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## **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

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# The MICROBIOLOGY DAY - BORDEAUX



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

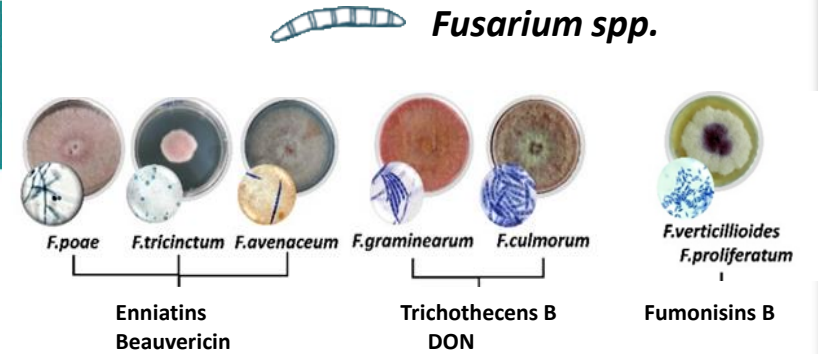
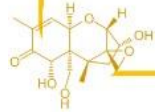
  
RÉPUBLIQUE  
FRANÇAISE  
Liberté  
Égalité  
Fraternité

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

➤ Field of research



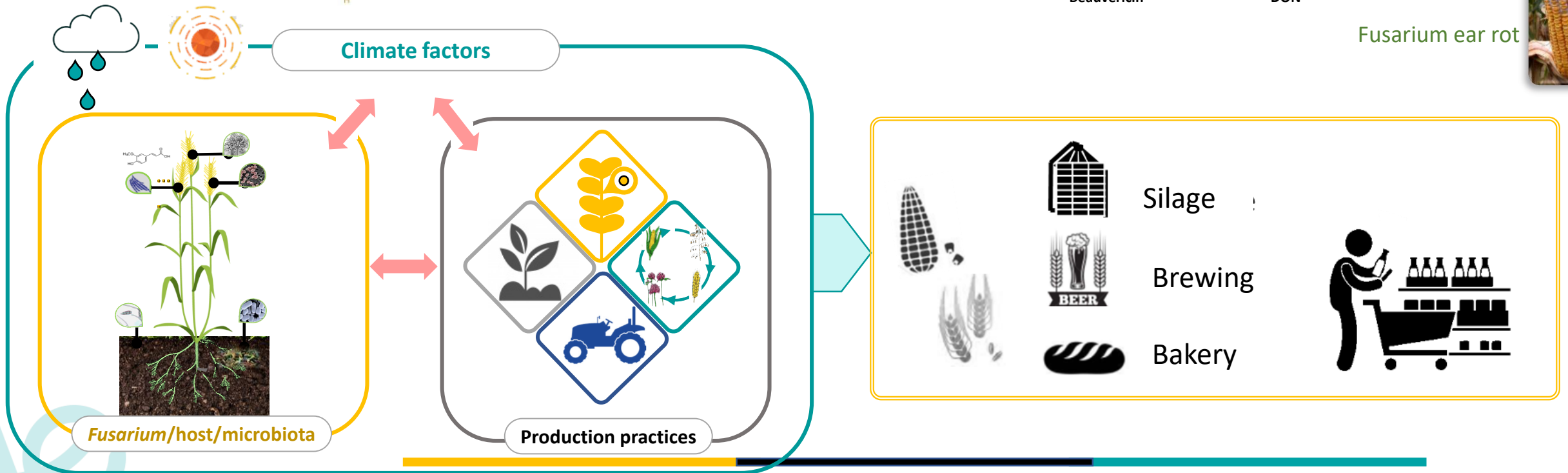
Focus on mycotoxins in cereal crops and particularly on mycotoxins produced by *Fusarium* species



Fusarium head blight



Fusarium ear rot



# Controlling the fungi and their production of mycotoxins

Mycotoxins = secondary metabolites

↓

Production enhanced by stress

↓

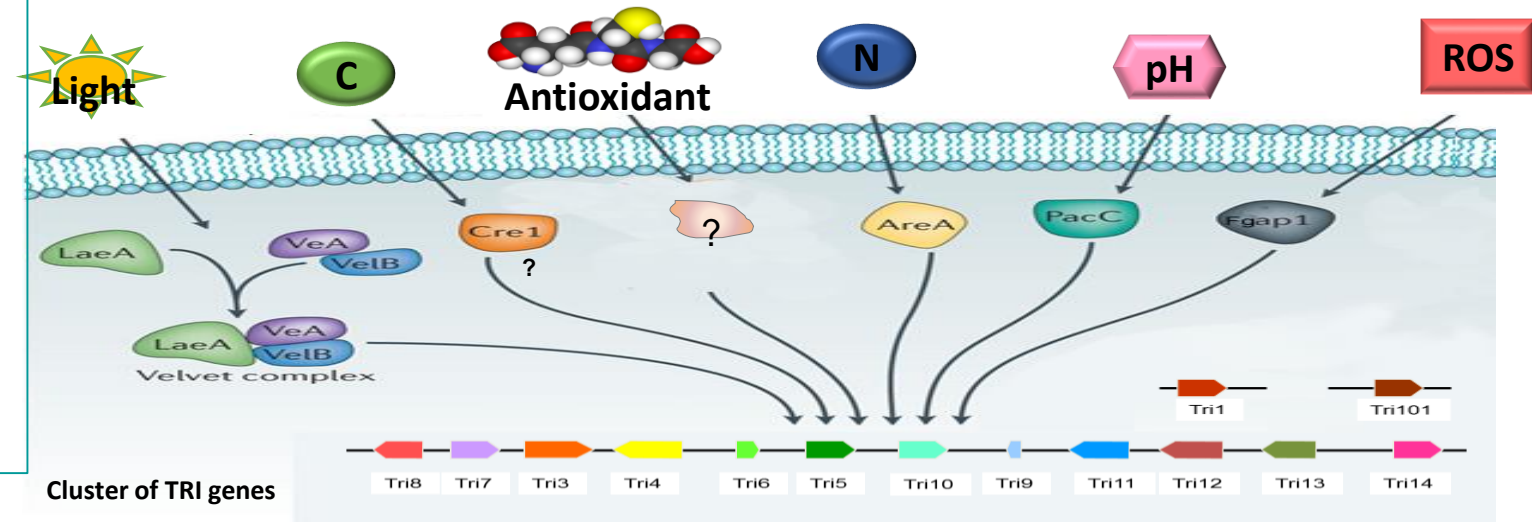
Affecting the mycelial growth may increase the synthesis of mycotoxins

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

↓

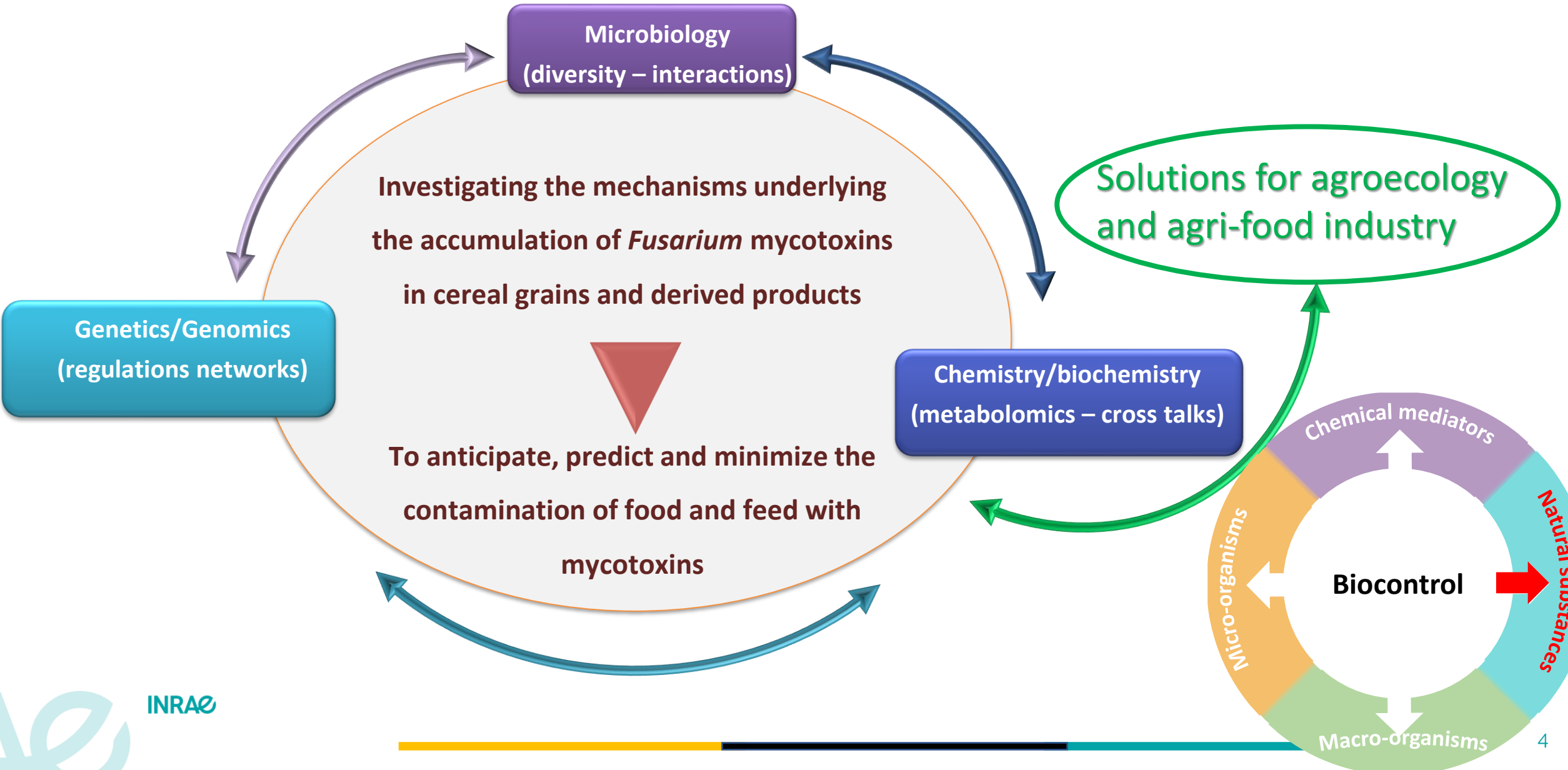
Spatio-temporal window for controlling

## Regulation of the synthesis of mycotoxins (DON)



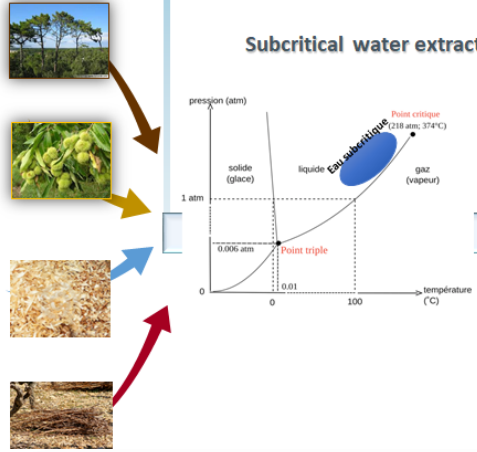
Colombo et al. 2019

# Core research objective

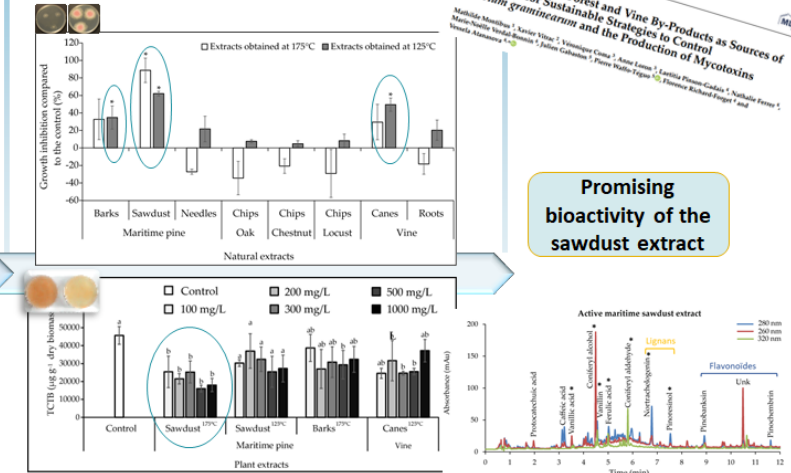


# Biocontrol solutions → bio-sourced antimycotoxin molecules

## Tick defensins and plant extracts



## Antifungal and anti-mycotoxin activity

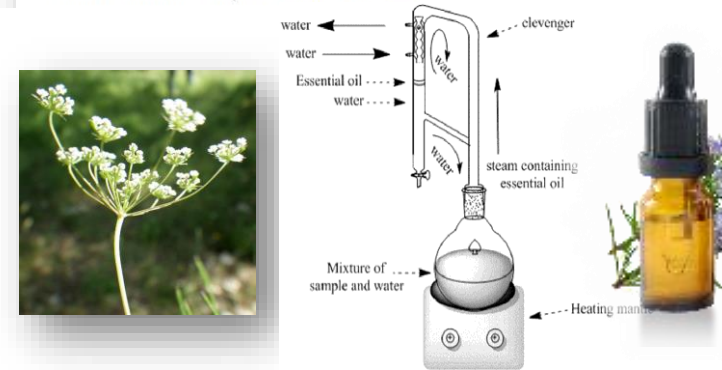


Promising bioactivity of the sawdust extract

## molecules

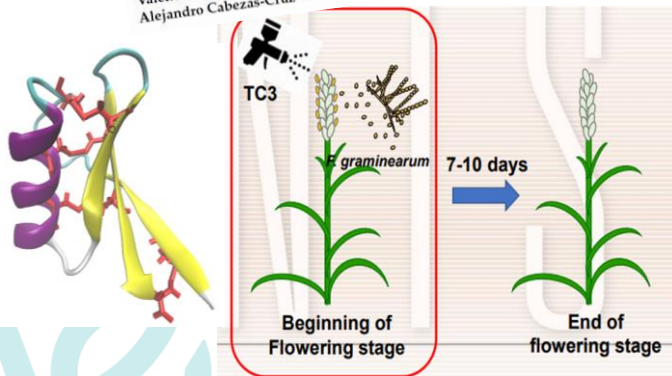
### Article: *Ammoides pusilla* Essential Oil: A Potent Inhibitor of the Growth of *Fusarium avenaceum* and Its Enniatin Production

Yasmine Chakroun<sup>1,2</sup>, Souheib Oueslati<sup>2</sup>, Vessela Atanasova<sup>1</sup>, Florence Richard-Forget<sup>1</sup>, Manef Abderrabba<sup>2</sup> and Jean-Michel Savoie<sup>1,\*</sup>



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

Valentin Leanne-Rialland<sup>1</sup>, Vessela Atanasova<sup>2</sup>, Sylvain Chereau<sup>2</sup>, Miray Tonk-Rügen<sup>3,4</sup>, Alejandro Cabezas-Cruz<sup>5,\*</sup> and Florence Richard-Forget<sup>2,\*</sup>



### <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] Topsisin-M (thiophanate-methyl)	Beginning of flowering stage	700	94	90

DAT : days after treatment

Active purified products or extracts

**Formulation** =

- stabilisation
- protection
- carrier





a solution useable in fields

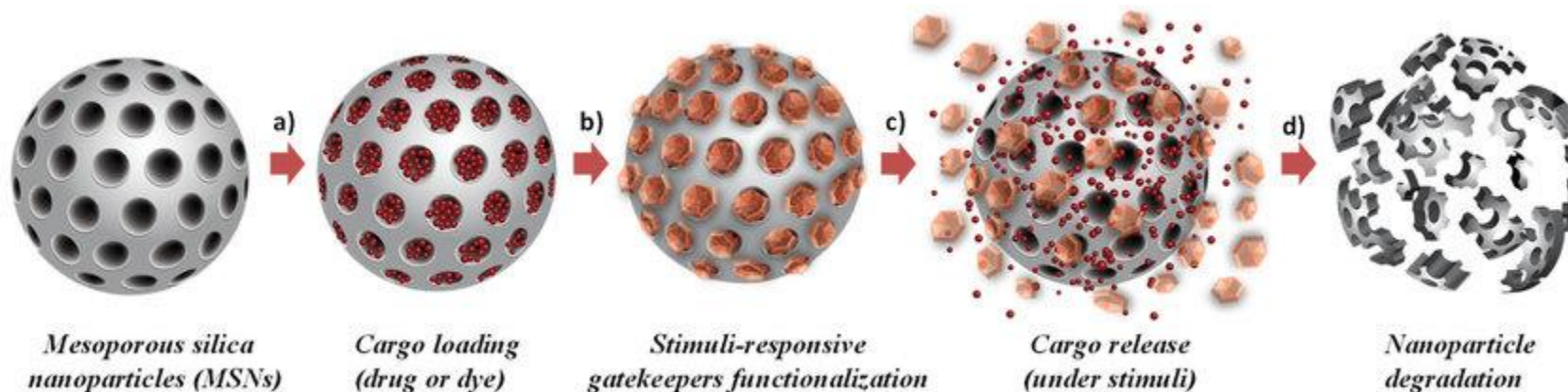
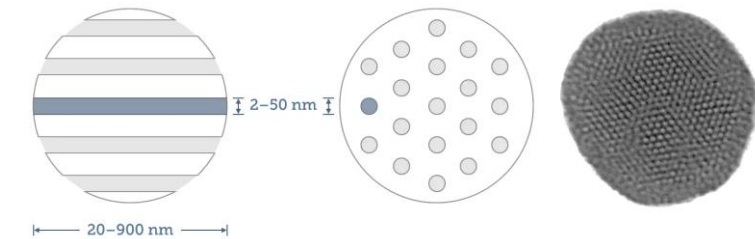
 *molecules*



Article

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

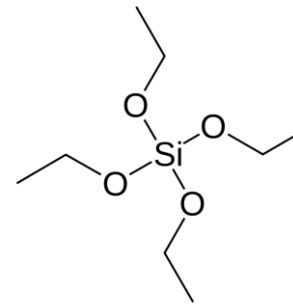
Yasmine Chakroun <sup>1,2</sup>, Youssef Snoussi <sup>2,3</sup>, Mohamed M. Chehimi <sup>3,4</sup>, Manef Abderrabba <sup>2</sup>, Jean-Michel Savoie <sup>1,\*</sup> and Souheib Oueslati <sup>2,\*</sup>



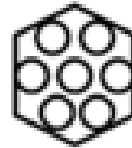
Poscher & Salinas 20200

# ➤ Synthesis of Mesoporous Silica Particles (MSP)

- Precursor : tetraethyl orthosilicate TEOS ( $\text{Si}(\text{OCH}_2\text{CH}_3)_4$ )
- 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



A quasi-neat silica, similar to calcined mesoporous silica

Specific surface = 487 m<sup>2</sup> / g

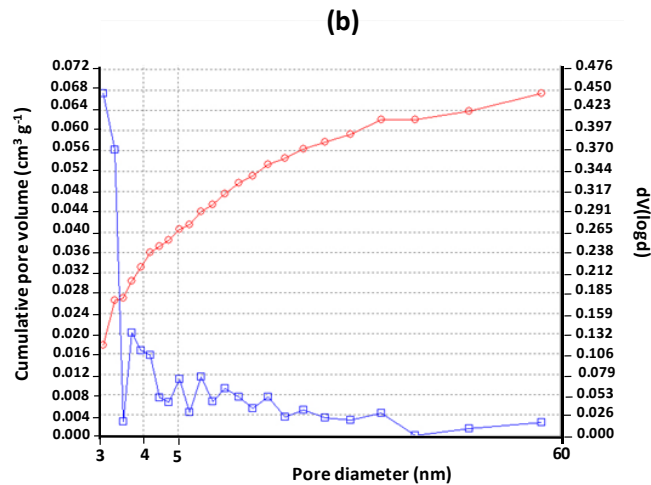


Figure 1: Pore size distribution of empty mesoporous silica particles (MSPs) (blue line) and cumulative pore volume (red line).

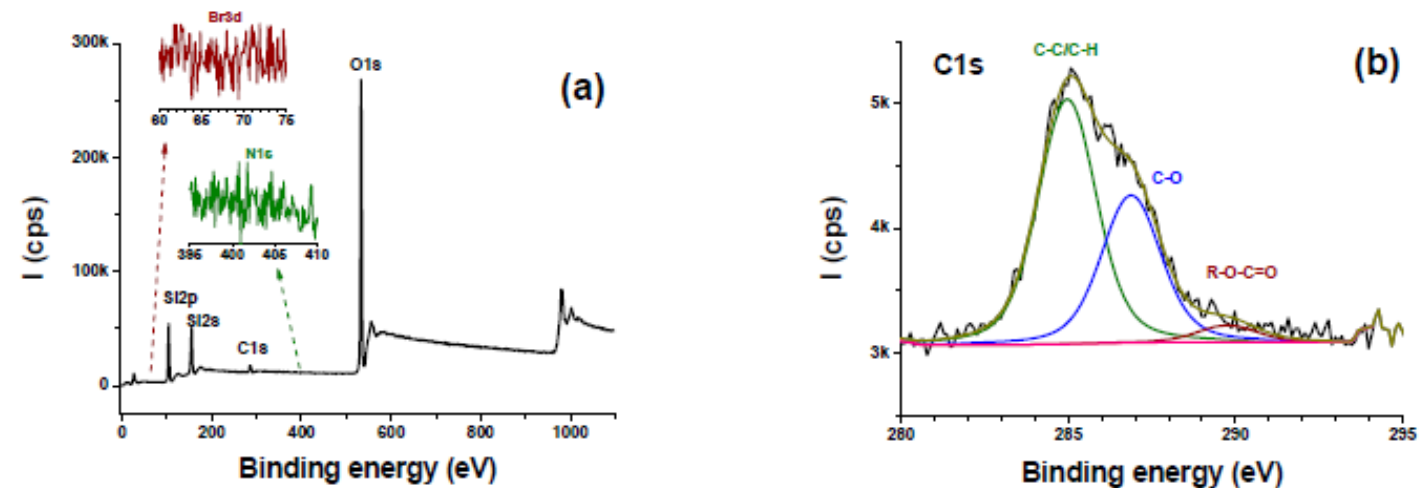
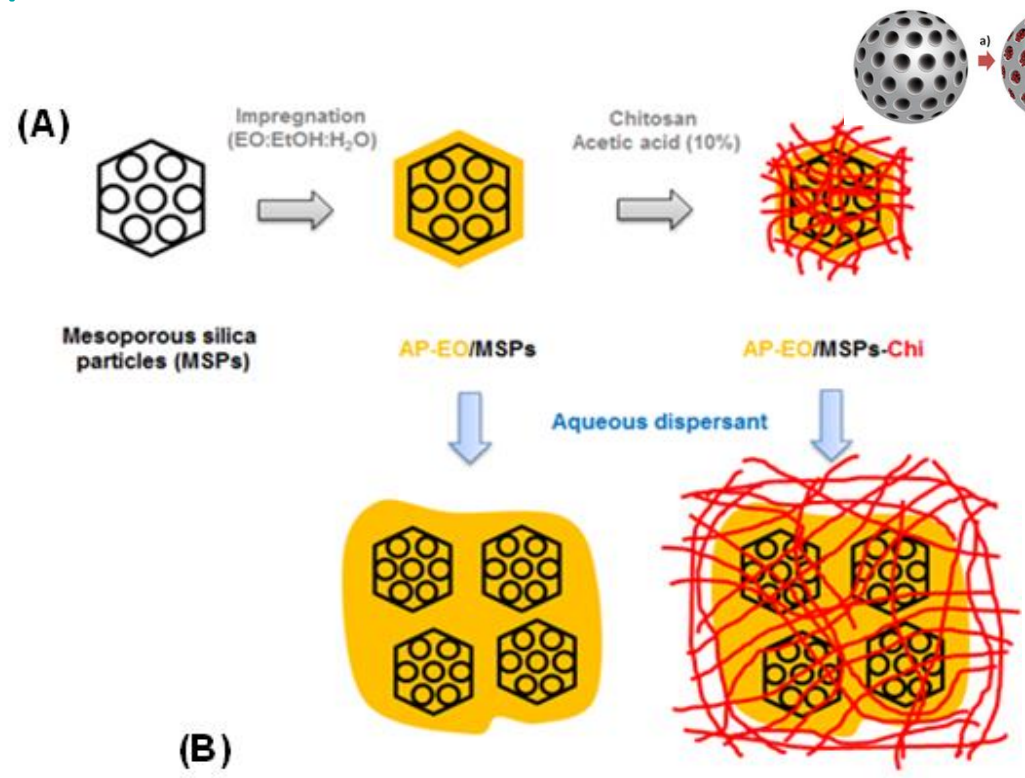


Figure 2. Survey (a), and peak-fitted C1s (b) spectra of MSPs. N1s (green) and Br3d (red) narrow regions are shown in inset of (a).

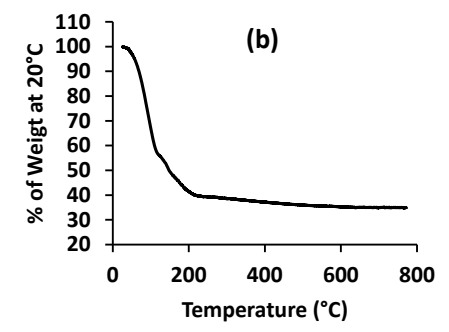
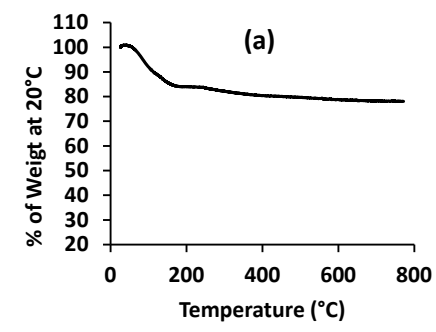
Apparent elemental composition determined by XPS : O, 61.3%; Si, 33.6%; C, ~5.1%.  
O/Si atomic ratio = 1.82 (theoretical value = 2)



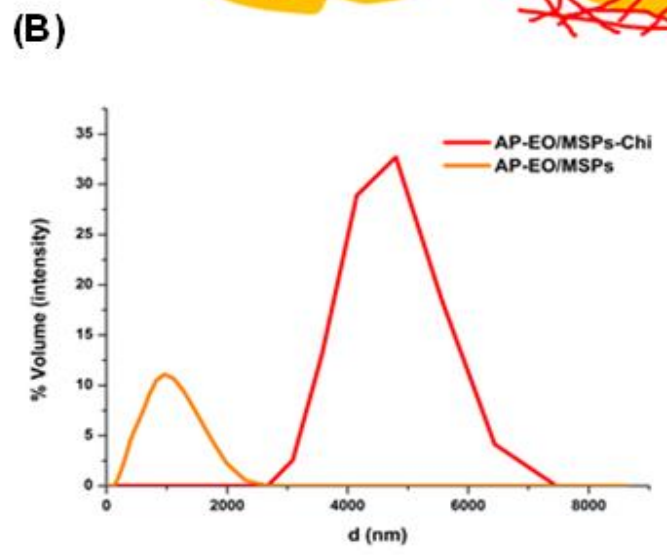
# Encapsulation of the essential oil in mesoporous silica and coating with chitosan



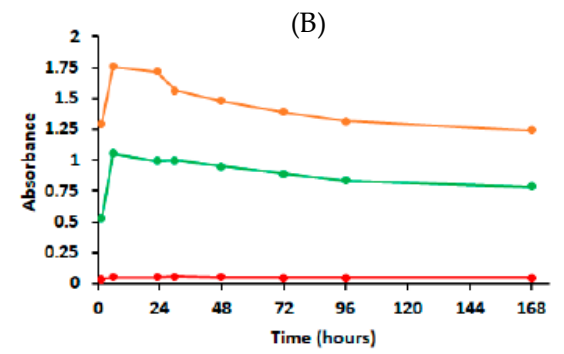
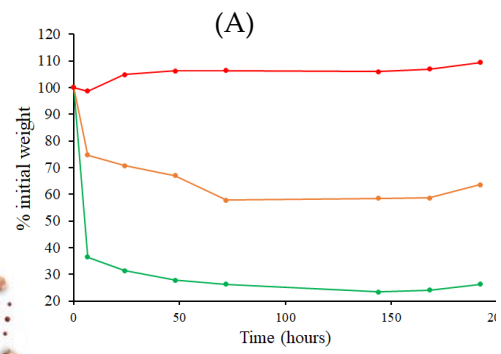
Yield of encapsulation of the EO = 45%



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



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

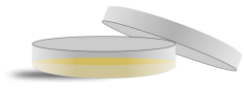


Release of *A. pusilla* EO in air (A) and in PBS buffer (B)

Encapsulation in MSP => slower release of EO

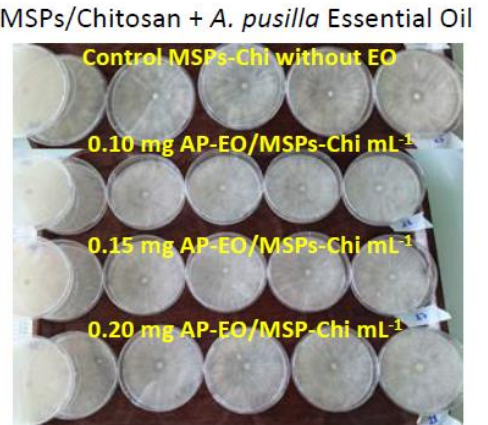
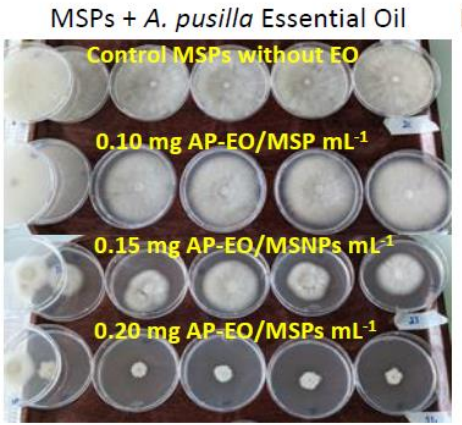
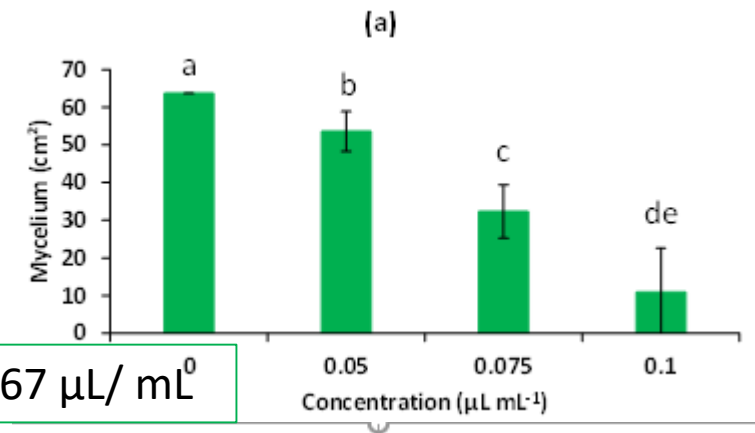
Coating with chitosan => protect from release of EO

# Antifungal and antimycotoxin activities



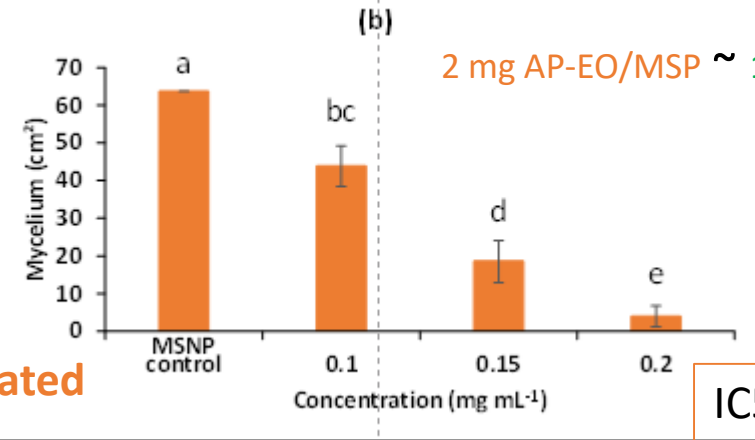
Free EO

IC50 = 0.067 μL/ mL<sup>0</sup>



Encapsulated

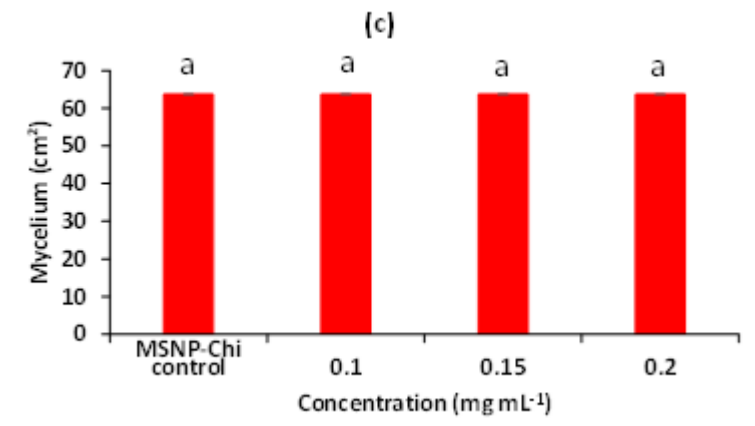
Efficacy >  
IC50 = 0.055 μL/ mL



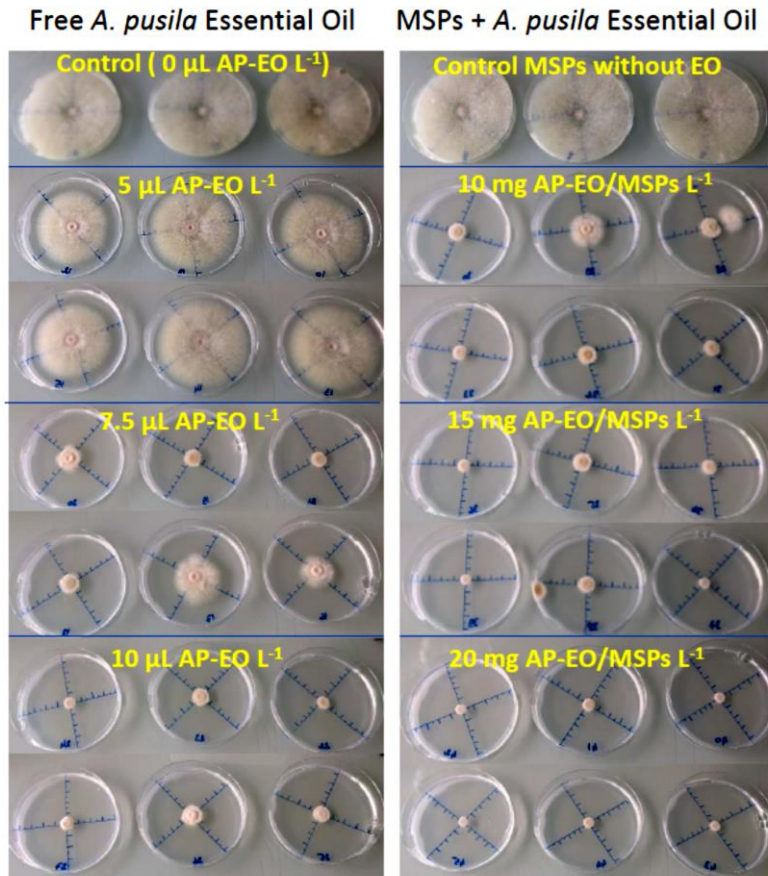
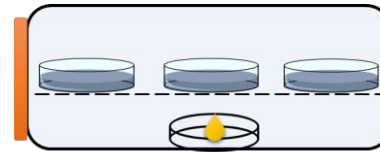
2 mg AP-EO/MSP ~ 1 μL AP-EO

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.

Coated with Chitosan

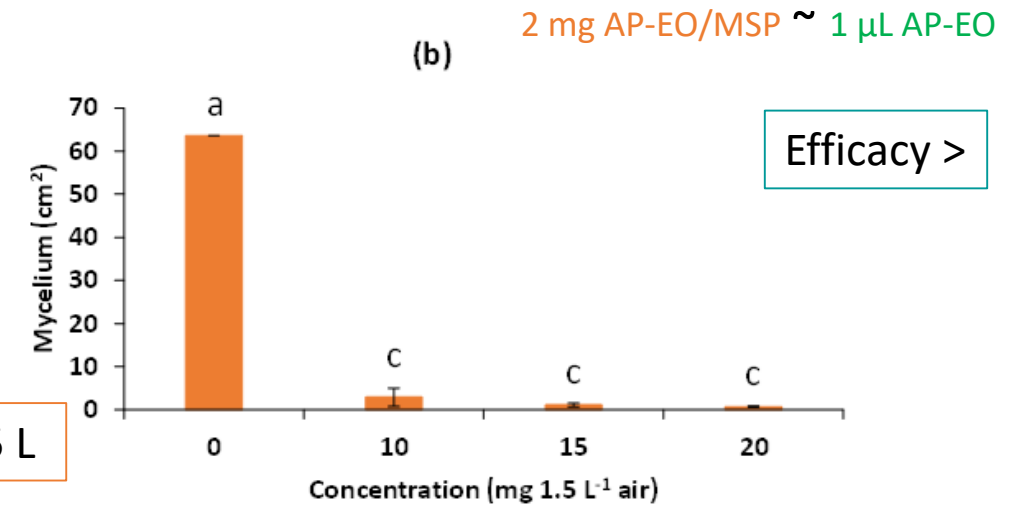
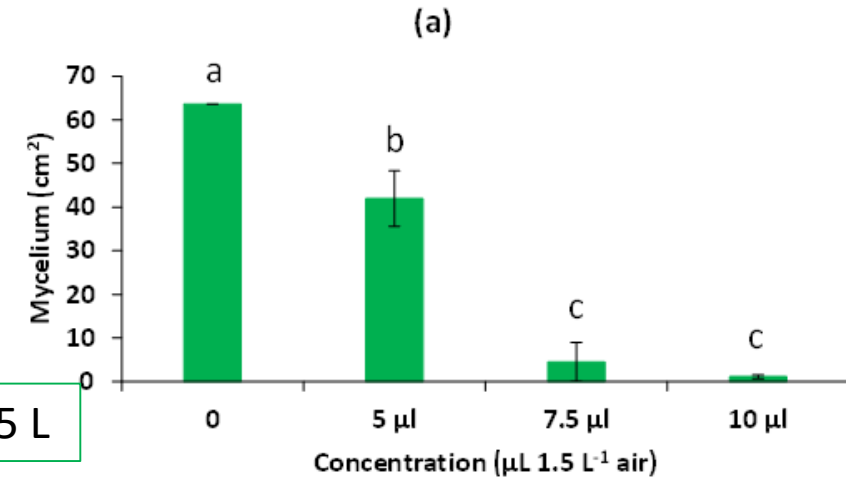


# Antifungal and antimycotoxin activities



IC50 = 4.86  $\mu\text{L}$  / flask 1.5 L

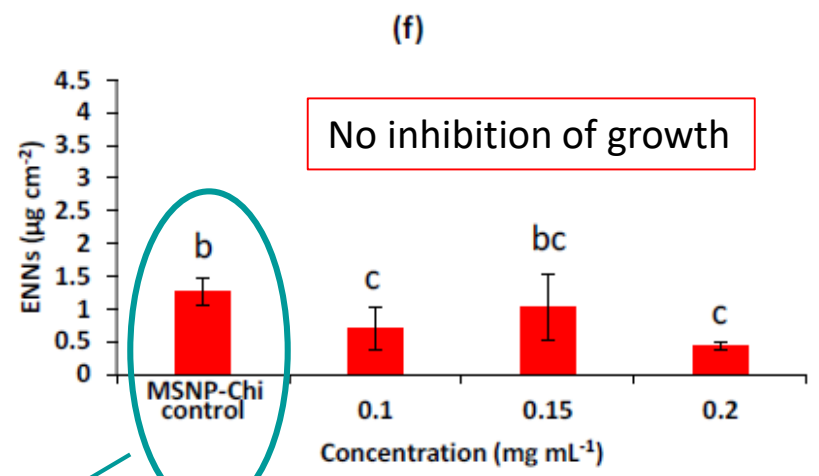
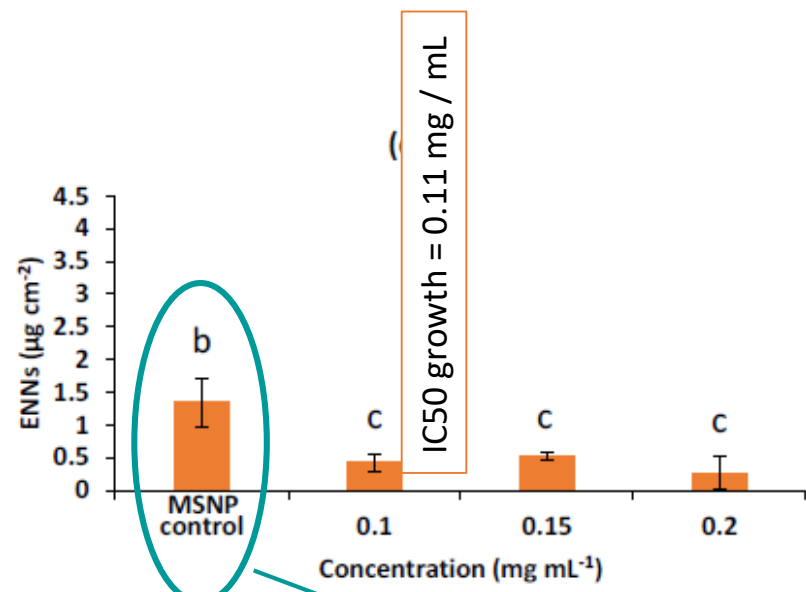
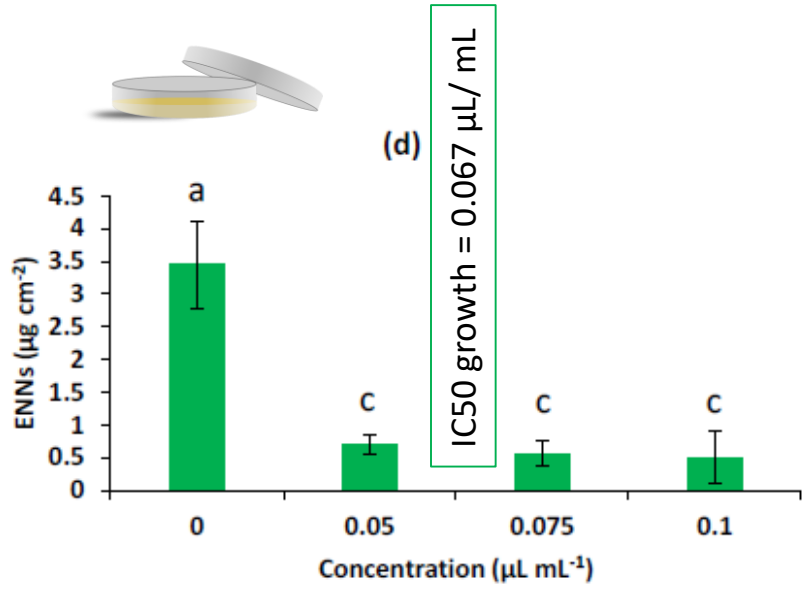
IC50 = 3.15  $\mu\text{L}$  / flask 1.5 L



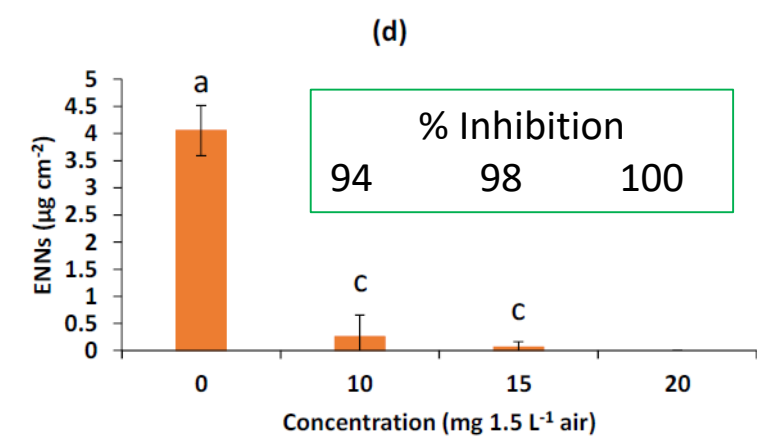
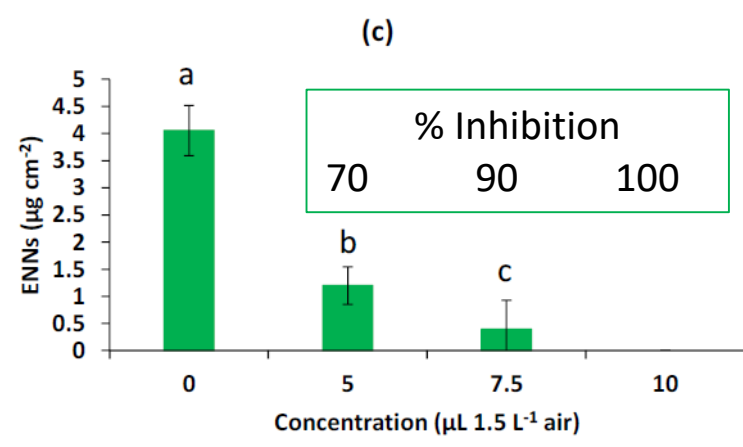
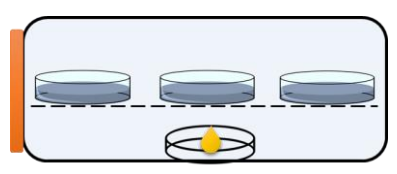
Effects of the **volatile compounds** released from AP-EO and AP-EO/MSPs on the mycelial growth of *Fusarium avenaceum* incubated for 10 days at 25 °C.

# Antifungal and antimycotoxin activities

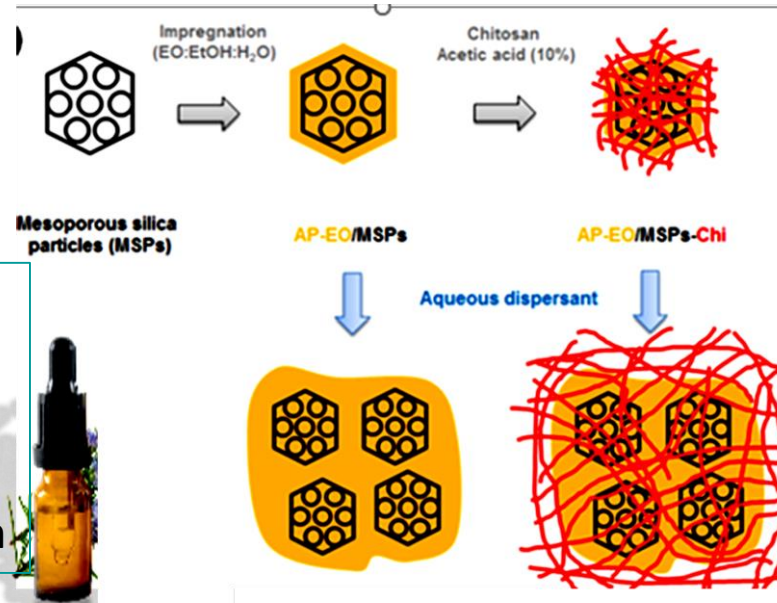
Enniatins concentration / Mycelial Biomass unit  
(decreases = inhibition of the synthesis)



> 60 % inhibition



## ➤ Conclusion



### *Ammoides pusilla* essential oil:

- Antifungal activity
- +
- Inhibition of Mycotoxin production



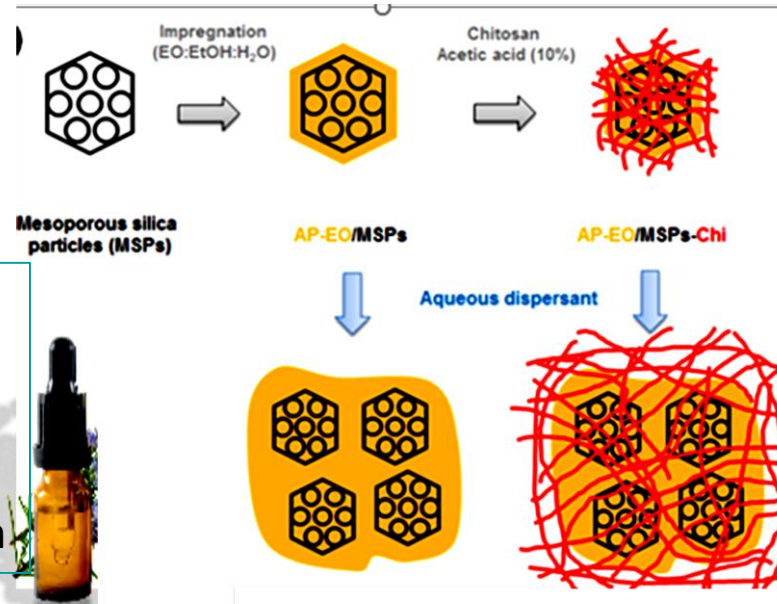
### Encapsulation in MSPs

- 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

Behaviour 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 ?

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

# ➤ Acknowledgments



université  
de BORDEAUX

+



CAMPUS  
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- Christophe Billette**

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