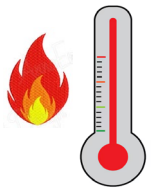
Exploitation of *Tenebrio molitor*, *Rhizopus oryzae* and *Trichoderma reesei* for the degradation of AFB1 in contaminated wheat bran

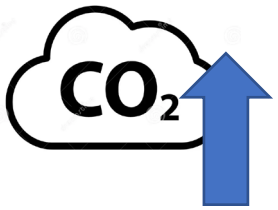
Enrico Daniso, Giulia Pascon, Costanza Uboni, Chiara Conchione, Sabrina Moret, *Francesca Tulli*

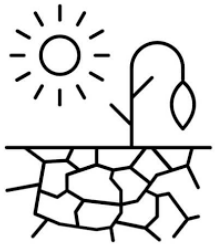
Dept. of Agriculture, Food, Environment and Animal Science, University of Udine, Italy

UNIVERSITÀ DEGLI STUDI DI UDINE

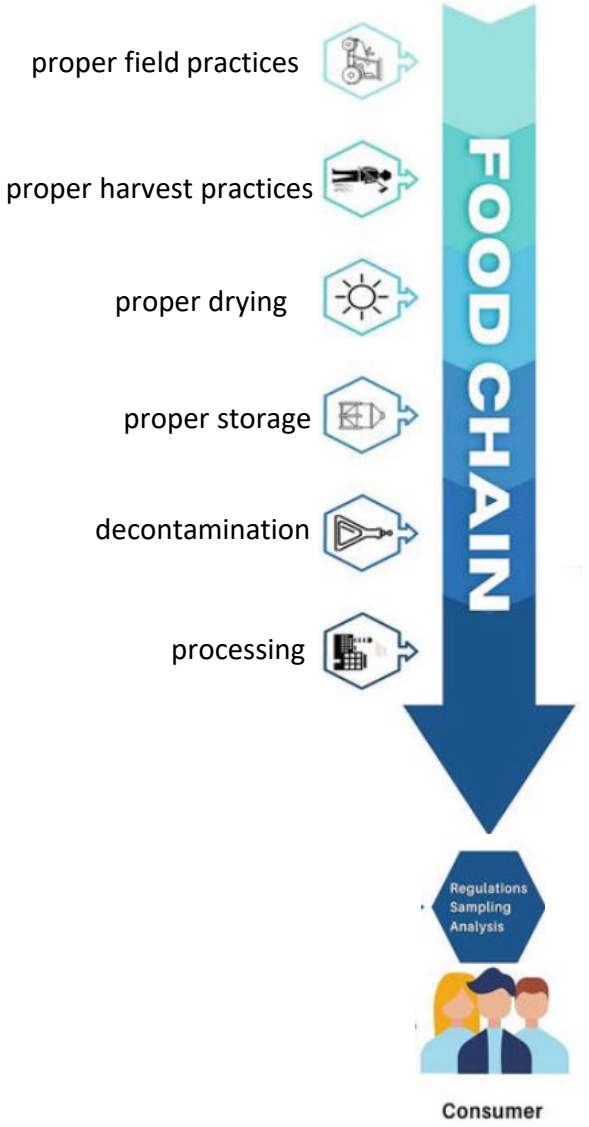
AFLATOXIN and climate change





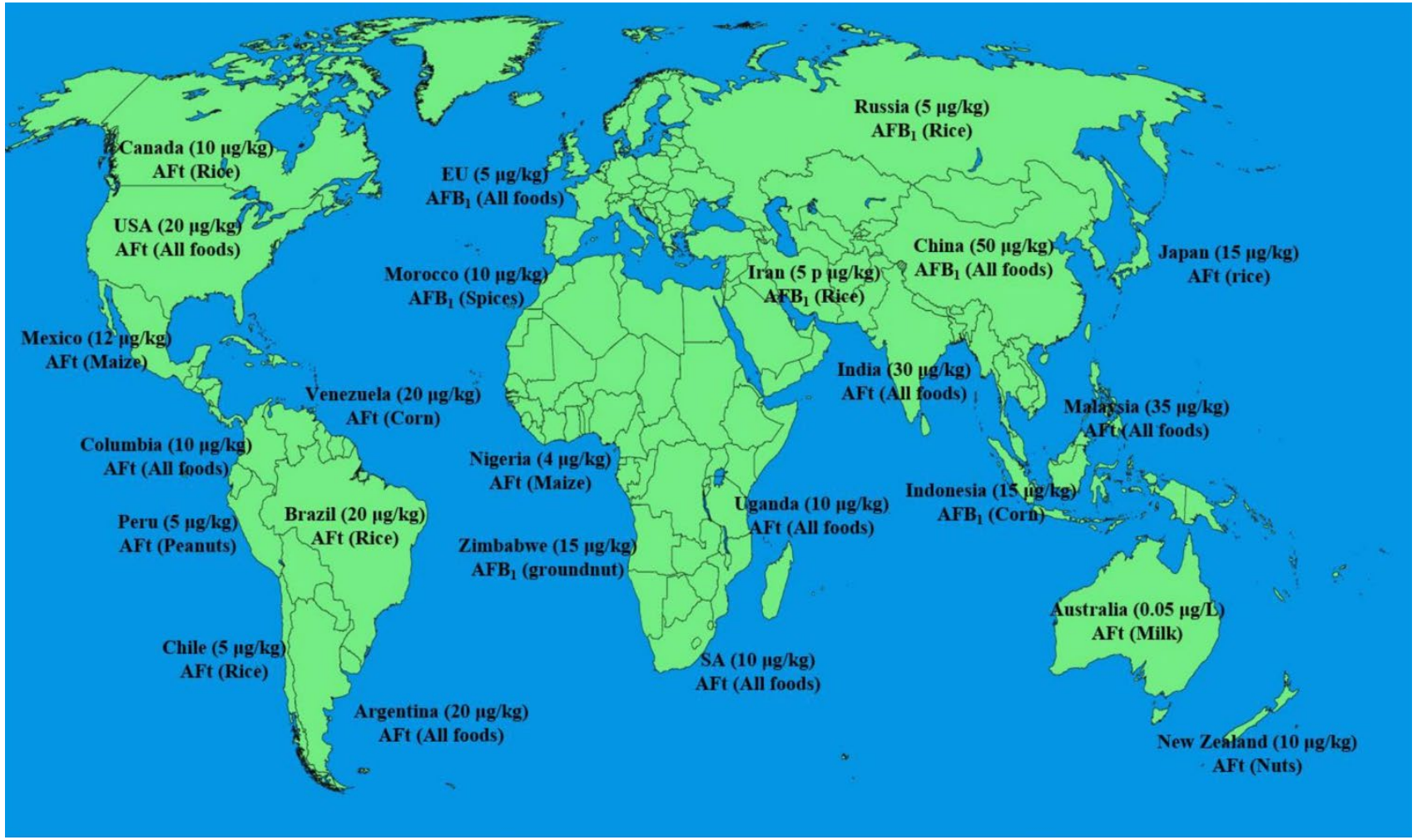


Aspergillus flavus



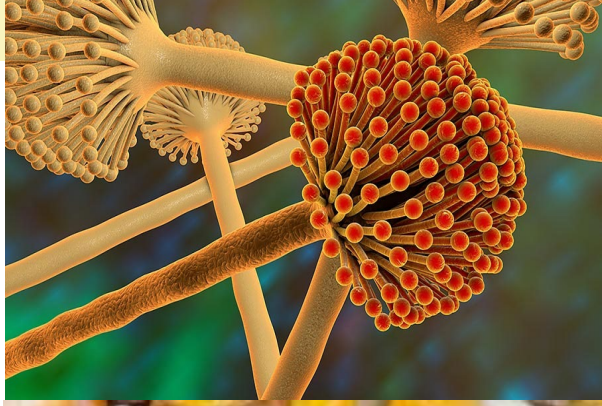
AFLATOXIN an emergency of global concern

Maximum permissible limit of aflatoxins in different countries



(from Balan et al., 2024)

Natural Mitigation of Mycotoxins:
Insects a true game-changer?



Different decontamination methods have been proposed over the years :

- Chemical
- Physical
- Biological

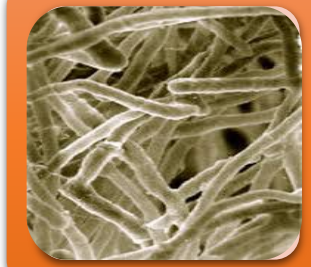
but their effectiveness or feasibility prevents their use

New detoxification techniques are required

Aim of the study

Utilization of aflatoxin-contaminated crop as rearing substrate for *Tenebrio molitor*

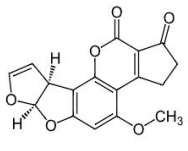
Evaluation of the degradation efficacy of AFB1 contaminated substrates from *Tenebrio molitor*



Combining the aflatoxin-degrading capacity of fungal strains of *Rhizopus oryzae* and *Trichoderma reesei* to improve food safety and bioremediation

Experimental Design

1
SUBSTRATE PREPARATION

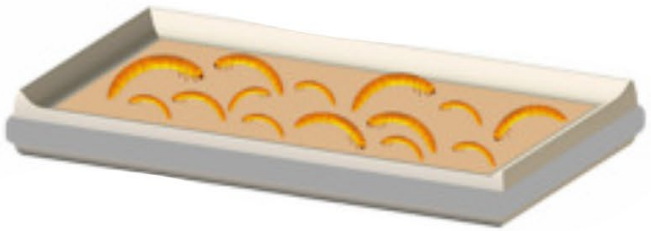


Aflatoxin

Contamination with **0, 125, 250, 500** µg/kg

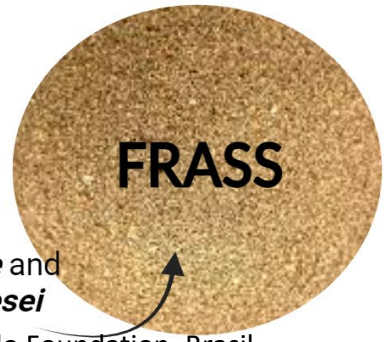
ELISA method used for AFB1 evaluation
AGRAQUANT, Romer Labs, Austria

2
TENEBRIO MOLITOR REARING



Insects reared on the contaminated substrates for **44 days** under controlled conditions (T°C: 25; RH: 50-60%)

3
TREATMENT WITH *RHIZOPUS ORYZAE* AND *TRICHODERMA REESEI*



Rhizopus oryzae and
Trichoderma reesei
from André Tosello Foundation, Brasil

4×10^6 spores g^{-1} for culture at 30 °C for 5 days

Can these fungi further degrade the AFB_1 contained in the frass?

All the experimental treatments were tested in triplicate

T. molitor growth parameters

Larval Growth

AFB1 level did not affect TM final weight. Mean weight (11mg) increased to a mean ind. weight of 117mg. The highest final weight was registered with AFB1-250

SGR

Negligible differences were observed in the specific growth rate with higher values observed in AFB1-250 group

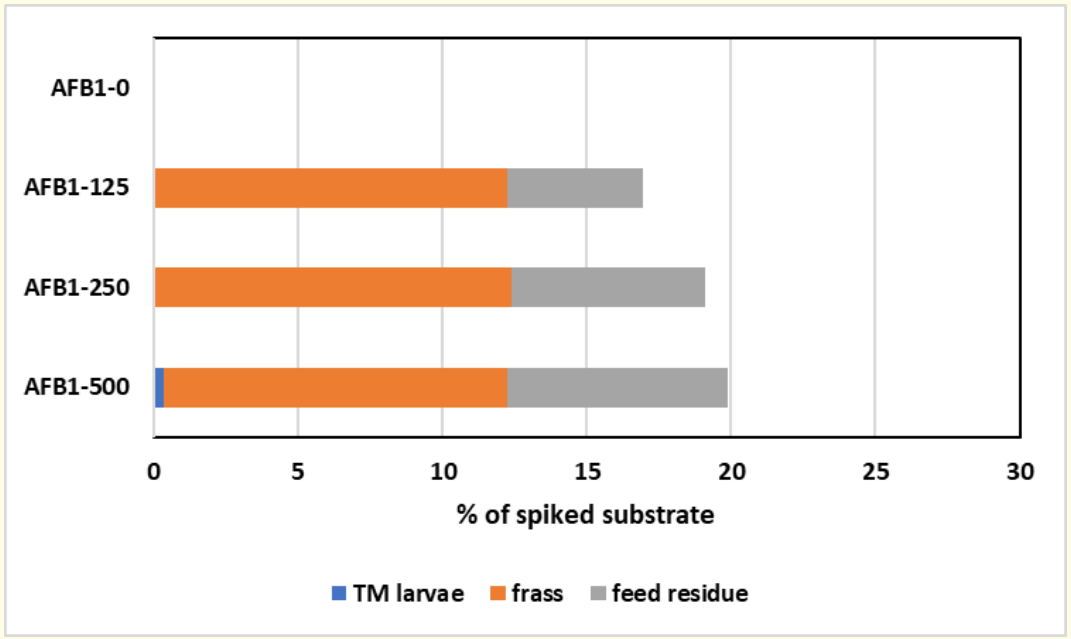
Mortality

Mortality rate was higher in the AFB1-0, considered as the control diet confirming TM larvae to be quite tolerant to AFB1 concentration

	AFB1-0	AFB1-125	AFB1-250	AFB1-500	*p value*
Final weight (g)	0.119	0.110	0.125	0.116	0.211
SGR (%)	6.20^{b}	6.19^{b}	6.47^{a}	6.35^{ab}	0.024
Mortality (%)	12.22^{a}	6.54^{b}	7.28^{ab}	7.90^{ab}	0.044

a, b $P<0.05$

AFB1 Degradation by *T. molitor molitor*



Mass balance (d.m. basis) of AFB1 in TM larvae, frass and feed residue after 44 days of larval rearing

1 **Substrate Degradation**

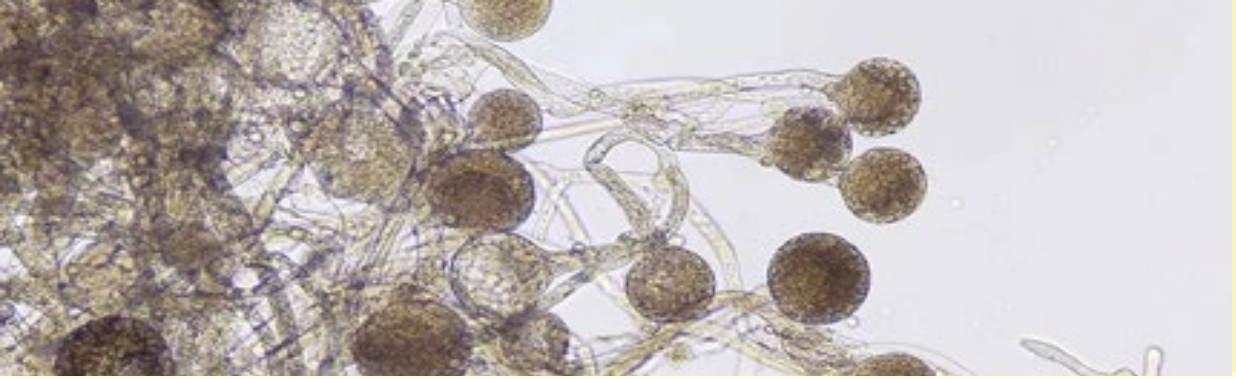
The aflatoxin B1 mass balance at the end of the breeding trial recovered between 16.9 and 19.9 % of the AFB1 initially inoculated in the different treatments. .

2 **Larval Accumulation**

AFB1 were detected ($4.10 \pm 0.91 \mu g/kg$) in TM larvae only with the highest contamination level, corresponding to 0.35% of the spiked AFB1

3 **Frass Contamination**

12.2% of the initial AFB1 content was detected in frass after larval growth independently from the contamination level.



Fungal Degradation of frass from TM rearing trial

	Initial	Final		
		RO	TR	RO-TR
AFB1-0	ND	ND	ND	ND
AFB1-125	17.0±1.68	ND	ND	ND
AFB1-250	32.99±0.49	ND	ND	ND
AFB1-500	67.4±5.19	3.1±1.79	4.7±2.35	ND

1

Initial Frass Contamination

Frass from TM rearing contained AFB1 levels up to 67.39 µg/kg.

2

Individual Fungal Treatment

R. oryzae and *T. reesei* individually reduced AFB1 level to level to 3.12 and 4.70 µg/kg in the highest contaminated contaminated frass

3

Combined Fungal Treatment

R. oryzae + T. reesei combination resulted in undetectable (< 2 ppb) AFB1 in all frass samples.

Findings: valorization of unsuitable AFB1 contaminated substrate

Insect Bioconversion

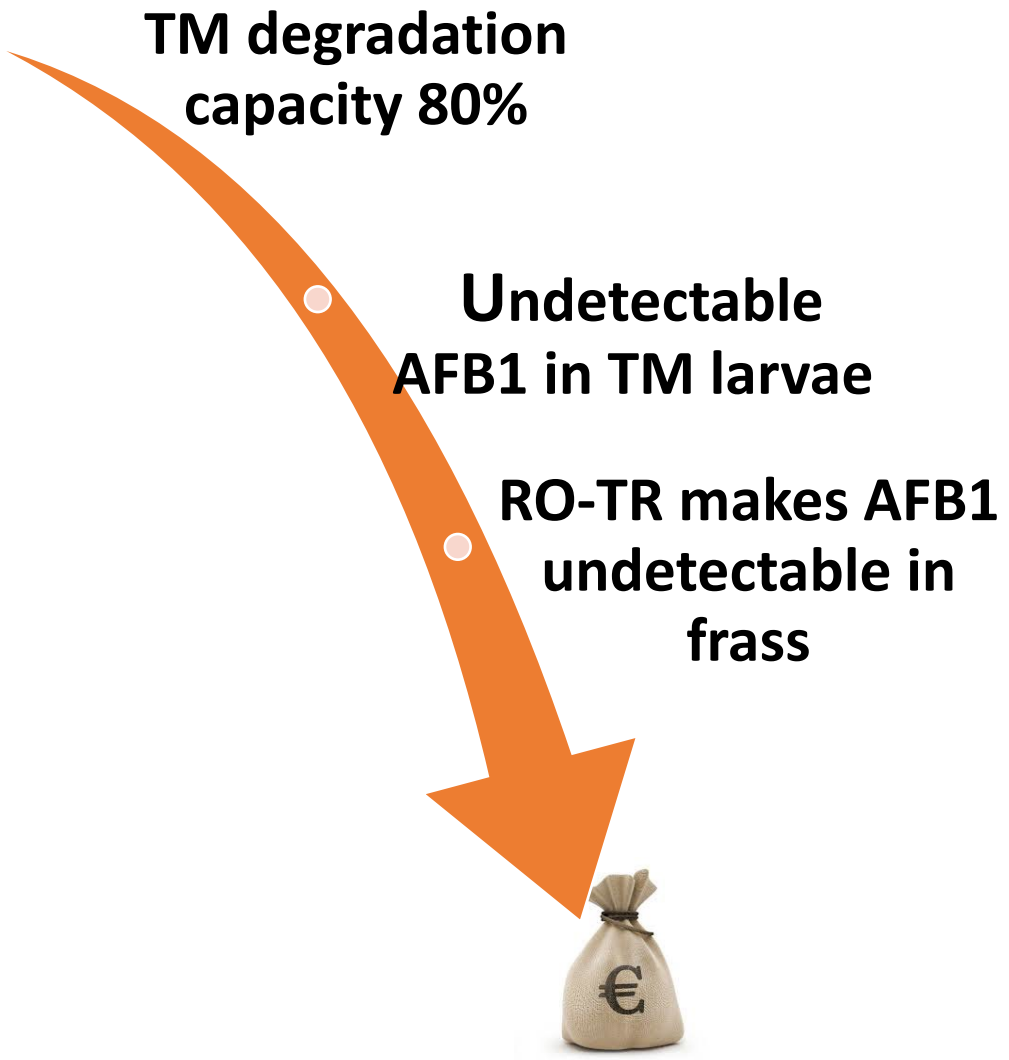
Aflatoxin B1 did not affect survival or body weight in TM, indicating a high tolerance and no accumulation of AFB1 from AFB1 contaminated substrate up to 500 ug/kg. TM larvae could convert AFB1 contaminated substrates into valuable protein while degrading ~80% of AFB1.

Fungal Degradation

The combination of *R. oryzae* and *T. reesei* further degrade remaining AFB1, reaching undetectable level of AFB1 in TM rearing frass.

Complete Decontamination

The combined approach results in AFB1-free insect protein and decontaminated for decontaminated for agricultural use.



Conclusions and Future Directions

Further Research

Expanded trials to evaluate other aflatoxins tolerance and and metabolites analyses are are needed.

Waste Valorization

The approach envisages the the reuse of contaminated crops for valuable protein production.

Sustainable Solution

Combines bioconversion and bioremediation to re-valorize unsuitable crops and to guarantee guarantee food safety

Thank you for your attention

Degradation of AFB1 by *R. oryzae and T. reesei reesei*

	Microorganism			Growth Substrate	
µg/kg of AFB1	RO	TR	RO-TR	ME Broth	Frass
0	ND	ND	ND	ND	ND
65	2.28±2.28 ^{B}	3.25±3.59 ^{C}	ND ^{A}	ND ^{A}	3.68±2.97 ^{B}
125	38.41±4.86 ^{B}	41.54±9.58 ^{B}	8.97±3.47^{A}	26.52±12.90 ^{a}	32.77±19.38 ^{b}
250	51.69±3.36 ^{B}	67.68±4.79 ^{C}	24.48±3.08^{A}	49.04±20.50	46.79±17.80
500	74.27±10.31 ^{B}	93.68±10.05 ^{C}	38.90±4.60^{A}	62.46±21.52 ^{A}	75.42±27.25 ^{B}

A, B, C $P < 0.01$; a, b $P<0.05$