



**2nd Mediterranean Workshop on
Mycotoxins and Toxigenic Fungi**

**12-14th October 2011
Istanbul, Turkey**

**Mycotoxins in Moroccan Foods :
Current Situation and Main Perspectives**

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1: National Hygiene Institute, Rabat

2: SUP'AGRO, Casablanca



Introduction

Mycotoxins



Aspergillus



Penicillium



Fusarium

- Secondary metabolites produced by moulds in/on foods and feeds
- Heat-stable
- **300-400** mycotoxins are known and identified
- Only about **20** mycotoxins are of great interest

Morocco

- Mediterranean country with 3500 km of coasts
- Warm climate characterized by high Temperature and Humidity levels
- 70% of population lives near coasts
- Nowadays, apparition of diseases with unknown etiology (i.e. cancers ?)
- Chronical Renal Insufficiency, Liver Cancer ??
- **Mycotoxins ?**



Background

Earliest reference (1945) : Ninard & Hinterman (NHI, Rabat): acute intoxication of pigs due to mouldy feed intake

First period (1977-2000): Hassan II AVI team: investigations on moulds and mycotoxins in foods and feeds

Second period (Since 2000): NHI team under Dr Zinedine's direction; more research including emerging mycotoxins

2011: Creation of the Moroccan Society for Mycotoxicology

Publications (First period)



Olives and olive oil

- A. Tantaoui Elaraki, B. Le Tutour & A. Aboussalim. *Rev. Fr. Corps Gras*, 1983, 30, 473-6
- B. Le Tutour, A. Tantaoui Elaraki & L. Ihlal. *JAOCS*, 1983, 60, 835-7
- A. Tantaoui Elaraki, B. Le Tutour, M. Bouzid & M.J. Keddani. *Ind. Alim. Agr.*, 1983, 997-1000
- B. Le Tutour, A. Tantaoui Elaraki, A. Aboussalim. *JAOAC*, 1984, 67, 611-2
- H. Gourama, A. Tantaoui Elaraki, M. Fares. *Actes Inst. Agro. Vét.*, 1985, 5, 51-7
- A. Tantaoui Elaraki & B. Le Tutour. *Oléagineux*, 1985, 40, 451-4
- H. Gourama, B. Le Tutour, A. Tantaoui Elaraki, B. Benbya & L.B. Bullerman. *J. Food Prot.* 1989, 52, 264-6
- A. Tantaoui Elaraki, S. Samane, M-F. Roquebert. *Micobio. Alim. Nutr.*, 1990, 8, 257-64
- S. Samane, A. Tantaoui Elaraki, M. Essadaoui. *Micobio. Alim. Nutr.*, 1991, 9, 335-52
- A. Tantaoui Elaraki. *Proceedings first CNCPRST-CSIC Seminar on Oleaginous Plants*, Rabat, October 19-21, 1993; pp 129-33
- A. Tantaoui Elaraki & A. Mennioui. *Micobio. Alim. Nutr.*, 1996, 14, 5-14

Publications (First period)



Citrus fruits

- M. Faid & A. Tantaoui Elaraki. *J. Food Prot.*, 1989, 52, 194-7
- M. Lemrani, A. Tantaoui Elaraki & M. Samih. *Micobio. Alim. Nutr.*, 1992, 10, 407-14
- A. Tantaoui Elaraki, M. Lemrani & R. Taousse. *Lebensm.-Wiss.u.-Technol.*, 1992, 353-6
- A. Tantaoui Elaraki, M. Lemrani & F.J. Gonzalez-Vila. *Micobio. Alim. Nutr.*, 1994, 12, 225-30

Wheat flour and derivatives

- B. Le Tutour, A. Tantaoui Elaraki & A. Belhelba. *Actes Inst. Agro. Vét.*, 1983, 3, 65-9
- A. Tantaoui Elaraki, F. Sebti, A. Breton, A. Kaanane & M. Faid. *Micobio. Alim. Nutr.*, 1993, 11, 195-202

Spices

- A. Tantaoui Elaraki & H. Bartine. *Micobio. Alim. Nutr.*, 1994, 12, 231-6
- H. Bartine & A. Tantaoui Elaraki. *J. Environm. Pathol. Toxicol. Oncol.*, 1997, 16, 61-5

Publications (First period)



General surveys on foods and feeds

- A. Tantaoui Elaraki, L. Benabdellah, M. Majdi, M.R. Elalaoui & A. Dahmani. *Actes Inst. Agro. Vét.*, 1994, 14, 11-6
- A. Tantaoui Elaraki, M. Majdi, A. Dahmani, & M.R. Elalaoui. *Actes Inst. Agro. Vét.*, 2000, 20, 65-70

Animal feeds

- B. Le Tutour, R. El Yazghi & A. Tantaoui Elaraki. *Actes Inst. Agro. Vét.*, 1987, 7, 91-100
- A. Tantaoui Elaraki, S.L. Mekouar, M. El Hamidi & M. Senhaji. *Vet. Human Toxicol.*, 1994, 36, 93-6
- S. Benkerroum & A. Tantaoui Elaraki. *Revue Méd. Vét.*, 2001, 152, 335-42

Publications (Second period)



Review papers

- A. Zinedine (2010). *Toxins*, 2010, 2, 1121-1133
- A. Zinedine, J. Mañes, *Food Control* 20 (2009), 334-344
- A. Zinedine, L. Idrissi.. *Les Technologies de laboratoire*, 2007, N° 7: 10-18.

Emerging *Fusarium* mycotoxins

- A. Sifou, *Food Control*. 22 (2011) 1826-1830.
- A. Zinedine , *Food control* , 22 (2011), 1-5.
- N. Mahnine, *Food Chemistry*, 124 (2011) 481–485.

Publications (Second period)

Traditional mycotoxins (AFs, OTA, ZEA, Fumonisins)

(Cereals, Rice, Bread, Milk, Spices, Poultry feeds, Dried fruits, etc.)



- A. Zinedine, et al., *Food Control* 21 (2010) 132–135
- C. Juan, et al., *International Journal of Food Microbiology* 126 (2008), 83-85
- C. Juan et al., *Food Control* 19 (2008), 849–853
- A. Zinedine et al. *Microchemical Journal* 87 (2007), 154-158
- A. Zinedine et al. *International Journal of Food Microbiology* 115 (2007) : 124-127
- A. Zinedine et al. *International Journal of Food Microbiology* 114 (2007): 25-29
- A. Zinedine et al. *Food Additives and Contaminants* 24 (2007), 285-291
- A. Zinedine et al. *Food and Chemical Toxicology* 45 (2007), 1-18
- A. Zinedine et al. *Cahiers Agricultures*, 16 (2007), 11-15
- A. Zinedine et al. *Food Control* 17 (2006), 868-874
- A. Zinedine et al. *Alimentaria* 359 (2004), 73-76

1- General Survey I

Aflatoxins

Products	analysed	Contaminated	Amount
Wheat	107	0	-
Barley	75	0	-
Corn	50	1	18*
Wheat bran	54	0	-
Raisins	44	0	-
Peanuts	6	1	820**
Total	286	2	2

* µg/kg Aflatoxin B1

** µg/kg Total aflatoxins B1+B2+G1+G2

Ochratoxin A & Zearalenone

Products	analysed	Contaminated	
		OTA	ZEA
Wheat	87	0	0
Barley	75	3*	0
Corn	43	0	0
Wheat bran	14	0	0
Total	219	3	0

* 1,13 – 2,83 µg/kg

Tantaoui Elaraki *et al.*, *Actes Inst. Agron. Vét.*, 1994, **14**, 11-6

1- General Survey II

Aflatoxin B1

Products	Anlysed	Contaminated	Limit amounts (ppb)
Peanuts	47	6	25-120
Almonds	27	3	18-30
Nuts	27	1	20
Pistachios	24	0	-
Raisins	42	0	-
Dryed figs	38	0	-
Prunes	38	1	20
Dates	35	0	-
Total	280	11	18-120

Tantaoui Elaraki *et al.*, *Actes Inst. Agron. Vét.*, 2000, **20**, 65-70

2- Mycotoxins in olives & olive oil

Products	Samples analysed	Aflatoxin B1		Ochratoxin A	
		Samples contaminated	Limit amounts (µg/kg)	Samples contaminated	Limit amounts (µg/kg)
Greek style black olives	103	12	5-37	5	40-80
Virgin olive oil	60	0	-	3	40

Tantaoui Elaraki & Le Tutour, *Oléagineux*, 1985, **40**, 451-4

3- Spices

Toxigenic moulds

Isolation of moulds from various spices :

- *Aspergillus flavus* (aflatoxins)

- *A. ochraceus* (ochratoxin A)

Tantaoui Elaraki & Bartine, *MAN*, 1994, **12**, 231-6)

Aflatoxin production by *A. flavus* :

- weak on cumin and hot paprika,

- very weak on curcumin, sweet paprika and ginger,

- nil on pepper (black and white) and cinnamon

Bartine & Tantaoui Elaraki, *JEPTO*, 1997, **16**, 61-5

Aflatoxins



Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco

A. Zinedine ^a, C. Brera ^{b,*}, S. Elakhdari ^d, C. Catano ^b, F. Debegnach ^b,
S. Angelini ^b, B. De Santis ^b, M. Faid ^c, M. Benlemlih ^d, V. Minardi ^b, M. Miraglia ^b

Spices

Matrix	Mycotoxin	Positive samples (%)	Average contamination ^a (µg/kg)	Maximum level (µg/kg)
Black Pepper	AFB ₁	47	0.09	0.30
	AFs	93	0.21	0.55
Ginger	AFB ₁	86	0.63	3.50
	AFs	86	1.47	9.10
Red Paprika	AFB ₁	100	2.88	5.40
	AFs	100	5.23	9.68
Cumin	AFB ₁	57	0.03	0.08
	AFs	57	0.05	0.18

A. Zinedine et al. / Food Control 17 (2006) 868–874

4- Mycotoxins in Cereals and cereal products

Wheat flour

Aflatoxin B1, Ochratoxin A & Zearalenone; 70 samples

Mycotoxin	Number of samples contaminated	Level ($\mu\text{g}/\text{kg}$)
Aflatoxin B1	0	-
Ochratoxin A	1	50
Zearalenone	0	-

Le Tutour *et al.*, *Actes Inst. Agron. Vét.*, 1983, **3**, 65-9

Cereal grains

Fumonisin B1, Ochratoxin A & Zearalenone



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Food Control 17 (2006) 868–874

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Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco

A. Zinedine ^a, C. Brera ^{b,*}, S. Elakhdari ^d, C. Catano ^b, F. Debegnach ^b,
S. Angelini ^b, B. De Santis ^b, M. Faid ^c, M. Benlemlih ^d, V. Minardi ^b, M. Miraglia ^b

Cereals

Matrix	Mycotoxin	Positive samples (%)	Average contamination ^a (µg/kg)	Maximum level (µg/kg)
Corn	OTA	40	1.08	7.22
	FB ₁	50	1930	5960
	ZEA	15	14	17
Wheat	OTA	40	0.42	1.73
Barley	OTA	55	0.17	0.80



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Microchemical Journal 87 (2007) 154–158

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Occurrence of ochratoxin A in bread consumed in Morocco

A. Zinedine ^{a,*}, C. Juan ^b, L. Idrissi ^a, J. Mañes ^b

Ochratoxin A in bread



Bread is the most consumed food in Morocco



WHO recommends 250 g of bread /day (starch, vitamins & proteins ...)

Average consumption in Morocco: 210 kg/year (570 g/day/person)

Occurrence of OTA in bread from Morocco

Bread origin	Number of samples	Positive samples ^a	Mean ^b ± RSD ^c (ng/g)	Range of OTA (ng/g)
Rabat	32	17	17.00 ± 2.35	0.53–89.50
Témara	11	6	7.10 ± 0.40	1.14–10.10
Méknes	36	12	5.40 ± 1.54	1.92–21.83
Salé	8	3	10.00 ± 1.68	0.14–136.10
Casablanca	13	10	25.50 ± 2.14	1.70–149
Total	100	48	13.00 ± 1.50	0.14–149

A. Zinedine et al. / Microchemical Journal 87 (2007) 154–158

% of contamination: 48% (0.14 - 150 ng/g)



26 % of samples > European Maximum Limit (3ng/g)

Bread from Casablanca was the most contaminated (77%)

The presence of OTA in bread is due to the contamination of cereals used for bread production

DAIly INTAKE FROM BREAD

- **Average contamination : 13 ng/g**
- **Average Daily Consumption : 577 g**
- **For 1 adult (60 kg): DI = 126 ng/kg bw**
- **7 times > TDI (17.1 ng/kg bw) : EFSA (2006)**
- **9 times > TDI (14 ng/kg bw) : FAO/WHO committee (2001)**



Moroccan population is probably exposed to OTA via bread consumption

This exposure is probably responsible for the apparition of some kidney diseases: Chronical Renal Insufficiency (4000 new cases/year)

This hypothesis needs to be checked: OTA in biological fluids (serum, breast milk, urine) (Cases-control Study)

5- Mycotoxins in animal feeds

Aflatoxin B1, Ochratoxin A & Zearalenone ?

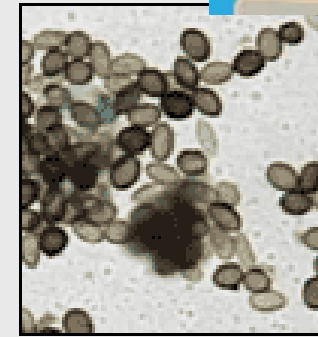
Products	Analysed	AFB1	OTA	ZEA	References
Varied poultry feed	70	1	0	0	Benkerroum & Tantaoui Elaraki, <i>Revue Méd. Vét.</i> , 2001, 152 , 335-42
Soya cake	12	1	-	-	Tantaoui Elaraki <i>et al.</i> , <i>Actes Inst. Agron. Vét.</i> , 2000, 20 , 65-70
Sunflower cake	18	4	-	-	
Processed poultry feeds	53	5	-	-	

Stachybotrys toxins ?

- 1991 (Gharb region), an apparent stachybotryotoxicosis outbreak: 113 mules, 85 donkeys and 18 horses died after being fed mouldy straw

- *Stachybotrys atra* isolated and shown to be toxigenic through biological tests:

- . mouse;
- . rat skin;
- . *Artemia salina* larvae



Stachybotrys atra





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International Journal of Food Microbiology 114 (2007) 25 – 29

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Presence of aflatoxin M1 in pasteurized milk from Morocco

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6- Aflatoxin M1 in pasteurized milk





Occurrence of AFM1 in pasteurized milk from Morocco

Dairies	No. of samples	Incidence of AFM1 (%)	Range of AFM1 ($\mu\text{g/L}$)
A	10	100	0.012–0.050
B	13	92.3	0.002–0.099
C	10	90	0.001–0.066
D	12	83.3	0.002–0.016
E	9	77.7	0.006–0.117
Total	54	88.8	0.001–0.117

A. Zinedine et al. / International Journal of Food Microbiology 114 (2007) 25–29

AFM1 in Milk

Argentina
Brazil
India
Iran
Italy
Japan
Korea
Kuwait
Libya
Portugal
Spain
Turkey

**Percentages of
contamination**

53 – 84 %

DAILY INTAKE OF AFM1

- Average contamination: **0.0186 µg/L**
- Consumption: **64 L/person/year**
- **DI = 3.2 ng AFM1/person/day**

	DI (AFM1) ng/person/day
European diet	6.8
Latin American diet	3.5
Oriental diet	12
Middle East diet	0.7
African Diet	0.1
Moroccan diet	3.2 (Zinedine et al.)

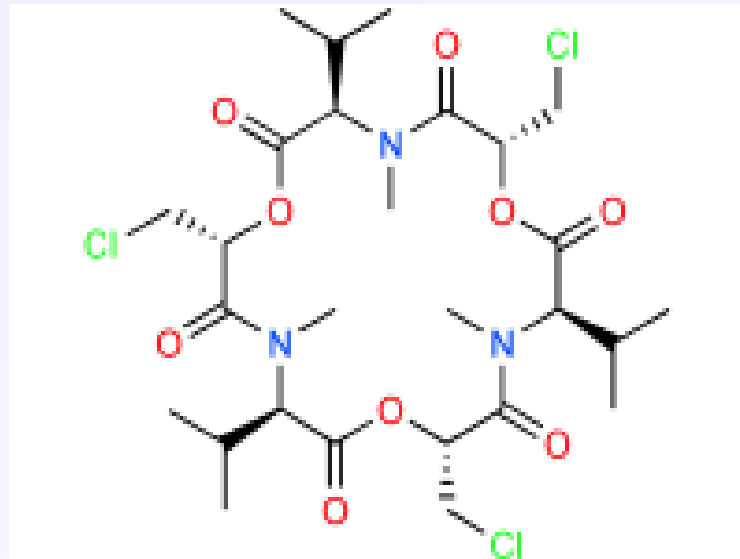


7- Emerging mycotoxins

Enniatins (ENs)

Enniatins (ENs) : *Fusarium* species

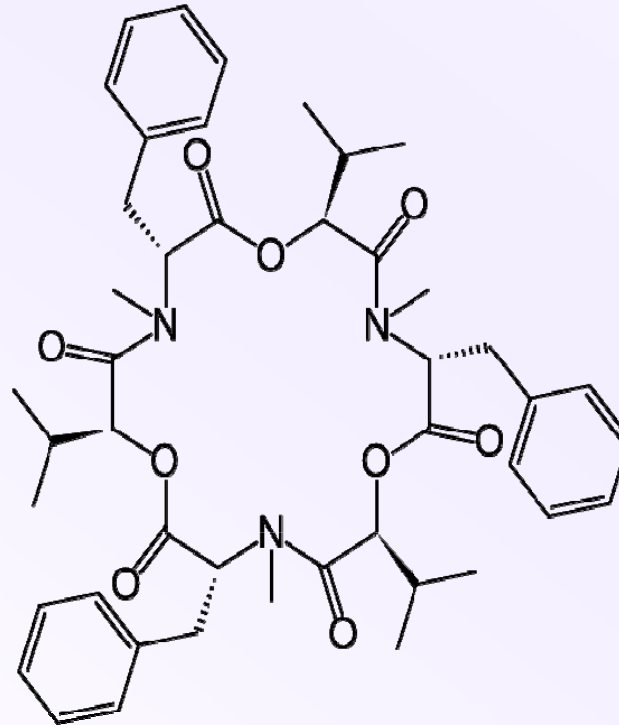
Biological activity: phytotoxic and cytotoxic.



Hexadepsipeptide

Beauvericin (BEA)

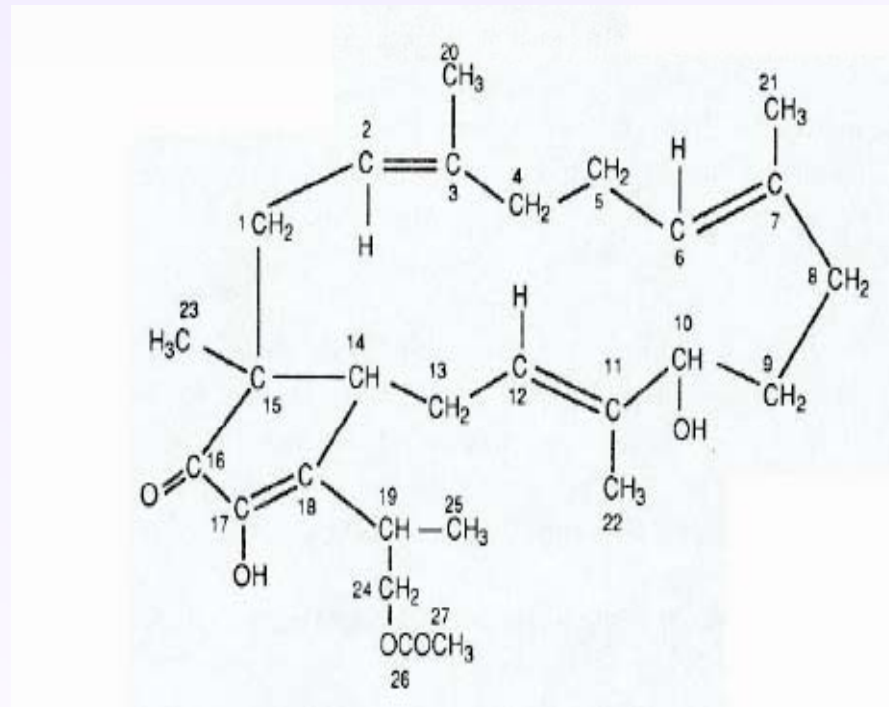
- *Fusarium* species and *Beauveria bassiana*.
- Toxic to several human cell lines and can induce DNA fragmentation.
- Antibacterial activity



Fusaproliferin (FUS)

-Biological activity:

- brine shrimp *Artemia salina*
- cytotoxic: B-lymphocyte cell line
- teratogenic: chicken embryos





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Further data on the occurrence of *Fusarium* emerging mycotoxins enniatins (A, A1, B, B1), fusaproliferin and beauvericin in raw cereals commercialized in Morocco

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ABSTRACT

In this study, 64 samples of raw cereals (wheat, maize and barley) purchased from local markets in Rabat–Salé area from Morocco were analyzed for the occurrence of six emerging mycotoxins: four

Cereals

Presence of enniatins (ENA, ENA1, ENB, ENB1) in analyzed samples.

Cereal	Number of samples	Enniatins ENs (mg/kg)								
		Positive samples for total ENs and frequency (%)	ENA		ENA1		ENB		ENB1	
			Mean ^a	Maximum level	Mean ^a	Maximum level	Mean ^a	Maximum level	Mean ^a	Maximum level
Wheat	25	12 (48)	34	34	105	209	5	11	19	19
Corn	31	13 (42)	ND	ND ^b	207	445	54	100	8	8
Barley	8	7 (87.5)	ND	ND	84	220	18	49	22	32

Cereals

Presence of beauvericin and fusaproliferin in analyzed samples.

Cereal	Number of samples	Beauvericin (mg/kg)			Fusaproliferin (mg/kg)		
		Positive samples	Mean value ^a	Maximum value	Positive samples	Mean value	Maximum value
Wheat	25	7	2	4	4	2	2
Corn	31	6	17	59	1	0.6	0.6
Barley	8	4	3	5	ND ^b	ND	ND

Cereals products

Food Chemistry 124 (2011) 481–485



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Further data on the levels of emerging *Fusarium* mycotoxins enniatins (A, A1, B, B1), beauvericin and fusaproliferin in breakfast and infant cereals from Morocco

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Cereals products

Cereal product	Major composition subsample (cereal product)	Number of samples (samples positive for total ENs)	Enniatins ENs (mg/kg)							
			ENA		ENA1		ENB		ENB1	
			Mean*	Maximum level	Mean*	Maximum level	Mean*	Maximum level	Mean*	Maximum level
Breakfast cereals (n = 48)	Maize (Cornflakes and other)	17(7)	nd	nd	113	423.6	nd	nd	20.1	20.1
	Wheat (other)	7(1)	nd	nd	469	469	nd	nd	795	795
	Rice (other)	9(2)	nd	nd	55.1	61.4	1.05	1.05	0.6	0.6
	Other (Fruits rings)	2(0)	nd	nd	nd	nd	nd	nd	nd	nd
	Other (Muesli)	10(9)	20	29.7	140.4	688	10.6	38.3	17.2	68.6
	Other (Fitness)	2(0)	nd	nd	nd	nd	nd	nd	nd	nd
	Oat	1(1)	ND	ND	99.1	99.1	81.1	81.1	21.4	21.4
Infant cereals (n = 20)	Wheat	14(0)	nd	nd	nd	nd	nd	nd	nd	nd
	Rice	6(2)	nd	nd	52	52	5.7	5.7	11.2	14.5

Cereals products

Cereal product	Major composition of subsample (cereal product)	Number of samples	Beauvericin (mg/kg)			Fusaproliferin (mg/kg)		
			Positive samples	Mean*	Maximum level	Positive samples	Mean*	Maximum level
Breakfast cereals	Maize (cornflakes and other)	17	nd	nd	nd	nd	nd	nd
	Wheat (other)	7	nd	nd	nd	nd	nd	nd
	Rice (other)	9	nd	nd	nd	1	3.9	3.9
	Other (fruit rings)	2	nd	nd	nd	nd	nd	nd
	Other (muesli)	10	2	3.7	5.3	4	1.9	5.5
	Other (fitness)	2	nd	nd	nd	nd	nd	nd
	Oat	1	nd	nd	nd	1	4.4	4.4
Infant cereals	Wheat (flour)	14	nd	nd	nd	nd	nd	nd
	Rice (flour)	6	2	5.6	10.6	1	7.4	7.4



8- Moroccan legislation

Comparison between European limits set for mycotoxins (Regulation CE No. 1881/2006 and Regulation CE No. 1126/2007) and maximum limits proposed by the Moroccan project in human foods

Mycotoxin	European limits		Moroccan limits	
	MRL ($\mu\text{g}/\text{kg}$)	Commodity	MRL ($\mu\text{g}/\text{kg}$)	Commodity
AFB1	2	Cereals and cereal products	10	All foods, wheat bran
	0.1	Baby foods including cereals	1	Peanuts, pistachios, nuts, almonds, vegetable oils in pasta, children foods
	5	Spices	3	Wheat meal
			5	Vegetable oils, cereals, wheat meal (complete)
AFs	4	Cereals and cereals products	-	-
	10	Spices		
AFM1	0.05	Liquid milk	0.05	Milk (product)
			0.03	Milk (product) for infant under 3 years
	0.025	Baby Milk	0.5	Milk powder
			0.03	Milk powder for infant under 3 years
OTA	2	Wine	30	Cereals
	5	Cereals		
	3	Cereal products		
	10	Dried raisins		
	10	Soluble coffee		
	0.5	Cereal products for babies		
ZEA	100	Cereals	200	Cereals, vegetable oils
	200	Corn		
	50	Bread		
	20	Cereal products for babies		
Fumonisin (B1 + B2)	4000	Unprocessed maize	-	-
	1000	Maize and maize-based foods intended for direct human consumption		
	800	Maize-based breakfast cereals and maize-based snacks		
	400	Processed maize-based foods and baby foods for infants and young children		
PAT	50	Fruits juices	50	Apple juices (products)
	10	Baby foods		
DON	1250	Unprocessed cereals other than durum wheat, oats and maize	-	-
	1750	Unprocessed durum wheat and oats and maize		
	750	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption		
	500	Bread, pastries, biscuits, cereal snacks and breakfast cereals		
	200	Processed cereal-based foods and baby foods for infants and young children		

AFs: total aflatoxins, MRL: maximum residues limits, DON: deoxynivalenol, PAT: patulin, OTA: ochratoxin A and ZEA: zearalenone.



Conclusion

More importance should be given to Mycotoxins: A Moroccan programme needed

Study the impact of the contamination on the population

Harmonize the Moroccan legislation project with international (European regulations)

Develop research on toxigenic Fungi

MSM (Moroccan Society of Mycotoxicology) should take part in the coordination between all activities related to Mycotoxins

MSM seeks for support and cooperation

Thank you



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