

## RESEARCH

### INSECT AND MYCOFLORA INTERACTIONS IN MAIZE FLOUR

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#### ABSTRACT

Maize flour treated with or without *Tribolium castaneum* was investigated for the presence of some fungi. *Fusarium moniliforme* had the highest occurrence of 36.7%, 28.1% and 33.3% while *Aspergillus flavus/parasiticus* had a frequency of 3.2%, 3.1% and 3% on primary isolation media of czapek dox agar (CDA), potato dextrose agar (PDA) and sabouraud dextrose agar (SDA) respectively, in maize flour without *T. castaneum*. The frequency of *F. moniliforme* reduced in maize flour with *T. castaneum* to 11.1%, 12.1% and 18.8% on CDA, PDA and SDA while *A. flavus/parasiticus* increased in occurrence after introducing *T. castaneum* to 22.2%, 18.2% and 12.3% on the three respective media. Fourteen and 7 fungal genera were isolated from maize flour with and without *F. castaneum* respectively. Two fungal species isolated from maize

flour without *T. castaneum* were *Cladosporium cladosporioides* and *C. lunata*. Ten species isolated from maize flour with *T. castaneum* were *A. pullulans*, *Auxarthron* spp., *C. herbarum*, *Eurotium* sp., *Phoma glomerata*, *Neosauorya* spp., *Scopulariopsis brevicaulis*, *Rhizopus oryzae*, *R. stolonifer* and *Wallemia sebi*. These results suggest an association and a synergistic interaction between important spoilage and mycotoxigenic fungi with *T. castaneum* such as *A. flavus/parasiticus* and some mildly parasitic fungal colonies but an antagonistic interaction with *F. moniliforme*.

*Tribolium castaneum*; storage fungi;  
synergistic/antagonistic interactions;  
mycotoxins.

#### INTRODUCTION

Maize (*zea mays*) and sorghum are the principal staple crops in Botswana with a total production of 1833.10 and 3597.18 metric tonnes respectively compared to millet with less than 50 metric tonnes (Botswana Ministry of Agriculture, 1995).

Insect and fungal infestation of food commodities is a common problem due to some agricultural practices that lead to fungal contamination. These spoilage agents lead to the deterioration of the food commodities manifested by loss of weight, nutritional value and toxicity by production of mycotoxins.

*Tribolium castaneum* (Herbst) is one of the most common tropical beetle pests of stored products and a major pest of cereals in particular. Under favourable conditions it has a short generation time of about 20 days and a high rate of multiplication. It disperses readily by flight, and is, therefore, not dependent on humans for its dispersal. *T. confusum* (J. du Val), the other species found in Botswana (Allotey *et al.*, 1980) also occur widely on stored products and the two species may be found together. Allotey and Mokhuni (unpublished results) found that under ambient laboratory conditions (24-28°C, 61-77% RH), *T. castaneum* out competes *T. confusum* when introduced at the same time in maize meal and standard

medium (maize/sorghum/glycerol, 8:8:1, w/w). Booth *et al.* (1990) reported that even though the two species occur widely in stored products, *T. confusum* is less numerous than *T. castaneum*. Therefore, in the present study *T. castaneum* was utilised.

Normally, freshly harvested grains, even before being milled into flour, are already contaminated with a range of potentially deteriorative agents, particularly, insects and fungi from the field and storage facilities. Allotey and Odamtten (1996) reported that mycoflora such as *Aspergillus*, *Penicillium* and *Cladosporium*, rest hidden in maize grains that can serve as nutrient sources for insect development. The storage fungi normally accompany or follow insect infestation (Miller, 1995). Botswana is a sub-tropical country, with a warm climate that favors the multiplication of microorganisms and destructive pests of stored products.

Contamination of food commodities is further aggravated by poor practices of post-harvest handling (Mpuchane and Siame, 1998). The generation of metabolic heat and water by insects in stored foods also increases the water activity (aw) and temperature of the maize flour to levels suitable for fungal growth and multiplication (Mills, 1986; Milton and Pawsey, 1988; Sauer, 1988). The presence and activities of these spoilage agents lead to deterioration of the food commodity, which is manifested by off-odours, discolouration, heating, caking, rancidity and toxicity through production of mycotoxins (Sinha *et al.*, 1988).

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*A. flavus/parasiticus* are known to produce aflatoxins associated with hepatocellular carcinoma while *F. moniliforme/proliferatum* produce fumonisins linked to oesophageal cancer in humans and equine leukoencephalomalacia (ELEM) in horses. The two groups of mycotoxins are among the five most important fungal toxins (aflatoxins, deoxynivalenol (a trichothecene), fumonisins, ochratoxin A and zearalenone), in terms of economic importance, incidence and toxicity in agricultural produce. The food commodity infested with insects and fungi will lose weight, nutritional value and elements necessary for its market quality, food and feed value. Insect and fungi spoilage represents an irreversible quality loss in the food product and leads to downgrading of the food commodity.

The relationship between insect and fungal infestation of stored products is still an area of active research in attempts to elucidate possible synergistic and/or antagonistic interactions between insects and fungi in food spoilage. Allotey and Odamtten (1996) have reported that storage insects infest grains and that the increase in insect population results in dissemination of different molds including potentially mycotoxigenic fungi. Additionally, insects can act as vectors of fungi, serving as internal and external carriers of spores. This includes serving as a mobile source of fungal metabolites and mycotoxins (Barney *et al.*, 1995). Therefore the risks of mold contamination of food commodities depend on a complex interaction of factors such as moisture content, temperature, storage time and conditions, fungal species and their interactions with insects and previous storage history (Mills, 1986).

This study was conducted to:

- (1) Determine the presence of fungi associated with spoilage in a popular retail quality maize flour, and
- (2) Determine the effect and interactions of *T. castaneum* infestation of the maize flour on fungal population and species.

## MATERIALS AND METHODS

### Sampling

Forty-two maize meal packets of 1kg each of a popular brand from a large milling concern in Botswana with a high turn-over rate were purchased from different retail shops in Gaborone and surrounding areas of Gabane and Tlokweng, all located in the central district of Botswana. Some of the samples were procured from the Botswana Marketing Board (BAMB) within Gaborone.

### Isolation of fungi from maize flour

One gram from each of the 42 packets was weighed after thorough mixing of the maize flour and suspended in 99ml of diluent of Ringer's solution (Beuchat, 1992). From the suspension 100 $\mu$ l was then inoculated in duplicate by spread plating onto 3 different media, namely, czapek dox agar (CDA), potato dextrose agar (PDA) and sabouraud dextrose agar (SDA) and incubated at 250C for 5 to 7 days.

### Development of *Tribolium castaneum* in maize flour

Twenty grams of maize flour was weighed and placed into 50 Petri dishes into which 20 adults *T. castaneum* were introduced and shaken to ensure even mixing. The introduced *T. castaneum* were removed from the Petri dishes after 2 weeks by which time they had laid sufficient eggs. The offsprings from the laid eggs were monitored. The insect cultures in the Petri dishes were then left undisturbed for 3 months after which the contents were recorded. The insect populations in the cultures including the cast skins were counted at the end of the three months. The insects were maintained under ambient laboratory conditions (temperature range 24—300C, relative humidity (RH) 50-70%). The results were analysed using Student-Newman-kuels pairwise comparison method.

Ten Petri dishes with enough number of adult *T. castaneum*, larvae and cast skins were selected for mycological examination (Table 4). To isolate fungi from *T. castaneum*, live insects and larvae were first killed by placing them in jars of ethyl acetate fumes for 5 min and then plated onto CDA, PDA and SDA. Wherever the numbers of specimens were sufficient, 8 to 10 insects, larvae and cast skins were plated each separately onto the 3 media (CDA, PDA and SDA) and incubated at 250C for 5 to 7 days.

### Identification of fungi

The fungi from the primary cultures were transferred onto malt extract agar (Pitt and Hocking, 1985) and incubated at 250C for about 7 to 10 days while *Fusarium* species was subcultured on carnation leaf agar (Nelson *et al.*, 1983) and incubated at 250C in alternating 12h light and 12h darkness for 6 to 10 days before identification. Identification was achieved with the help of a number of textbooks. For *Aspergillus*

Samson and van Reenen-Hoekstra (1988) and for *Fusarium*, Nelson *et al.* (1983). For other asexual genera, the primary text was Samson and van Reenen-Hoekstra (1988). For some of the fungi, reference of additional texts was required. These included Domsch *et al.* (1993), Ellis (1971, 1976) and Samson *et al.* (1986).

## RESULTS

The isolation rate of fungi from maize flour without *T. castaneum* was 59.5% (25/42) (Table 1) while maize flour samples inoculated with *T. castaneum* were all positive for fungi (Table 2).

For maize flour without *T. castaneum*, the isolation rate ranged from 3.2% for *A. alternata*, *A. flavus/parasiticus*, *A. ochraceus*, and *Penicillium* sp. to 77.5% for *Fusarium* spp on CDA. Similarly, on PDA the rate ranged from 3.1% for *A. flavus/parasiticus*, *A. niger*, *M. racemosus* and *Mucor* spp. to 75% for *Fusarium* spp.

While SDA isolation rate was 3% for *A. alternata*, *A. flavus/parasiticus*, *A. ochraceus*, *A. niger*, *C. sphaerospermum*, *C. lunata*, *Mucor* sp. to 66.7% for *Fusarium* spp. Clearly for all the 3 media the predominant

mycoflora was *Fusarium* spp. with *F. moniliforme* as the dominant species. Statistically the combined frequency of *Fusarium* spp. was significantly higher than the combined frequency of *Aspergillus* spp. *Fusarium* spp. was 77.5% (95% CI 60.4-89.6%) on CDA, 75% (95% CI 58.0-87.7%) on PDA and 66.7% (95% CI 49.5-81.1%) on SDA compared to *Aspergillus* spp. with 9.7% (95% CI 2.5-24.1%) on CDA, 18.8% (95% CI 8.0-35.0%) on PDA and 15.2% (95% CI 5.8-30.4%) on SDA (Table 1).

In maize flour with *T. castaneum*, the isolation rate was 3.7% for species of *A. niger*, *A. pullulans*, *C. herbarum*, *Penicillium* sp., *P. glomerata*, *S. brevicaulis*, *R. oryzae*, *R. stolonifer* and Perithecial fungi to 22.2% each for *Fusarium* spp. and *A. flavus/parasiticus* on CDA. On PDA, the isolation frequency was 3% for *A. fumigatus*, *A. niger*, *C. herbarum*, *P. glomerata* and fungi sterilia to 40.4% for *Fusarium* spp. and 18.2% for *A. flavus/parasiticus*. While on SDA the lowest isolation rate was 2.0% for the species of *A. alternata*, *A. niger*, *Auxarthron* sp., *C. herbarum*, *Eurotium* sp., *F. oxysporium*, *P. glomerata*, *Mucor* sp. and *S. brevicaulis* while the highest was 40.8% for *Fusarium* spp. with *F. moniliforme* (18.4%) as the dominant species. For *Aspergillus* spp., the isolation was 30.7%, with *A. flavus/parasiticus* (12.3%) being of the highest occurrence in insect infested maize flour. Maize flour with *T. castaneum* had a higher fungal diversity than that without *T. castaneum* (Tables 1 and 2). But there was no significant difference between the combined frequency of *Fusarium* spp. with 22.2% (95% CI 9.54-0.6%) on CDA, 39.4% (95% CI 24.0-56.6%) on PDA and 40.8% (95% CI 27.8-54.9%) on SDA compared to that of *Aspergillus* spp. with 25.9% (95% CI 12.1-44.7%) on CDA, 30.3% (95% CI 16.5-47.4%) on PDA and 30.6% (95% CI 19.0-44.5%) on SDA (Table 2).

Table 1

Frequency of occurrence of mycoflora in maize flour without *Tribolium castaneum* (Herbst) infestation

Fungi record	Number of isolates (%) of mycoflora in grain and medium type		
	CDA	PDA	SDA
<i>Alternaria alternata</i>	1(3.2)	0(0)	1(3.0)
<i>Aspergillus flavus/parasiticus</i>	1(3.2)	1(3.1)	1(3.0)
<i>A. ochraceus</i>	1(3.2)	2(6.3)	1(3.0)
<i>A. fumigatus</i>	0(0)	2(6.3)	2(6.1)
<i>A. niger</i>	0(0)	1(3.1)	1(3.0)
<i>Aspergillus</i> sp.	1(3.2)	0(0)	0(0)
<i>Cladosporium cladosporioides</i>	2(6.5)	0(0)	0(0)
<i>C. sphaerospermum</i>	0(0)	0(0)	1(3.0)
<i>Curvularia lunata</i>	0(0)	0(0)	1(3.0)
<i>Fusarium</i> spp.	11(35.5)	9(28.1)	6(18.2)
<i>Fusarium moniliforme</i>	11(35.5)	9(28.1)	11(33.3)
<i>F. oxysporium</i>	2(6.5)	6(18.8)	5(15.2)
<i>Penicillium</i> sp.	1(3.2)	0(0)	0(0)
<i>Mucor</i> spp.	0(0)	1(3.1)	1(3.0)
<i>Mucor racemosus</i>	0(0)	1(3.1)	2(6.1)
Total	31	32	33

Table 2

Frequency of occurrence of mycoflora in maize flour without *Tribolium castaneum* (Herbst) infestation

Fungi record	Number of isolates (%) of mycoflora in grain and medium type		
	CDA	PDA	SDA
<i>Alternaria alternata</i>	0(0)	0(0)	1(2.0)
<i>Aspergillus flavus/parasiticus</i>	6(22.2)	6(18.2)	6(12.3)
<i>A. ochraceus</i>	0(0)	2(6.1)	4(8.2)
<i>A. fumigatus</i>	0(0)	1(3.0)	2(4.1)
<i>A. niger</i>	1(3.7)	1(3.0)	1(2.0)
<i>Aspergillus</i> spp.	0(0)	0(0)	2(4.1)
<i>Aureobasidium pullulans</i>	1(3.7)	0(0)	0(0)
<i>Auxarthron</i> sp.	0(0)	0(0)	1(2.0)
<i>Cladosporium herbarum</i>	1(3.7)	1(3.0)	1(2.0)
<i>C. sphaerospermum</i>	3(11.1)	0(0)	3(6.1)
<i>Eurotium</i> sp.	0(0)	0(0)	1(2.0)
<b>Fungi sterilia</b>	0(0)	1(3.0)	0(0)
<i>Fusarium</i> spp.	3(11.1)	9(27.3)	10(20.4)
<i>F. moniliforme</i>	3(11.1)	4(12.1)	9(18.4)
<i>F. oxysporium</i>	0(0)	0(0)	1(2.0)
<i>Penicillium</i> spp.	1(3.7)	0(0)	2(4.1)
<b>Perithecial fungi</b>	1(3.7)	0(0)	0(0)
<i>Phoma glomerata</i>	1(3.7)	1(3.0)	1(2.0)
<i>Mucor</i> spp.	0(0)	2(6.1)	1(2.0)
<i>Mucor racemosus</i>	3(11.1)	0(0)	2(4.1)
<i>Neosartorya</i> spp.	0(0)	2(6.1)	0(0)
<i>Scopulariopsis brevicaulis</i>	1(3.7)	3(9.1)	1(2.0)
<i>Rhizopus oryzae</i>	1(3.7)	0(0)	0(0)
<i>R. stolonifer</i>	1(3.7)	0(0)	0(0)
<i>Wallemia sebi</i>	0(0)	0(0)	0(0)
Total	27	33	49

Fourteen and 7 fungal genera were isolated from maize flour with and without *T. castaneum*, respectively. Two species isolated only from maize flour without *T. castaneum* were *C. cladosporioides* and *C. lunata*, while 10 species were isolated from maize with insects, these were; *A. pullulans*, *Auxarthron* spp., *C. herbarum*, *Eurotium* sp., *P. glomerata*, *Neosartorya* spp., *S. brevicaulis*, *R. oryzae*, *R. stolonifer* and *W. sebi* indicating insect contamination of maize flour.

The increase in *A. flavus/parasiticus* when *T. castaneum* was introduced in maize flour seems to negatively affect *Fusarium* spp., which decreased in frequency. *Fusarium* spp. was significantly at higher frequency on all three media in maize flour without *T. castaneum* compared to maize flour with *T. castaneum*. However, *A. flavus/parasiticus* increased in *T. castaneum* infested maize flour.

There was an increase in the number of live adults and larvae compared to the pupae and the number dying in the three developmental stages of *T. castaneum* life cycle. (Table 3)

Table 3

*Tribolium castaneum* population in maize flour under ambient conditions without mycological analysis

Species	Adult alive	Adult dead	Larvae alive	Larvae dead	Pupae alive	Pupae dead	Total population
<i>T. castaneum</i>	64.36±3.89 (15-125)	12.3±1.44 (0-48)	27.2±5.122 (0-141)	0.12±0.12 (0-6)	0.44±0.32 (0-16)	0.0 (0)	104.1±5.78 (35-230)

Numbers in parenthesis refer to range (%); Values are mean±S.E, n=50

Table 4

*Tribolium castaneum* population in maize flour used for mycological analysis

Species	Adult alive	Adult dead	Larvae alive	Cast Skins
<i>T. castaneum</i>	43.0±7.79 (15-100)	17.9±3.96 (1-45)	3.0±0.95 (0-10)	55.0±9.46 (20-100)

Numbers in parenthesis refer to range (%)  
Values are mean±S.E, n=10

In general, plates with eight or more of *T. castaneum* adults (Table 4) were selected for mycological analysis. However, only the larvae were in insufficient numbers for plating.

Table 5

Summary composition of *Tribolium castaneum* population from results in table 3 using Student-Newman-kuels method

Species	Stages compared	Comments
<i>T. castaneum</i>	adults (a) vs pupae	(d) s
	adults (a) vs larvae	(d) s
	adults (a) vs pupae	(a) s
	adults (a) vs larvae	(a) s
	adults (a) vs adults	(d) s
	adults (d) vs pupae	(d) s
	adults (d) vs larvae	(d) s
	adults (d) vs pupae	(a) s
	adults (d) vs larvae	(a) ns
	larvae (a) vs pupae	(d) s
	larvae (a) vs larvae	(d) s
	larvae (a) vs pupae	(a) s
	pupae (a) vs pupae	(d) ns
	pupae (a) vs pupae	(d) ns
	larvae (d) vs pupae	(d) ns

a= alive, d=dead, s= significant, ns=not significant

Most plates had significantly more adults than larvae and pupae while the number of larvae and pupae were mostly insignificant (Table 5).

## DISCUSSION

The risk of mold and mycotoxin contamination of a food commodity depends on a complex interaction of several factors, which include: moisture content, temperature, fungal species composition, their interactions with insects

and previous storage history (Mills, 1986; Miller, 1995). Moisture has also been found to be an important factor in attracting *T. castaneum* to wheat flour (Willis and Roth, 1950). The interaction of insects with each other and their environment under favourable temperature and grain moisture content usually maximises biological activity, leading to rapid floral and fauna succession and deterioration of stored products. From this study, prolonged activity of *T. castaneum* population and their interactions generated additional moisture in the environment, which in turn promoted greater microbial activity.

For maize flour without *T. castaneum*, *F. moniliforme* had the highest occurrence on all the three media of CDA, PDA and SDA with 36.7%, 28.1% and 33.3%, respectively. *Fusarium moniliforme* and the closely related species of *F. proliferatum* are among the most common fungi associated with maize (Wicklow, 1995). However, the frequency of *F. moniliforme* reduced in maize flour with *T. castaneum* to 11.1% each on CDA, and PDA, and 18.8% on SDA. The metabolic activities and/or metabolites produced by *T. castaneum* favours *A. flavus/parasiticus* that increased in frequency compared to *F. moniliforme* with a reduced frequency. On the other hand, *A. flavus/parasiticus* that was at very low frequency in maize flour without *T. castaneum* increased in occurrence after introducing *T. castaneum*.

In the case of *A. flavus/parasiticus*, *T. castaneum* either promotes its multiplication or is a carrier of its spores and hyphae/mycelium thus encouraging its dissemination. Clerk and Badu-Yeboah (1996) reported that *A. flavus* and *A. parasiticus* are among the *Aspergilli* found in the gut of *T. castaneum*. They also noted that the longer a species of fungi survives in the gut the greater the chance of it being carried over considerable distance before being voided in faecal pellets.

The co-occurrence of toxigenic fungi on maize, namely, aflatoxin-producing *A. flavus/parasiticus* and fumonisin-producing *F. moniliforme* and/or *F.*

*proliferatum* has been reported in many places (Miller, 1995). The genera of *Aspergillus* and *Fusarium* were the predominant species while *Penicillium* was negligible. The success of such fungi as *Aspergillus* and *Fusarium* as food colonists is attributed to their ability to produce specific toxic metabolites that make the food objectionable or unusable to other organisms. This also protects them and their colonised substrate from predation (Janzen, 1977; Wicklow, 1995). The presence of insect contamination in maize flour, which in this study favoured the proliferation and/or introduction of more *Aspergillus* species and especially *A. flavus/parasiticus* in maize flour would have implications for the co-occurrence of mycotoxins in a food commodity in light of the high frequency of *Fusarium* species in the same maize flour. Other studies have also demonstrated a high incidence of *A. flavus* and aflatoxin contamination in insect damaged kernels including edible categories of peanuts. The findings by other researchers of the association of insects with fungus sporulation leading to increased amounts of aflatoxins implicates insects as playing a major role in mold and aflatoxin contamination (Diener *et al.*, 1987).

In Botswana as well as other parts of sub-Saharan Africa, most farmers practice rain-fed farming which is subject to unreliable and short rainy seasons and drought (Oniang'o and Allotey, 1999). Crop plants exposed to drought and/or temperature stress are more susceptible to colonisation by *A. flavus/parasiticus* and *F. moniliforme* in the field that are then carried over into storage (Mpuchane *et al.*, 1997). The two genera were also dominant in maize flour with *A. flavus/parasiticus* being more frequent after introducing *T. castaneum*

Other mildly parasitic colonists isolated in both maize flour with and without *T. castaneum* at low frequency of 6.5% or less were *A. alternata*, *A. pullulans*, *Auxarthron* spp., *C. herbarum*, *C. sphaerospermum*, *C. cladosporioides*, *C. lunata*,

*Eurotium* sp., Fungi sterilia, *M. racemosus*, *Mucor* spp., *Neosartorya* spp., *Penicillium* spp., Perithecial fungi, *P. glomerata*, *R. stolonifer*, *R. oryzae*, *S. brevicaulis* and *Wallemia sebi* (Tables 1 and 2).

From the present study the role and importance of *T. castaneum* in affecting fungal population and number of species is evident. The red flour beetle, *T. castaneum*, a major insect pest of stored food was particularly associated with *A. flavus/parasiticus*. Other fungal species isolated after introducing *T. castaneum* into the maize flour were *A. pullulans*, *Auxarthron* spp., *C. herbarum*, *Eurotium* sp., *P. glomerata*, *Neosartorya* spp., *R. stolonifer*, *R. oryzae*, *S. brevicaulis* and *Wallemia sebi*. This suggests a synergistic interaction between *T. castaneum* and *A. flavus/parasiticus* but an antagonistic interaction with *F. moniliforme*.

To control *T. castaneum* population in maize flour and cereals in general, and in turn reduce the mycoflora population, *T. castaneum* can be controlled by use of low temperatures. For example, at 90C *T. castaneum* has been found to have an LT50 of 0.9 weeks for non-acclimatised

adults and an LT50 of 3.1 weeks for acclimatised adults (Evans, 1983). Exposure at 150C was found to kill all newly hatched *T. castaneum*, but they were more resistant at 2 weeks with a survival rate of 80%. At four weeks they had a survival rate of 20%. (Howe, 1962).

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