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Fumonisins in South African subsistence maize - A single kernel approach

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Introduction:

Fumonisins are a group of naturally occurring, polyketide-derived mycotoxins produced mainly by *Fusarium verticillioides* and *Fusarium proliferatum*. Recently fumonisins B_2 and B_4 have also been detected from *Aspergillus niger* and *Tolypocladium*. Fumonisins constitute an important health risk because they are carcinogenic and cause various toxicoses in humans and animals. *Fusarium* species occur world-wide in maize where they infect the cob during flowering. They can produce high amounts of fumonisins in tropical and subtropical regions.

Maize is the staple cereal food grown and consumed by the rural farming communities of Africa and especially in the Transkei region in South Africa. The Transkei region has one of the highest esophageal cancer incidence rates in the world which seems to be associated with the fumonisin intake.

In this study we survey the fumonisin content in single maize kernels to establish the effects of manual visual sorting as well as determine if these kernels actually contain fumonisins.

Infected/damaged Undamaged/uninfected

Results & Discussion:

From visibly infected or damaged kernels primarily only *F. subglutinans* and *F. verticillioides* were isolated, although *P. concavorugulosum*, *A. wentii*, *Eurotium* sp., *P. aurantiogriseum*, *P. crustosum*, *P. pittii* and *P. brevicompactum* were also present at a low number

When single kernels were analyzed, all 10 batches (5 good and 5 moldy as sorted by the farmers) contained positive kernels. Of the 400 tested kernels, 59 (15%) were positive for fumonisins (FB_1, FB_2, FB_3, and FB_4) and 15 (<4%) of these were at levels above 100 mg/kg. The total fumonisin concentration (FB_1, FB_2, FB_3 and FB_4) of single kernels in all the batches was 1.8–1428 mg/kg **(up to 1.4 ‰ !)**. A theoretical calculation of the effect of removing the highly infected kernels (4%) showed that the average fumonisin concentration decreased by 71% after a simple sorting. The strategy of sorting out visibly infected kernels has recently been successfully applied in an intervention study in the same rural Transkei area resulting in a mean fumonisin reduction of 84% by removing a mean of 3.9% by weight. A more thorough sorting of the subsistence grown maize kernels is therefore essential in order to decrease the fumonisin concentration.

Table 1: Infection rate of batches and fumonisin (FB) content in uninfected and infected maize kernels (n_{total}=400).

Batch	GM416	GM438	GM440	GM447	GM448	MM404	MM429	MM431	MM432	MM455
Quality[a]	Good	Good	Good	Good	Good	Moldy	Moldy	Moldy	Moldy	Moldy
Infection rate[b],[c]	66	66	83	66	100	100	83	66	83	83
Dominating FB-producing fungi (%) [b],[d]	*F. sub(17)*	*F. vert (17) F. sub (17)*	*F. vert (17)*	*F. vert (50)*	*F. sub (33), F. vert (17)*	*F. vert (50), F. sub (33)*	*F. vert (17).*	*F. vert (17)*	*F. sub (33)*	*F. vert (17)*
Other fungi[b,c,d]	*P. conca, A. wentii, P. auran*	*P. conca, P. auran*	*P. conca*	*A, wentii*	*F. vert*	*F. sub., Eurotium sp.*		*P. crustosum, A. wentii*	*P. pitt, F. vert, P. brevi*	*P. conca*
Visibly infected or damaged (n=100)	2%	3%	3%	3%	3%	6%	14%	7%	19%	12%
Uninfected kernels contaminated with FB (n=10)	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Visually infected kernels contaminated with FB (n=30)	20%	27%	27%	13%	10%	23%	10%	40%	23%	3%
FB contents in infected kernels (min-max, mg/kg)	34.4-1428	4.0-90	1.9-715	1.8-432	5.8-929	2.1-257	39-736	3.2-968	4.6-713	8.37
Mean FB content in infected kernels (mg/kg)[e]	330	49	114	149	328	54.8	297	166	159	8.37
Median FB in infected kernels (mg/kg)	53	42	30	82	50	4.8	117	33	41	8.4
Total FB content (mg/kg)[f]	1.1	0.28	0.79	0.52	0.85	0.67	3.7	4.1	6.2	0.03

Note: Fumonisin content is the total FB_1, FB_2, FB_3 and FB_4 content.
Abbreviations: *F. vert: F. verticillioides; F. sub: F. subglutinans; P. conca: P. concavurugulosum, P. auran.: P. aurantiogriseum; P. pitt.: P.pittii; P. brevi: P. brevicompactum.*
[a]As sorted by the five subsistence farmers.
[b] Surface sterilization in hypochlorite prior to plating for a total of 180 randomly selected kernels (18 per batch with 6 on DG18%, 6 on CZID, and 6 on PCMA).
[c] Plating of 60 visibly infected or damaged kernels on water agar per batch.
[d] Not including sterile mycelia and species found only once among the 240 kernels.
[e] LC-MS/MS on Ultima QQQ. LOD_{FB1} of 0.16 mg/kg and LOD_{FB2} of 0.057 mg/kg
[f] Total FB content were calculated from the infection rate, mean kernel weight, batch weight, and mean fumonisin concentration

Conclusion

- Fumonisin contamination is primarily caused by *F. verticillioides* and *F. subglutinans*.

-Single kernels contained up to 1.4 ‰ Fumonisin B_1, B_2, B_3 and B_4.

-The fumonisin concentration could be lowered by 71% by removal of 4% of the kernels

-A more thorough sorting of the subsistence grown maize kernels is essential in order to decrease the fumonisin concentration.

Methods:

Maize samples

Ten batches of home grown maize were obtained from subsistence farmers in Transkei, South Africa: each consisted of approximately 500 g dried maize. Five batches were classified as high quality maize (GM) to be used for human consumption, and five as low quality (moldy) maize (MM) to be used for beer brewing. The average rate of infected/damaged kernels of each of the batches was determined by random selection of 100 kernels, from which infected kernels were counted.

Microbiology

Kernels were surface sterilized in 0.4% sodium hypochlorite for 2 minutes; afterwards the kernels were washed in water. Six kernels were placed on each of the following media: Dichloran 18% Glycerol agar (Hocking & Pitt 1980), Czapek Iprodione Dichloran Agar and Potato-Carrot-Manganese. In addition, 6 visibly infected or damaged kernels from each batch were placed on 2% water agar, overall resulting in an analysis of 24 kernels per batch and a total of 240 kernels. Plates were incubated for 7 days at 25°C in darkness. The *Fusarium*, *Penicillium* and *Aspergillus* species were subcultured onto appropriate media for the specific genus and identified by morphology, secondary metabolite profiling and by comparison to ex-type cultures.

Extraction of kernels

From each maize batch, kernels were divided into a group of undamaged/uninfected kernels and a group of damaged/infected kernels. Based on a visual inspection of each batch 10 uninfected kernels and 30 infected kernels were selected from each batch for chemical analysis, thus in total 400 kernels were analyzed. A single kernel was transferred to a 5-mL cryo-tube containing 10 steel balls (D=3mm) and 1.5 mL distilled water added. The cryo-tubes were shaken for 5 minutes by a Mini Bead-beater (Biospec Products Inc, Bartlesville, OK). Afterwards 1.5 mL acetonitrile was added and the tubes were shaken on a shaking desk for 30 minutes. The mixture was centrifuged at 6000 g and 1.5 mL supernatant was filtrated through a PTFE 0.45 µm filter and used directly for LC-MS/MS analysis.

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