in industrial productions, maize and sorghum, sampled in Zaria and Kaduna metropolis area of Kaduna State in Nigeria were found to have high levels of *Fusarium* species (31.86%). This indicates that *Fusarium* is a real threat to the health and safety of consumers of malted drinks,

alcoholic drinks and grain based food products since it can produce toxins which cause any diseases of humans and animals.

Recommendations

1). There should be a mass enlightenment of farmers where they will be taught good agricultural practices that will minimize the



invasion of the grains by the fungus during cultivation, harvest and processing.

- 2). The grains should only be stored at moisture content values below their individual threshold values required to keep fungal growth minimal,
- 3). The grains should be stored under

REFERENCES

- Allah, Fadl, M.E., (1998): Occurrence and toxigenicity of *Fusarium moniliforme* from freshly harvested maize ears with special references to fumonisin production in Egypt. *Mycopathologia* 140: 99-103.
- Baba-Moussa AAMT (1998): La microflore associee aux deats des lepidopteres foreus de ties et mineours d'epis demais dans la regions Sud du Benin aavec reference speciale a *Fusarium moniliforme* Sheldon. *Memoire d'Ingenieur Agronome*.
- Calvin, B. M. and L. R. Harrison (1992): Fumonisin induced pulmonary edema and hydriothorax in swine. *Mycopathology*. 117: 79-82.
- Chen, J.P. Mirocha, C. J., Xie, W., Hogge, L. and Olson, D. (1992): Production of the mycotoxin Fumoonisin B1 by *Alternaria. alternate f. sp. Lycopersici*. *Appl. Environ. Microbiol.* 58:3928-3931.
- Doko, M.B., C. Canet, N. Brown, E. W., Sydenham, S. Mpuchane, and B.A. Siame (1996): Natural Occurrence of fumonisins and zearalenone in cereals and cereal-based foods in eastern and southern Africa. *J. Agric. Food Chem.* 44:3240-3243.
- FAO (2002): FAOSTAT Database, Food and Agriculture Organisation, Roma, Italy URL:<http://apps.fao.org/lim500/nph-wrap.pl>.
- Gupta, Rajni and Singh, S. (1996): Natural Occurrence of *Fusarium* and zearalenone in sorghum grains during field and storage. *Geobios* 21:251-256.
- Kedera C.J., Plattner R.D., Desjardins A.E. (1999); Incidence of *Fusarium* and levels of Fumonisin B1 in maize in Western Kenya. *Appl. Environ. Microbiology* 65: 41- 44.
- Leslie, J.F. (2002): *Agroecology and Biotechnology of Stalk Rot Pathogens of Sorghum and Millet*. Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506-5502.
- Marin, S., Sanchis, V., Saenz, R., Ramos, A.J., Vinas, I. & Magan, N. (1998): Environmental factors, in vitro interactions and niche overlap between *Fusarium moniliforme* F. *proliferatum* and *F. graminearum*, *Penicillium*, and *Aspergillus* species from maize grain. *Mycol. Res.* 102:831-837.
- Nelson, P.F., T.A. Toussoun and W.F.O. Marasas, (1983): *Fusarium* species. An illustrated manual for identification. State University Press, Pennsylvania, USA.
- Ominski, K.H., (1994): Ecological aspects of growth and toxin production by storage fungi. In: Miller J.d. Trenholm H.S. eds. *Mycotoxins in grains. Compounds other than aflatoxin*. Eagen Press, USA. Pp. 287-305.
- Onyike, N.B. and Nelson, P. (1992): *Fusarium* species Associated with Sorghum Grain From Nigeria, Lesotho, and Zimbalwe, *Mycologia*, . 84 (3), Pp. 452-458.
- Orsi, R.B., Correa, B., Pozzi, c, and Schamass, E. (2000): Mycoflora and occurrence of fumonisins in freshly harvested and stored hybrid maize. *J. Stor. Prod.Res.* 36:75- 87.
- Samson R.A. (1991): Identification of food-borne *Penicillium*, *Aspergillus* and *Fusarium* Species. In: Champ BR, Highly E, et al., Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand. 23-26, April, 1991.
- Sweets, L. (2000): *Stored grain fungi*. Missouri State University, G44440, Missouri. Missouri.edu/storage/disease/sgfungi.
- Voss K.A., C.W. Bacon, F.I. Meredith, and W.P. Norred. (1996): Comparative subchronic toxicity studies of nixtamalized and water extracted *Fusarium moniliforme* culture material. *FoodChem. Toxicol.* 34: 623-632.



P

* De

* De

ABST
Tradit
to tre
on the
prese
aquec
used.

Keyw
studie

INTR
Stachy
family
up to 4
rather
or with

known
(verba
uses o
glucos
proper
herb is
cooled
search
previo

to inve
the ac
toxicity

MATE
Plant

moist a
in the
Depart