An essential oil in mesoporous silica particles for inhibiting the production of mycotoxins by a phytopathogenous fungus, Fusarium avenaceum

Yasmine Chakroun, Youssef Snoussi, Mohamed M. Chehimi, Manef Abderrabba, Souheib Oueslati, Jean-Michel Savoie

The MICROBIOLOGY DAY - BORDEAUX

MycSA UR1264 Mycologie et Sécurité des aliments INRAE

An essential oil in mesoporous silica particles for inhibiting the production of mycotoxins by a phytopathogenous fungus, *Fusarium avenaceum*

May 2023

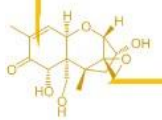
INRAE Centre Nouvelle Aquitaine

RÉPUBLIQUE FRANÇAISE
Liberté Égalité Fraternité

Context: Elaborating food and feed safety from the field to the consumer

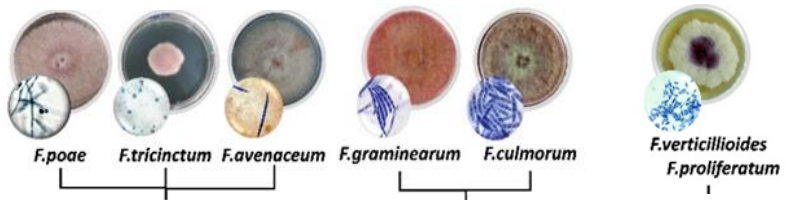
Field of research

Focus on mycotoxins in cereal crops and particularly on mycotoxins produced by *Fusarium* species



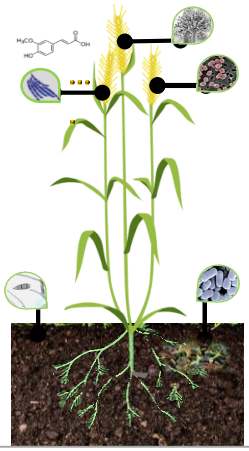
Fusarium head blight

Fusarium spp.



Fusarium ear rot

Climate factors



***Fusarium*/host/microbiota**

Production practices

Silage

Brewing

Bakery

Controlling the fungi and their production of mycotoxins

Mycotoxins = secondary metabolites

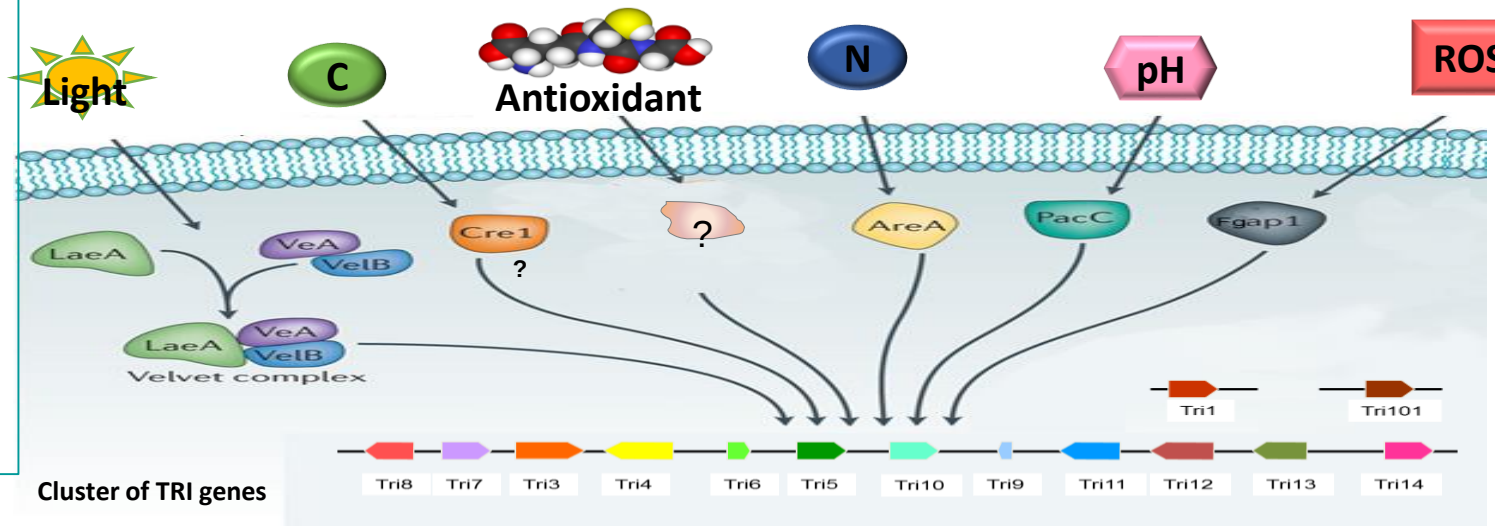
↓

Production enhanced by stress

↓

Affecting the mycelial growth may increase the synthesis of mycotoxins

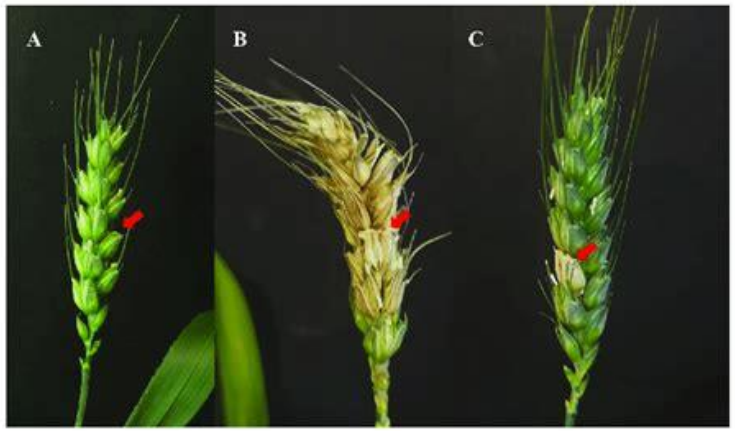
Regulation of the synthesis of mycotoxins (DON)



Infection at flowering and spreading over the spikes favoured by certain mycotoxins

↓

Spatio-temporal window for controlling



Colombo et al. 2019

Core research objective

Microbiology
(diversity – interactions)

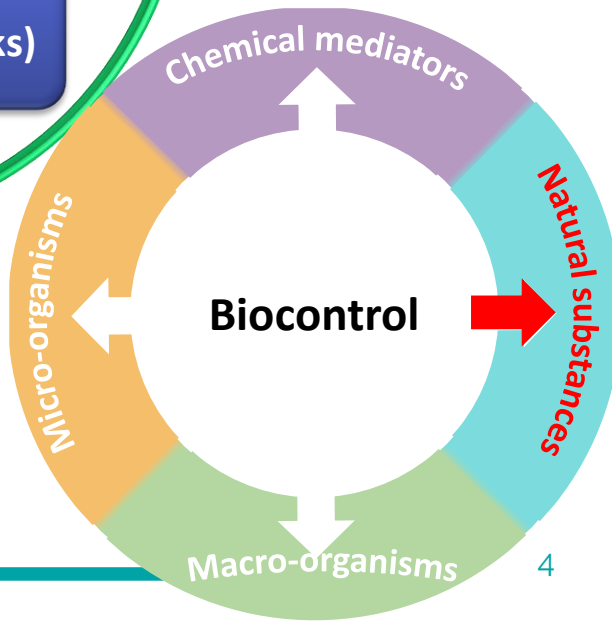
Genetics/Genomics
(regulations networks)

Chemistry/biochemistry
(metabolomics – cross talks)

Investigating the mechanisms underlying the accumulation of *Fusarium* mycotoxins in cereal grains and derived products

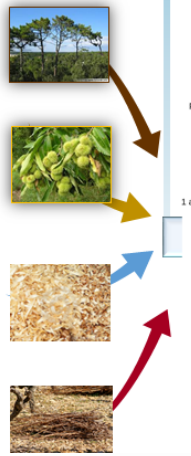
To anticipate, predict and minimize the contamination of food and feed with mycotoxins

Solutions for agroecology and agri-food industry

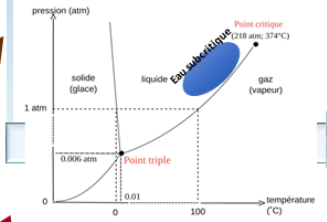


Biocontrol solutions –> bio-sourced antimycotoxin molecules

Tick defensins and plant extracts

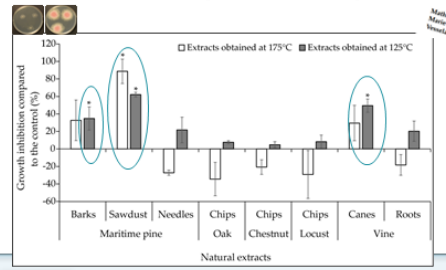


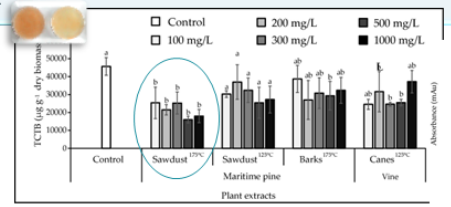
Subcritical water extraction

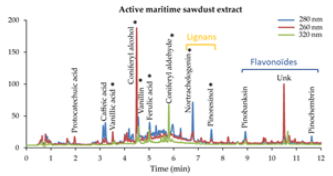


extracts

Antifungal and anti-mycotoxin activity







Promising bioactivity of the sawdust extract

molecules

Screening of Wood/Forest and Vine By-Products as Sources of New Drugs for Sustainable Strategies to Control *Fusarium graminearum* and the Production of Mycotoxins

molecules

Article

Ammoides pusilla Essential Oil: A Potent Inhibitor of the Growth of *Fusarium avenaceum* and Its Enniatin Production

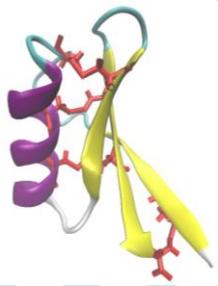
Yasmine Chakroun [1,2], Souheib Oueslati [2], Vessela Atanasova [1], Florence Richard-Forget [1], Manef Abderrabba [2] and Jean-Michel Savoie [1,*]

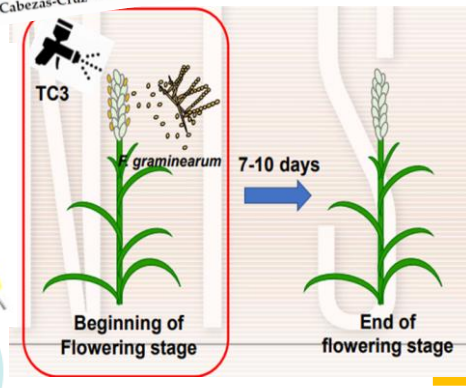
Journal of Fungi

Review

Use of Defensins to Develop Eco-Friendly Alternatives to Synthetic Fungicides to Control Phytopathogenic Fungi and Their Mycotoxins

Valentin Leannec-Rialland [1], Vessela Atanasova [2], Sylvain Chereau [2], Miray Tonk-Rügen [3,4], Alejandro Cabezas-Cruz [5,*] and Florence Richard-Forget [2,*]





<Preventive test against wheat head blight>

Chemical	Application and Inoculation timing	Concentration (ppm)	%, Control	
			7DAT	10DAT
TC3	Beginning of flowering stage	300	42	32
		100	36	4
【Reference】Topsin-M (thiophanate-methyl)		700	94	90

DAT : days after treatment

Active purified products or extracts

Formulation =
- stabilisation
- protection
- carrier

a solution useable in fields

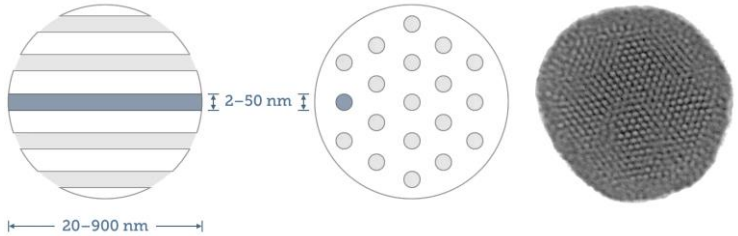
Biocontrol solutions –> bio-sourced antimycotoxin formulations

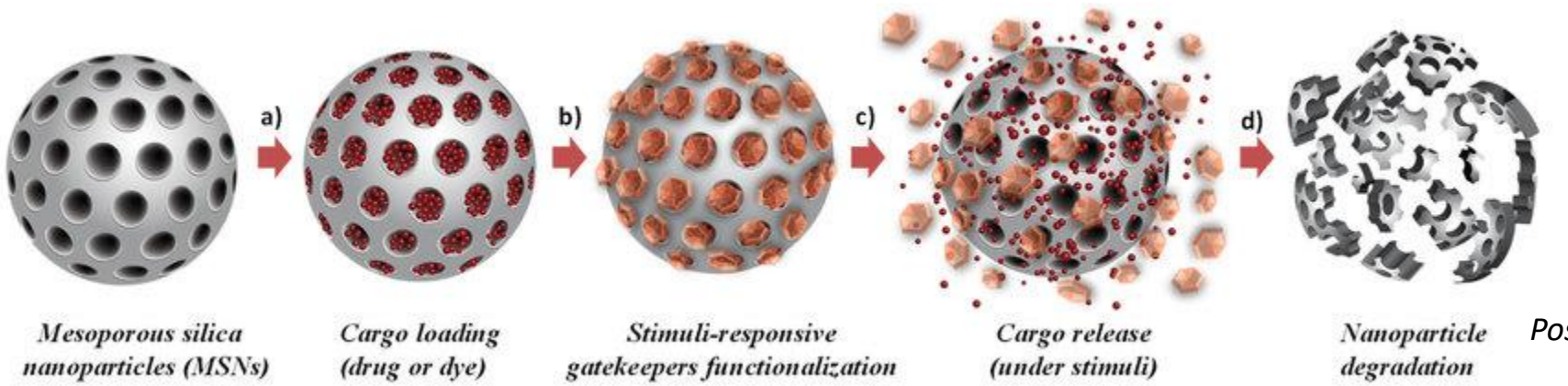
molecules

Article

Encapsulation of *Ammoides pusilla* Essential Oil into Mesoporous Silica Particles for the Enhancement of Their Activity against *Fusarium avenaceum* and Its Enniatins Production

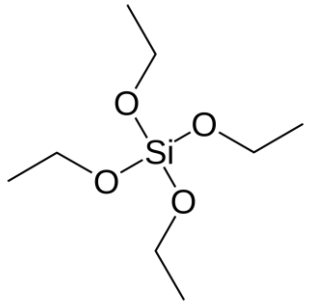
Yasmine Chakroun [1,2], Youssef Snoussi [2,3], Mohamed M. Chehimi [3,4], Manef Abderrabba [2], Jean-Michel Savoie [1,*] and Souheib Oueslati [2,*]





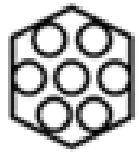
Poscher & Salinas 20200

Synthesis of Mesoporous Silica Particles (MSP)



- Precursor : tetraethyl orthosilicate TEOS (Si(OCH2CH3)4)
- Surfactants : CTAB and Pluronic P123
- 70°C for 16h and decantation 48h with ethanol as solvent

Pore size distribution of MSPs = narrow multimodal distribution, average pore size 3.1 nm



Specific surface = 487 m² / g

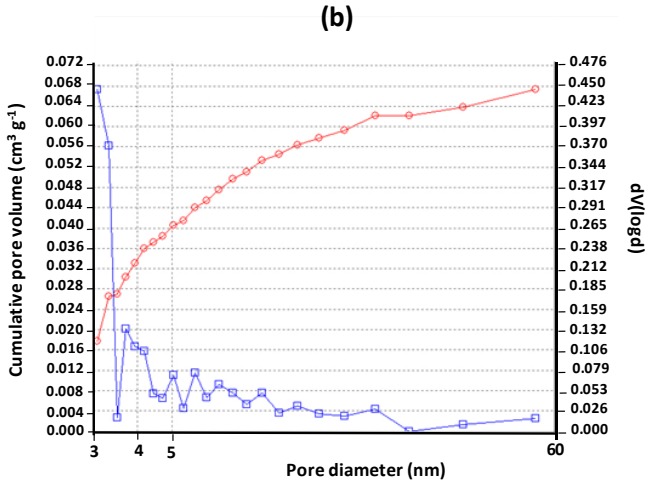


Figure 1: Pore size distribution of empty mesoporous silica particles (MSPs) (blue line) and cumulative pore volume (red line).

A quasi-neat silica, similar to calcined mesoporous silica

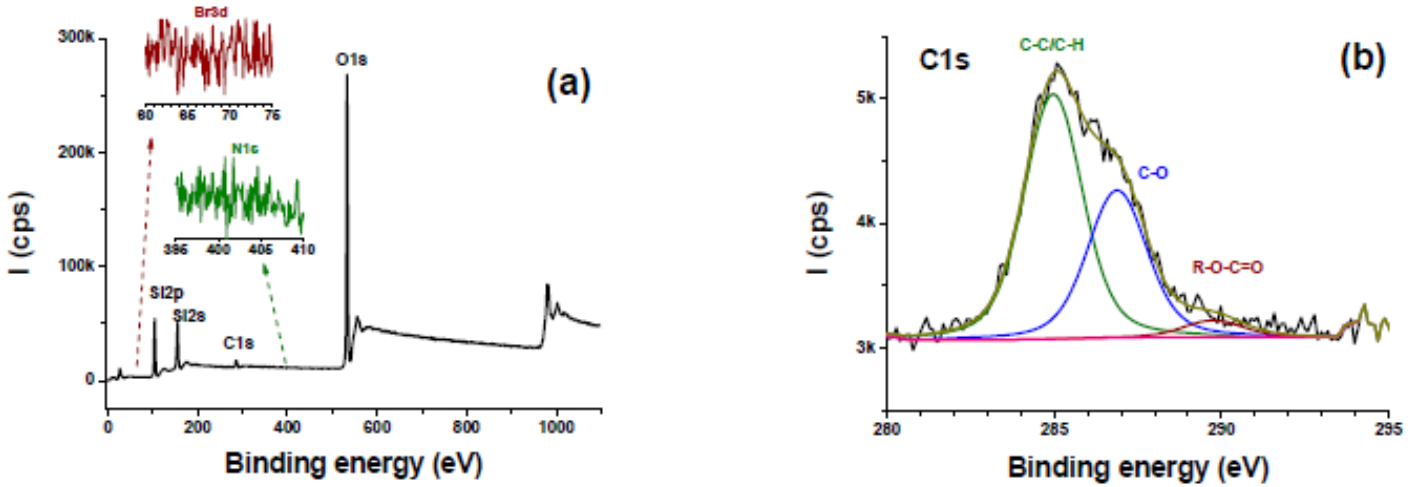
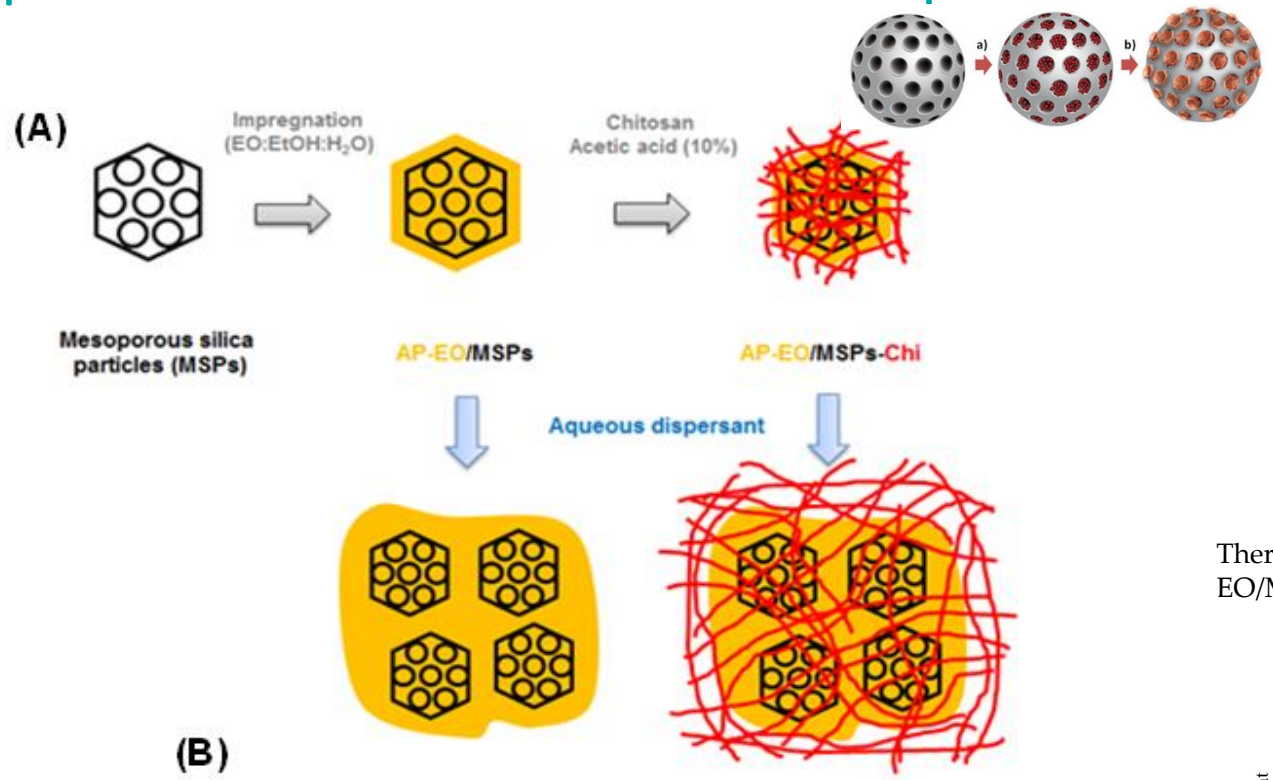


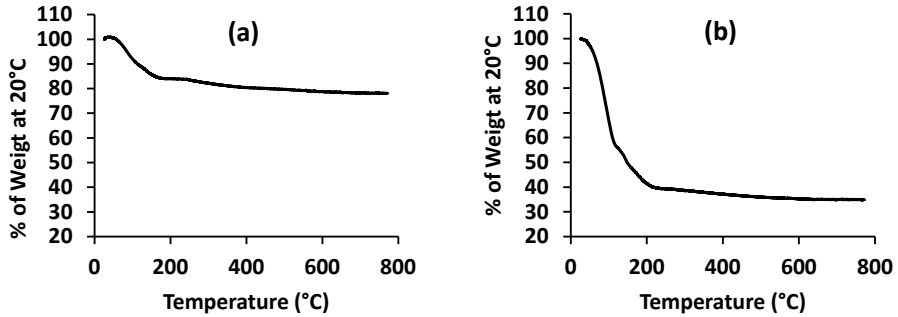
Figure 2. Survey (**a**), and peak-fitted C1s (**b**) spectra of MSPs. N1s (green) and Br3d (red) narrow regions are shown in inset of (**a**).

Apparent elemental composition determined by XPS : O, 61.3%; Si, 33.6%; C, ~5.1%.
O/Si atomic ratio = 1.82 (theoretical value = 2)

Encapsulation of the essential oil in mesoporous silica and coating with chitosan

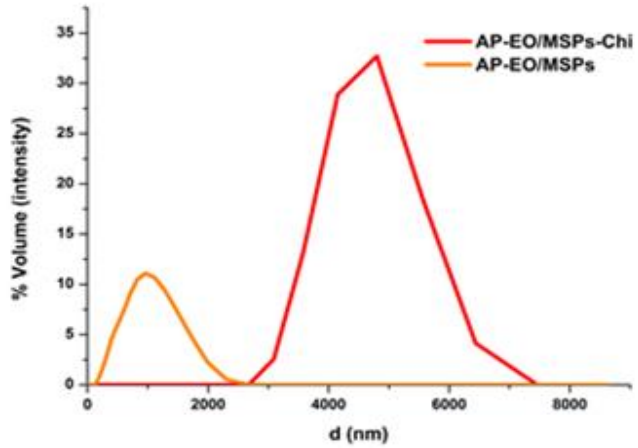


Yield of encapsulation of the EO = 45%

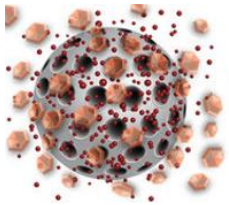


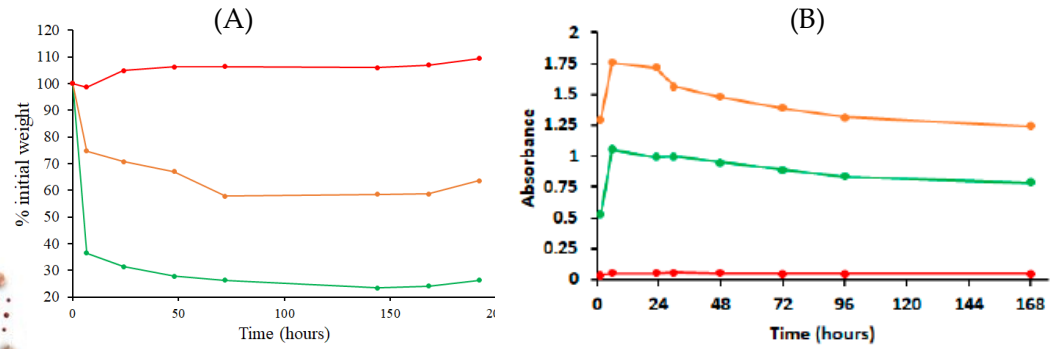
Thermogravimetric analysis (TGA) of MSPs, (**a**) empty mesoporous silica particles, (**b**) AP-EO/MSPs, mesoporous silica particles loaded with *A. pusilla* EO

(B)



Dynamic Light Scattering (DLS) characterization of the mesoporous-silica-encapsulated EO





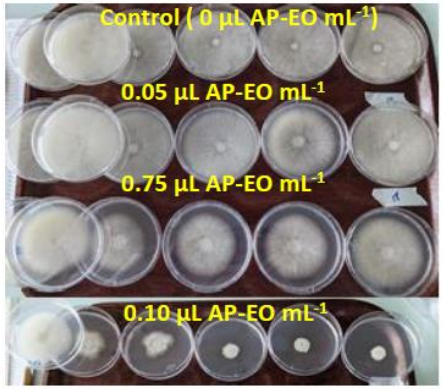
Release of *A. pusilla* EO in air (A) and in PBS buffer (B)

Encapsulation in MSP => slower release of EO

Coating with chitosan => protect from release of EO

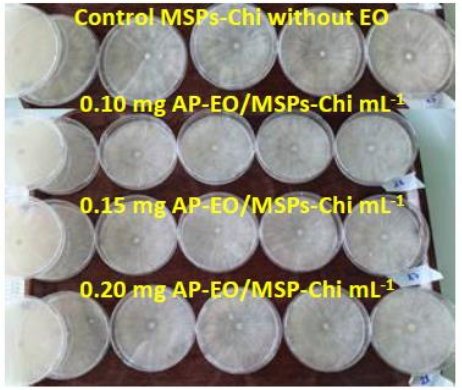
Antifungal and antimycotoxin activities

Free *A. pusilla* Essential Oil



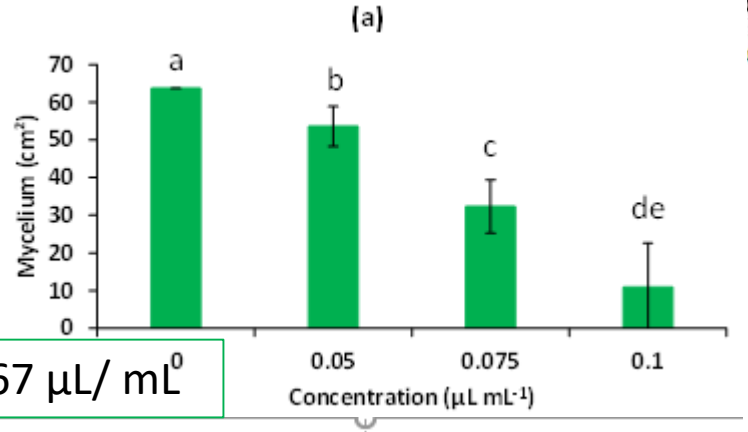
MSPs + *A. pusilla* Essential Oil

MSPs/Chitosan + *A. pusilla* Essential Oil



Effects of MSPs and AP-EO introduced **into the culture medium** on the mycelial growth of *Fusarium avenaceum,* incubated for 10 days at 25 °C.

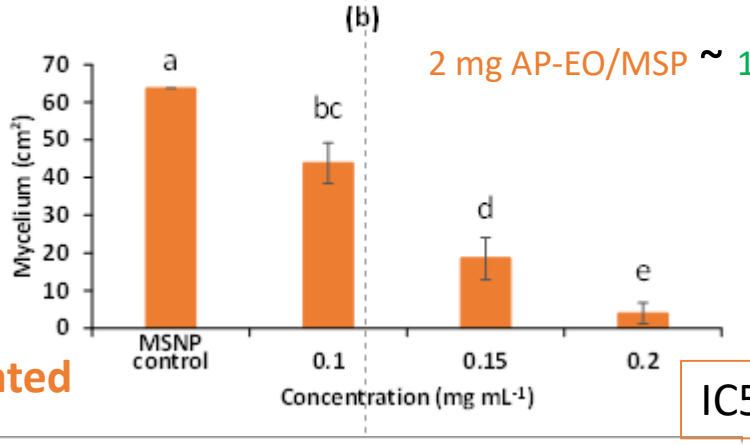
Free EO



IC50 = 0.067 µL/ mL

2 mg AP-EO/MSP ~ 1 µL AP-EO

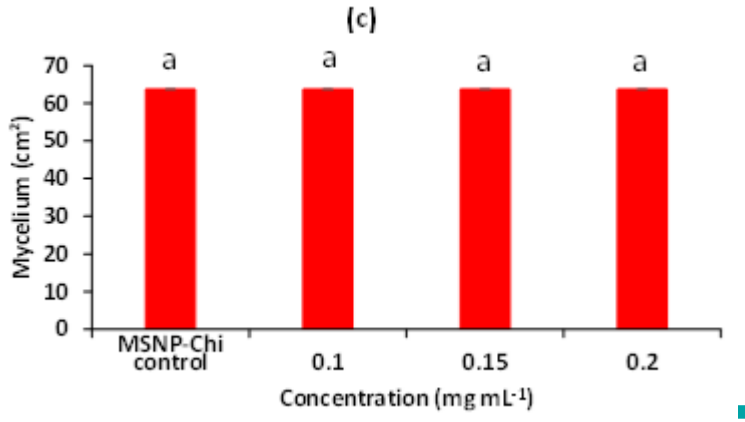
Encapsulated



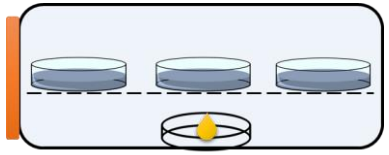
Efficacy >

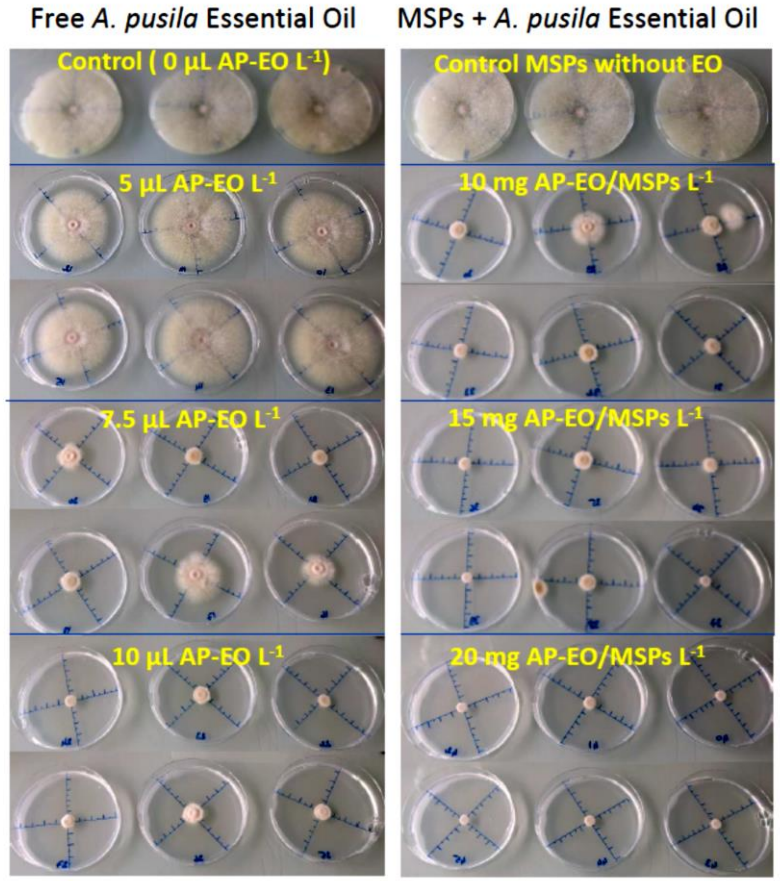
IC50 = 0.055 µL/ mL

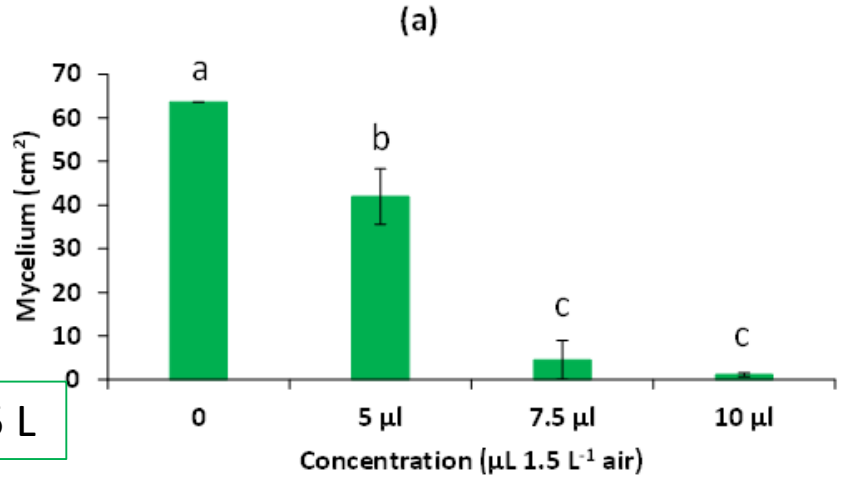
Coated with Chitosan



Antifungal and antimycotoxin activities

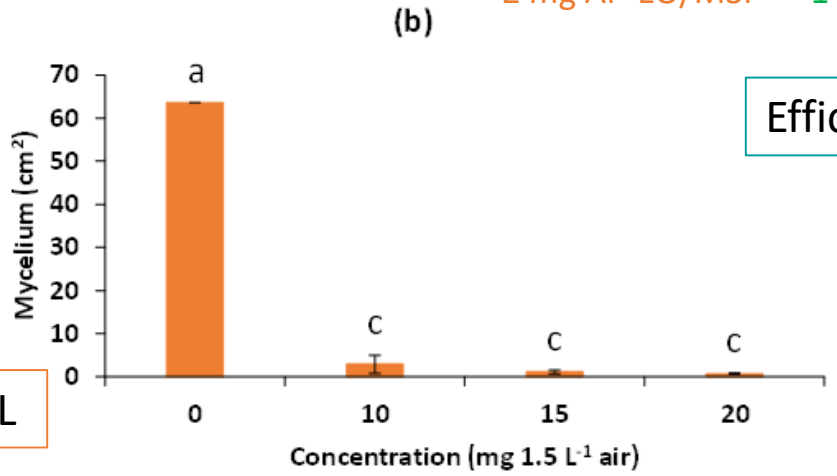






IC50 = 4.86 µL / flask 1.5 L

2 mg AP-EO/MSP ~ 1 µL AP-EO



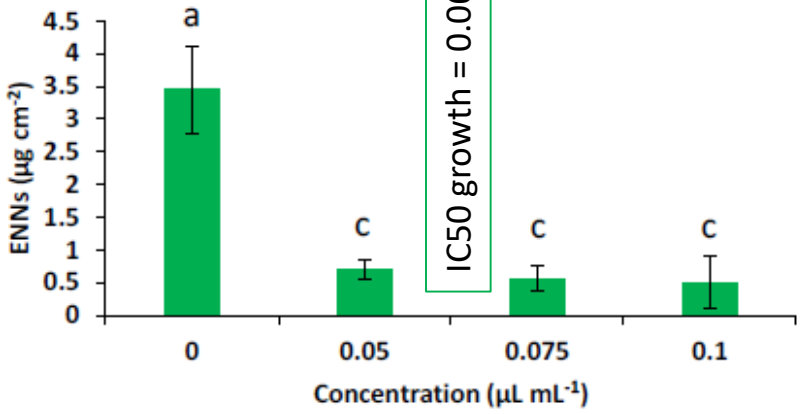
Efficacy >

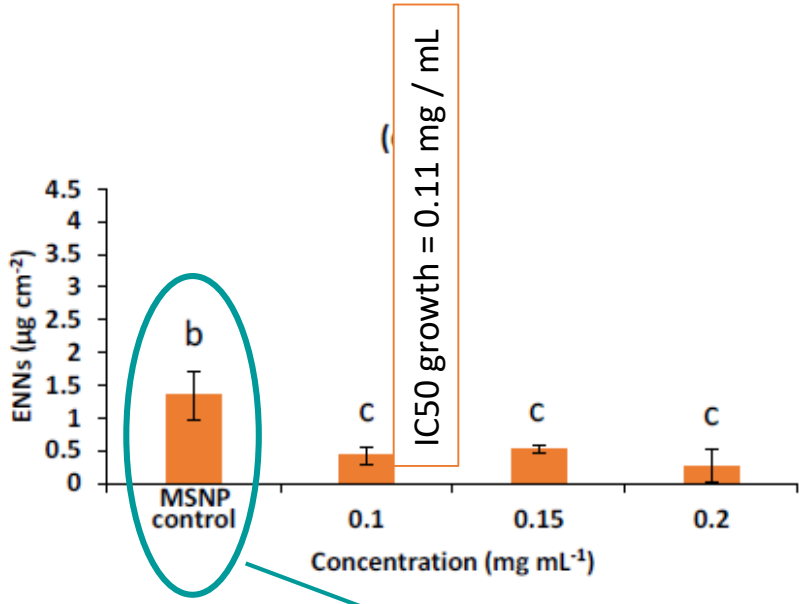
IC50 = 3.15 µL / flask 1.5 L

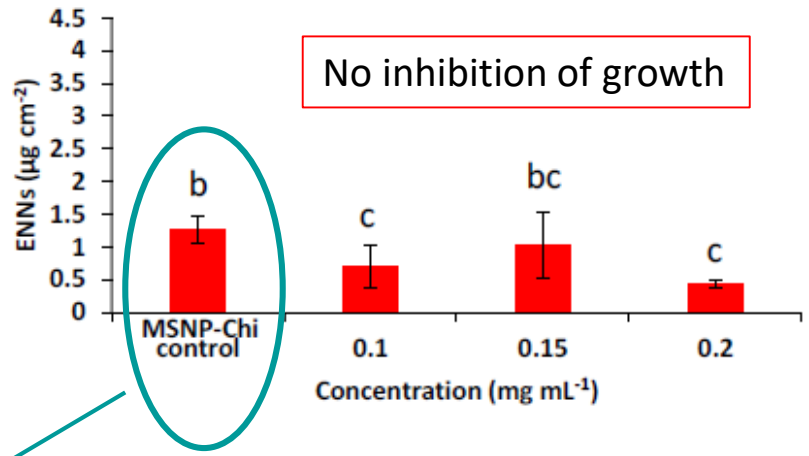
Effects of the **volatile compounds** released from AP-EO and AP-EO/MSPs on the mycelial growth of *Fusarium avenaceum* incubated for 10 days at 25 °C.

Antifungal and antimycotoxin activities

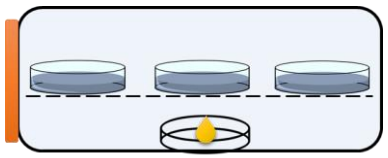
Enniatins concentration / Mycelial Biomass unit
(decreases = inhibition of the synthesis)

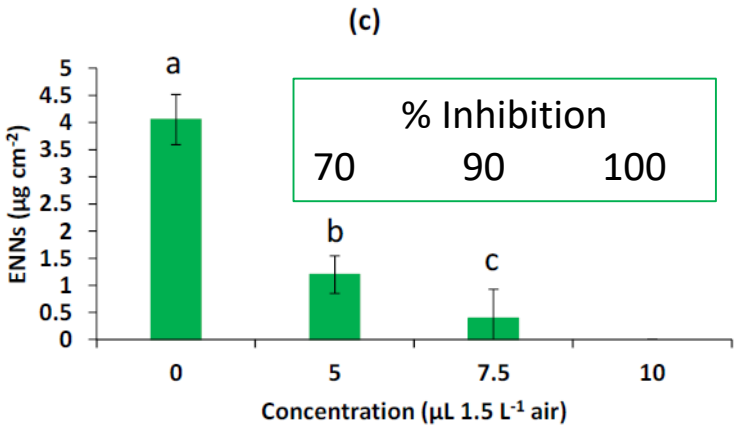


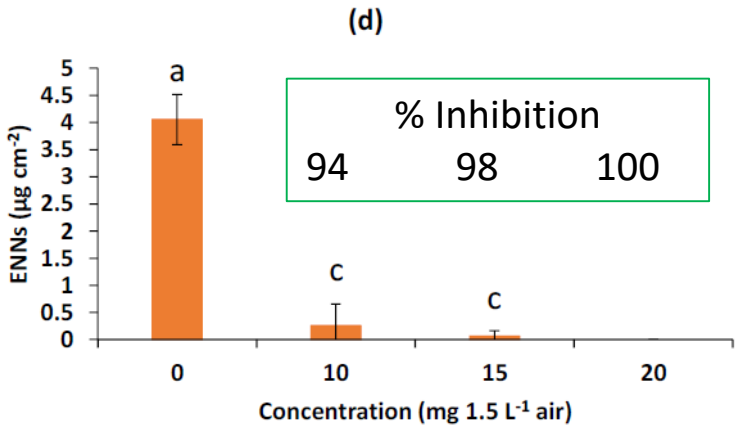




> 60 % inhibition



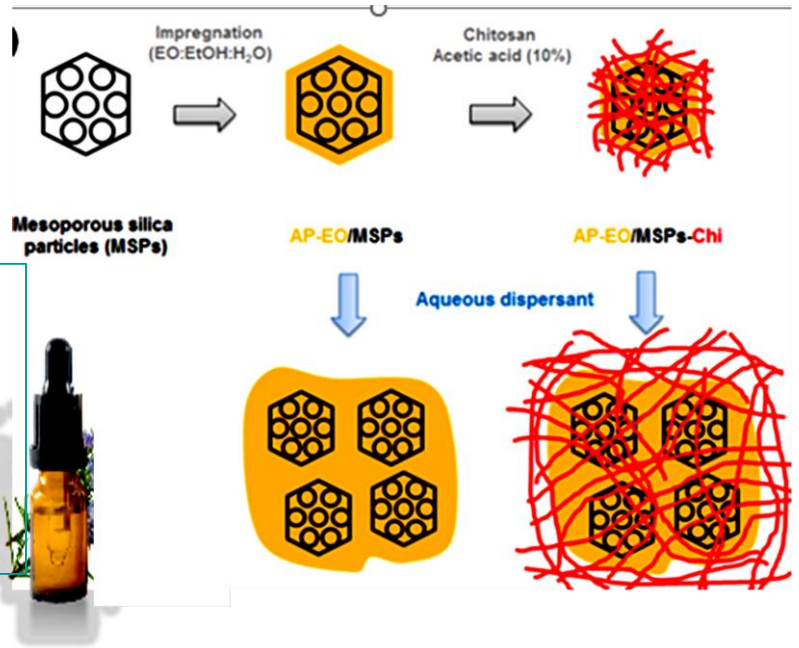




Conclusion

Ammoides pusilla essential oil:

- Antifungal activity
+
- Inhibition of Mycotoxin production



Encapsulation in MSPs

- Delay in release of EO and prolonged time of activity
- Improvement of the biological activities
- Carrier for field dispersion

Coating with chitosan

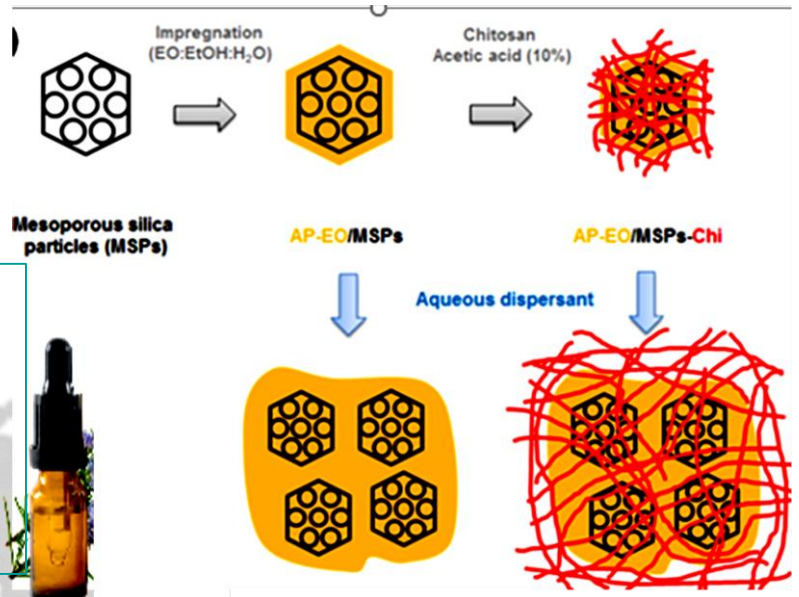
Protection for long term storage

Behavirour and efficacy of treatment on spikes ?
(in planta – field trials)

Conclusion

Ammoides pusilla essential oil:

- Antifungal activity
+
- Inhibition of Mycotoxin production



Encapsulation in MSPs

- Delay in release of EO and prolonged time of activity
- Improvement of the biological activities
- Carrier for field dispersion

Coating with chitosan

Protection for long term storage

- No antifungal activity
but
- Inhibition of Mycotoxin production

How does it work in fungal cells ?

Behavirour and efficacy of treatment on spikes ?
(in planta – field trials)

Acknowledgments

Florence Forget
Marie Foulongne Oriol
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Youssef Snoussi
Mohamed M. Chehimi

Manef Abderrabba
Souheib Oueslati