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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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DISCUSSION PAPER ON MYCOTOXINS IN SORGHUM

(Prepared by the electronic working group led by Tunisia[1])

Background

At the 2nd session of the Committee on Contaminants in Foods, held in The Hague, The Netherlands, from 31 March-4 April 2008, it was agreed to establish an electronic working group led by Tunisia and open to all members and working in English, to prepare a discussion paper on mycotoxins in sorghum (ALINORM 08/31/41, paragraph 186).

This discussion paper should include an overview of available data on mycotoxins in sorghum with a view of possible evaluation by JECFA.

At the meeting, the following member countries and organisations expressed their willingness to participate in the electronic working group: Australia, Brazil, China, Eritrea, Indonesia, Nigeria, Sudan, Sweden, Switzerland and Thailand.

Tunisia invited participants, including participants from the member countries and organisations who had already expressed their willingness to assist in this task, to send data concerning the subject through the "Kick off Message" sent 21 August 2008.

SORGHUM: DATA OF A GENERAL NATURE

I- <u>Introduction</u>

Sorghum is a genus of numerous species of grasses, some of which are raised for grain and many of which are used as fodder plants either cultivated or as part of pasture. The plants are cultivated in warmer climates worldwide. Species are native to tropical and subtropical regions of all continents in addition to the South West Pacific and Australasia. Sorghum is in the subfamily Panicoideae and the tribe Andropogoneae (the tribe of big bluestem and sugar cane).

Sorghum is crucial to the world food economy because it contribute to household food security in many of the world's poorest, most food-insecure regions. In the main production regions in Africa and Asia, more than 70 percent of the sorghum crop is consumed as food. A large proportion of farm households aim simply to produce enough grain to meet household requirements - and many often fail to meet even this limited goal. Only a small proportion of the harvest is traded, mostly on local food markets.

[1] with data provided by Brazil and Sudan

The plant produces a drawn up stem from 50 to 70 cm for the current cultivated forms and of the lengthened sheets similar to those of corn. At the end, a panicle of fruit flowers then containing of the seeds develops which come to maturity in autumn.

There are several kinds of sorghum corresponding to various uses:

- the fodder sorghum which is a hardy perennial cut on foot for the animal feeds

- the sorghum-grain: annual plant which one cultivates many varieties and especially more resistant and more productive hybrids adapted to the animal or human feeds.

- the sugar sorghum whose size can exceed 5 m

- sorghum with paper

- broom millet

The presence of deteriorative fungi with ability to produce mycotoxin in grains and food represents a great hazard for human and animal health, and it has been reported for sorghum in many countries with a high frequency of Aspergillus and Fusarium genera. Aflatoxins are bifuranocumarin mycotoxins produced by A. flavus and A. parasiticus, with aflatoxin B1 (AFB1) being the most hepatotoxic, showing mutagenic and carcinogenic and, probably, teratogenic properties in animals. According to the International Agency for Research on Cancer, AFB1 is classified as a human carcinogen class 1. Fumonisins are mycotoxins produced mainly by F. verticillioides Sacc Nirenberg (= F. moniliforme Sheldon), and F. proliferatum in several agricultural products worldwide, especially maize and sorghum. The toxic effects of fumonisins depend on the animal specie and the toxigenicity of Fusarium strains. This toxin causes leukoencephalomalacia in equines and rabbits, pulmonary edema in swine, and it has been reported as a probable cause of esophageal cancer in humans.

II-<u>World production</u>

Roughly 90 percent of the world's sorghum area and 95 percent of the world's millet area lie in the developing countries, mainly in Africa and Asia. These crops are primarily grown in agroecologies subject to low rainfall and drought. Most such areas are unsuitable for the production of other grains unless irrigation is available. Sorghum is widely grown both for food and as a feed grain, while millet is produced almost entirely for food.

Developed countries produce nearly one-third of the world's sorghum. In North America, it is cultivated in the central and southern plains of the United States (mainly in Kansas, Texas and Nebraska), where rainfall is low and variable. The United States is the world's largest producer, with over 25 percent of global output. Production in Europe is limited to small areas in France, Italy and Spain. In Oceania, Australia is the only producer of significance. (Table1).

World production of sorghum in 2005

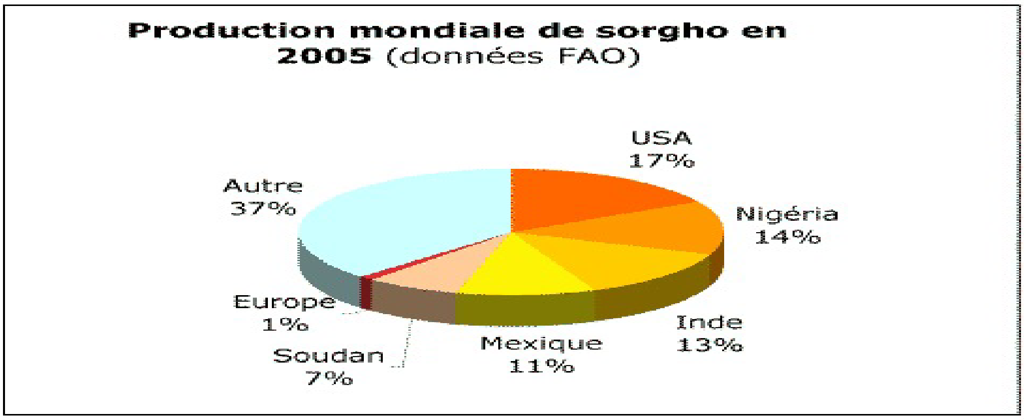


Figure 1. The world's major sorghum producers.

	Area (million ha)			Yield (t/ha)			Production (million tons)		
	1979-81	**1989-91**	**1992-94**	**1979-81**	**1989-91**	**1992-94**	**1979-81**	**1989-91**	**1992-94**
Developing countries	**38.60**	**38.30**	**40.00**	**1.14**	**1.04**	**1.11**	**43.90**	**40.00**	**44.20**
Africa	13.40	18.30	21.80	0.89	0.75	0.78	11.90	13.78	17.10
Northern Africa	3.29	4.07	5.95	0.90	0.67	0.69	2.94	2.73	4.10
Sudan	3.05	3.90	5.77	0.74	0.53	0.58	2.27	2.09	3.32
Western Africa	5.70	10.00	11.30	0.89	0.76	0.82	5.10	7.60	9.30
Burkina Faso	1.05	1.32	1.40	0.59	0.75	0.89	0.62	0.99	1.25
Mali	0.43	0.77	0.95	0.78	0.87	0.77	0.34	0.68	0.73
Niger	0.82	2.04	2.26	0.42	0.19	0.18	0.35	0.39	0.42
Nigeria	2.70	4.90	5.70	1.22	0.98	1.07	3.30	4.80	6.10
Central Africa	0.93	1.09	1.21	0.68	0.72	0.74	0.64	0.79	0.89
Eastern Africa	3.23	2.95	3.08	0.95	0.88	0.89	3.08	2.59	2.75
Ethiopia	1.05	0.81	0.91	1.35	1.09	1.27	1.42	0.88	1.16
Kenya	0.17	0.13	0.12	0.95	0.88	1.05	0.16	0.11	0.12
Mozambique	0.29	0.42	0.38	0.63	0.40	0.33	0.18	0.17	0.12
Somalia	0.48	0.45	0.40	0.35	0.54	0.36	0.17	0.24	0.14
Tanzania	0.71	0.53	0.66	0.76	0.99	0.90	0.54	0.53	0.59
Uganda	0.17	0.24	0.26	1.78	1.49	1.50	0.31	0.36	0.38
Zimbabwe	0.14	0.14	0.13	0.61	0.58	0.52	0.09	0.08	0.07
Southern Africa	0.17	0.22	0.19	0.50	0.37	0.39	0.09	0.08	0.07
Asia	20.78	16.56	15.11	0.95	1.03	1.19	19.69	17.00	17.98

Near East	0.92	0.60	0.60	0.81	0.95	1.06	0.75	0.58	0.64
Saudi Arabia	0.28	0.13	0.15	0.44	1.31	1.18	0.12	0.17	0.18
Yemen	0.63	0.47	0.45	0.98	0.85	1.02	0.62	0.40	0.46
Far East	19.85	15.95	14.51	0.95	1.03	1.19	18.94	16.42	17.34
China	2.83	1.55	1.36	2.49	3.31	4.12	7.03	5.13	5.61
India	16.36	13.79	12.55	0.70	0.78	0.89	11.38	10.79	11.23
Pakistan	0.40	0.41	0.40	0.58	0.59	0.59	0.23	0.24	0.24
Thailand	0.22	0.19	0.17	1.07	1.28	1.35	0.24	0.24	0.23
Central America and the Caribbean	1.96	2.07	1.73	2.82	2.73	2.87	5.54	5.64	4.95
El Salvador	0.13	0.12	0.14	1.15	1.27	1.48	0.15	0.16	0.20
Guatemala	0.04	0.06	0.07	1.95	1.41	1.19	0.08	0.08	0.08
Haiti	0.16	0.13	0.11	0.76	0.74	0.78	0.12	0.10	0.09
Mexico	1.49	1.61	1.28	3.35	3.17	3.43	4.99	5.10	4.38
Nicaragua	0.05	0.05	0.05	1.55	1.61	1.80	0.08	0.08	0.09
South America	2.48	1.40	1.38	2.77	2.59	3.08	6.86	3.61	4.23
Argentina	1.87	0.65	0.70	3.02	2.95	3.72	5.64	1.92	2.60
Brazil	0.08	0.16	0.15	2.13	1.54	1.87	0.17	0.25	0.28
Colombia	0.22	0.26	0.22	2.22	2.87	3.08	0.49	0.74	0.69
Uruguay	0.06	0.03	0.04	2.01	2.48	2.97	0.11	0.08	0.11
Venezuela	0,23	0.25	0.21	1.61	2.13	2.10	0.37	0.53	0.44
Developed countries	**6.48**	**5.05**	**4.99**	**3.33**	**3.38**	**3.94**	**21.58**	**17.08**	**19.66**
Australia	0.55	0.46	0.50	1.98	2.16	1.96	1.08	1.00	0.98
EC	0.13	0.11	0.12	4.58	4.90	5.61	0.59	0.56	0.70
South Africa	0.38	0.22	0.18	1.43	1.58	2.05	0.54	0.34	0.37
United States	5.27	4.06	4.05	3.63	3.69	4.32	19.16	14.97	17.50
CIS[2]	0.09	0.15	0.10	1.14	0.76	0.73	0.10	0.11	0.07
World	45.10	43.30	45.00	1.45	1.32	1.42	65.50	57.10	63.90

1. Each figure is a 3-year average for the respective period, e.g., 1979-81.

2. Until 1991, area of the former USSR.

Source: FAO

III- Utilization

Food use

Worldwide, approximately 27 million tons of sorghum were consumed as food each year during the 1992-94 period, almost the entire amount in Africa and Asia. It is a key staple in many parts of the developing world, especially in the drier and more marginal areas of the semi-arid tropics. Per caput food consumption of sorghum in rural producing areas is more stable, and usually considerably higher, than in urban centres. And within these rural areas, consumption tends to be highest in the poorest, most food-insecure regions.

Sorghum is eaten in a variety of forms that vary from region to region. In general, it is consumed as whole grain or processed into flour, from which traditional meals are prepared. There are four main sorghum-based foods:

- flat bread, mostly unleavened and prepared from fermented or unfermented dough in Asia and parts of Africa;
- thin or thick fermented or unfermented porridge, mainly consumed in Africa;
- boiled products similar to those prepared from maize grits or rice;
- preparations deep-fried in oil.

The composition in nutritive elements of the sorghum, the millets compared with other cereals (for 100 of edible portion; 12 percent of moisture) are given in the following table:

a Nx6.75.
Sources: Hulse. laing et Pearson. 1980: United States National Research Council/National Academy of Sciences. 1982: USDA HNIS 1984.

III.2. Animal feed

Cereal	Protein [a] (g)	Fat content	Ashes (g)	Rough fibre	Carbohydrates	Energy (kcal)	Ca (mg)	Fe (mg)	Thiamine (mg)	Riboflavine (mg)	Niacine (mg)
Rice (brun)	7,9	2,7	1,3	1,0	76,0	362	33	1,8	0,41	0,04	4,3
wheat	11,6	2.0	1,6	2,0	71,0	348	30	3,5	0,41	0,10	5,1
but	9,2	4,6	1,2	2,8	73,0	358	26	2,7	0,38	0,20	3,6
Sorghum	10,4	3,1	1,6	2,0	70,7	329	25	5,4	0,38	0,15	4,3
Millet candle	11,8	4,8	2,2	2,3	67,0	363	42	11,0	0,38	0,21	2,8
Eleusine	7,7	1,5	2,6	3,6	72,6	336	350	3,9	0,42	0,19	1,1
Millet of the birds	11,2	4,0	3,3	6,7	63,2	351	31	2,8	0,59	0,11	3,2
Common millet	12,5	3,5	3,1	5,2	63,8	364	8	2,9	0,41	0,28	4,5
Small millet	9,7	5,2	5,4	7,6	60,9	329	17	9,3	0,30	0,09	3,2
Moha of Japan	11,0	3,9	4,5	13,6	55.0	300	22	18,6	0,33	0,10	4,2
Indigenous millet	9,8	3,6	3,3	5,2	66,6	353	35	1,7	0,15	0,09	2,0

About 48 percent of world sorghum grain production is fed to livestock (human food use constitutes about 42 percent). In contrast to food utilization, which is relatively stable, utilization for feed sorghum changes significantly in response to two factors: rising incomes, which stimulate the consumption of livestock products, and the price competitiveness of sorghum vis-a-vis other cereals, especially maize. While sorghum is generally regarded as an inferior cereal when consumed as food, the income elasticities for livestock products (and hence the derived demand for feed) are generally positive and high.

Demand for animal feed is concentrated in the developed countries and in middle-income countries in Latin America and Asia, where demand for meat is high and the livestock industry is correspondingly intensive. Over 85 percent of sorghum feed use occurs in three countries (United States, Mexico and Japan) together absorb nearly 70 percent of the world total.

Sorghum utilization by type and region.

	1979-81 average (million tons)	**1984-86 average (million tons)**	**1989-91 average (million tons)**	**1992-94 average (million tons)**
United States	10.5	14.7	10.9	11.1
Mexico	6.7	6.6	8.1	7.1
Japan	4.1	4.2	3.5	2.6
China	2.4	2.1	1.5	1.9

Argentina	2.1	2.5	0.9	1.5
EC	1.8	0.5	0.8	0.9
Australia	0.4	0.3	0.8	0.8
Colombia	0.5	0.5	0.7	0.7
Venezuela	0.7	1.3	0.6	0.4
CIS[1]	2.5	0.9	0.3	0.1
Others	3.4	3.1	3.2	3.2
World	**35.1**	**36.7**	**31.3**	**30.6**

1. Until 1991, area of the former USSR.
Source: FAO

III.3. Other uses

Another significant aspect of the sorghum grain, especially in Africa, is its use in the production of alcoholic beverages. The grain is used for malt or like complement in the production of two types of beer: lager and brown ale (weak beer traditional African out of alcohol which contains fine suspended particles). The sweetened juice of its stem can be used to produce alcohol (ethanol).

The fibres of the plant are used to manufacture boards wall used in construction or many biodegradable packing

IV- Conservation of sorghum

The foodstuffs can be the seat various deteriorations during their production, collects, transport, storage, transformation or distribution. These deteriorations are varied. They can be the fact of living organisms, of physicochemical or biochemical modifications, enzymes or microbial substances. It is rare that these deteriorations intervene separately one of the other. In general, they occur at the same time or follow one another without transition.

On the practical level, these deteriorations result in:

- Modifications of the organoleptic characteristics (colour, taste, appearance / aspect), with like consequences the degradation of the commercial value and even of the economic losses;
- health risks of the consumers (diseases, dead).

The goal of the conservation is to prolong the shelf life of products and to allow their best distribution in time and space; and to ensure their qualities hygienic and commercial.

Degradation of seeds of sorghum is a function of the temperature of storage. At low temperature, degradation remains slow but it accelerates quickly as soon as the temperature rises. It is thus necessary to dry the wet collected sorghums quickly. The moisture of safeguard for a long duration storage is 13-13,5 %.

IV-1 Drying

a. Natural drying

After harvest, generally manual, the ears of millet and sorghum are generally laid out in boots or grinding stones and are dried with the sun. One can improve this natural drying by building surfaces of drying or trays. In the traditional zones of culture, natural drying does not present in general a difficulty.

b. Artificial drying

The two principal techniques of drying of the sorghum are ventilation under great thickness with ambient air or slightly heated and fast drying with the hot air out of drier.

IV-2 Storage

Very often, on the level of the villages, the sorghums and the millets are stored out of ears in the traditional attics. The candles of millet are sometimes broken in several pieces, before being ensiled to increase the apparent unit weight.

The producers generally consider that storage out of ears ensures a better conservation. It should however be noted that he requires a volume per kg of product stored more significant than storage in grain. In the centres of marketing, storage is generally carried out out of bags of 100 kg out of jute or polypropylene.

On the basis of measurement made in Senegal, the specific volume of the millet stored out of bags of 100 kg is of: 1,2 m³/t approximately whereas that of the sorghum is of 1,8 m³/t because of its larger granulometry.

Characteristic physics of the grains

	size	**Weight of 1000 grains**	**Unit weight in (kg/m³)**
Sorghum (Sorghum vulgare)	4-5 mm	15 à 35	685 à 760
Mil (Pennisetum typhoïdes)	2,5-3,5 mm		
Sonna Variety		4 à 7 g	760 à 840
Sanio Variety		6 à 9 g	790 à 860

(*Source: File document of FAO Title: Conservation of the grains in hot areas)*

Storage in bulk is practised in the horizontal or vertical general-purpose silos. In the moderated zones, ventilation is often employed to lower the temperature of the grain and to block the activity of the insects. In tropical zone, ventilation can be less of interest because the ambient air is often at a high temperature. However, in certain seasons, it is possible to use the night fresh air (with temperatures dropping until 15-18° C).

IV-3 Enemies of stocks

The principal insects depredators are the charançon (Sitophilus orizae), the trogoderme (Trogoderma granarium) which can create many damage in the stores in particular by its larvae which destroy the bags and tribolium. Other insects: the alucite (Sitotroga cerealella) and the tinea (Ephestia) are less frequent.

V- <u>Transformation of the product</u>

The dehusking and the grinding of the sorghum, hard operations by manual ramming, are the object of many mechanization test. Dry dehusking would make it possible in particular to lengthen the shelf life of the flour.

At the industrial level many studies treat partial substitution of corn but without full-scale applications; one of the brakes is the difficulty in providing to the large mills a homogeneous product of satisfactory quality with the current techniques of storage out of bags.

VI- <u>Conditions of storage supporting the Mycotoxins</u>

The Aspergillus moulds are opportunist hyaline moulds with fast proliferation, generally present in the ground and the matters in decomposition. Their colonies are usually of yellow colour, green yellow, brown or green; of granulous aspect, velvety or duveteux; and present a white peripheral edge and a net contour.

The species Aspergillus producing of aflatoxines and thus causes contamination of food by aflatoxines, are ubiquists in the areas of the world to the hot and wet climate. Aspergillus flavus / A. parasiticus cannot develop nor to produce aflatoxines when the activity of water is lower than 0,70, the relating humidity to 70 percent and the temperature of 10° C . Under conditions of stress, for example in the event of dryness or of infestation of insects, the contamination by aflatoxines is likely to be high.

The two essential physical factors which influence the growth of moulds and the production of mycotoxins are the temperature and moisture. Indeed, the moulds of fields require a high moisture content in the substrate (20-25 %) compared to moulds of storage (10-18 %). Each species has its optimal level of moisture (or activity of water, A_W and of temperature for the production of mycotoxin.

We can distinguish according to the temperature various species:

- Thermophilous: develop well with 50°C, with a minimal temperature of growth higher to 20°C: *Byssochlamys nivea Absidia ramosa Aspergillus fumigatus* ..
- Thermotolerantes, with a maximum temperature of growth close to 50°C, but a minimal temperature much lower than 20°C: *Aspergillus Niger*.
- Mésophiles: develop between 10°C and 40°C: *Aspergillus versicolor...*
- Cryophiles: whose optimal germination is carried out at lower temperatures à10°C.

Below of A_W 0,65 no mould can develop, some that is the nature of food because it does not have sufficient water. Thus, according to requirements' of the water moulds we distinguishes from the species:

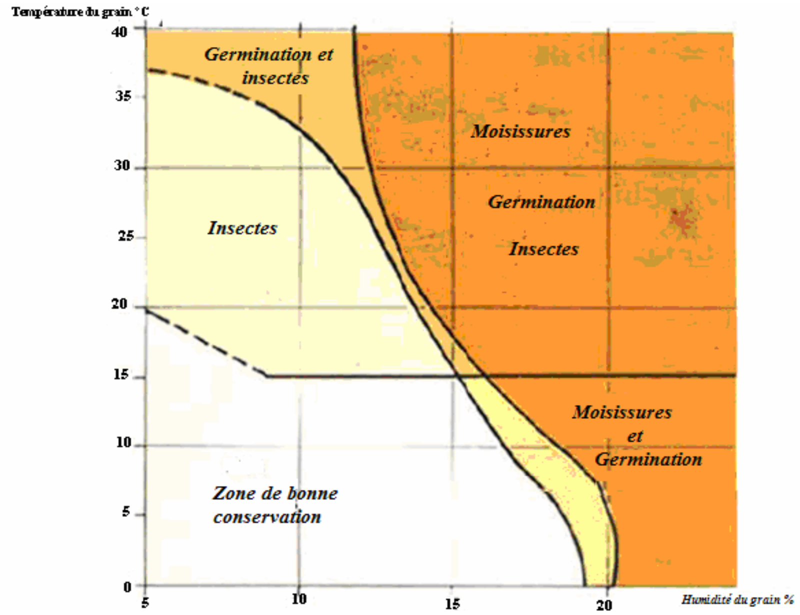
- Xerophilous: at which the germination of the spores is possible with water contents lower than 80%: they are mainly the species of the Aspergillus
- Mésophiles: with a water requirement ranging between 80 and 90%: *Penicillium cyclopium, P.expansum....*
- Hygrophile (higher than 90%): *Fusarium spp* Mucorales...

These optima can differ appreciably from their optima for the growth.

Thus the conditions most favorable for an optimal growth and a production of aflatoxines by *A. flavus* are, an A_W relatively weak (0,84 - 0,86) as well as a high temperature between 25 and 40 °C. A. flavus can develop with 15°C with an A_W of about 0,90.

The optimal temperature of production of the OTA by *Aspergillus ochraceus* is 28°C, this production is strongly reduced to 15°C or 37°C. In the cold areas, the OTA thus is rather produced by of *Penicillia* whereas in the hot areas, it is rather *Aspergillii* which manufacture it. The OTA is formed preferentially on acid food. The production of zéaralénone (ZEA) or toxin F-2 is supported by low temperatures located between 10 and 15°C.

Growth of the moulds according to the water content and the temperature



At side of A_W and the temperature, the pH can have a critical effect for the fungal growth and the production of mycotoxin.

Indeed, the acidity of the **medium** has a significant role for the growth of moulds as for the production of toxins. For example that Fumonisin B1 is formed with pH 3,7, whereas the optimal growth takes place with pH 5,6.

The majority of the moulds need oxygen to develop. It appears as well as the reduction of oxygen and especially the increase in the CO 2 content as well has very significant a depressor effect on the formation of mycotoxins as on the moulds growth.

IV- ***Contamination Data***

Considering the significant risk of the mycotoxins as well on human and animal health several research and investigations were carried out in several countries in order to evaluate the risk of exposure has these contaminants.

All the countries do not have truly the same risks according to dominant climatic conditions'. In the areas moderated like France, England, part of the United States, the major mycotoxicological risk will be due to toxins of Fusarium (trichothecenes, moniliforme, fumonisines, zéaralénone). In France, those are required in cereals such as corn, corn and the barley. Other toxins of Penicillium and Aspergillus, such as ochratoxin the citrinine and aflatoxins (toxins *of Aspergillus flavus* are also subject of research). For the hot and wet countries like sub-Saharan Africa and the Latin America, the most dreaded mycotoxins are aflatoxins.

IV-1. Case of Tunisia

Tunisia is a country favourable to the proliferation of moulds toxinogenes, because of its hot and wet climate and of the precarious conditions of storage.

The research of the mycotoxins on the level of the food products Tunisian began since the Seventies.

Materials and methods

Sampling of sorghum grains

A total of 36 sorghum samples in 2008, 16 samples in 2007, 21 samples in 2005, 15 samples in 2004 and 195 samples in 2003 were collected from different areas of Tunisia. The analyzed products were the grains of sorghum in bulk, in bulk ground sorghum, prepackaged ground sorghum, cooked sorghum, Sorghum with the sesame grains, Sorghum with the dry fruits, Sorghum with the grains of pine of Alep.

Mycotoxins identification:

The extraction was done by ethanol - water and cleaned up on packed Silica Gel column and the HPLC (with fluorescence detector VOD-C - length 25cm , Diameter 4-6 nm column) was used for the detection of aflatoxin .

The most recent results concerning the sorghum and which enter within the framework of programme of monitoring of the contamination of the foodstuffs by the mycotoxins, revealed the results of the following table:

Average contents of aflatoxines in the sorghum expressed in ppb

Year	Product	Aflatoxine B1	G2+G1+B2+B1
2008	bulk ground sorghum	**2,23(**0,4-10,2**)**	**1,83**(0,1-8)
2007	Ground sorghum p	**25,22**(2,5-55,1)	**23,557(**2,5-51,4**)**
	bulk ground sorghum	**11,58**(0-31,3)	**10,65**(0-29,2)
	Sorghum with the sesame grains	**3,8**	**3,8**
	Sorghum with the dry fruits	**5,9**	**5,2**
2005	Sorghum with the dry fruits	**2,49**(0-6,1)	**2,5(**0,1-6,1)
	Sorghum with the sesame grains	**2,2**	**2,2**
	Sorghum with the grains of pine of Alep	**0**	**0,1**
	Sorghum in grains	**0**	**0,1**
	Ground sorghum p	**2**	**2,0**
	bulk ground sorghum	**3,62(**0-10,7)	**3,55(**0,1-10,4)
2004	Sorghum in grains	**54,42**(0-181,3)	**51,5**(0-171,7**)**
	bulk ground sorghum	**26,05(**0,97-72)	**22,92(**0,97-64,4**)**
	Cooked sorghum	**2,12(**0-5,4)	**2,12**(0-5,4)
2003	Sorghum in grains	**24,29(**0-410,2)	**23,34(**0-381)
	Ground sorghum p	**34,49**(0-221,3)	**32,14(**0-783)
	bulk ground sorghum	**26,08(**0-291,72)	**24,58(**0-273,48)

*** The figures between brackets indicate the minimal and maximum values**

The results show a rather increased contamination of the sorghum by aflatoxins B1, B2, G1 and G2. This finds its origin under the climatic conditions characterized by significant moisture (strong rate of pluviometry) like at temperatures varying of 6 to 45°C.

IV-2.Case of Sudan

Mycotoxins in sorghum *(Sorghum bicolor) in Sudan*

Background:

Sorghum (*Sorghum bicolor* (L.) is the most important staple food crop in Sudan. It provides the principal source of calories in the country. Sorghum resides in 75% of the total cultivated areas under cereals and contributed 85 % of the food crops produced (Ibrahim *et al.*2006).

Sorghum grains are used primarily as a major source of food for 85 % of the population. The grains and stalks are important feed resource for livestock and poultry in Sudan (Ejeta, 1988, Habash, 1990). In addition sorghum grains are also exported to African, Arab neighbouring countries especially Saudi Arabia and Asian countries.

Sorghum is principally produced under rain-fed mechanized and traditional farming systems in Sudan mainly in a mono cropping system (Affan 1984; Mahmoud 1992). About 8.4 million ha is under the traditional rain-fed sorghum while 5.4 and 1.9 million ha are under mechanized and irrigated farming (Ibrahim *et al*. 2006).

In the rain-fed sector a number of traditional sorghum varieties and land races (Feterita type) are very popular and preferable for being short maturing and drought tolerant. However, a choice of traditional, improved varieties and hybrid are realized and cultivated (Ahmed *et a*l., 1992).

Sorghum is grown in July and harvested in October. Out of the total sorghum production, 80% is stored in different storage structures. The major storage area in Sudan is Gedarif city situated in the traditional sorghum production sector and comprises the main sorghum market in the country. At Gedarif, the crop is stored in governmental good standards concrete silos, private sector metal silos or in jute bags in private sector stores or trader's stores. **(**Underground pits were popular in Gedarif area and are still in use in other areas**).** Inadequate storage methods observed were the main cause of grains losses due to store pests and /or fungal contamination. There is a risk of mycotoxin contamination of stored sorghum grains in all the storage types in Gedarif area.

Storage fungi are known to be the dominant cause of post harvest deterioration of cereals mainly by *Aspergillus* and *Penicillium* species (Gonzalez *et al*.,1997). A post harvest research on grain loss due to fungi is very scanty in Sudan and some research has been conducted with the prime objectives of identifying mycoflora associated with stored sorghum grains and their role in changing the chemical components (Abdalla, 1998, Ahmed *et al*., 2008). A number of *Aspergillus* and *Penicillium* species were found associated with sorghum causing severe grain deterioration. The average losses due to fungal contamination amounted to more than 10% (Ahmed *et al*., 2008). In this study we identified the kind and amount of mycoflora associated with stored sorghum grains in the main storage types in Sudan and their significance in mycotoxin production.

Materials and methods

Sampling of sorghum grains

A total of 86 sorghum grain samples each weighing 2 kg were collected from different storage types in Gedarif city. Random method of sampling was used for the collection of samples such that equal number of sacks was used for each sample and variety. These samples were well combined to 28 collective samples with regard to the variety and type of storage. They were analyzed for fungal contamination, germination and mycotoxins contents.

Germination tests:

Seed germination tests were conducted using standard procedure described by Mathur and Jorgensen, (1992).

Isolation of fungi

The seed plating method (ISTA, 1985) using potato dextrose agar (PDA) medium was used to determine seed borne fungi. A similar number of seeds were surface sterilized using 5% sodium hypochlorite.

Excess surfactant was drained off and the seeds were rinsed three times with sterile distilled water. Excess water on the seeds was mopped using sterile filter paper. The fungi were identified on the bases of their macroscopic and microscopic characters using the keys of Booth, 1971, Ellis, 1971 and Pitt 1979.

Mycotoxins identification:

Aflatoxins were experimented for at the National Chemical Laboratories in Khartoum. The extraction was done by ethanol - water and cleaned up on packed Silica Gel column and the HPLC (with fluorescence detector VOD-C - length 25cm , Diameter 4-6 nm column) was used for the detection of aflatoxin .

Aflatoxin contamination in sorghum sample from Gedarif area, Sudan.

No	Sample	Variety	Type of Storage	Aflatoxin µ / kg			
				B1	B2	G1	G2
1	116FSS	WadAhmed	traditional	1	nd	nd	nd
2	117FSS	Mukhalafat	Traditional	nd	nd	nd	nd
3	115FSS	Arfagadamak	traditional	nd	nd	nd	nd
4	107SBS	Daber	Improved	nd	nd	nd	nd
5	108SBS	Tetron	Improved	2.4	nd	nd	nd
6	105SBS	Wad Aker	Improved	nd	nd	nd	nd
7	196SBS	Feterita	Improved	nd	nd	nd	nd
8	112TS	Mugud	traditioanl	7	1.5	nd	nd
8	123ABS	Feteriat	Grain silos	nd	nd	nd	nd
10	118ABS	Wad Aker	Silos	nd	nd	nd	nd
11	122ABS	Feterita	Grain silos	1.2	nd	nd	nd
12	109KBS	Wad Aker	Improved	nd	nd	nd	nd
13	111KBS	B. Fet0000erita	Inproved	nd	nd	nd	nd
14	110KBS	Harira	Improved	nd	nd	nd	nd
15	101FBS	Arfagadamak	Improved	nd	nd	nd	nd
16	102FBS	Korokolo	Improved	nd	nd	nd	nd
17	104FBS	Biased Feteriata	Improved	nd	nd	nd	nd
18	103FBS	Feterita 2006	Improved	2.4	nd	nd	nd
19	120ABS	Korokolo	Improved	nd	nd	nd	nd
20	121ABS	Daber	Silos	nd	nd	nd	nd
21	130FBS	Biased	improved	nd	nd	nd	nd
22	131FBS	Left after sieving	improved	nd	nd	nd	nd
23	114TS	Feterita	Traditional	nd	nd	nd	nd
24	113TS	Wad Aker	Traditional	nd	nd	nd	nd
25	132KBS	Random	improved	nd	nd	nd	nd
26	133FBS	Start cleaning	improved	nd	nd	nd	nd
27	134FBS	Before sieving	improved	nd	nd	nd	nd
28	135FBS	Biased 16 sacks	improved	nd	nd	nd	nd

Sorghum samples collected from Gedarif area (August 2008) Sudan.

National Chemical Laboratories, Ministry of Health, Khartoum, Sudan.

RESULTS AND DISCUSSION'

Experiments for determination of mycotoxin contamination as shown was only done for aflatoxin. It was found that aflatoxin had low concentration in all the samples. The highest record was detected in a traditional store on Mugud variety (B1) which was 7µg/Kg. However, B2 was only detected in the same store with1.5 µg/kg. This may be mainly because the sorghum produced in the same season (2007/2008) which was put in storage not more than six months. As for strains G1 and G2 they are generally not found on cereals. However, aflatoxin is more serious on oil seeds which were not analyzed here. A more thorough research on presence of ochratoxin in sorghum is underway. This will be done on a national level and testing the difference storage methods. Results and appropriate interventions will be communicated later.

Some technical help may be needed and consequently requested from those who have experience with such research.

IV-3.Case of Brazil

Taking into account the lack of mycotoxigenicity studies of Aspergillus and Fusarium strains isolated from Brazilian sorghum, the objective of the present study was to determine the toxigenic potential of A. flavus, F. verticillioides and F. proliferatum strains isolated from both freshly harvested and stored sorghum in São Paulo State, Brazil.

MATERIALS AND METHODS

Aspergillus and Fusarium strains

Fifty-nine A.flavus, 35 F. verticillioides, and 3 F. proliferatum isolates, obtained from freshly harvested (l0) and stored (l30) sorghum grains cultivated in Nova Odessa, Silo Paulo State, were evaluated. Samples (lOg) were collected monthly, and the grains were ground and homogenized in 90 mL water. Decimal dilutions of up to 10-6 were accomplished and I-mL aliquots of the dilutions were inoculated onto potato dextrose agar. After incubation (5 days at 25°C), the colonies were counted, isolated, and identified. Aspergillus and Fusarium strains were identified according to Rapel and Fennell (27) and Nelson et al. (21,23) methods, and stored on Sabouraud dextrose agar (SDA) slants at4-8°C.

Fumonisin production by *Fusarium verticillioides* and *F. proliferatum* strains isolated from freshly harvested and stored sorghum kernels in Brazil

Fusarium	Fumonisin concentration (ug/g)		
Strain	FEI	FE_2	Total
FH-17[a]	0.15	ND	0.15
FH-20	0.76	0.00	0.85
FH-29	0.16	ND	0.16
FH-31	0.83	ND	0.83
FH-32	0.67	0.39	1.06
FH-34	0.82	0.13	0.95
FH-35	0.52	0.15	0.67
FH-36	0.24	ND	0.24
FH-39	4.38	1.00	5.38
FH41	0.37	ND	0.37
FH42	1.79	0.36	2.15
FH47	0.29	ND	0.29
FH49	0.16	ND	0.16
FH-S4	0.15	ND	0.15
FH-55	0.12	ND	0.12
FH-57	0.13	ND	0.13
FH-61 *	0.12	ND	0.12

FH-74	0.12	0.06	0.18
FH-75	0.40	0.08	0.48
SI-80[b]	0.12	ND	0.12
SI-81 *	0.16	0.02	0.18
SI-83*	ND	ND	ND
SI-84	0.65	ND	0.65
SI-85	0.19	ND	0.19
SI-86	0.14	ND	0.14
SI-87	0.15	ND	0.15
SI-89	0.15	ND	0.15
S2-143	0.06	ND	0.06
S2-145	0.19	ND	0.19
S2-169	0.67	ND	0.67
S4-259	0.51	0.11	0.62
S4-260	0.23	ND	0.23
S4-293	ND	ND	ND
S4-295	0.23	ND	0.23
S4-296	ND	ND	ND
S5-319	ND	ND	ND
S7407	1.18	ND	1.18
S7416	0.35	ND	0.35

a Freshly harvested; b stored samples. ND = not detected.

* *Fusarium proliferatum.*

Table 1. Aflatoxin production by *Aspergillus flavus* strains isolated from freshly harvested and stored sorghum kernels in Brazil.

A. flavus	Aflatoxin concentration (µg/kg)		
Strain	B_1	B_2	Total
FH-06[a]	63.70	33.00	96.70
S7-393[b]	ND	ND	ND
S7-397	ND	ND	ND
S7-400	ND	ND	ND
S7-402	788.40	23.30	811.70
S7-404	467.00	173.00	640.00
S7-405	29.00	ND	29.00
S7-408	27.00	ND	27.00
S7-409	ND	ND	ND
S7-413	320.00	9.50	329.50
S7-415	559.00	207.00	766.00
S7-417	12.00	ND	12.00
S8-420	89.00	ND	89.00
S8-421	1139.00	84.50	1223.50
S8-423	22.00	ND	22.00
S8-425	1422.00	528.00	1950.00
S9-426	94.00	ND	94.00
S8-427	25.00	ND	25.00
S8-429	750.00	16.00	766.00
S8-436	723.50	5.40	728.90
S9-440	ND	ND	ND
S9-441	ND	ND	ND
S9-446	3258.00	24.50	3282.50
S9-449	769.00	57.00	826.00
S9-452	ND	ND	ND
S9-456	ND	ND	ND
S10-462	439.00	6.50	445.50
S10-463	591.00	ND	591.00
S10-464	52.00	ND	52.00
S10-465	568.00	ND	568.00
S10-468	320.00	9.50	329.50
S10-469	ND	ND	ND
S10-470	198.00	ND	198.00
S10-471	ND	ND	ND
S10-473	527.00	86.00	613.00
S10-477	72.00	ND	72.00
S10-479	ND	ND	ND
S10-480	ND	ND	ND
S10-483	ND	ND	ND
S11-484	ND	ND	ND
S11-485	878.50	326.00	1204.50
S11-494	ND	ND	ND
S11-505	ND	ND	ND
S12-511	559.00	ND	559.00
S12-515	615.00	9.00	624.00
S12-517	27.00	ND	27
S12-518	45.50	ND	45
S12-519	ND	ND	N
S12-520	56.50	21.00	77
S12-521	35.50	7.50	43
S12-527	42.00	ND	42
S12-529	ND	ND	N
S12-533	ND	ND	N
S13-536	574.00	42.50	61
S13-539	439.00	163.00	60
S13-541	ND	ND	N
S13-547	94.50	35.00	12
S13-552	189.00	42.00	23
S13-556	ND	ND	N

[a] Freshly harvested; [b] stored samples. ND = not detected.

Fumonisin-producing *F. verticillioides* strains
been analyzed by other investigators (6,16), who det
fumonisin producer strains in corn, but low pro
sorghum. According to Nelson *et al.* (22), the low pro
fumonisins by *F. verticillioides* strains from sorgh
may be related to the substrate and/or to the geograp

The higher production of FB_1, when compared t
also been reported (4,8,9), with FB_1 accounting for
fumonisins both in culture and in naturally contamin
FB_2 and FB_3 concentrations detected in foods or pr
culture by *F. verticillioides* strains are approximately
of the produced FB_1. However, Apsimon (2) is
verticillioides strains producing more FB_2 than FB_1.

Moretti *et al.* (20) concluded all strains isolated fror
belonged to the "F" mating population characterized
no FB_1 and FB_2 production. In contrast, majority
isolated from maize belonged to the "A" mating p
which produces moderate to high levels of FB_1 and F

Two strains of the 3 *F. proliferatum* isolates produ
FB_2 at concentrations of 0.12 and 0.18 µg/g (Table 2).
production by other *Fusarium* species, mainly *F. pro*
has been reported (23,38); however, *F. verticillioides*
to be the main producer of this toxin.

In the present study, a small number of *A. flavus* s
isolated from freshly harvested sorghum samples
although a larger number of toxigenic strains were iso
stored sorghum (S7-S13). This result might be explai
fact that *Aspergillus* is classified in the literature as
fungus, which has already been detected in the field. C
F. verticillioides, which is typically considered to
fungus, a larger number of strains was detected
harvested samples. Nevertheless, this fungus was iso
the seventh month of storage.

Table 1. Aflatoxin production by *Aspergillus flavus* strains isolated from freshly harvested and stored sorghum kernels in Brazil.

A. flavus	Aflatoxin concentration (µg/kg)		
Strain	B_1	B_2	Total
FH-06[a]	63.70	33.00	96.70
S7-393[b]	ND	ND	ND
S7-397	ND	ND	ND
S7-400	ND	ND	ND
S7-402	788.40	23.30	811.70
S7-404	467.00	173.00	640.00
S7-405	29.00	ND	29.00
S7-408	27.00	ND	27.00
S7-409	ND	ND	ND
S7-413	320.00	9.50	329.50
S7-415	559.00	207.00	766.00
S7-417	12.00	ND	12.00
S8-420	89.00	ND	89.00
S8-421	1139.00	84.50	1223.50
S8-423	22.00	ND	22.00
S8-425	1422.00	528.00	1950.00
S9-426	94.00	ND	94.00
S8-427	25.00	ND	25.00
S8-429	750.00	16.00	766.00
S8-436	723.50	5.40	728.90
S9-440	ND	ND	ND
S9-441	ND	ND	ND
S9-446	3258.00	24.50	3282.50
S9-449	769.00	57.00	826.00
S9-452	ND	ND	ND
S9-456	ND	ND	ND
S10-462	439.00	6.50	445.50
S10-463	591.00	ND	591.00
S10-464	52.00	ND	52.00
S10-465	568.00	ND	568.00
S10-468	320.00	9.50	329.50
S10-469	ND	ND	ND
S10-470	198.00	ND	198.00
S10-471	ND	ND	ND
S10-473	527.00	86.00	613.00
S10-477	72.00	ND	72.00
S10-479	ND	ND	ND
S10-480	ND	ND	ND
S10-483	ND	ND	ND
S11-484	ND	ND	ND
S11-485	878.50	326.00	1204.50
S11-494	ND	ND	ND
S11-505	ND	ND	ND
S12-511	559.00	ND	559.00
S12-515	615.00	9.00	624.00
S12-517	27.00	ND	27.00
S12-518	45.50	ND	45.50
S12-519	ND	ND	ND
S12-520	56.50	21.00	77.50
S12-521	35.50	7.50	43.00
S12-527	42.00	ND	42.00
S12-529	ND	ND	ND
S12-533	ND	ND	ND
S13-536	574.00	42.50	616.50
S13-539	439.00	163.00	602.00
S13-541	ND	ND	ND
S13-547	94.50	35.00	129.50
S13-552	189.00	42.00	231.00
S13-556	ND	ND	ND

[a] Freshly harvested; [b] stored samples. ND = not detected.

Fumonisin-producing *F. verticillioides* strains have also been analyzed by other investigators (6,16), who detected high fumonisin producer strains in corn, but low producers in sorghum. According to Nelson *et al.* (22), the low production of fumonisins by *F. verticillioides* strains from sorghum grains may be related to the substrate and/or to the geographical area.

The higher production of FB_1, when compared to FB_2, has also been reported (4,8,9), with FB_1 accounting for 70% of all fumonisins both in culture and in naturally contaminated corn. FB_2 and FB_3 concentrations detected in foods or produced in culture by *F. verticillioides* strains are approximately 15 to 25% of the produced FB_1. However, Apsimon (2) isolated *F. verticillioides* strains producing more FB_2 than FB_1.

Moretti *et al.* (20) concluded all strains isolated from sorghum belonged to the "F" mating population characterized by little or no FB_1 and FB_2 production. In contrast, majority of strains isolated from maize belonged to the "A" mating population, which produces moderate to high levels of FB_1 and FB_2.

Two strains of the 3 *F. proliferatum* isolates produced $FB_1 + FB_2$ at concentrations of 0.12 and 0.18 µg/g (Table 2). Fumonisin production by other *Fusarium* species, mainly *F. proliferatum*, has been reported (23,38); however, *F. verticillioides* continues to be the main producer of this toxin.

In the present study, a small number of *A. flavus* strains was isolated from freshly harvested sorghum samples (1 strain), although a larger number of toxigenic strains were isolated from stored sorghum (S7-S13). This result might be explained by the fact that *Aspergillus* is classified in the literature as a storage fungus, which has already been detected in the field. Concerning *F. verticillioides*, which is typically considered to be a field fungus, a larger number of strains was detected in freshly harvested samples. Nevertheless, this fungus was isolated until the seventh month of storage.

RESULTS AND DISCUSSION

Aflatoxin analysis showed that 38 (64.4%) of 59 tested *A. flavus* strains produced detectable levels of aflatoxins at concentrations ranging from 12.00 to 3282.50 ug/kg (AFB_I + AFB_2). Fifteen strains produced only AFB[, while 23 produced both AFB_I and AFB_2 (Table 1). Aflatoxin group B (AFB_I and AFB_2), producing *A. flavus* strains, has also been described by Pier (24) and Pitt (25), who identified 10% AFB_I producer strains and 90% strains producing both AFB_I and AFB_2• In addition, other researchers (13,15) have also been reported higher AFB 1 levels compared to AFB_2• Our results agree with those ones reported by Kichou *et al.* (l4), who demonstrated that 23% of *A. flavus* strains isolated from sorghum in Morocco produced AFB_I and AFB_2• In India, Sashidhar *et al.* (30), analysing 150 sorghum grain samples, found high rates of contamination by *A.flavus* (67%) and *Fusarium* (59%); however, only two strains produced AFB 1 at concentrations of 16 and 40 flg/kg. Production of AFB_I and AFB_2 in sorghum and wheat inoculated with *A. flavus* was also reported (39).

Fumonisin analysis showed that 32 (91.5%) of 35 tested *F. verticillioides* strains produced detectable levels of fumonisins at concentrations ranging from 0.12 to 5.38 ug/g (FB_I + FB_2). Twenty-three strains produced only FB_I and 9 produced FB_I + FB_2 (Table 2). The mean recovery rate for fumonisins was approximately 85%. Fumonisin production by almost every *F. verticillioides* strains (28,38) has been observed in 100% of *F. verticillioides* strains isolated from corn.

The occurrence of toxin production by strains isolated from foods and animal feed does not necessarily imply the presence of mycotoxins. However, it indicates a potential risk for a possible contamination with mycotoxins. Furthermore, if these foods represent a good substratum for mycotoxin production and if the abiotic factors (especially moisture and temperature) are appropriate, the contaminant hazard tends to increase.

V- Preventive measures

The prevention of the contamination by the mycotoxins of the sorghum starts from semi until the release to the market. Good husbandries represent the first line of defence against the contamination of cereals by the mycotoxins, followed by the implementation good manufacturing practice during handling, storage, the transformation and the distribution of cereals intended for the human and animal consumption. The code of practices as regards prevention and reduction of the contamination of cereals by the mycotoxins, is a good reference for this prevention (codex- CAC/RCP 51-2003).

APPENDIX

Result of analyses relating to research and proportioning of aflatoxines B1, B2, G1 and G2 in the sorghum in Tunisia

Denomination of the product	Period of the analysis	G2+G1+B2+B1	Aflatoxine B1
bulk ground sorghum	2008	7.08	6,48
bulk ground sorghum	2008	3,4	3,1
bulk ground sorghum	2008	4,8	4,5
bulk ground sorghum	2008	3.3	3.0
bulk ground sorghum	2008	8,26	7,6
bulk ground sorghum	2008	4,1	3,6
bulk ground sorghum	2008	10.2	8
bulk ground sorghum	2008	5,7	4,9
bulk ground sorghum	2008	3,5	3,2
bulk ground sorghum	2008	2,4	2,1
bulk ground sorghum	2008	2.2	1,9
bulk ground sorghum	2008	1.9	1,6
bulk ground sorghum	2008	1,4	1,1
bulk ground sorghum	2008	1,1	0,8
bulk ground sorghum	2008	1	0,7
bulk ground sorghum	2008	1,3	1
bulk ground sorghum	2008	1,02	0,72
bulk ground sorghum	2008	2	1,7
bulk ground sorghum	2008	1,5	1,2
bulk ground sorghum	2008	2	1,7
bulk ground sorghum	2008	1,9	1,6
bulk ground sorghum	2008	1,7	1,4

bulk ground sorghum	**2008**	1,23	0,93
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	0,7	0,4
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	<0.4	<0.1
bulk ground sorghum	**2008**	1,3	1
bulk ground sorghum	**2008**	1,3	1
bulk ground sorghum	**2008**	<0.4	<0.1
Sorghum with the sesame grain	2007	3.8	3.8
Ground sorghum P	2007	8.0	8.0
Ground sorghum P	2007	2.5	2.5
Ground sorghum P	2007	26.0	24.0
Ground sorghum P	2007	55.1	51.4
Ground sorghum V	2007	16.7	15.3
Ground sorghum V	2007	31.3	29.2
Sorghum with the dry fruits	2007	5.9	5.2
Ground sorghum V	2007	0	0
Ground sorghum V	2007	1.48	1.48

Ground sorghum P	2007	28.1	26.1
Ground sorghum P	2007	30	27.9
Ground sorghum V	2007	12.4	11.8
Ground sorghum V	2007	19.2	16.8

Denomination of the product	**Period of the analysis**	**G2+G1+B2+B1**	**Aflatoxine B1**
Sorghum with the dry fruits	2005	0,66	0,66
Ground sorghum V	2005	0	0,1
Sorghum with hazel nuts	2005	1,2	1,2
Sorghum with the sesame grain	2005	2,2	2,2
Sorghum with the dry fruits	2005	1,8	1,8
Ground sorghum v	2007	0	0
Sorghum with the grains of pine of alep	2005	0	0,1
Sorghum with the dry fruits	2005	0	0,1
Sorghum grains	2005	0	0,1
Ground sorghum P	2005	2‹0	2‹0
Ground sorghum v	2005	4‹83	4‹56
Sorghum with the dry fruits	2005	5‹0	5‹0
Sorgho aux fruits secs	2005	2‹68	2‹68
Ground sorghum v	2005	4‹6	4‹6
Ground sorghum v	2005	0‹6	0‹6
Ground sorghum v	2005	10‹7	10‹4
Ground sorghum v	2005	9‹3	9‹0
Ground sorghum v	2005	0	0‹1<
Sorghum with hazel nuts	2005	6‹1	6‹1
Ground sorghum v	2005	2‹06	2‹06
Ground sorghum v	2005	0‹54	0‹54
Ground sorghum v	2005	0	0‹1<

Denomination of the product	**Period of the analysis**	**G2+G1+B2+B1**	**AflaB1 en ppb**
Sorghum grains v	**2004**	5,6	**5,6**
Sorghum grains v	**2004**	181,3	**171,7**
Sorghum grains v	**2004**	135,2	**80,2**
Ground sorghum v	**2004**	72	**64,4**
Sorghum grains v	**2004**	0	**0**
Ground sorghum v	**2004**	5,2	**3,4**
Ground sorghum v	**2004**	0,97	**0,97**
Sorghum grains v	**2004**	0	**0**
Cooked sorghum	**2004**	4,2	**4,2**
Cooked sorghum	**2004**	0	**0**
Cooked sorghum	**2004**	0	**0**
Cooked sorghum	**2004**	0	**0**
Cooked sorghum	**2004**	4,1	**4,1**
Cooked sorghum	**2004**	1,2	**1,2**
Cooked sorghum	**2004**	5,4	**5,4**
Sorghum grains	2003	32,12	29,34
Ground sorghum P	2003	7,25	7,25
Ground sorghum P	2003	7,83	7,83
Ground sorghum P	2003	11,35	9,96
Ground sorghum P	2003	**1,87**	1,87
Ground sorghum v	2003	7,12	7,12
Sorghum grains v	2003	**0**	0
Ground sorghum P	2003	54,33	54,33
Ground sorghum P	2003	**1,65**	1,65
Ground sorghum P	2003	12,26	12,26
Sorghum grains v	2003	3,34	3,34
Ground sorghum v	2003	25,1	25,1
Ground sorghum v	2003	24,85	24,85
Sorghum grains v	2003	**1,84**	1;84
Sorghum grains v	2003	**0**	
Ground sorghum P	2003	6,73	5,9
Ground sorghum P	2003	6,33	5,5
Ground sorghum P	2003	2	2
Ground sorghum P	2003	10,17	9,36
Ground sorghum P	2003	4,02	4,02
Ground sorghum P	2003	**1,22**	1,22
Ground sorghum P	2003	4,74	4,05
Ground sorghum P	2003	5,17	4,78
Ground sorghum P	2003	4,62	4,62
Ground sorghum P	2003	11,99	10,8
Sorgho grains v	2003	9,9	8,74
Sorgho grains v	2003	**0,66**	0,66
Ground sorghum P	2003	*4,1*	4,1
Ground sorghum P	2003	*5,2*	5,2
Ground sorghum P	2003	*0,89*	0,89
Ground sorghum P	2003	*4,5*	4,5
Ground sorghum P	2003	*99,2*	91,5
Ground sorghum P	2003	*12,7*	11,2
Ground sorghum P	2003	*11,3*	10,5
Ground sorghum P	2003	*1,2*	1,2
Ground sorghum P	2003	*6,0*	6,0
Ground sorghum P	2003	*21,13*	19,8

Sorghum grains v	2003	**1,98**	1،98
Sorghum grains v	2003	**0**	0
Sorghum grains v	2003	46,6	46،6
Sorghum grains v	2003	0	0
Ground sorghum P	2003	17,26	15,6
Ground sorghum P	2003	41,45	37,7
Ground sorghum P	2003	63,58	59,66
Ground sorghum P	2003	49,35	45,6
Ground sorghum P	2003	3,6	3,6
Ground sorghum P	2003	5,35	5,1
Ground sorghum P	2003	12,86	12,2
Ground sorghum P	2003	12,14	11,5
Ground sorghum P	2003	10,1	10,1
Ground sorghum P	2003	58,92	55,5
Ground sorghum P	2003	3,2	3,2
Ground sorghum P	2003	1,72	1,72
Ground sorghum P	2003	22,89	21,81
Ground sorghum P	2003	37,83	36
Ground sorghum P	2003	2,57	2,57
Ground sorghum P	2003	5,68	5,42
Ground sorghum P	2003	12,16	9,8
Ground sorghum P	2003	40,42	35,8
Ground sorghum P	2003	70,3	64
Ground sorghum P	2003	6,57	6,1
Ground sorghum P	2003	13,52	10,8
Ground sorghum P	2003	16,05	15,2
Ground sorghum P	2003	4,08	3,82
Ground sorghum P	2003	4,5	4,14
Ground sorghum P	2003	0	0
Ground sorghum P	2003	4,92	4,6
Ground sorghum P	2003	0,93	0,93
Ground sorghum P	2003	14,64	14,3
Ground sorghum P	2003	46,8،	44,8
Ground sorghum P	2003	64,17	55,3
Ground sorghum P	2003	99,79	95,96
Ground sorghum P	2003	30,77	29,7
Ground sorghum P	2003	18,75	15,9
Ground sorghum P	2003	4,48	2,36
Ground sorghum P	2003	8,89	8,89
Ground sorghum P	2003	6,98	6,98
Ground sorghum P	2003	15,17	14,5
Ground sorghum P	2003	0	0
Ground sorghum P	2003	0	0
Ground sorghum P	2003	2،42	2,42
Ground sorghum P	2003	44،26	40,8
Ground sorghum P	2003	17,65	16,7
Ground sorghum P	2003	4,6	4,6
Ground sorghum P	2003	1,5	1,5
Ground sorghum P	2003	30,72	29,3
Ground sorghum P	2003	101,9	73,8
Ground sorghum P	2003	78,4	74,4
Ground sorghum P	2003	14,04	13,4
Ground sorghum P	2003	13,16	12,5
Ground sorghum P	2003	77,4	73,4

Ground sorghum P	2003	0	0
Ground sorghum P	2003	53,6	50,4
Ground sorghum P	2003	8,5	8,5
Ground sorghum P	2003	2,1	2,1
Ground sorghum P	2003	11,8	11,8
Ground sorghum P	2003	37,6	35,6
Ground sorghum P	2003	107,7	102,4
Ground sorghum P	2003	0	0
Ground sorghum P	2003	35,18	33,5
Ground sorghum P	2003	53,13	49,5
Ground sorghum P	2003	6,1	6,1
Ground sorghum P	2003	7,4	7,4
Ground sorghum P	2003	3,5	3,5
Ground sorghum P	2003	232,6	221,6
Ground sorghum P	2003	،15,18	14,5
Ground sorghum P	2003	76,8	72,8
Ground sorghum P	2003	63,3	59
Ground sorghum P	2003	13,2	12,5
Ground sorghum P	2003	0	0
Ground sorghum P	2003	83,4	66,7
Ground sorghum P	2003	18	17
Ground sorghum P	2003	12,2	11,7
Ground sorghum P	2003	821,43	783
Ground sorghum P	2003	444,8	408,6
Ground sorghum P	2003	9,75	9,75
Ground sorghum P	2003	3,41	3,41
Ground sorghum P	2003	5,51	5,51
Ground sorghum P	2003	6,52	6,52
Ground sorghum P	2003	3,78	2,63
Ground sorghum P	2003	16.75	16.75
Sorghum grains v	2003	24،36	22,5
Sorghum grains v	2003	54،36	49,87
Sorghum grains v	2003	9،18	8,32
Sorghum grains v	2003	10،38	9,6
Sorghum grains v	2003	3،83	3,83
Sorghum grains v	2003	5،46	5,46
Sorghum grains v	2003	11،04	10,45
Sorghum grains v	2003	6،25	5,5
Sorghum grains v	2003	291،72	273,48
Sorghum grains v	2003	0،57	0,57
Sorghum grains v	2003	7,55	6,9
Sorghum grains v	2003	17،98	17,1
Sorghum grains v	2003	0،47	0,47
Sorghum grains v	2003	0،61	0,61
Sorghum grains v	2003	20،56	16,7
Sorghum grains v	2003	14،92	12,36
Sorghum grains v	2003	0	0
Sorghum grains v	2003	0	0
Sorghum grains v	2003	0،87	0,87
Sorghum grains v	2003	0	0
Sorghum grains v	2003	0	0
Sorghum grains v	2003	1،13	1,13
Sorghum grains v	2003	32,91	32,3
Sorghum grains v	2003	23,24	22,31

Sorghum grains v	2003	0	0
Sorghum grains v	2003	4,94	4,94
Sorghum grains v	2003	8,7	8,27
Sorghum grains v	2003	1,57	1,57
Sorghum grains v	2003	35,99	34,2
Sorghum grains v	2003	10,77	10
Sorghum grains v	2003	7,09	6,7
Sorghum grains v	2003	410,2	381
Sorghum grains v	2003	3,5	3,5
Sorghum grains v	2003	8,6	8,6
Sorghum grains v	2003	45‹6	43,6
Sorghum grains v	2003	37‹92	36
Sorghum grains v	2003	0	0
Sorghum grains v	2003	58,1	54,4
Sorghum grains v	2003	10‹06	9,5
Sorghum grains v	2003	4‹6	4,6
Sorghum grains v	2003	8‹16	8,16
Sorghum grains v	2003	44‹7	42,5
Sorghum grains v	2003	1‹273	259
Sorghum grains v	2003	7‹2	7,2
Sorghum grains v	2003	2,11	2,11
Sorghum grains v	2003	12‹7	12
Sorghum grains v	2003	23‹23	21,97
Sorghum grains v	2003	21‹23	19,98
Sorghum grains v	2003	0	0
Sorghum grains v	2003	0	0
Sorghum grains v	2003	9,11	7,9
Sorghum grains v	2003	0	0
Sorghum grains v	2003	1	1
Sorghum grains v	2003	62,4	56,4
Sorghum grains v	2003	9,3	9,3
Sorghum grains v	2003	24,36	22,5
Sorghum grains v	2003	13,2	7,8
Sorghum grains v	2003	4,94	4,94
Sorghum grains v	2003	0	0