STUDIES ON FUNGI CAUSING GRAIN MOULD OF SORGHUM VARIETIES IN NORTHERN NIGERIA WITH SPECIAL EMPHASIS ON SPECIES CAPABLE OF PRODUCING MYCOTOXINS

BY

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A thesis submitted in partial fulfilment of
the requirements for the Degree of
MASTER OF SCIENCE

Department of Crop Protection
Ahmadu Bello University
Zaria, Nigeria.

Nov. 1979.

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D E C L A R A T I O N

I hereby declare that the whole of the work now submitted as Thesis for the Master of Science Degree in Crop Protection of Ahmadu Bello University, is the result of my investigation (except where reference is made to published literature and where assistance is acknowledged) and has not been part of any presentation for any other qualification.

DADA J. D.
(M.Sc. Candidate)

To the Memory of

James Aroninuola

Kabai Kamun.

ACKNOWLEDGEMENTS

I wish to express my profound appreciation to Dr. I. D. Erinle, Head of the Department of Crop Protection, Dr. P. D. Tyagi, Cereals Pathologist and Professor J. A. Fewster, members of my advisory Committee for supervising this work. I also thank the Head of the Department for allowing the use of research facilities in the Department for this work.

I also wish to thank the following for their various contributions to the success of this work: Professor D. MacDonald who initiated the project, Mr. Salifu for his help in the mycotoxin analysis, Messrs Gumel and Auta of their assistance in the identification of the fungal isolates, the technical and administrative staff of the Department of Crop Protection for their services and friendship, Mr. Oyejola for his help in the statistical analysis and Mr. Hassan Salihu for typing the thesis.

I am indebted to Professor L. J. Egler, Head of Department of Microbiology for giving me the opportunity to undertake this study and Dr. J.B. Gaultney for his personal encouragement.

Finally I am most greatful to Mbo-o my true helpmate, to Lydia, Ruth, Demas and Oluwafunmilola for making the burden lighter and to Mami and Femi for standing by me as usual.

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1.0. INTRODUCTION

Sorghum, also known as Guinea corn (Sorghum bicolor L. Moench), is one of the most important cereals of the semi-arid tropics, and provides the staple food for large populations in Africa and Asia. It is typically well adapted to the climate of the Savanna zones because it tolerates heat and drought. Sorghum is well suited to regions with erratic, uncertain rainfall at planting time, because even as a seedling, it soon recovers after limited periods of drought.

In North America and Australia Sorghum is grown commercially on large farms with grain yield averaging 3053 kg/ha and 2,222 kg/ha, respectively, (Anon, 1977) and all of it is used for livestock feed. This contrasts sharply with the average annual production in West Africa and India estimated at 572 kg/ha and 544 kg/ha respectively, most of which is used for human consumption. This fact and the forecast

(Mensah, 1977) that world demand for food will increase at the annual rate of 2.4% with a concomitant annual increase in population of 2.7% during the next ten years, makes imperative the need to raise and stabilize sorghum yields in the less developed tropical nations.

In northern Nigeria, sorghum is grown either as a sole crop or inter-cropped with other crops. From 1960 to 1975, it accounted for about 49 per cent of the total production of cereals in Nigeria and, on average, 46 per cent of the annual average devoted to cereals (Abalu, 1978). Estimated total production of sorghum in Nigeria in 1977 was 3.5 million tons from an area of about 5 million ha. corresponding to a yield of about 610 kg/ha. Sorghum is grown primarily as a grain crop for human consumption, but it is also used for making beer, medicines and as livestock feed.

There are four ecological zones within the areas cultivated with sorghum in northern Nigeria (Fig.1; Table[1]).

Traditional sorghum cultivars are normally photosensitive with flowering timed so that grains fill and mature after the rains have ceased. These cultivars are generally low to moderate yielders and often fail to mature fully if the rains cease early. During the drought of the early 1970's in Nigeria, these cultivars had very poor yields because the rains did not last long enough to allow for full growth and development of the crop. In order to ensure good grain filling without the interference of drought stress, non photosensitive cultivars of relatively short duration and with wide adaptability are being developed. However these short duration cultivars which mature during the wet weather, are vulnerable to mould attack, and this has become a major problem in their adoption. Grain moulds reduce grain quality and such grain is especially undesirable for human consumption because these fungi have the potential to produce metabolites, known as mycotoxins, which can be toxic to man and animals. Studies on fungi causing grain moulds and

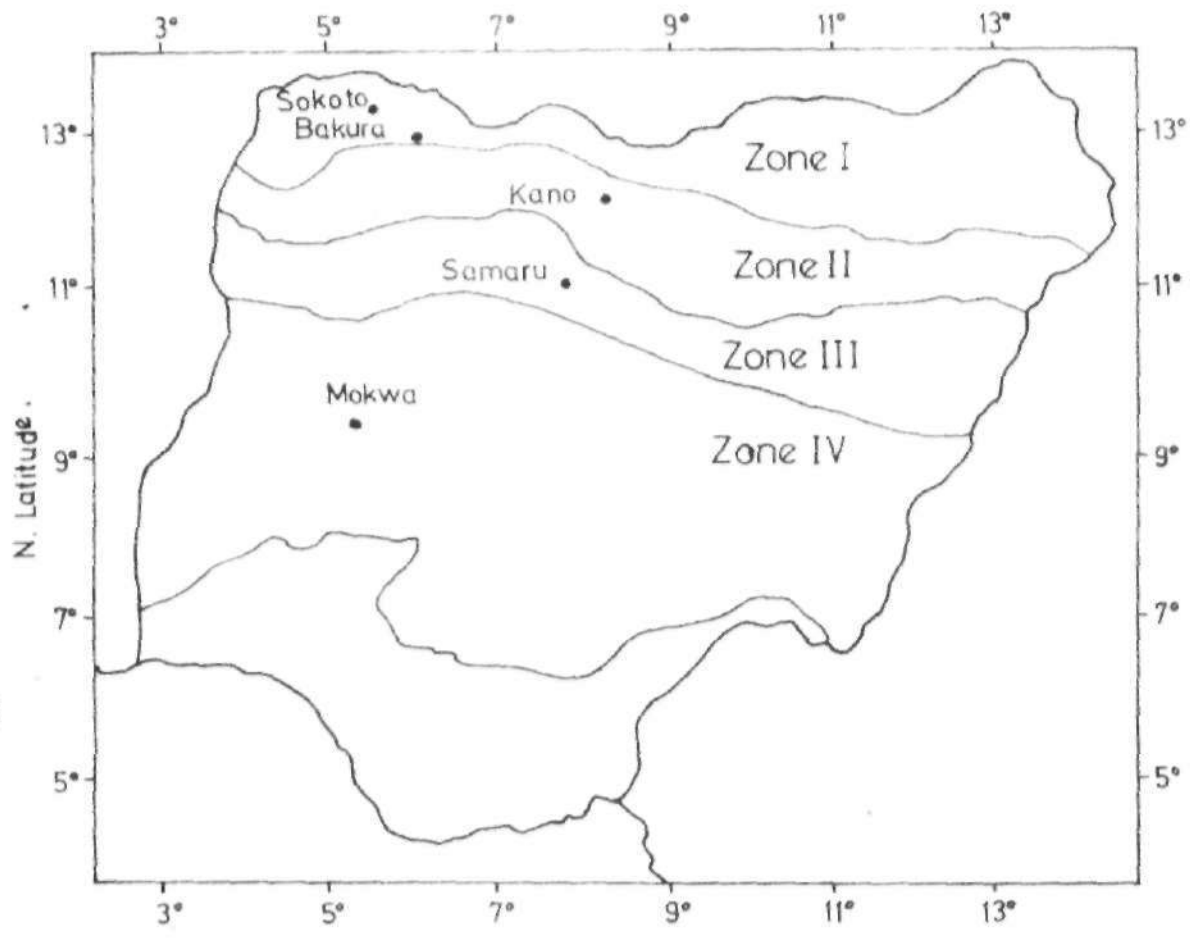


Fig. 1 Sorghum-growing ecological zones in northern Nigeria.
Zone I northern sudan savanna, Zone II sudan savanna,
Zone III northern guinea savanna and Zone IV southern guinea savanna.

Table 1: Sorghum growing ecological zones.
(After Tyagi, 1978)

Ecological zone	Growing Season (days)	Average rainfall (mm)
Northern Sudan Savanna	90 - 110	600
Sudan Savanna	120 - 150	750
Northen Guinea Savanna	150 - 180	1000
Southern Guinea Savanna	180 - 220	1200

their potential to produce mycotoxins in sorghums are therefore essential, not only in exploring these causative fungi but also in the human health problems that are likely to be caused by the mycotoxins.

The aim of this study, therefore, is (1) to make a quantitative assessment of the mycotoxin producing fungi (Aspergillus flavus, A. niger, Fusarium spp. e.g. F. moniliforme, F. semitectum and F. equiseti, and Phoma sorghina) associated with the different stages of grain development of sorghum varieties grown in different ecological zones of northern Nigeria and

(II) to investigate possible mycotoxin(s) contamination in grains of these samples.

2.0. LITERATURE REVIEW

2.1. Grain moulds:

The term grain or head mould describes the diseased appearance of sorghum grains resulting from infection of the developing grain by one or more parasitic fungal species while in the field. In broad sense, the term also applies to sorghum grains infected with fungi while in storage (Williams and Rao, 1978).

The symptoms which develop as a result of field infection of the developing grain by parasitic fungi depend upon the fungus or fungi, and the time and severity of infection. Grains severely infected appear to be completely covered with pink and/or black mould and they disintegrate into powder in the threshing process. Lightly infected grain may appear almost completely normal except for slight pink or black discolouration on a small portion of the surface, and internally the grain appears normal (Williams and Rao, 1978).

2.2. Importance of grain mould

Grain moulds occur as a major and widespread disease of sorghum. They cause considerable yield loss and reduce quality of harvested grain.

2.2.1. Yield loss: In Central Kentucky, sorghum yields of less than 641 kg/acre was recorded when yields of 2,316 kg/acre or more were expected (Gray, Lacefield and Lowe, 1971). The low yields were the result of the failure of heads to produce mature seed which was believed to be due to grain moulds. Bhatnagar (1971) reported a marked reduction in the size and weight of sorghum grain artificially infected with Curvularia lunata. Denis and Girard (1978) observed that when there were few other limiting factors to plant development, grain moulds had a clear negative effect on yield. Castor (1977) reported reduced yield, test weight, and 1000 - kernel weight in sorghum due to grain mould caused by Fusarium spp. In a survey of millet and sorghum

diseases in India, Sundaram, Palmer, Nagarajan and Prescott (1972) recorded losses of up to 50% due to grain moulds in hybrid sorghum. According to Glueck, Rooney, Rosenow and Miller (1977) significant losses due to grain moulds occurred in Texas in 1974 and 1976.

2.2.2. Losses in quality

2.2.2.1. Market value: In the case of grains produced for direct human consumption, the quality of the harvested product is of considerable importance. Grain moulds discolour the grain, reducing its acceptability and thus the value of the grain. von Oppen and Jambunathan (1978) in surveys conducted in villages in Central India, found that the market prices of sorghum grains are significantly related to the degree of mould infection and the price of mouldy grain was about 20% less than that of the cleanest grain.

2.2.2.2. Nutritional value: Sorghum grain deterioration has been attributed to physical, physiological, and chemical changes within the grain. Glueck et al. (1977) reported that starch granules of deteriorated grains have considerable pitting on the surface (resulting from enzyme degradation), and the protein matrix is weakened and partially hydrolysed. In deteriorated grain, the soluble carbohydrates are usually decreased for they are utilized to provide energy for the growth and development of the fungi. The proteins are hydrolysed and partially utilized in the synthesis of fungal protein which remain in the grain.

2.2.2.3. Loss of viability: There is a loss in viability and reduction in seedling vigour in moulded sorghum grains. Various levels of reduction in viability were reported by Narasimhan and Rangaswamy (1969), Tripathi (1974), Castor (1977), Rao and Williams (1977) and Denis and Girard (1977). Arif and Ahmed (1969) found that all fungi isolated

from sorghum grain reduced germination and that Fusarium was the most harmful, followed by Aspergillus, Penicillium and Helminthosporium. Mathur, Mathur, and Neeragaard (1975) frequently detected Fusarium moniliforme in embryos of sorghum grain samples and they observed that F. moniliforme affected germination and seedling growth of the samples.

2.3. Fungi associated with sorghum grain mould

Many reports on sorghum grain mould deal generally with the mycoflora of the seeds. Records of such studies have been obtained from India (Narasimhan and Rangaswami, 1969; Ravindranath, 1976) Pakistan (Junejo and Malik,, 1967), U.S.A. (Hansing and Hartley, 1962; Castor, 1977), Senegal (Denis and Girard, 1977) and Nigeria (Zummo, Salifu, 1977; Elegbede, 1978; and Tyagi, 1978).

Fungi isolated from sorghum grains include the following genera: Acrothecium, Alternaria, Aspergillus, Bipolaris, Chaetomium, Chaetopsis, Cladosporium,

Cladotrichum, Cochliobolus, Colletotrichum, Cunninghamella, Curvularia, Cylindrocarpon, Fusarium, Helminthosporium, Mucor, Nigrospora, Olpitrichum, Pellicularia, Penicillium, Phoma, Pestalotia, Pycnidium, Ramularia, Rhizopus, Sordaria, Thielavia, and Trichothecium. Of these, the most frequently encountered genera are Fusarium, Curvularia, Aspergillus, and Phoma.

Rao and Williams (1977) reported that not all the fungi associated with sorghum grain moulds can be implicated as the real causative agents, and they found that Fusarium moniliforme, F. semitectum, and Curvularia lunata were involved in the development of the highest levels of grain moulds. Castor (1977) inoculated sorghum heads in the field with F. moniliforme, F. semitectum, C. lunata, C. protuberata, Alternaria spp., and Helminthosporium spp., and concluded that Fusarium and Curvularia were the principal pathogens that caused discolouration and reduction in viability of grains. Castor (1977) found F. moniliforme

to be more pathogenic than F. semitectum. Among the fungi isolated from field-collected sorghum grains in Nigeria, the most common in decreasing order of frequency were Phoma sorghina, Fusarium and Curvularia (Tyagi, 1978).

These three fungi were detected from the "milk" stage of grain development and their numbers generally increased as the grain developed.

2.4. Mycotoxins produced in sorghum grains

In considering the nutritive value of mouldy sorghum grain, a most important area of concern is the possibility that the fungi will produce mammalio - toxic chemicals known as mycotoxins.

The known mycotoxins, the fungi which produce them and the nature of the diseases they cause are listed in Tables 2 and 3.

Initial work on mycotoxins was concentrated on the aflatoxin group of chemicals. The aflatoxins, produced by Aspergillus flavus group and A. parasiticus,

Table 2: Some important mycotoxins, organisms which produce them and crops with which they are commonly associated. (After Jarvis, 1975).

Toxin	Organisms	Crop
Aflatoxins	Aspergillus flavus, A. parasiticus.	Oilseeds, nuts, cereals
Ochratoxin A	A. ochraceus, A. sulphureus, A. nelleus, Penicillium viridicatum.	Cereals oilseeds
Rubratoxins	P. rubrum, P. purpurogenum	Cereals
Citrinin	P. citrinum, P. citreo -viride, A. terreus, A.candidus	Cereals
Penicillic acid	P.puberuhum, P.cyclopium, P. martensii, A. ochraceus.	Cereals
Zearalenone (F-2 toxin)	Fusarium graminearum (Giberella zea).	Cereals
Patulin	P. urticae, P. patulum, P. clav-iforme, A. clavatus, A. terreus, Byssochlamys nivea.	Fruits and fruit juices

Table 3: Compounds involved in mycotoxicoses (After Ciegler, 1975).

Toxin	Biological effect	Susceptible host
Aflatoxin	Hepatotoxin, Cancer	Mammals, fish, birds
T - 2	Dermal necrosis, hemorrhage	Cattle, man?
F - 2	Vulvovaginitis, abortion	Swine
Ochratoxin A	Nephrotoxin	Swine man?
Maltoryzine	Death	Cattle
Sporidesmins	Hepatotoxin, facial eczema	Cattle sheep
Psoralens	Dermotoxin	Man
Diplodiatoxin	Nephritis, mucoenteritis	Cattle, sheep
Rubratoxins	Hepatitis x	Dogs
	Hemorrhage	Swine, poultry

are highly toxic to animals at very low concentrations, and they have been detected in sorghum and other cereals. Coady (1965) demonstrated the presence of aflatoxin in millet, sorghum grain, and sorghum flour in Ethiopia. Alpert, Hutt and Davidson (1971) detected aflatoxin in Ugandan sorghum grains. In a survey of the natural occurrence of aflatoxins in the U.S.A. and Canada, Shotwell, Hesseltine and Goulden (1969) found aflatoxin in excess of 10 ug/kg in 6 of 533 sorghum samples. In northern Nigeria, Salifu (1978) detected aflatoxin in excess of 20 ug/kg in two mould - susceptible varieties of sorghum and showed that mycotoxin production in the field starts at the hard dough stage of grain maturity. However, Elegbede (1978) did not detect any aflatoxins in stored sorghum samples collected from farmers in Zaria area. In an extensive study of aflatoxins in Nigerian groundnuts, MacDonald and Harkness (1963) found appreciable levels of aflatoxin in groundnuts collected from widely separated locations.

In a survey of South African foodstuffs, Martin, Gilman and Keen (1971) and Martin (1974) reported the presence of aflatoxin in excess of 10 ug/kg in 3 of 39 good quality sorghum samples examined. In North India, Tripathi (1973) isolated aflatoxins B_1, B_2, G_1, and G_2 from mouldy sorghum.

Rukmini and Bhat (1978) found a toxic metabolite in sorghum grains which they called T-2 toxin. They isolated Fusarium incarnatum from these grain samples.

Ochratoxin A is one of the three chemically related metabolites isolated from Aspergillus ochraceus. It was detected in maize offered for sale in the U.S.A. (Shotwell, Hesseltine and Goulden, 1969). It has also been reported in wheat samples in Canada (Scott, Van Nalbeek, Kennedy, and Anyeti, 1972) and in stored sorghum samples in Nigeria (Elegbede, 1978).

Zearalenone (F-2 toxin) is produced by Fusarium roseum, F. tricinctum, F. oxysporum, and F. moniliforme. It was detected in Swaziland in 4 samples of mouldy

sorghum and 27 pooled samples of sour fermented porridge and beer made from maize and sorghum (Martin, 1974). A similar study was carried out in Lesotho and Zearalenone (up to 50 ug/kg) was found in 16 of 71 beer samples. Schroeder and Hein (1975) detected Zearalenone in samples of grain sorghum which were heavily infected with F. roseum "Gibbosum" and F. roseum f. sp. semitectum. Grains from selected infected heads contained twice as much zearalenone as grain from a combined bulk. They concluded that zearalenone contamination may be a significant factor where there is severe Fusarium attack on maturing grain sorghum during warm and highly humid weather. Stipanovic and Schroeder (1975) investigated the zearalenone - producing potential of Fusarium roseum cultures isolated from mouldy sorghum grains. From one culture grown on grain sorghum they isolated zearalenone and two related compounds, zearalenol and 8^1 - hydroxyzearalenone. They suggested that the presence of these compounds in grain sorghum may

constitute a health hazard. In Zaria (Nigeria), the grains of short-season sorghums grown and harvested in 1976 contained up to 200 ug/kg zearalenone (Salifu, 1978). However, Elegbede (1978), working on stored samples of local cultivars obtained from farmers around Zaria, detected only very low levels of zearalenone in 2 out of 17 samples.

3.0 MATERIALS AND METHODS

3.1. Study of fungi associated with stored grains of different varieties.

3.1.1. Varieties: The seeds of 19 sorghum varieties used for this study were obtained from the National Seed Service, Federal Department of Agriculture (F.D.A.), Samaru and Plant Breeding Section, Institute for Agricultural Research, Ahmadu Bello University, Zaria. The varieties used were:

1.	KSV 2	11.	MSV 1
2.	KSV 3	12	MSV 2
3.	KSV 4	13.	MSV 3
4.	KSV 5	14.	75/76
5.	KSV 6	15.	KL 123
6.	SSV 2	16.	CK 60
7.	SSV 3	17.	Serena
8.	SSV 6	18.	Is 9289
9.	SSV 7	19.	6788B
10.	SSV 8		

The seeds had been in store for about six months.

3.1.2. Grain mould rating: 5 grades of intensity can be distinguished from a given lot of mouldy grains. This type of grading is based on the quantum of discolouration found on the seed that can be observed by an unaided eye (Anon, 1976, 1978). The grades are presented in Table 4.

3.1.3. Isolation of fungi from grains: Seeds were soaked for 1 minute in sterile water and then surface sterilized by immersion for three minutes in a 0.5% aqeous solution of sodium hypochlorite (NaOcl). The seeds were then rinsed in sterile water three times. Following these treatments, seeds were plated out aseptically on Potato Dextrose Agar plus sterptomycin. Each petri dish contained 10 seeds. Plating of seeds was carried out in a hood which was presterilized with fumes of formaldehyde. The fumes were produced when formalin (in a petri dish placed in the hood) is added to few grains of potassium permanganate. The petri dishes were incubated at room temperature (27 - 32^{o}C) and examined after 4 and 8 days for growth of fungi from the seeds.

Table 4: Grain mould rating

Grade	Degree of mouldiness
1	Grains mould free and free from discolouration.
2	1 - 10% of grains with some mould and discolouration.
3	11 - 25% of grains with mould and discolouration.
4	26 - 50% of grains with mould and discolouration.
5	More than 50% grains severely moulded and discoloured.

3.1.4. Identification of fungi: The fungi isolated from the seeds were identified after comparing them with authentic fungal cultures available in the culture collection of the Crop Protection Department. This culture collection consists of fungal cultures identified by staff of the Commonwealth Mycological Institute, Kew, London. Whenever necessary, standard monographs on different groups of fungi were consulted.

3.2. Study of fungi associated with field collected grains.

3.2.1. Samples collected from farmers: 39 samples of sorghum collected from different sorghum growing areas of Nigeria were supplied by the Cereals Pathologist, ICRISAT, Samaru.

The method of isolation of fungi from the grains and their identification was the same as that given in Section 3.1.3. and 3.1.4.

3.2.2. Samples from trials planted in the four sorghum growing ecological zones of Nigeria.

3.2.2.1. Location of trials: The field trials were laid out at four locations: Bakura (Northern Sudan Savanna), Kano (Sudan Savanna), Samaru (Northern Guinea Savanna) and Mokwa (Southern Guinea Savanna). The Northern Sudan zone trial was to have been sited at Tungan Tudu, a few kilometres north of Sokoto, but communication problems made this inconvenient. Bakura, which is located at the boundary of the Northern Sudan and Sudan ecological zones, has research facilities. It was therefore chosen as the Northern Sudan location.

3.2.2.2. Varieties: For this aspect of the experiment, seeds of different varieties were also obtained from the National Seed Service, Federal Department of Agriculture, Samaru and the Plant Breeding Section, Institute for Agricultural Research, Ahmadu Bello University, Zaria. These varieties were those

recommended for the different ecological zones and also four short reason varieties (CK 60, Serena, Is9289, and 6788B) which had been used by Salifu (1978) in his studies on mycotoxin contamination of sorghum (See Table 5). 19 varieties were planted at Samaru. At other locations, the Institute for Agricultural Resarch (I.A.R.) - recommended varieties for each zone together with the four short season varieties were planted.

3.2.2.3. Plot size, design of experiment and fertilizer application.

At each location, randomized complete block design was used for the trials. The plantings were done as soon as the rains were established in the zone (Table 5).

Each variety was planted on five ridges, 0.9 metres apart x 7.7 metres long, 12 plantings per ridge in four replicates.

Superphosphate was applied at the rate of 125 kg/ha before ridging. Nitrochalk was applied at 62.5 kg/ha two weeks after planting, followed by a second

Table 5: Varieties planted at different locations

	LOCATION			
	Bakura (Northern Sudan Savanna)	Kano (Sudan Savanna)	Samaru (Northern Guinea Savanna)	Mokwa (Southern Guinea Savanna)
Date of planting	27th June 1978	20th June 1978	13th June 1978	9th June, 1978
Variety	CK 60	CK 60	CK 60	CK 60
	Serena	Serena	Serena	Serena
	Is9289	Is 9289	Is 9289	Is 9289
	6788B	6788B	6788B	6788B
	KSV 3	KSV 2	KSV 2	MSV 1
	KSV 4	KSV 6	KSV 3	MSV 2
		KL 123	KSV 4	MSV 3
			KSV 5	
			KSV 6	
			KL 123	
			SSV 2	
			SSV 3	
			SSV 6	
			SSV 7	
			SSV 8	
			75/76	
			MSV 1	
			MSV 2	
			MSV 3	

identical dose six weeks later, i.e. eight weeks after planting.

3.2.2.4. Grain mould ratings, isolation and identificatio of fungi.

The methods used were the same as those described in sections 3.1.2., 3.1.3., and 3.1.4.

3.3. Study of mycotoxin contamination of field - collected grains.

3.3.1. Sampling: Ripe grains from about 20 sorghum heads of each variety were thoroughly mixed and divided into two lots. One lot was used for isolation of fungi (See 3.2.2.4) and the other was analysed for mycotoxin contamination. The seeds for mycotoxin analysis were dried in a forced draft oven at 60°C for 24 hours, ground in a mechanical mill and 50g of each sample were weighed out to be used for toxin extraction.

3.3.2. Extraction: Mycotoxins were extracted by the AOAC method (Anon, 1970). Samples of ground sorghum (50g) were extracted for 30 minutes with methanol:

water (55:45 v/v, 150 mls) using a wrist-action shaker (Gallenkamp, England) and filtered through Whatman No.1 filter paper.

3.3.3. Cleaning - up of extract: The filtrate were prepared for thin layer chromatography as described by Pons and Goldblatt (1965).

To 75 ml of a filtrate, 10ml 20% lead acetate and 30 ml distilled water were added and the mixture evaporated on a water bath (97°C) to the original volume (75ml). This was filtered through Whatman filter paper No.1. The filtrate was shaken with chloroform (3 x 20ml) in a separating funnel. The lower (chloroform) layer was filtered through a bed of anhydrous sodium sulphate to dry the chloroform of moisture. The combined chloroform extract was evaporated to dryness on a water bath, dissolved in 1 ml of chloroform and transferred quantitatively into a vial. The extract in the vial was re-evaporated to dryness on an electric hot plate/magnetic stirrer (Techne Cambridge Limited,

Duxford, England) and then dissolved in 0.5ml chloroform. The extract was kept for t.l.c.

3.3.4. Extraction of P. sorghina cultures for t.l.c.

P. sorghina was plated on five PDAS plates and incubated for 6 days at room temperature. The mycelia from all the plates were scraped into a sterilized flask and extracted for t.l.c. as described in sections 3.3.2. and 3.3.3.

3.3.5. Thin layer chromatography.

3.3.5.1. Preparation of chromatoplates: Thirty grams of silica gel GH-R (Machery, Nagel & Co., 516 DORGN, West Germany) were mixed with distilled water (60ml) and shaken for one minute. The slurry was then spread on glass plates, with the aid of a spreader (Shandon Scientific Co. Ltd., London, N,W.10, England) to give a layer of adsorbent, 0.25mm thick. The plates were dried at room temperature for ten minutes, activated at 105^{o}C for one hour, and stored in a desiccator.

Table 6: Spotting pattern on t.l.c. plates.

Spot No.	1	2	3	4	5	6	origin
Extract No.		1		2		3	
Mycotoxin Standard	B_1		B_2		Zea		

Each plate had six spots, each spot containing either an extract, i.e. sample, or mycotoxin standard.

B_1 = Aflatoxin B_1

B_2 = Aflatoxin B_2

Zea = Zearalenone.

grain each. SSV 7 had the lowest level of contamination, with 8% infected grains.

Among the mycotoxing producing fungi, Phoma sorghina, and Fusarium spp. (F. semitectum, F. moniliforme and F. equiseti) were the most common (Plates 1 - 6). Grains of KSV 4, KSV 5 and SSV 8 were contaminated with A. flavus while A. niger was associated only with 2 varieties: SSV 7 and SSV 8. SSV 7, MSV 3 and KL 123 were free from contaminations by Fusarium spp. while Is9289 and KSV 3 had high levels of the fungus. All the varieties were contaminated with P. sorghina, the highest level of contamination being in KSV 2, KSV 3, SSV 3, MSV 3, KL 123 and CK 60.

4.1.2. Mycotoxing-producing fungi associated with field collected grains.

4.1.2.1. Mycotoxin-producing fungi associated with grains collected from farmers.

In all the 39 samples, the percentage of infected grains ranged from 10 - 100, the lowest being found in

Table 7: Grain mould ratings, percent infected grains and numbers of mycotoxin producing fungi isolated from stored grains of different varieties of sorghum.
(Each test sample consisted of 50 grains)

Variety	Grain mould rating	Infected Seeds (%)	Number of mycotoxin producing fungi			
			A. flavus	A. niger	Fusarium spp.	P. sorghina
KSV 2	3	50	0	0	2	5
KSV 3	3	34	0	0	5	7
KSV 4	2	22	1	0	1	2
KSV 5	2	20	1	0	3	1
SSV 2	1	12	0	0	1	1
SSV 3	2	56	0	0	2	6
SSV 6	2	28	0	0	1	4
SSV 7	1	8	0	1	0	1
SSV 8	2	18	1	1	1	1
MSV 1	1	15	0	0	1	2
MSV 2	2	18	0	0	1	2
MSV 3	2	24	0	0	0	5
75/76	2	14	0	0	1	3
KL 123	3	42	0	0	0	5
CK 60	3	34	0	0	3	7
Serena	2	24	0	0	1	4
Is 9289	3	34	0	0	8	3
6788B	2	18	0	0	1	2

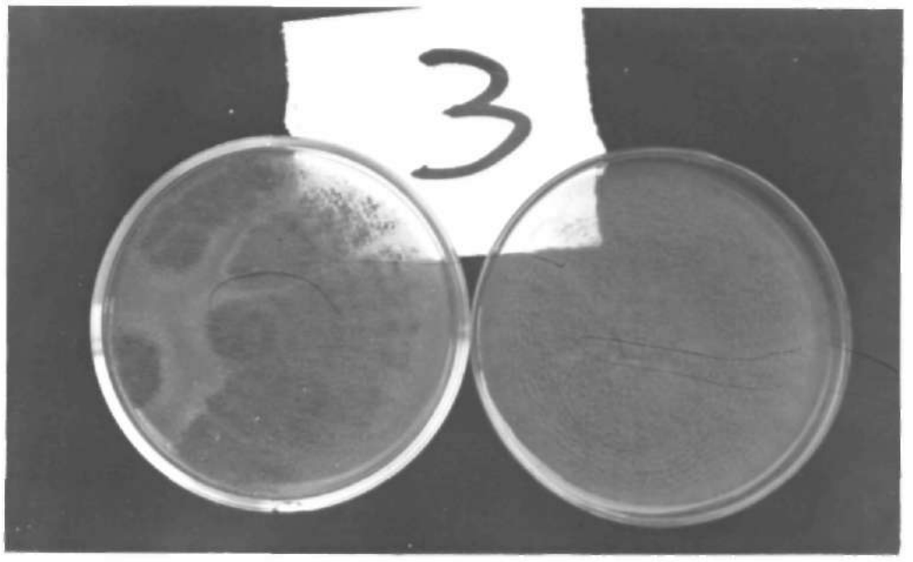


Plate 1. A. flavus growing on PDAS.

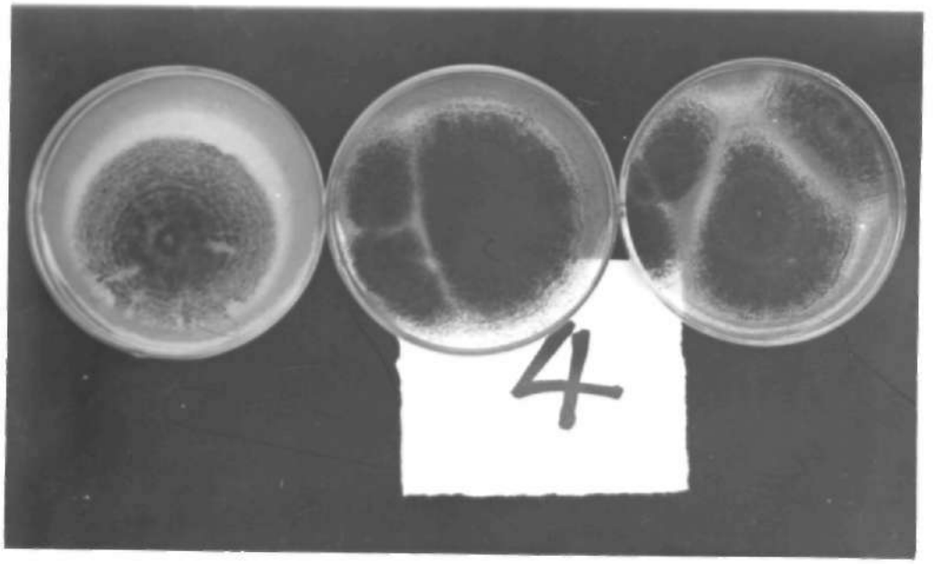


Plate 2. A. niger growing on PDAS.

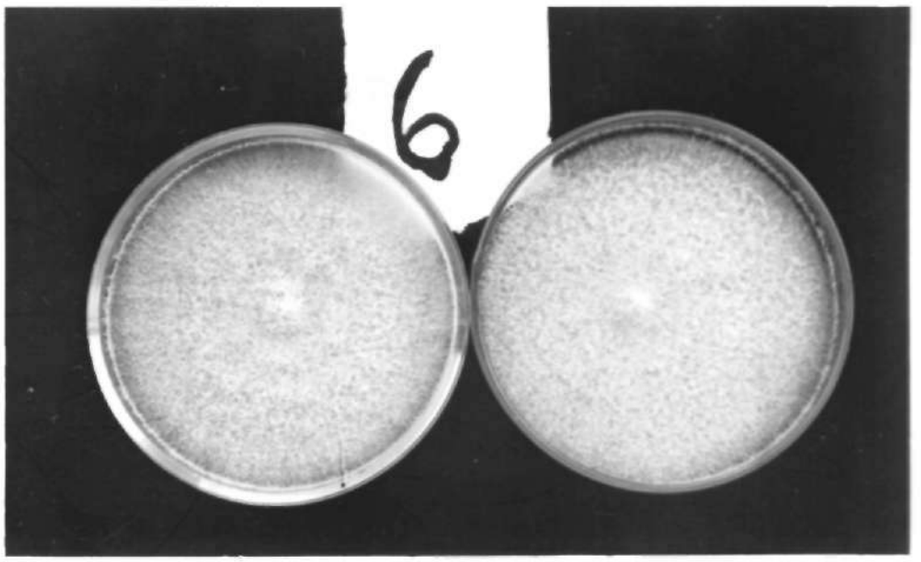


Plate 3. F. moniliforme growing on PDAS.

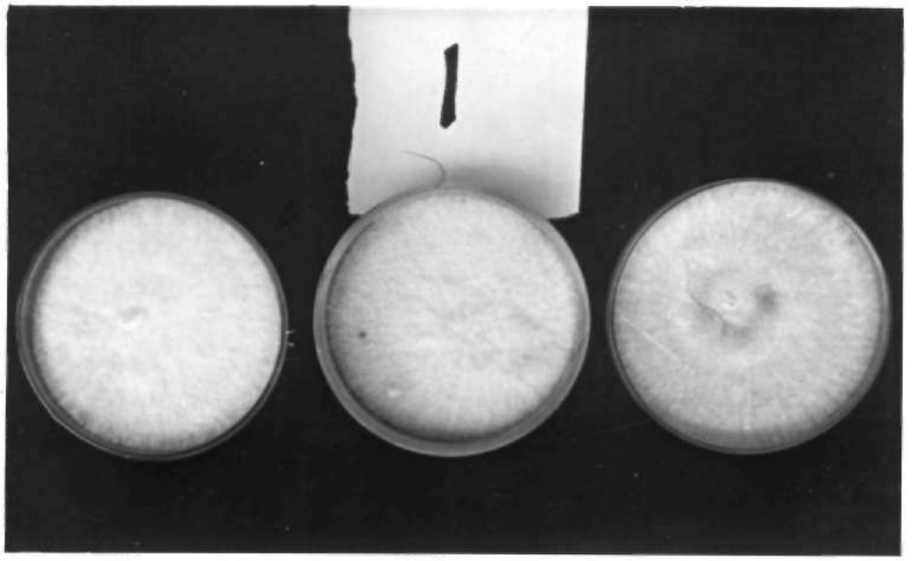


Plate 4. F. equiseti growing on PDAS.

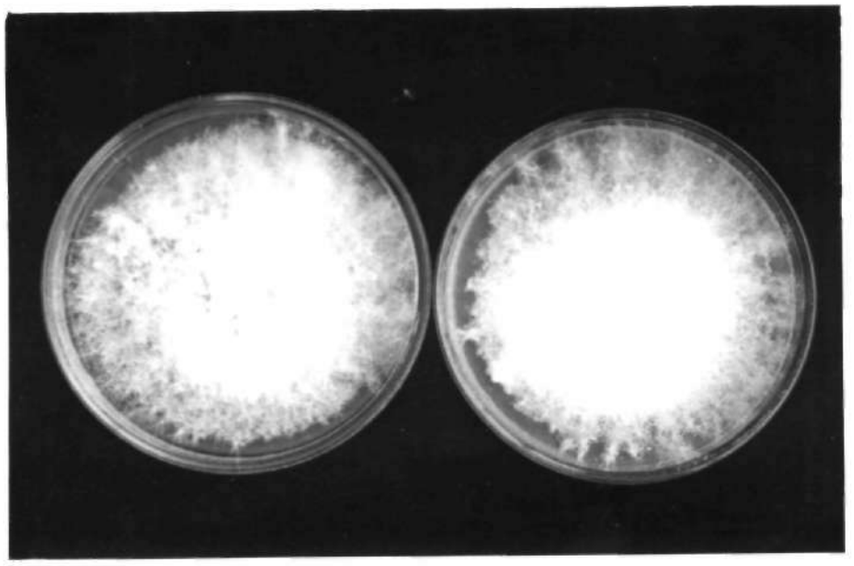


Plate 5. F. semitectum growing on PDAS

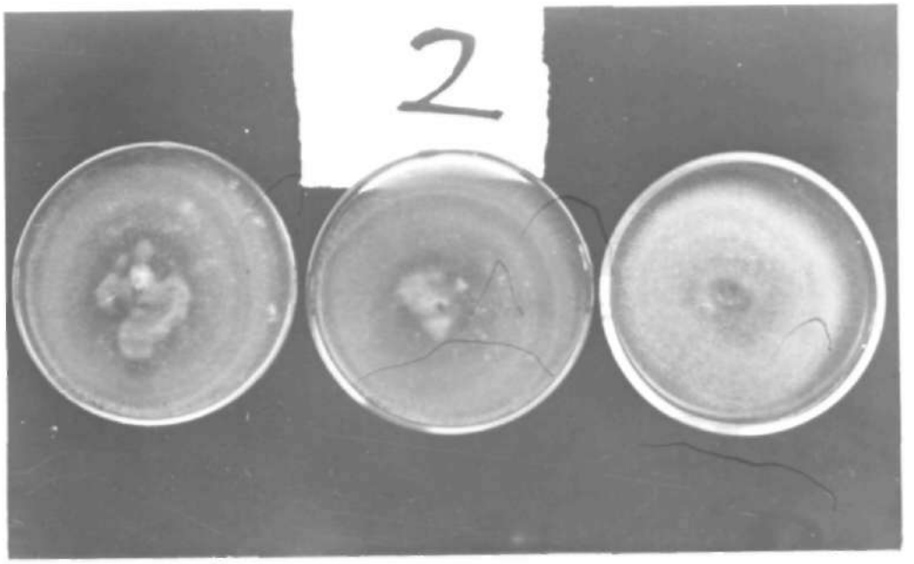


Plate 6. P. sorghina growing on PDAS.

sample 38 and the highest in sample 23 (Table 8). Thirty of the samples had at least 50% infected grains. This shows how serious fungal contamination can be in farmers seeds although this does not necessarily imply mycotoxin contamination. In almost all the samples, Fusarium spp. (F. semitectum, F. moniliforme, F. equiseti) were predominant. However, samples 2, 3, 5, 6, 7, 9, 12, 20 and 38 did not have Fusarium contamination. In general, contamination of all the samples with A. flavus, A. niger and P. sorghina was infrequent. Of the 39 samples tested, A. flavus was isolated from 13 samples, A. niger from 15 samples and P. sorghina from 18 samples.

4.1.2.2. Mycotoxin-producing fungi associated with grains in the four sorghum growing zones.

Bakura:

Varieties KSV 3 and KSV 4 were free from grain mould whereas serena and 6788B had a grain mould rating of 3 (Table 9). KSV 4 had the highest percentage

Table 8: Percentage of infected seeds and numbers of mycotoxin producing fungi isolated from grain samples collected from different localities in northern Nigeria.

(Each test sample consisted of 50 grains)

Collection number	Location	Infected Seeds (%)	Number of mycotoxin producing fungi			
			A. flavus	*A. niger*	*Fuserium* spp.	*P. sorghina*
1	K/Dangora	26	7	1	1	0
2	Kuganga	46	0	1	0	0
3	Likoro	36	1	1	0	0
4	Sundu	54	1	6	2	0
5	Sundu	32	0	3	0	3
6	Kiru	44	1	1	0	2
7	Sobe	54	2	2	0	0
8	Kauru	18	1	2	3	0
9	Ikara	32	4	9	0	0
10	Lafiya	56	4	1	1	3
11	Dangora	60	0	4	4	1
12	Makarfi	62	2	9	0	0
13	Falgoe	42	4	2	4	0
14	Dutsin Wai	68	0	0	45	1

Table 8 Cont'.

15	Zaria area	58	0	0	29	5
16	"	60	0	0	19	5
17	"	62	0	0	23	0
18	"	54	0	0	8	1
19	"	60	0	0	17	0
20	"	50	0	0	0	4
21	"	68	0	0	21	0
22	"	66	0	0	37	2
23	"	100	0	0	10	3
24	"	86	0	0	38	0
25	Not known	76	1	2	32	0
26	"	90	0	0	31	7
27	"	96	0	0	37	0
28	"	54	2	0	22	0
29	"	90	0	1	28	11
30	"	96	0	0	30	4
31	"	86	0	0	27	0
32	"	92	0	0	34	6
33	"	76	0	0	37	0
34	"	86	0	0	25	0
35	"	90	0	0	33	3
36	"	76	0	0	29	0
37	"	80	0	0	37	1
38	"	10	2	0	0	0
39	"	70	0	0	25	7

of infected seeds, (100%), closely follwed by 6788B (84%), Serena and Is 9289 had 66% infected seeds each while KSV 3 had the lowest, 56%.

Among the mycotoxin - producing fungi A. flavus was the most predominant followed by A. niger, Fusarium spp and P. sorghina in that order (Table 10). The frequency of A. flavus and A. niger was highest on 6788B. A. flavus was also present in sufficiently high numbers on KSV 4. Fusarium spp were recorded in highest numbers on Serena followed by Is9289 and 6788B. The frequency of P. sorghina was low on all the varieties, Is9289 being free from P. sorghina.

The highest numbers of mycotoxin producing fungi were recorded on 6788B, followed by KSV 4, KSV 3, Serena and Is9289 in that order.

From the results obtained, no definite conclusions could be reached as to which stage of grain development had the highest numbers of fungi.

Table 9: Grain mould ratings and percentage of infected seeds in different varieties of sorghum grown at Bakura.

Variety	Grain mould rating	Infected seeds (%)
KSV 3	1	56
KSV 4	1	100
Serena	3	66
Is 9289	2	66
6788B	3	84

Table 10: Numbers of mycotoxin producing fungi isolated from seeds of different varieties of sorghum grown at Bakura.
(Each test sample consisted of 50 seeds)

Variety	Stages of grain development	Number of mycotoxin producing fungi			
		A. flavus	A. niger	Fusarium spp.	P. sorghina
KSV 3	M	7	3	0	0
	S	0	0	1	0
	H	0	0	0	4
	R	0	0	0	2
KSV 4	M	0	0	0	0
	S	35	10	1	0
	H	0	0	0	2
	R	0	0	0	1
Serena	M	1	0	1	1
	S	1	0	5	0
	H	0	0	10	0
	R	3	3	11	0
Is9289	M	8	11	0	0
	S	1	0	4	0
	H	0	0	4	0
	R	1	0	5	0
6788B	M	9	8	0	1
	S	22	20	0	0
	H	0	0	10	0
	R	20	8	3	0

M = Milk stage S = Soft dough stage
H = Hard dough stage R = Ripe stage.

Kano.

The varieties CK 60, Is9289 and 6788B had higher grain mould ratings than KSV 2 and KSV 6 (Table 11). KL 123 was free from grain mould. Three varieties, KSV 6, Is9289 and 6788B had 100% infected grains, while in the others the percentage of infected grains was more than 60.

In general, P. sorghina was the most predominant fungus, followed by A. niger, A. flavus and Fusarium spp. in that order (Table 12). P. sorghina was recorded in high proportions on 6788B, but its incidence on grains of KSV 6 and Serena was very low. The Aspergillus spp appeared in their highest frequencies on Serena. The numbers of both A. flavus and A. niger were low on KL 123. Fusarium spp were present in small numbers in the varieties.

The highest numbers of all the fungi were recorded on 6788B followed by Serena. In general the frequency of all the fungi was low on KSV 6, KL 123 and CK 60.

Table 11. Grain mould ratings and percentage of infected seeds in different varieties of Sorghum grown at Kano.

Variety	Grain mould rating	Infected seeds (%)
KSV 2	2	62
KSV 6	2	100
KL 123	1	88
CK 60	3	80
Serena	2	80
Is 9289	3	100
6788B	3	100

Table 12: Numbers of mycotoxin-producing fungi isolated from seeds of different varieties of sorghum grown at Kano.
(Each test sample consisted of 50 seeds).

Variety	Stage of grain development	Number of mycotoxin producing fungi			
		A. flavus	A. niger	Fusarium spp.	P. sorghina
KSV 2	M	4	10	5	0
	S	3	5	6	6
	H	0	0	0	9
	R	8	8	4	1
KSV 6	M	-	-	-	-
	S	-	-	-	-
	H	-	-	-	-
	R	13	9	8	1
KL 123	M	0	2	2	0
	S	0	3	1	1
	H	0	0	0	0
	R	3	0	0	14
CK 60	M	-	-	-	-
	S	-	-	-	-
	H	-	-	-	-
	R	1	0	3	25

Table 12 Cont'.

Serena	M	0	0	0	0
	S	10	20	0	0
	H	10	22	0	1
	R	4	10	7	0
Is9289	M	3	0	2	15
	S	6	0	5	8
	H	0	0	0	0
	R	2	0	4	19
6788B	M	11	21	0	4
	S	0	7	15	3
	H	0	0	0	48
	R	0	0	0	48

M = Milk stage
S = Soft dough stage
H = Hard dough stage
R = Ripe stage
- = Samples were not available

Samaru.

Serena had the highest grain mould rating, 4, while KSV 2 had the lowest, 1 (Table 13). In other varieties the grain mould ratings ranged from 2 - 3. Grains of varieties KSV 4, KSV 5, KSV 6, SSV 8, MSV 1 and CK 60 were severely infected, showing 100% infection, followed by SSV 3 (94%), 6788B (90%), KSV 3 (90%), KL 123 (80%) and KSV 2 (78%). SSV 6 had the lowest percentage of infected seeds (20%).

Table 14 shows the numbers of various mycotoxin - producing fungi isolated from different varieties at the four stages of grain development. Fusarium spp. were the most predominant and had the highest frequency of occurrence.. They occurred on all the varieties, their numbers being high on KSV 5, KSV 6, KL 123, SSV 2, SSV 6, and SSV 8 as compared to other varieties. The occurrence of P. sorghina was next in importance. The incidence of P. sorghina was highest on CK 60 followed by KSV 4, KSV 3 and SSV 3 in that order.

Table 13: Grain mould ratings and percentage of infected seeds in different varieties of sorghum grown at Samaru.

Variety	Grain mould rating	Infected seeds (%)
KSV 2	1	78
KSV 3	2	90
KSV 4	2	100
KSV 5	2	100
KSV 6	2	100
KL 123	2	80
SSV 2	2	56
SSV 3	2	94
SSV 6	2	20
SSV 7	2	60
SSV 8	2	100
75/76	2	68
MSV 1	2	100
MSV 2	2	50
MSV 3	2	50
CK 60	3	100
Serena	4	56
6788B	3	90

Table 14: Numbers of mycotoxin-producing fungi isolated from seeds of different varieties of sorghum grown at Samaru.
(Each test sample consisted of 50 seeds)

Varieties	Stages of grain development	Number of mycotoxin producing fungi			
		A.flavus	A.niger	Fusarium spp.	P.sorghina
KSV 2	M	1	0	11	4
	S	0	0	33	1
	H	0	0	34	1
	R	1	2	6	2
KSV 3	M	0	0	18	20
	S	0	0	0	8
	H	0	0	13	30
	R	0	2	15	21
KSV 4	M	5	0	20	12
	S	0	0	0	21
	H	0	0	5	43
	R	0	0	14	9
KSV 5	M	1	1	31	5
	S	0	0	48	2
	H	2	1	41	0
	R	1	1	46	2

Table 14 Cont'

KSV 6	M	0	0	1	15
	S	0	0	38	4
	H	0	0	42	2
	R	2	1	30	12
KL 123	M	0	0	40	6
	S	0	0	38	8
	H	0	0	31	9
	R	4	1	10	0
SSV 2	M	2	0	41	8
	S	2	2	39	7
	H	2	1	23	0
	R	3	3	2	0
SSV 3	M	7	4	24	14
	S	3	1	28	18
	H	1	1	20	28
	R	3	3	13	0
SSV 6	M	6	2	19	8
	S	3	0	46	1
	H	2	1	39	2
	R	1	0	2	1

Table 14 Cont'

SSV 7	M	0	0	0	7
	S	0	0	5	21
	H	0	0	23	22
	R	2	0	0	4
SSV 8	M	0	0	28	15
	S	0	0	33	8
	H	0	0	24	14
	R	3	1	25	0
75/76	M	2	1	46	4
	S	7	3	30	4
	H	4	2	7	21
	R	0	0	4	0
MSV 1	M	-	-	-	-
	S	-	-	-	-
	H	0	0	0	1
	R	0	0	3	32
MSV 2	M	13	13	8	6
	S	0	0	33	0
	H	0	0	29	0
	R	0	0	2	0

Table 14 Cont'

MSV 3	M	0	0	38	1
	S	1	4	17	18
	H	0	1	10	0
	R	6	1	2	2
CK 60	M	0	0	9	6
	S	2	1	6	28
	H	1	4	1	36
	R	0	0	10	40
Serena	M	1	6	0	2
	S	1	0	0	0
	H	7	13	1	0
	R	0	0	7	0
6788B	M	0	0	31	4
	S	0	0	10	11
	H	0	0	19	26
	R	1	0	6	0

M = Milk stage
S = Soft dough stage
H = Hard dough stage
R = Ripe stage
- = Samples were not available.

In general, A. flavus and A. niger occurred in low levels on all the varieties. A. niger was not present in varieties KSV 4, SSV 7, MSV 1 and 6788B while A. flavus was not present in KSV 3 and MSV 1. Of all the varieties highest numbers of fungi were recorded on KSV 5 followed by SSV 3.

Mokwa.

The highest grain mould ratings were recorded in varieties CK 60, Is 9289 and 6788B while MSV 1, MSV 2 and MSV 3 had the lowest (Table 15). CK 60 and Serena had 100% grain infection followed by Is 9289 (78%) and 6788B (64%).

Of the mycotoxin producing fungi, the incidence of P. sorghina was highest followed by Fusarium spp., A. niger and A. flavus in that order (Table 16). P. sorghina appeared in high numbers on CK 60, Serena, Is 9289 and 6788B but it was not present on MSV 1, MSV 2, and MSV 3. The numbers of Fusarium spp. were higher on CK 60 and serena than on other varieties;

they were not present on MSV 1 and MSV 3. A. flavus and A. niger were either absent or only present in small numbers. Of all the varieties, maximum numbers of fungi were recorded on Serena, closely followed by Is 9289 and 6788B.

Table 15: Grain mould ratings and percentage of infected seeds in different varieties of sorghum grown at Mokwa.

Variety	Grain mould rating	Infected seeds (%)
MSV 1	2	40
MSV 2	2	38
MSV 3	2	38
CK 60	4	100
Serena	3	100
Is 9289	4	78
6788B	4	64

Table 16: Numbers of mycotoxin producing fungi isolated from seeds of different varieties of sorghum at Mokwa.
(Each test sample consisted of 50 seeds).

Variety	Stage of grain development	Number of mycotoxin producing fungi			
		A.flavus	A.niger	Fusarium spp.	P.sorghina
MSV 1	M	-	-	-	-
	S	-	-	-	-
	H	-	-	-	-
	R	9	10	0	0
MSV 2	M	-	-	-	-
	S	-	-	-	-
	H	-	-	-	-
	R	4	1	2	0
MSV 3	M	-	-	-	-
	S	-	-	-	-
	H	-	-	-	-
	R	0	0	0	0
CK 60	M	0	0	27	11
	S	0	0	6	26
	H	0	0	5	0
	R	0	0	7	38

Table 16 Cont'

Serena	M	0	0	17	33
	S	0	0	12	25
	H	0	0	8	33
	R	0	0	9	39
Is 9289	M	0	0	2	42
	S	0	0	0	50
	H	0	0	0	39
	R	0	0	3	22
6788B	M	1	0	5	38
	S	0	0	1	49
	H	0	2	2	37
	R	2	5	1	0

M = Milk stage

S = Soft dough stage

H = Hard dough stage

R = Ripe stage

- = Samples were not available.

4.1.2.3. Statistical analysis of data.

Only eight varieties (KSV 2, KSV 3, KSV 4, KL 123, CK 60, Serena, Is 9289 and 6788B) were planted at two or more locations. In order to know if there were any interactions between different variants, the data were statiscally analysed.

The analysis of variances for the different fungi are given in Table 17. The means of the main effects are shown in Table 18, and that of the interactions in Tables 19 - 21.

i. A. flavus and A. niger.

Varieties: This variant had no significant effect on the numbers of A. flavus and A. niger.

Location: The frequency of occurrence of A. flavus and A. niger was affected by locations and the results are significant at P= 0.05.

Stage of grain development: This factor had no significant effect on the numbers of A. flavus and A. niger.

Interactions - None of the interactions was significant.

ii. Fusarium spp.

Varieties: The numbers of Fusarium spp. were affected by varieties and the results are significant at P= 0.05.

Locations: The incidence of Fusarium spp. was highly influenced by location and the results are significant at P = 0.01.

Stage of grain development: This factor did not affect the number of Fusarium spp.

Interaction: Location x variety interaction was significant at P = 0.001. Other interactions were at significant.

iii. P. sorghina.

Varieties: The frequency of occurrence of P. sorghina was influenced by varieties and the results are significant at P = 0.05.

Locations: The numbers of P. sorghina were affected by location and the results are significant at P = 0.001.

Stage of grain development: This factor did not affect the numbers of P. sorghina and the results are not significant.

Interactions: Location x variety interaction is significant at P = 0.01. Other interactions were not significant.

Table 17: Analysis of variance

Source of Variation	DF	Mean Squares			
		A. flavus	A. niger	Fusarium spp.	P. sorghina
Stage of grain development	3	32.1	10.9	35.9	115.8
Location	3	120.9*	95.5*	608.9***	2380.9***
Variety	7	26.3	37.4	124.9*	310.8*
Location x Stage	9	28.6	18.3	54.9	201.5
Stage x Variety	21	27.1	16.5	51.1	123.0
Location x Variety	11	43.1	39.3	157.5***	300.1**
Residual	30	29.6	22.8	39.0	99.7

* Significant at P = 0.05

** Significant at P = 0.01

*** Significant at P = 0.001

Table 18: Means of main effects.

A. Stages of grain development.

Stage	A. flavus	A. niger	Fusarium spp.	P. sorghina
Milk	2.4	2.9	10.0	9.5
Soft dough	4.9	3.2	6.7	11.7
Hard dough	0.9	2.0	9.6	15.2
Ripe	2.7	1.7	5.8	12.7

Table 18 Cont'

B. Locations

Location	A. flavus*	A. niger*	Fusarium*** spp.	P. sorghina***
Bakura	5.9 ± 1.2	3.2 ± 1.1	4.8 ± 1.9	0
Kano	3.1 ± 1.2	5.2 ± 1.0	0.6 ± 1.4	12.3 ± 2.2
Samaru	0.9 ± 1.0	1.2 ± 0.9	0.9 ± 1.2	13.1 ± 1.9
Mokwa	0.1 ± 1.4	0	7.6 ± 1.6	12.7 ± 2.5

* Significant at P = 0.05

*** Significant at P = 0.001

Table 18 Cont'

C. Varieties

Variety	A. flavus	A. niger	Fusarium spp.*	P. sorghina*
KSV 2	2.6	2.3	12.5 ± 2.2	2.6 ± 3.5
KSV 3	0	0.9	3.8 ± 2.2	17.4 ± 3.5
KSV 4	4.1	0.5	3.0 ± 2.2	17.8 ± 3.5
KL 123	1.3	0	15.4 ± 2.2	4.3 ± 3.5
CK 60	2.1	2.0	6.1 ± 2	15.6 ± 3.3
Serena	2.4	4.7	6.0 ± 1.6	7.6 ± 2.5
Is 9289	1.2	0.6	5.0 ± 1.8	15.5 ± 2.9
6788B	4.7	4.5	6.8 ± 1.6	16.1 ± 2.5

* Significant at P = 0.05.

Table 19: Interaction of location x stage of grain development over eight varieties.

Location	Stage	Means			
		A.flavus	A.niger	Fusarium spp	P.sorghina
Bakura	M	5.0	4.4	0.2	0.4
Bakura	S	11.8	6.0	2.2	0
Bakura	H	0	0	4.8	1.2
Bakura	R	6.8	2.2	3.8	0.6
Kano	M	3.6	6.6	1.8	3.8
Kano	S	3.8	7.0	5.4	3.6
Kano	H	2.0	4.4	0	11.6
Kano	R	3.0	3.0	3.0	17.8
Samaru	M	1.0	0.9	18.4	7.7
Samaru	S	0.4	0.1	12.4	11.0
Samaru	H	1.1	2.4	14.8	20.7
Samaru	R	0.9	0.7	9.7	10.3
Mokwa	M	0.3	0	12.8	31.0
Mokwa	S	0	0	4.8	37.5
Mokwa	H	0	0.5	3.8	27.3
Mokwa	R	0.5	1.3	5.0	24.8

Table 20: Interaction of stage of grain development x variety over four locations

Variety	Stage	Means			
		A.flavus	A.niger	Fusarium spp	P.sorghina
KSV 2	M	5.0	5.0	8.0	2.0
KSV 2	S	3.0	2.5	19.5	3.5
KSV 2	H	0	0	17.0	5.0
KSV 2	R	9.0	5.0	5.0	1.5
KSV 3	M	3.5	1.5	9.0	10.0
KSV 3	S	0	0	0.5	4.0
KSV 3	H	0	0	6.5	17.0
KSV 3	R	0	1.0	7.5	11.5
KAV 4	M	2.5	0	10.0	6.0
KSV 4	S	17.5	10.0	0.5	10.5
KSV 4	H	0	0	2.5	22.5
KSV 4	R	0	0	7.0	5.0
KL 123	M	0	2.0	21.0	3.0
KL 123	S	0	3.0	19.5	4.5
KL 123	H	0	0	15.5	4.5
KL 123	R	3.5	1.0	5.0	7.0

Table 20 Cont'

CK 60	M	0	0	7.1	12.3
CK 60	S	1.0	0.5	18.0	8.5
CK 60	H	0.5	2.0	6.0	27.0
CK 60	R	0.3	0	3.0	18.0
Serena	M	0.5	1.5	6.7	34.3
Serena	S	3.0	5.0	4.5	9.0
Serena	H	4.3	8.8	4.3	6.3
Serena	R	1.8	3.3	4.8	8.5
Is 9289	M	3.7	3.7	8.5	9.8
Is 9289	S	2.3	0	1.3	19.0
Is 9289	H	0	0	3.0	19.3
Is 9289	R	1.0	0	1.3	13.0
6788B	M	5.3	7.3	4.0	13.7
6788B	S	5.5	6.8	9.0	11.8
6788B	H	0	0.5	6.5	15.8
6788B	R	8.3	3.3	7.8	27.8

Table 21: Interaction of location x variety over four stages of grain development.

Variety	Location	Means			
		A.flavus	A.niger	Fusarium*** spp.	P.sorghina**
KSV 3	Bakura	1.8	0.8	0.3 ± 0.3	1.5 ± 1.0
KSV 4	Bakura	8.8	2.5	0.3 ± 0.3	0.8 ± 0.5
Serena	Bakura	1.3	0.8	6.8 ± 1.3	0.3 ± 0.3
Is9289	Bakura	2.3	2.8	3.3 ± 1.1	0
6788B	Bakura	15.3	9.0	3.3 ± 1.3	0.3 ± 0.3
KSV 2	Kano	3.8	5.8	3.8 ± 1.3	4.0 ± 2.2
KL 123	Kano	0.8	1.3	0.8 ± 0.5	3.8 ± 3.4
Serena	Kano	6.0	13.0	1.8 ± 1.8	0.3 ± 0.3
Is9289	Kano	2.8	0	2.8 ± 1.2	10.5 ± 4.2
6788B	Kano	2.8	7.0	3.8 ± 3.7	25.7 ±12.9
CK 60	Kano	1.0	0	3.0	25.0 ± 0
KSV 2	Samaru	0.5	0.5	21.0 ± 7.3	2.0 ± 0.7
KSV 3	Samaru	0	0.5	11.5 ± 4.0	19.8 ± 4.5
KSV 4	Samaru	1.3	0	9.8 ± 4.5	21.3 ± 7.7
KL 123	Samaru	1.0	0.3	29.8 ± 6.9	5.8 ± 2.1
CK 60	Samaru	0.8	1.3	6.5 ± 2.0	27.5 ± 7.6
Serena	Samaru	2.3	4.8	2.0 ± 1.7	0.5 ± 0.5
6788B	Samaru	0.3	0	16.5 ± 5.6	10.3 ± 5.7
CK 60	Mokwa	0	0	11.3 ± 7.3	18.3 ± 8.3
Serena	Mokwa	0	0	11.5 ± 5.3	32.5 ± 2.9
Is9289	Mokwa	0	0	1.3 ± 2.0	38.3 ± 5.9
6788B	Mokwa	0.8	1.8	2.3 ± 0.9	31.0 ±10.7

4.2. Mycotoxin contamination of sorghum grains.

In no case did the thin layer chromatography of the sorghum grain samples of the varieties grown in the four ecological zones show a fluorescence corresponding to those produced by the Aflatoxin B_1, Aflatoxin B_2 and Zearalenone standards used in this investigation (Tables 22 - 25). However, blue-green fluorescence (visible only at 360 nm) and black band (visible only at 255 nm) were observed in some sorghum varieties.

No fluorescence or black band were observed in the varieties grown at Bakura (Table 22). At Kano, varieties KL 123, Serena and 6788B showed black band only while CK 60 showed blue-green fluorescence only (Table 23). On the other hand KSV 2 and KSV 6 showed both black band and the blue - green fluorescence. Among the varieties grown at Samaru, KSV 4 and Serena showed both blue-green fluorescence and black band. Three varieties SSV 3, SSV 6 and SSV 8 showed only the black band, while KSV 3 and KSV 5 showed the blue-green fluorescent spots only (Table 24). At Mokwa

(table 25) MSV 3 and 6788B showed both blue-green fluorescence and black band, while CK 60 showed only the blue-green fluorescent spots and Is9289, the black band only.

When pure cultures of P. sorghina were extracted for t.l.c. and viewed under .U.V. light, the black band and blue-green fluorescence were observed. This suggests that the black band and blue-green fluorescence were characteristic of certain metabolite(s) produced by P. sorghina.

Table 22: Results of mycotoxin analysis in seeds of various sorghum varieties grown at Bakura.

Variety	Mycotoxin standard				
	Aflatoxin B_1	Aflatoxin B_2	Zearalenone	U_1	U_2
KSV 3	-	-	-	-	-
KSV 4	-	-	-	-	-
Serena	-	-	-	-	-
Is 9289	-	-	-	-	-
6788B	-	-	-	-	-

U_1 = unidentified mycotoxin /metabolite showing black band at short wavelength (255nm).

U_2 = unidentified mycotoxin/metabolite showing blue - green fluorescense at long wavelength (360nm).

\- = absent

\+ = present.

Table 23: Results of mycotoxin analysis in seeds of various sorghum varieties grown at Kano.

Variety	Mycotoxin				
	Aflatoxin B_1	Aflatoxin B_2	Zearalenone	U_1	U_2
KSV 2	-	-	-	+	+
KSV 6	-	-	-	+	+
KL 123	-	-	-	+	-
CK 60	-	-	-	-	+
Serena	-	-	-	+	-
Is 9289	-	-	-	-	-
6788B	-	-	-	+	-

U_1 = unidentified mycotoxin/metabolite showing black band at short wavelength (255nm)

U_2 = unidentified mycotoxin/metabolite showing blue-green fluorescense at long wavelength (360nm).

\- = absent

\+ = present

Table 24: Results of mycotoxin analysis in seeds of various sorghum varieties grown at Samaru.

Variety	Mycotoxin				
	Aflatoxin B_1	Aflatoxin B_2	Zearalenone	U_1	U_2
KSV 2	-	-	-	-	-
KSV 3	-	-	-	-	+
KSV 4	-	-	-	+	+
KSV 5	-	-	-	-	+
KSV 6	-	-	-	-	-
KL 123	-	-	-	-	-
SSV 2	-	-	-	-	-
SSV 3	-	-	-	+	-
SSV 6	-	-	-	+	-
SSV 7	-	-	-	-	-
SSV 8	-	-	-	+	-
75/76	-	-	-	-	-
MSV 1	-	-	-	-	+
MSV 2	-	-	-	-	-
MSV 3	-	-	-	-	-
CK 60	-	-	-	-	-
Serena	-	-	-	+	+
6788B	-	-	-	-	-

U_1 = unidentified mycotoxin/metabolite, showing black band at short wavelength (255nm)

U_2 = unidentified mycotoxin/metabolite, showing blue - green fluorescense at long wavelength (360nm).

\- = absent

\+ = present.

Table 25: Results of mycotoxin analysis in seeds of sorghum varieties grown at Mokwa.

Variety	Mycotoxin				
	Aflatoxin B_1	Aflatoxin B_1	Zearalenone	U_1	U_2
MSV 1	-	-	-	-	-
MSV 2	-	-	-	-	-
MSV 3	-	-	-	+	+
CK 60	-	-	-	-	+
Serena	-	-	-	-	-
Is 9289	-	-	-	+	-
6788B	-	-	-	+	+

U_1 = unidentified mycotoxin/metabolite showing black band at short wavelength (255nm)

U_2 = unidentified mycotoxin/metabolite showing blue - green fluorescense at long wavelength (360nm).

- = absent

+ = present.

5.0. DISCUSSION

Quantitative assessment of the toxigenic fungi associated with sorghum grain mould complex in Nigeria showed that in the Northern Sudan Savanna, Aspergillus spp. were the most predominant followed by Fusarium spp. and P. sorghina. In other zones the frequency of occurrence of these fungi was as follows:

Sudan Savanna: Aspergillus spp. > P. sorghina > Fusarium spp.

Northern Guinea Savanna: Fusarium spp. > P. sorghina > Aspergillus spp.

Southern Guinea Savanna: P. sorghina > Fusarium spp. > Aspergillus spp.

This shows that Aspergillus spp. were predominant in areas with comparatively less rainfall and Fusarium spp. and P. sorghina were more abundant in high rainfall areas. Probably, differences in temperatures during August and September at the four locations may have also influenced the incidence of these fungi (See Appendix 2 - 5).

The occurrence of Aspergillus spp, in high numbers on sorghum grains in the Northern Sudan Savanna and Sudan Savanna zones is interesting. According to Christensen and Kaufmann (1965) Aspergillus spp. are storage fungi, i.e. they do not invade grains to any appreciable degree before harvest. There is however, a report from India (Tripathi, 1974) which showed that Aspergillus spp. constituted 15 per cent of the total fungi isolated from sorghum grains directly collected from the field. It seems that under certain environmental conditions, Aspergillus spp. may be one of the important components of the field problem of sorghum grain mould.

As mentioned earlier, in the Northern Guinea Savanna and Southern Guinea Savanna zones the predominant fungi associated with grain mould were Fusarium spp. and P. sorghina. Various workers have made a detailed study of the fungi associated with mouldy sorghum grain (Hansing and Hartley, 1962; Junejo and Malik, 1967; Narasimhan and Rangaswami, 1969; Denis and Grard, 1977; Castor, 1977; Rao and Williams, 1977; Tyagi, 1978), and

the workers in both the U.S.A. and India (Castor, 1977; Rao and Williams, 1978) have reported that Fusarium moniliforme, F. semitectum, and Curvularia lunata are the most destructive pathogens in the filed problem of sorghum grain mould. In Nigeria, however, P. sorghina is also a common grain mould fungus (Zummo; Tyagi, 1978). Recently, Denis and Girard (1978) recognized the importance of P. sorghina in the etiology of grain mould complex in Senegal. In India, Williams and Rao (1978) noted that in advanced lines of sorghum which have been developed with resistance to Fusarium and Curvularia, Phoma caused considerable grain damage during the rainy season of 1978.

It is normally expected that seeds of long season varieties would harbour less numbers of fungi as compared to short season varieties. While this was true for samples obtained from Mokwa it was not so in the case of samples obtained from Samaru. From seeds of long season varieties such as SSV 2, SSV 3, SSV 6 and SSV 8 fairly high numbers of Fusarium spp and

P. sorghina were isolated although these varieties had less grain mould as compared to the short season varieties. Probably the four rainy days in October and abundant dew till 20th of October at Samaru favoured infection of heads by these fungi (Appendix 4). However their further development on the surface of the grains did not take place as evidenced by the low grain mould ratings.

There does not seem to be any correlation between per-centage of infected grains and grain mould ratings. Rao and Williams (1978) observed that even grains which externally show no symptoms of grain mould can be infected by grain mould fungi. They consistently isolated Fusarium spp. and P. sorghina from surface - sterilized clean-appearing sorghum grains. In many samples, similar results were obtained in the present study.

Although data were collected on numbers of fungi present in seeds at four stages of grain development, no definite conclusions can be drawn from these results.

It can only be said that the toxigenic grain mould fungi get established in grain as early as the milk stage. Tarr (1962) reported that moulds may develop in the sorghum inflourescence at any stage from the young inflorescence to the mature head, provided that climatic conditions are suitably moist.

The present results are based on data collected during 1978. The severity of grain mould in general and frequency of occurrence of the different mycotoxin - producing fungi differs from year to year depending on weather conditions. There is thus a need to define accurately, the conditions of temperature, rainfall and relative humidities associated with sorghum grain mould development.

In the present investigation, sorghum grains obtained from different varieties grown in the four ecological zones were tested for mycotoxin contamination. Aflatoxins B_1 and B_2, and Zearalenone were not detected in any of the samples, although fungi which are known to produce these mycotoxins under certain conditions were associated with the grain samples.

There are conflicting reports of the production of mycotoxins in sorghum grain. Several workers have found aflatoxins (Coady, 1969; Alpert et al., 1971; Tripathi, 1973), and zearalenone (Schroeder and Hein, 1975; Stipanovic and Schroeder, 1975; Martin and Gilman, 1976) being produced in sorghum grain and grain products. In the U.S.A., Shotwell et al. (1969) found aflatoxin in excess of 10 ug/kg in only 6 of 533 sorghum samples tested. In Nigeria, Salifu (1978) detected these mycotoxins in grains of short season sorghums only. However, Elegbede et al (In Press) did not find aflatoxins B, G or zearalenone in samples of stored sorghum collected from farmers in five villages around Zaria although the samples were contaminated by Aspergillus spp. and Fusarium spp. In India, Rukmini and Bhat (1978) did not detect aflatoxin in 64 samples collected from farmers although Aspergillus flavus was isolated from almost all the samples.

Jarvis (1971) observed that various physical, chemical and biological factors affect mycotoxin production; the most important being temperature, relative humidity, the strain of toxigenic organism and the occurrence of competitive growth. Ciegler (1978) reported that temperatures optimum for mycotoxin production need not correspond to those optimum for growth: Fusarium tricinctum grows well at 25°C but produces T - 2 toxin best rear freezing temperatures. It is likely that one of these factors might have contributed to the absence of mycotoxins (aflatoxin B_1, B_2, and zearalenone) in the samples tested in the present study. The actual load of a toxigenic fungus on the grain is also an important factor which affects elaboration of mycotoxin in detectable amounts.

Thin layer chromatography of the sorghum grain extracts showed some blue-green fluorescence (visible only at, 360 nm) and black bands (visible only at, 255 nm). Pure cultures of P. sorghina also produced such blue-green fluorescence and black bands.

Boerema et al. (1977) reported that various strains of P. sorghina produce a metabolite which is acutely toxic to rats and chickens and probably to other mammals and birds. Possible involvement of this metabolite in the etiology of "onyalai" disease (haemorrhagic bullae in the month) in South Africa has also been indicated (Rabiel et al., 1975). It is possible that Nigerian strains of P. sorghina produce a metabolite similar to the one reported by these workers. Further work is required to ascertain if the toxic metabolite(s) produced by P. sorghina in South Africa and the one(s) detected in the present work is the same or different, and if it is the one responsible for the toxicity.

6.0. SUMMARY

In this study, a quantitative assessment of the main mycotoxin producing fungi (*Aspergillus flavus*, *A. niger*, *Fusarium* spp. and *Phoma sorghina*) associated with grains of sorghum varieties in different ecological zones of Nigeria, was made. Possible mycotoxin contamination in grains of these varieties was also investigated. *Aspergillus* spp. were predominant in areas with comparatively less rainfall while *Fusarium* spp and *P. sorghina* were more abundant in high rainfall areas: In the Northern Sudan Savanna, *Aspergillus* spp were the most predominant followed by *Fusarium* spp. and *P. sorghina*. In Sudan Savanna, *Aspergillus* spp. were the most predominant followed by *P. sorghina* and *Fusarium* spp. In Northern Guinea Savanna, *Fusarium* spp. were the most predominant followed by *P. sorghina* and *Aspergillus* spp. while in Southern Guinea Savanna *P. sorghina* was the most predominant followed by *Fusarium* spp. and *Aspergillus* spp. *Fusarium* spp. was the most predominant

in grain samples collected from farmers in different localities of northern Nigeria. There is a need to define accurately, the conditions of temperature, rainfall and relative humidity in relation to the development of sorghum grain mould in northern Nigeria.

Although mycotoxin - producing fungi were associated with many of the sorghum varieties studied, mycotoxins such as Aflatoxin B_1, Aflatoxin B_2 and zearalenone were not detected in them. A metabolite(s) which showed black band (visible only at 255 nm) and blue - green fluorescence (visible only at 360 nm) was associated with P. sorghina. Further work is required to ascertain if the metabolite(s) is the same as that associated with some toxic syndromes in South Africa.

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APPENDIX 1

Days to heading of the varieties used

Variety	Days to heading
KSV 2	105
KSV 3	65
KSV 4	66
KSV 5	98
KSV 6	103
SSV 2	130
SSV 3	130
SSV 6	126
SSV 7	123
SSV 8	123
MSV 1	150
MSV 2	150
MSV 3	150
CK 60	100
Serena	100
Is 9289	100
6788B	75

APPENDIX 2

Weather Data for Bakura: Wet Season, 1978.

	June	July	August	Sept.	Oct.	Nov.
Number of rainy days	6	13	8	8	5	0
Rainfall (mm)	143.9	230.4	150.6	167.8	31.4	0
Relative humidity	DATA NOT AVAILABLE					
Temperature	DATA NOT AVAILABLE					

APPENDIX 3

Weather Data for Kano: Wet season, 1978.

Latitude $11^{o}57'$ N

Longitude $8^{o}34'$ E

Altitude 1594 ft. MSL.

	June	July	August	Sept.	Oct.	Nov.
Number of rainy days	6	15	13	6	3	0
Rainfall (mm)	129.3	221.5	273.8	46.0	7.5	0
Relative humidity (Max.)	77	92	88	83	81	65
Temperature ^{o}C						
max.	34.4	31.7	32.2	33.3	37.2	36.1
min.	21.1	20.6	20.6	18.3	25	16.7

APPENDIX 4

Weather Data for Samaru: Wet season, 1978.

Latitude 11° 11'N

Longitude 07° 38'E

Altitude 2250 ft MSL

	June	July	August	Sept.	Oct.	Nov.
Number of rainy days	7	13	20	15	4	0
Rainfall (mm)	164.7	145.3	324.9	207.2	33.9	0
Relative humidity (max)	77.6	82.4	79.9	78.6	67.1	31.8
Temperature (°C)						
max.	29.6	26.8	28.6	29.5	31.7	30.1
min.	19.8	19.7	19.7	19.5	19.1	14.4

APPENDIX 5

Weather Data for Mokwa: Wet season, 1978.

Latitude 09° N

Longitude 05° 04°E

	June	July	August	Sept.	Oct.	Nov.
Number of rainy days	14	12	12	15	7	0
Rainfall (mm)	159.0	145.3	97.0	187.3	58.3	0
Relative humidity (max)	25.8	80.5	81.7	81.8	89.7	62.8
Temperature °C						
max.	37.1	29.6	30.6	30.8	33.0	33.9
min.	20.4	21.6	21.8	21.6	20.7	27.4

APPENDIX 6

Potato Dextrose agar with streptomycin (PDAS).

Peeled Irish potato	-	200g.
Glucose	-	20g.
Agar powder	-	20g.
Streptomycin	-	2ml/200 ml PDA.

Streptomycin was prepared by dissolving 1gm streptomycin powder in 200ml distilled water.

APPENDIX 7

Analysis of variance for A. flavus

Source of variation	Sum of squares	Mean Square	Signif. of F
Location	362.723	120.908	.015*
Variety	183.866	26.267	.528
Stage of grain development	96.357	32.119	.370
2-Way interactions	1320.008	32.195	.409
Location x variety	474.084	43.099	.200
Location x stage	257.199	28.578	.486
Variety x stage	569.479	27.118	.575
Residual	887.384	29.579	

* Significant at P = .05

APPENDIX 8

Analysis of variance for A. niger

Source of variation	Sum of squares	Mean Square	Signif. of F.
Location	286.339	95.446	.014*
Variety	261.519	37.360	.163
Stage of grain development	32.911	10.970	.698
2-Way interactions	937.263	32.860	.503
Location x variety	432.634	39.330	.115
Location x stage	164.797	18.311	.616
Variety x stage	346.065	16.479	.778
Residual	683.640	22.788	

* Significant at P = .05

APPENDIX 9

Analysis of variance for Fusarium spp.

Source of variation	Sum of Squares	Mean Square	Signif. of F.
Location	1826.923	608.974	.001***
Variety	874.037	124.862	.012*
Stage of grain development	107.543	35.848	.444
2-Way interactions	3342.979	81.536	.019
Location x variety	1732.580	157.507	.001***
Location x stage	494.973	54.997	.228
Variety x stage	1071.967	51.046	.245
Residual	1170.381	39.013	.001

* Significant at P = .05

*** Significant at P = .001

APPENDIX 10

Analysis of variance for *P. sorghina*

Source of variation	Sum of Squares	Mean Square	Signif. of F.
Location	7142.854	2380.951	.001***
Variety	2175.582	310.797	.014*
Stage of grain development	347.325	115.775	.341
2-Way interactions	7041.871	171.753	.062
Location x variety	3300.990	300.090	.008**
Location x stage	1813.726	201.525	.072
Variety x stage	2582.609	122.981	.293
Residual	2989.669	99.656	

* Significant at P = .05

** Significant at P = .01

*** Significant at P = .001

APPENDIX II

Interaction of location x variety over four stages of grain development.

Variety	Location	Fusarium spp.		P. sorghina	
		STD. DEV.	VARIANCE	STD. DEV.	VARIANCE
KSV 3	Bakura	.50	.25	1.91	3.67
KSV 4	Bakura	.50	.25	.96	.92
Serena	Bakura	4.65	21.58	.50	.25
Is9289	Bakura	2.22	4.92	0	0
6788B	Bakura	4.72	22.25	.50	.25
KSV 2	Kano	2.63	6.92	4.24	18.00
KL 123	Kano	.96	.92	6.85	46.92
Serena	Kano	3.50	12.25	.50	.25
Is9289	Kano	2.22	4.92	8.35	69.67
6788B	Kano	7.50	56.25	25.70	660.25
CK 60	Kano	0	0	0	0
KSV 2	Samaru	14.58	212.67	1.41	2.00
KSV 3	Samaru	7.94	63.00	9.03	81.58
KSV 4	Samaru	8.96	80.25	15.37	236.25
KL 123	Samaru	13.72	188.25	4.03	16.25
CK 60	Samaru	4.04	16.33	15.18	230.33
Serena	Samaru	3.37	11.33	1.00	1.00
6788B	Samaru	11.09	123.00	11.44	130.92
CK 60	Mokwa	10.53	110.92	16.68	278.25
Serena	Mokwa	4.04	16.33	5.75	33.00
Is9289	Mokwa	1.5	2.25	11.79	138.92
6788B	Mokwa	1.89	3.58	21.37	456.67