

A qualitative and semi quantitative study of volatile organic compounds released by mycotoxin-producing strains of *Aspergillus flavus*.

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3rd International Webinar on Chemistry and Pharmaceutical Chemistry
October 29-30, 2021 | Webinar | Online Meeting



General information

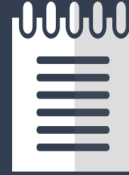
CONTEXT



25% of the world's foodstuffs
are contaminated with
mycotoxins

Sanitary control

Method of detection



New approach to detect
fungi and mycotoxin
contamination

Study of the VOCs
emitted by fungi

Study of the aflatoxin
biosynthesis



Contamination detection

Presence of fungi

Kind of genus

Kind of species

Production of mycotoxin

General information

WHAT IS A MYCOTOXIN? - WHO PRODUCE THEM?

Fungi metabolite = MYCO

Toxin for the vertebra (animals and humans) = TOXINE

400 Mycotoxins

Thermo-resistant

Non-volatil

ECONOMICAL & HEALTH ISSUES

FILAMENTOUS FUNGI



Aspergillus sp.



Fusarium sp.



Penecillium sp.

General information

ASPERGILLUS FLAVUS



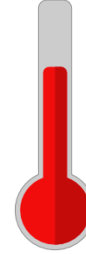
Peanuts, spices, cereals



Oral – Dermal - Respiratory



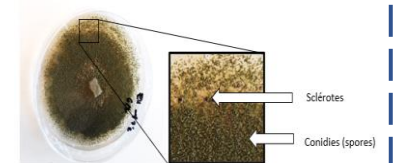
Green - Yellow



$T^{\circ}\text{C} = 25 - 42^{\circ}\text{C}$
 $\text{pH} = 7,5$
 $0.78 < a_w < 0.96$



Two categories
(type S and L)



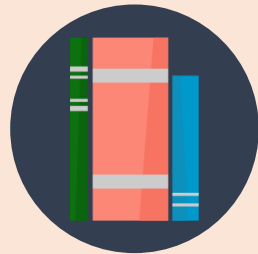
Aflatoxin B1, B2, G1, G2, M1
and M2

General information

AFLATOXIN



A. flavus
A. parasiticus



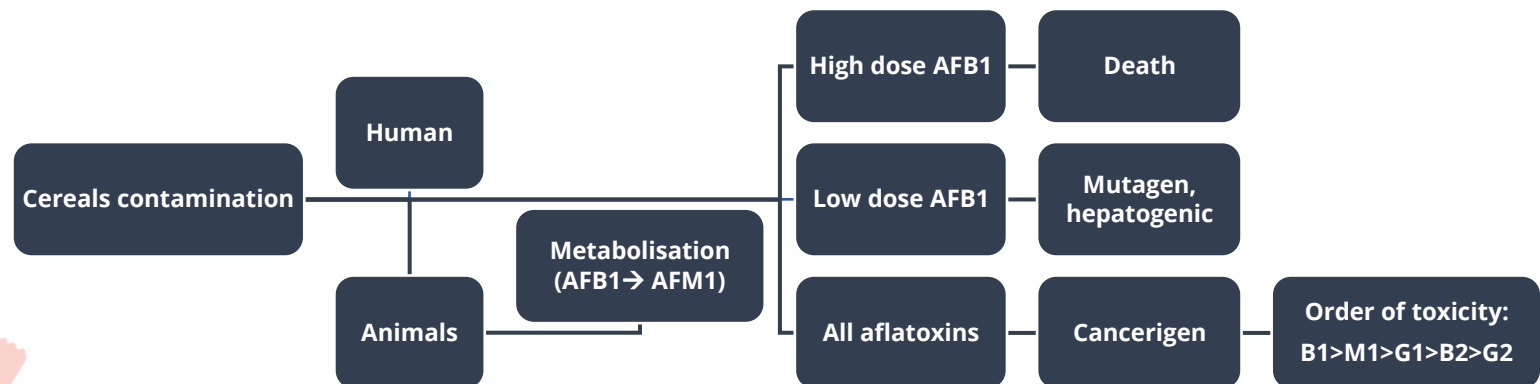
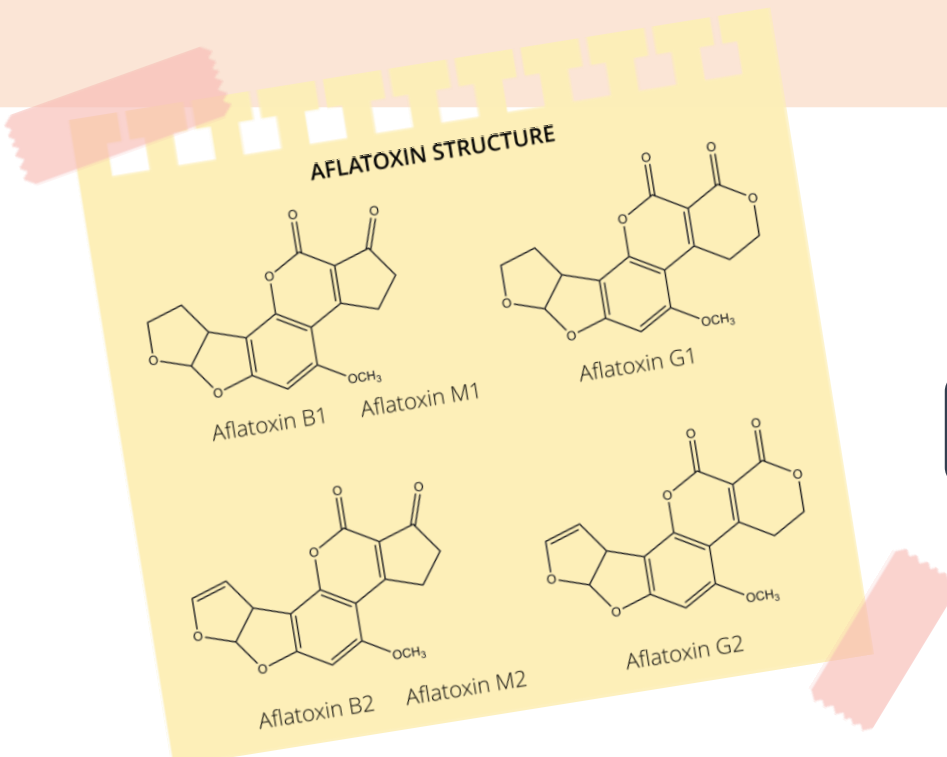
Etymology



Thermostable

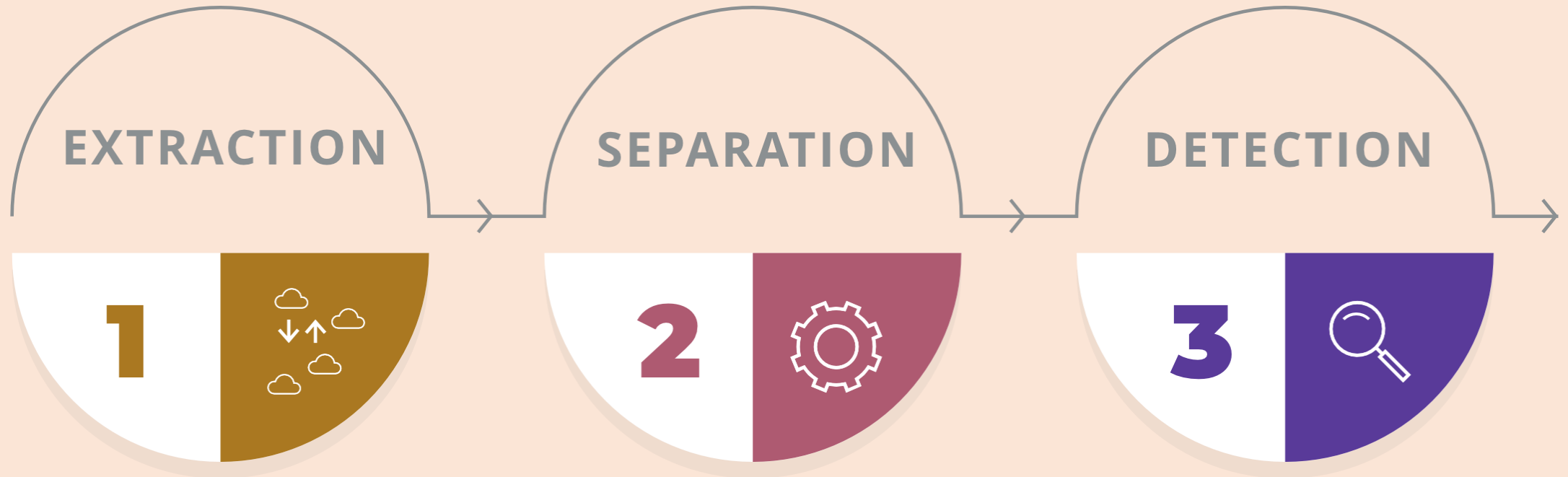


Blue or green fluorescence
at 365 nm



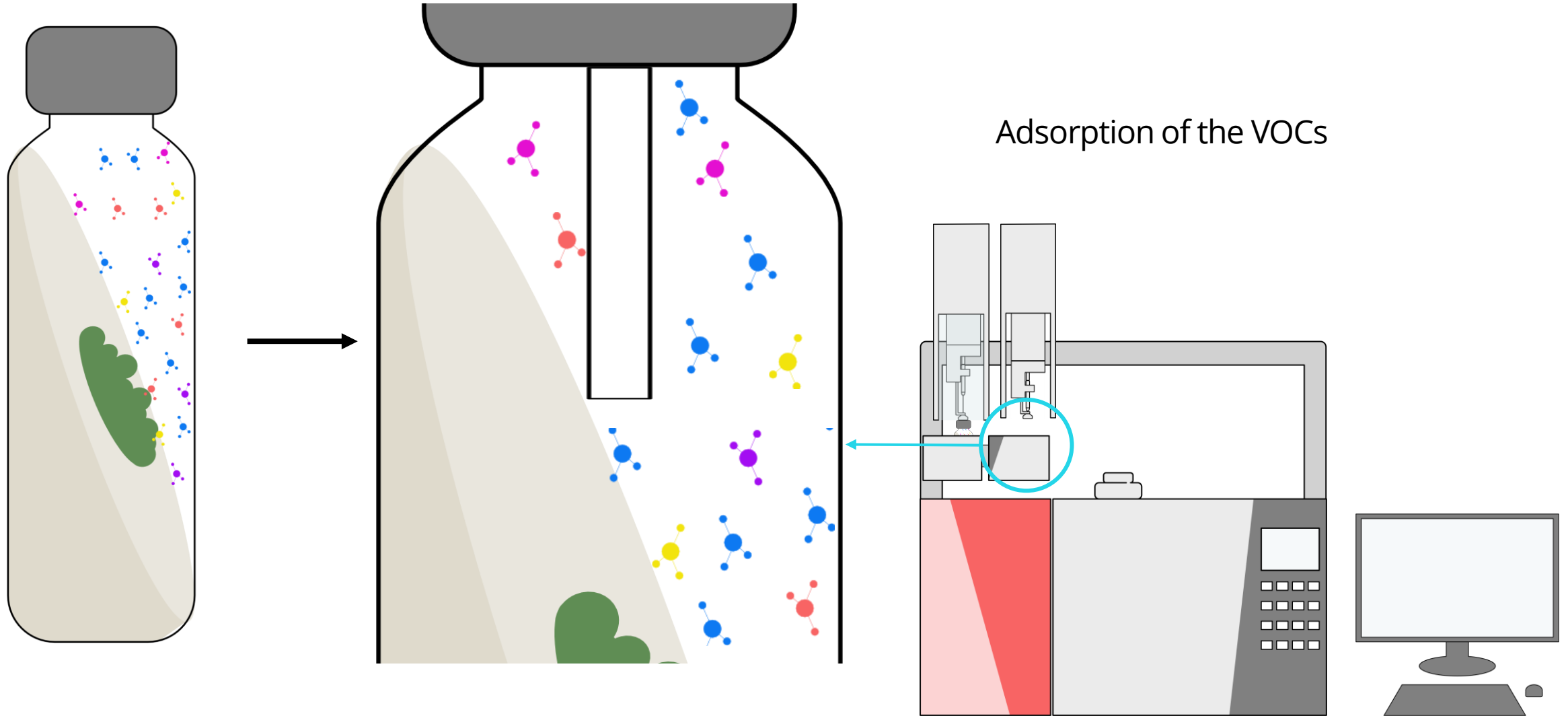
Method of analyse

GLOBAL



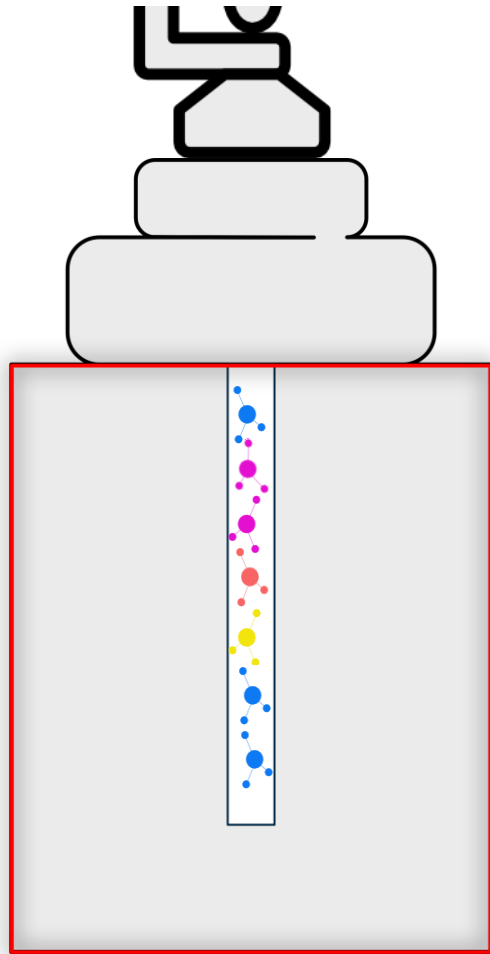
Method of analyse

EXTRACTION

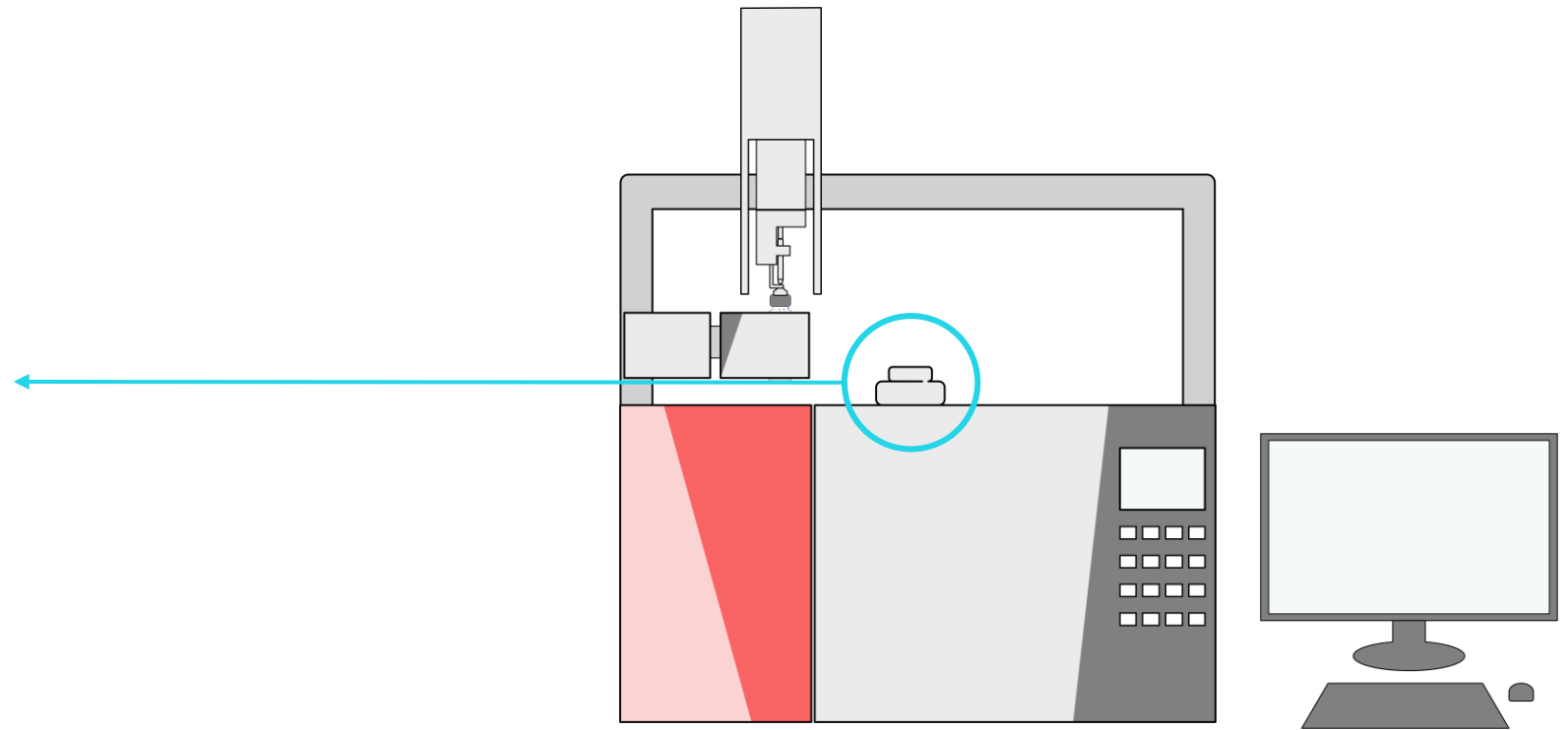


Method of analyse

SEPARATION



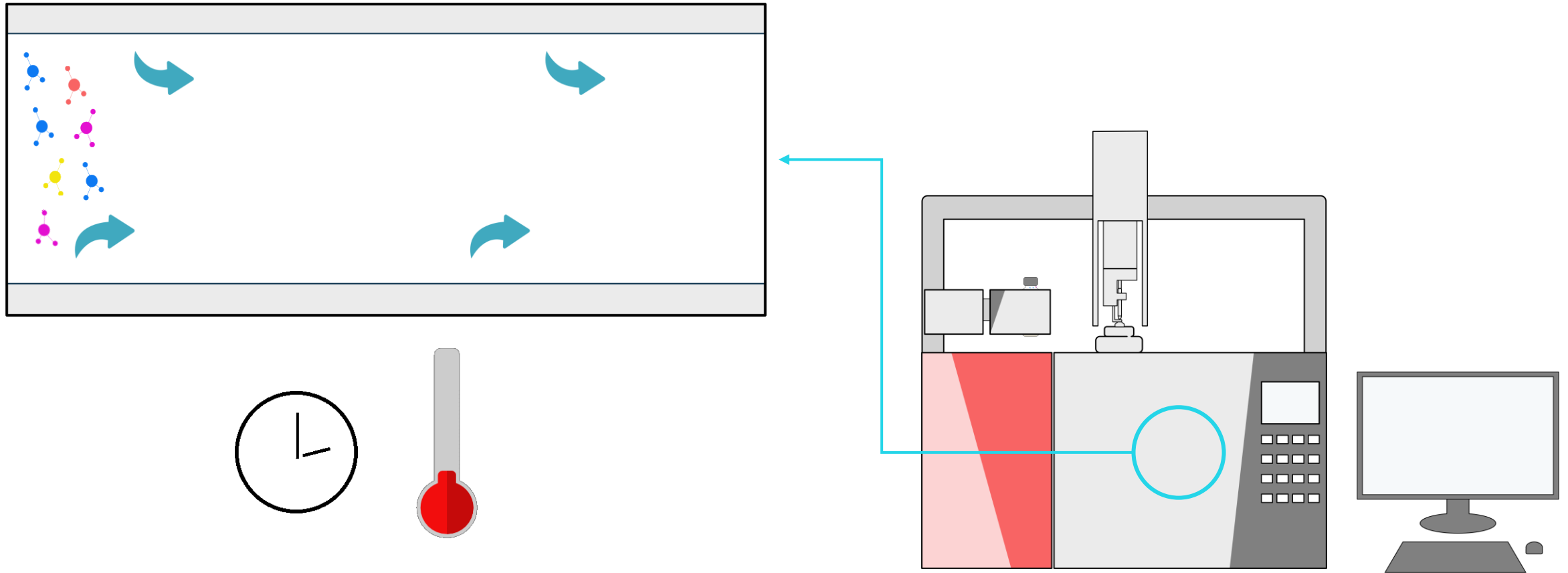
Injector
Desorption of the VOCs



Method of analyse

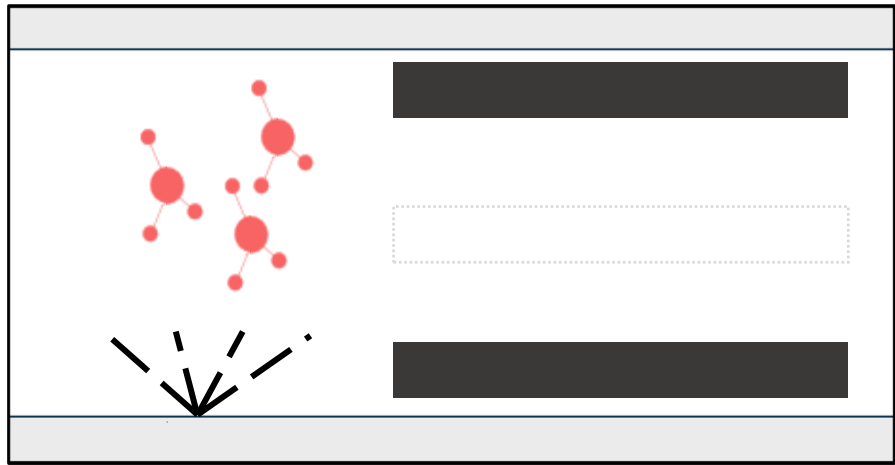
SEPARATION

Gas chromatography
Separation by polarity and volatility



Method of analyse

DETECTION

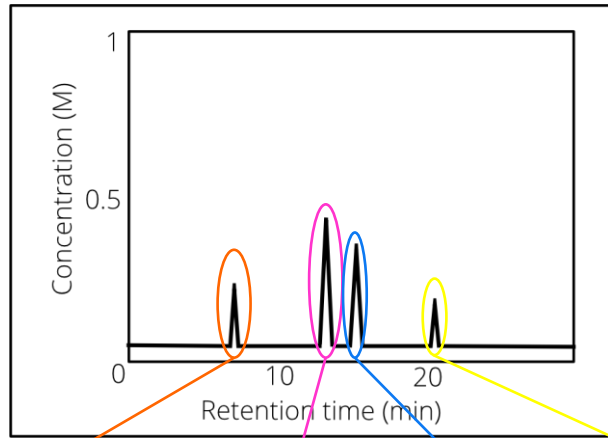


Mass spectrum (EI)



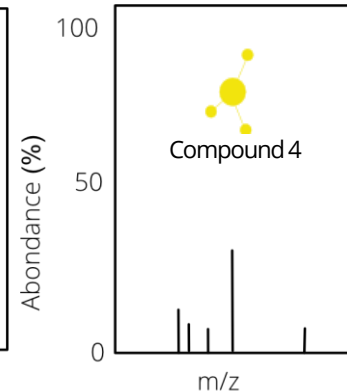
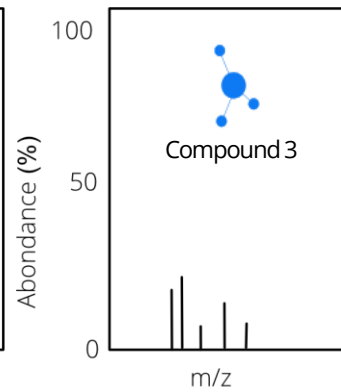
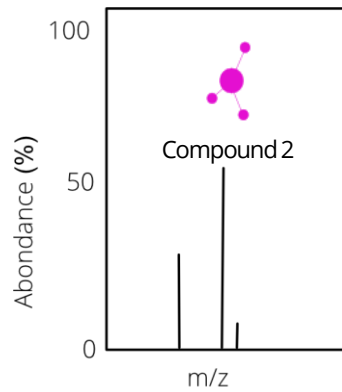
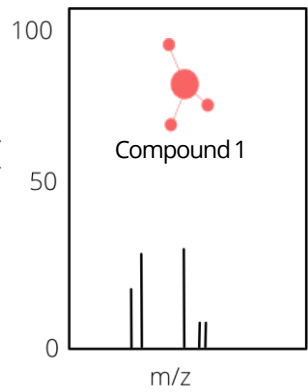
Method of analyse

IDENTIFICATION

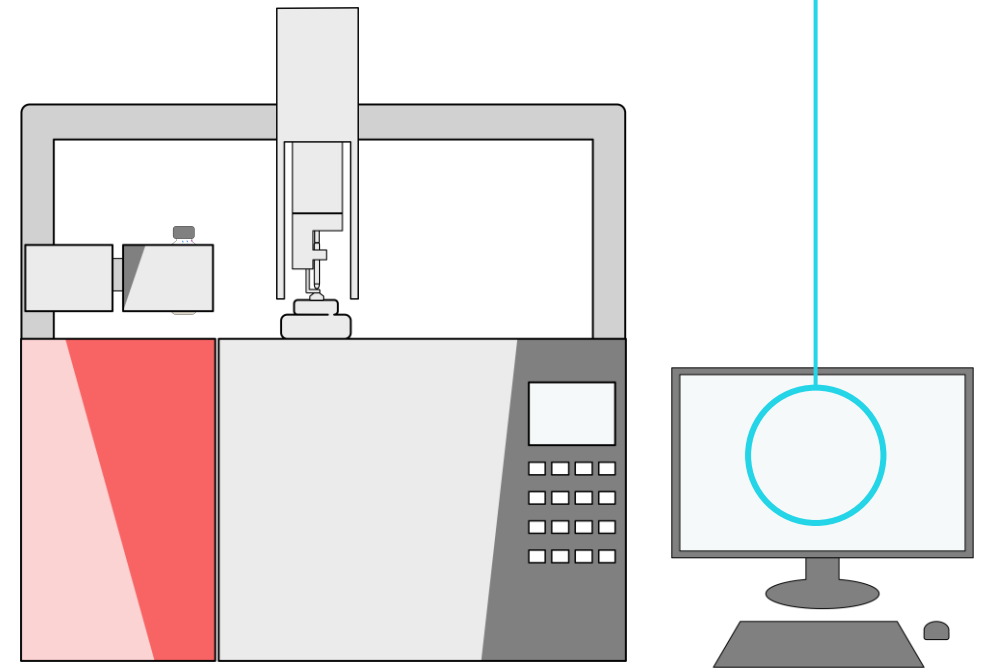


Chromatogram

Identification by comparison with mass spectra and retention index



Mass spectrum



Method of analyse

DETECTION OF AFLATOXIN

Unisensor kit and HPLC-MS/MS

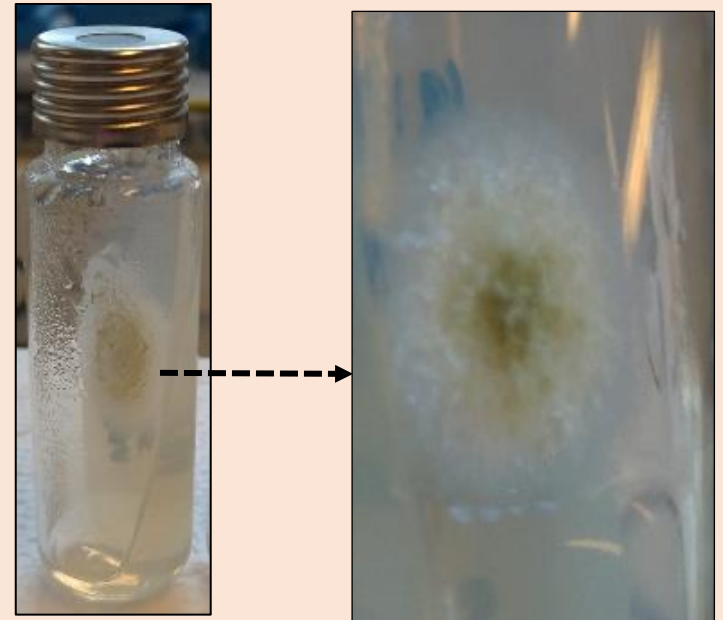
Volatile organic compounds as toxigenicity markers in *Aspergillus flavus*

Published work - Josselin et al. Toxins 2021

Material & Method

- ❑ ITEM 8111 (toxigenic strain – aflatoxin producer)
- ❑ ITEM 8111* (non-toxigenic strain – non aflatoxin producer)
- ❑ ITEM 8088: non-toxigenic strain

Study of the volatile organic compounds and the aflatoxin B1 on different days (3, 5, 7 and 9 days after inoculation)

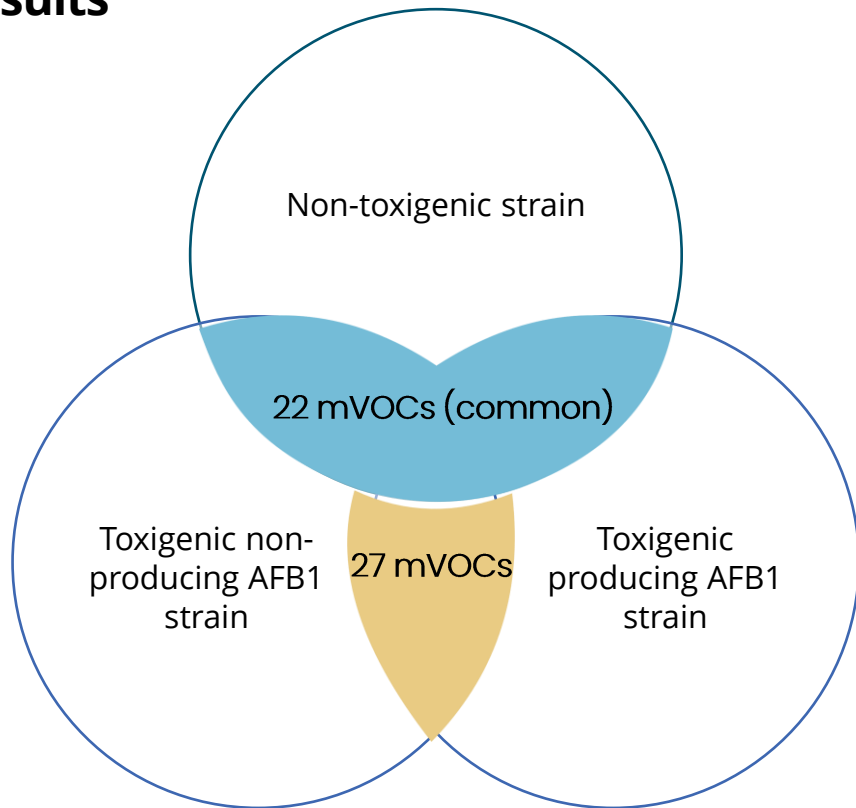


Growth of ITEM 8111 on slanted PDA medium in GCMS vials (photo)

Volatile organic compounds as toxigenicity markers in *Aspergillus flavus*

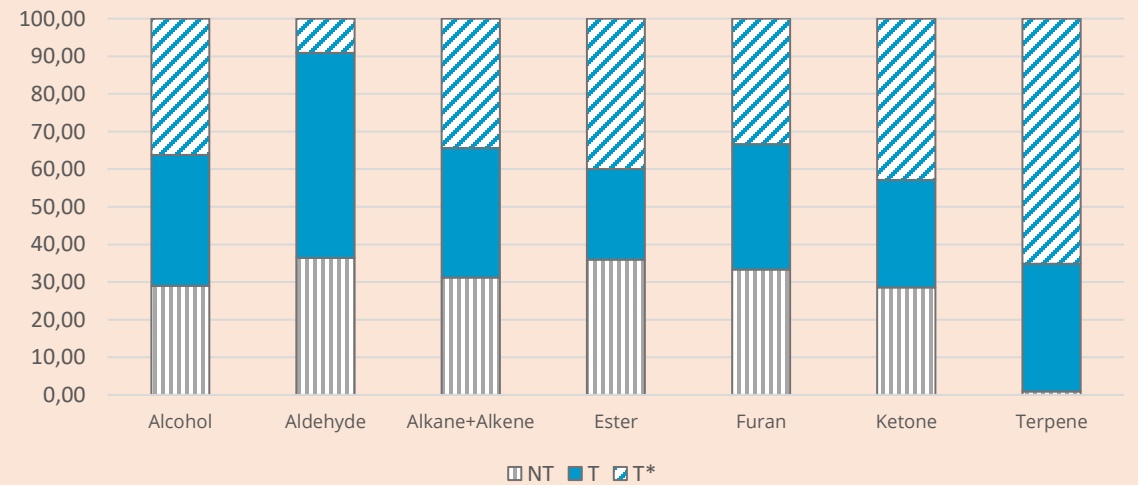
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Results



mVOCs emitted only by one of the three strains and compounds common to two or three strains - venny 2.1.0

Distribution of the relative proportions of each family of molecules



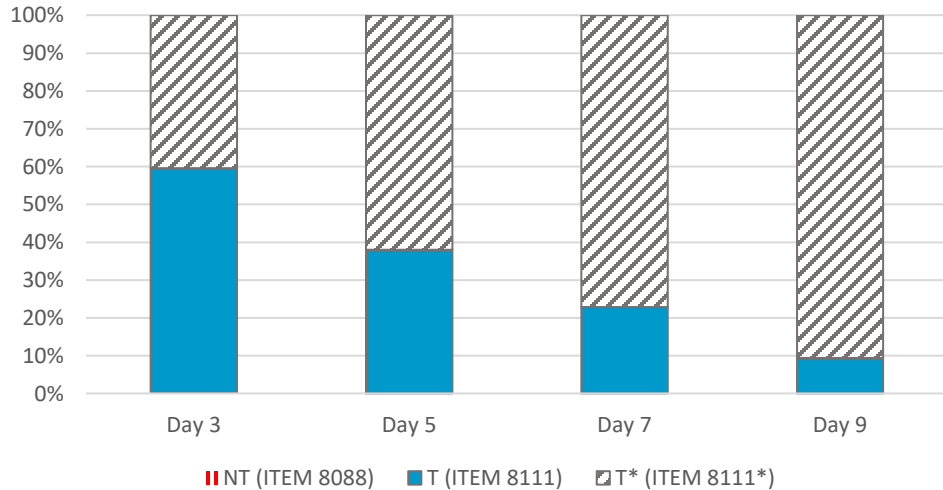
NT= ITEM 8088, T= ITEM 8111, T*=ITEM 8111* no producing

→ Difference mainly related to the terpene family

Volatile organic compounds as toxigenicity markers in *Aspergillus flavus*

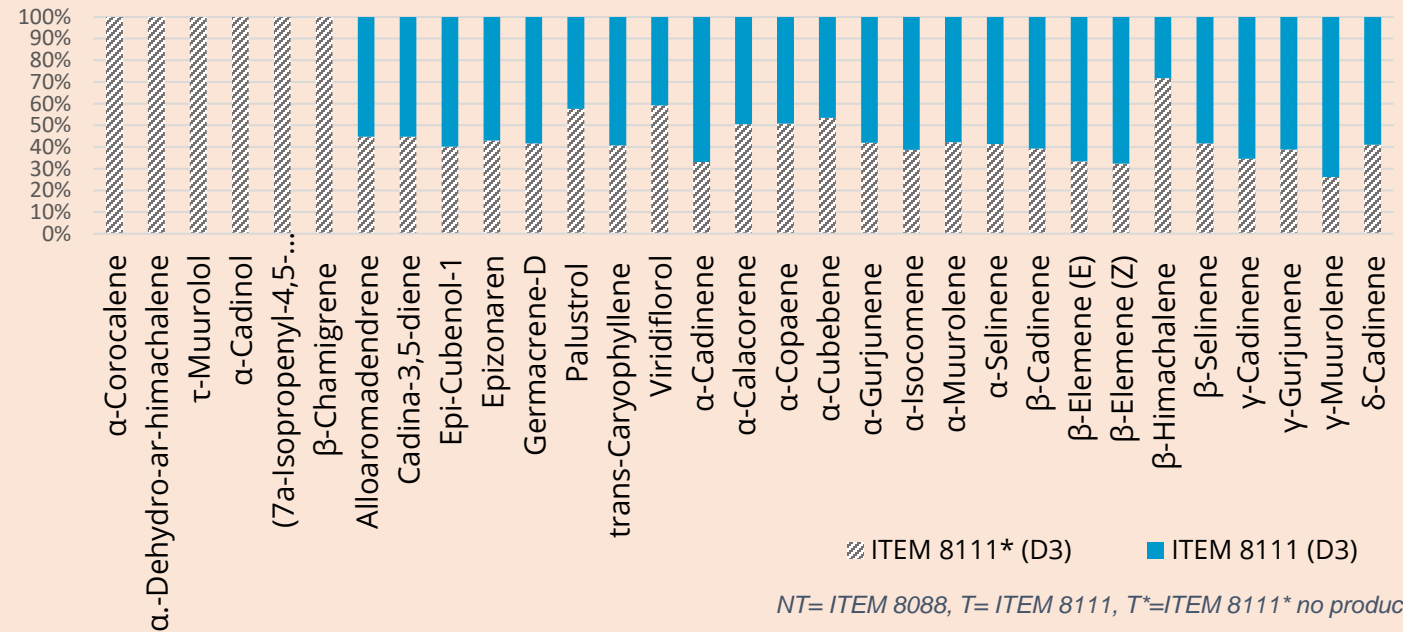
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Distribution of terpenes between strains over time



✓ Day 3 has the greatest abundance of terpenes.

Terpenes emitted during the 3rd day by the ITEM 8111 and 8111*



NT= ITEM 8088, T= ITEM 8111, T*=ITEM 8111* no producing

✓ 6 terpenes only during the absence of aflatoxin B1 production

→ Persistence of terpenes during the days when no aflatoxin B1 is produced

Volatile organic compounds as toxigenicity markers in *Aspergillus flavus*

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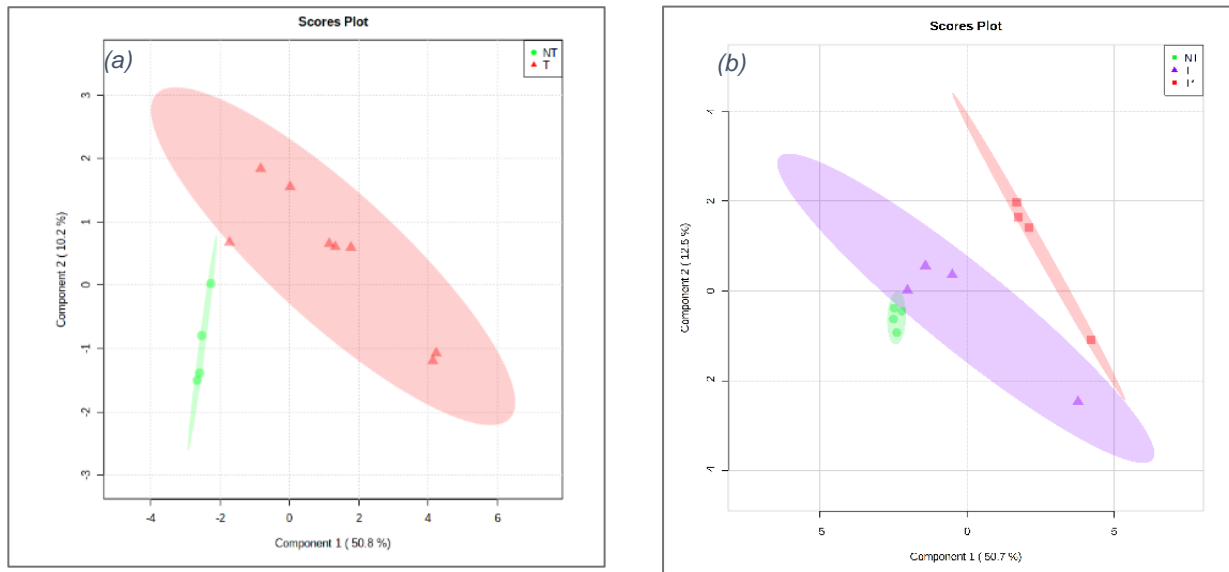


Figure 5: PLS-DA applied on the data (a) of the toxicogenic (T-Δ) and non-toxicogenic (NT-o) strains, (b) of the aflatoxin producing (T-Δ), the non-producing (T*-□) and non-toxicogenic (NT-o).

PLS-DA cross validation details:

Measure	1 comps	2 comps	3 comps	4 comps
Accuracy	0.66667	0.58333	0.66667	0.66667
R2	0.65163	0.93144	0.97249	0.99146
Q2	0.35946	0.59931	0.62235	0.61309

Toxicogenic



Non-toxicogenic

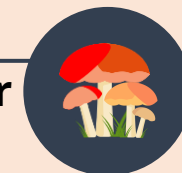
→ Epizonaren, δ -cadinene, germacrene-D, β -himachalene, γ -cadinene, β -selinene, γ -gurjunene, α -isocomene, α -cadinene

→ Ethyl 2-methylbutyrate and heptane

→ Styrene, β -selinene and γ -gurjunene

→ Terpenes only for the non-AFB1 strain
(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol, α -Dehydro-ar-himachalene, α -Corocalene, α -Cadinol, β -Chamigrene, τ -Muurolol

AFB1 producer



Non AFB1 producer

Volatile organic compounds as toxigenicity markers in *Aspergillus flavus*

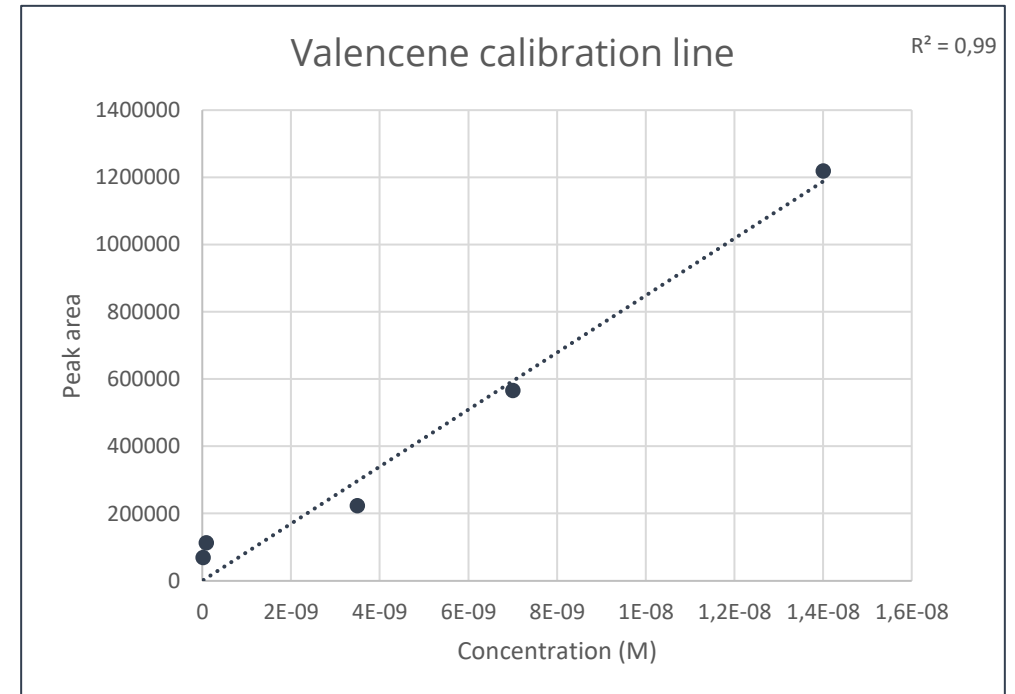
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Stock solution with the five most abundant molecules + molecules to quantify

→ to simulate the environment and the competition on the SPME fiber as samples

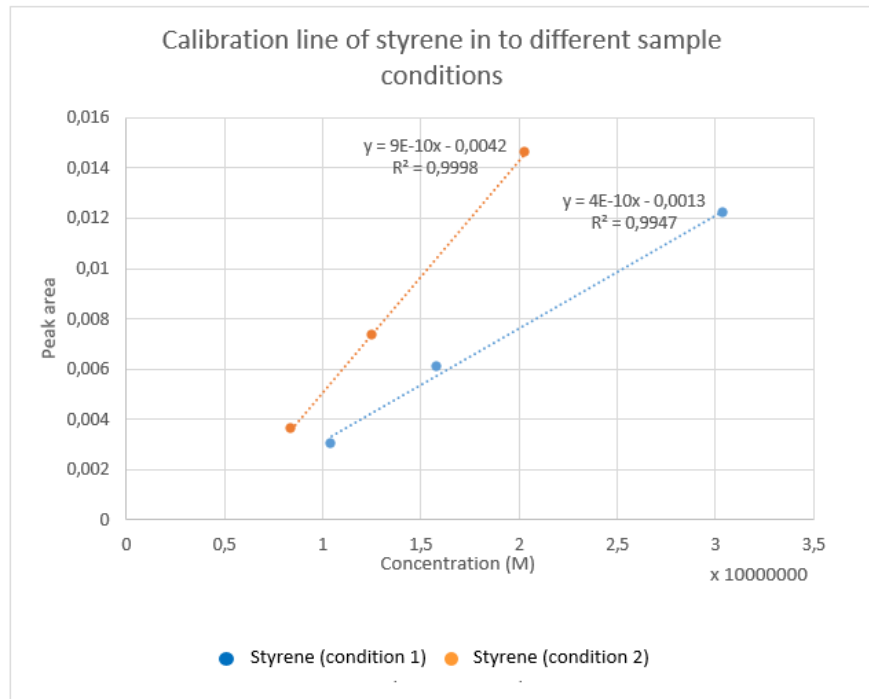
- ✓ At least 5 dilution in to the range of the sample
- ✓ 3 repetitions
- ✓ Same GCMS method as sample



Terpenes concentration was identified using the valence as standards.

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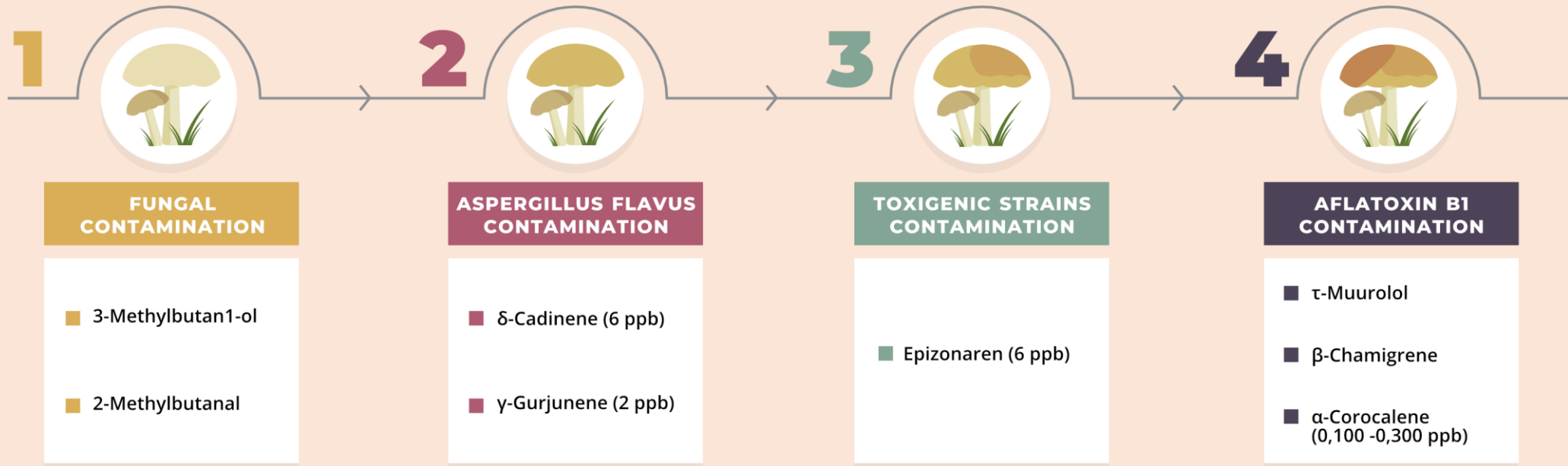


The calibration line is for one sample at one conditions.

Compound	ITEM 8111	ITEM 8111*
α -Cadinene	0.432	0.277
α -Cadinol	-	0.175
α -Isocomene	0.950	0.720
α -Muurolene	0.282	0.209
α -Selinene	1.817	1.565
β -Chamigrene	-	0.370
β -Elemene	8.897	5.181
β -Himachalene	0.737	2.590
δ -Cadinene	6.042	7.874
γ -Gurjunene	2.615	1.895
γ -Muurolene	0.769	0.381
τ -Muurolol	-	0.105
Aromadendrene	0.205	0.255
Epi-cuben-1-ol	0.311	0.360
Epizonaren	7.128	5.948
Germacrene-D	1.132	0.996
Styrene	261.75	29.8x10 ⁶
2-Methylbutan-1-ol	2.223	0.888
3-Methylbutan-1-ol	0.934	0.440

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THANKS FOR YOUR ATTENTION.

