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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

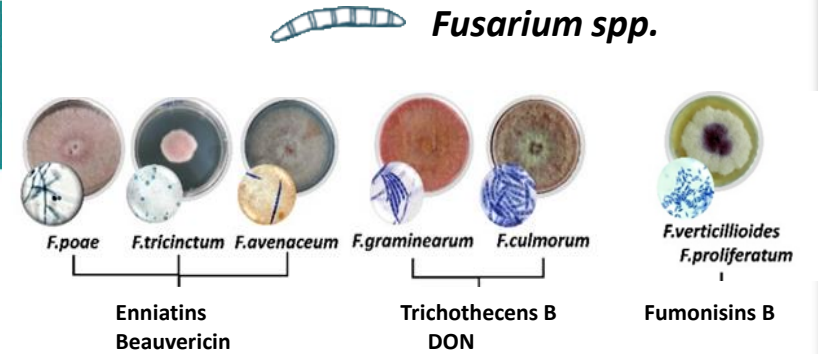
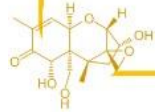


➤ Context: Elaborating food and feed safety from the field to the consumer

➤ Field of research



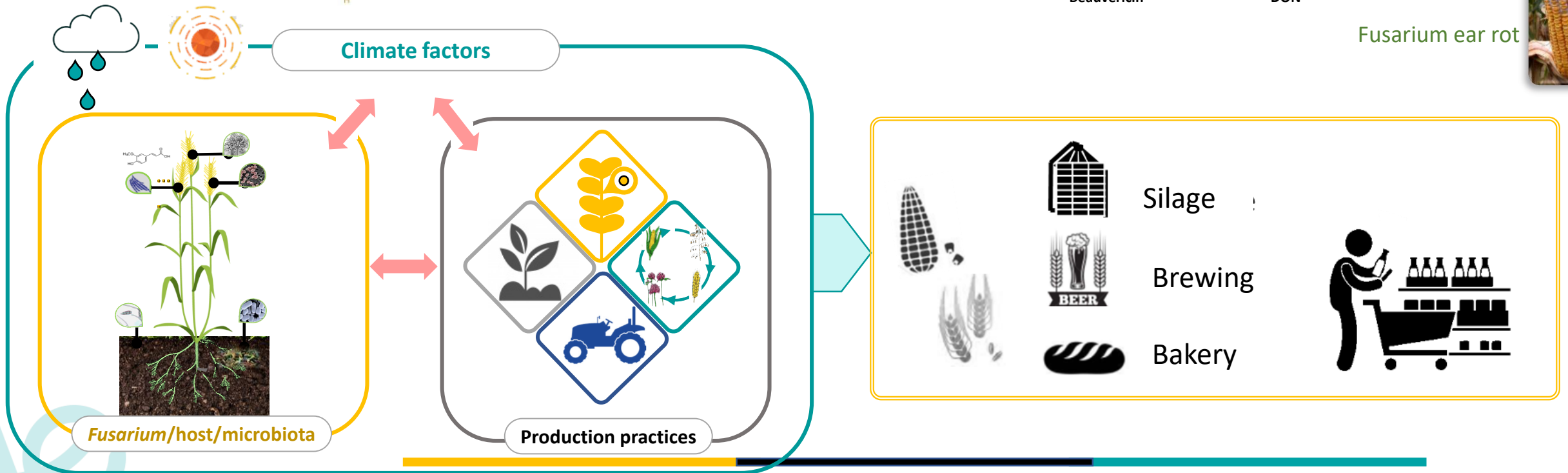
Focus on mycotoxins in cereal crops and particularly on mycotoxins produced by *Fusarium* species



Fusarium head blight



Fusarium ear rot



Controlling the fungi and their production of mycotoxins

Mycotoxins = secondary metabolites

↓

Production enhanced by stress

↓

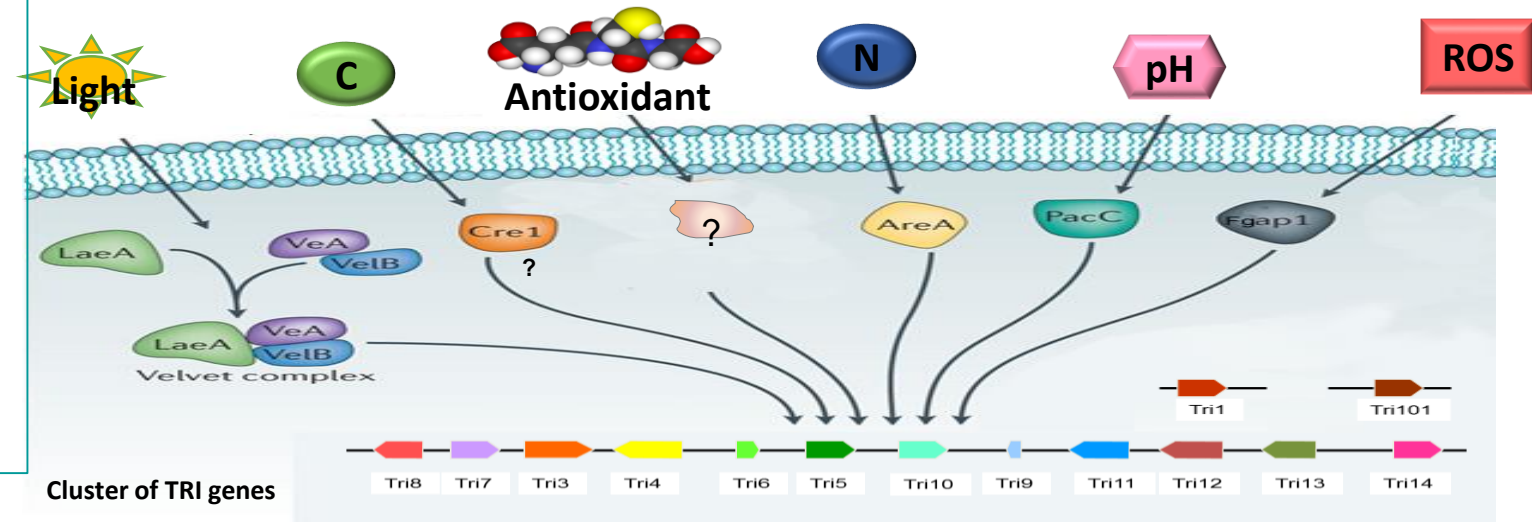
Affecting the mycelial growth may increase the synthesis of mycotoxins

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

↓

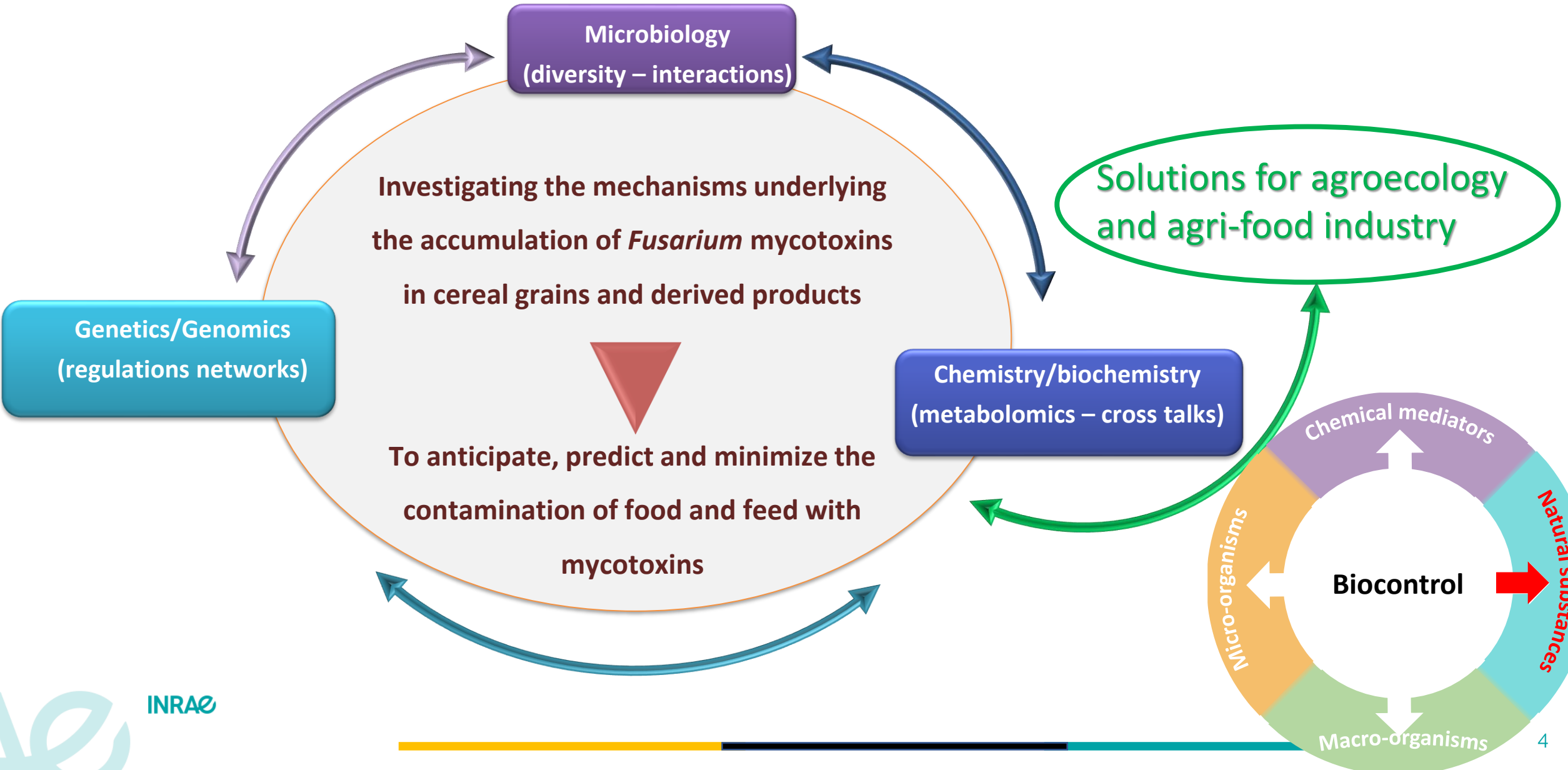
Spatio-temporal window for controlling

Regulation of the synthesis of mycotoxins (DON)



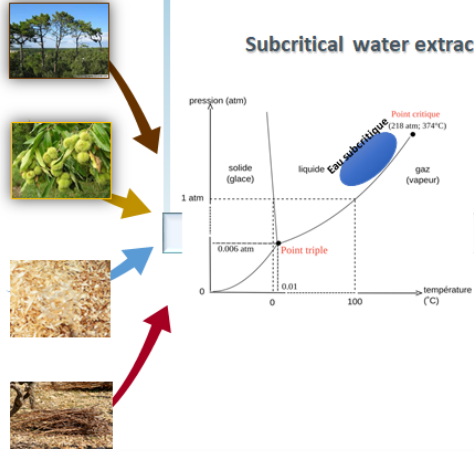
Colombo et al. 2019

Core research objective

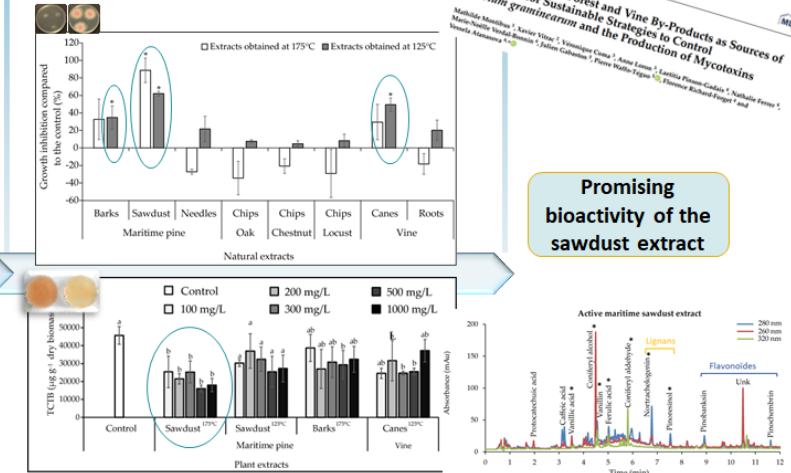


Biocontrol solutions → bio-sourced antimycotoxin molecules

Tick defensins and plant extracts



Antifungal and anti-mycotoxin activity

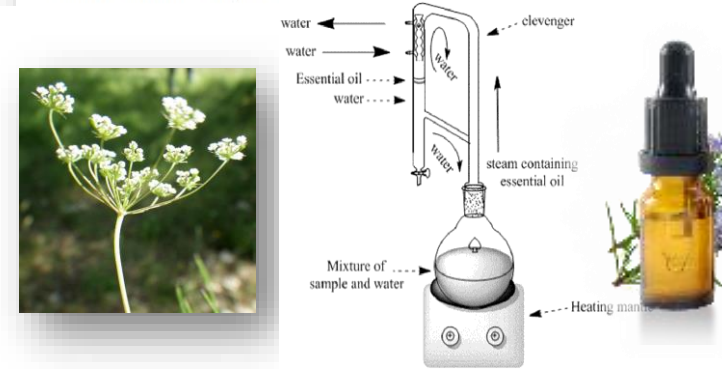


Promising bioactivity of the sawdust extract

molecules

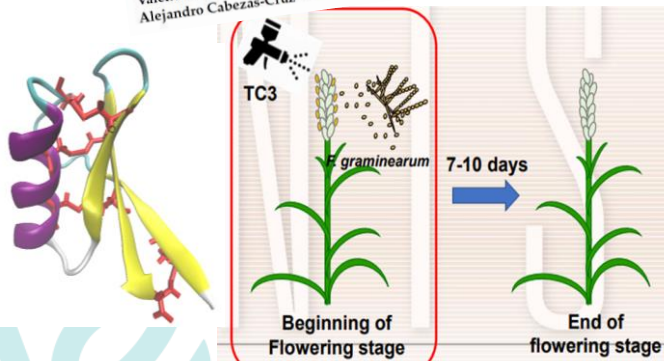
Article: *Ammoides pusilla* Essential Oil: A Potent Inhibitor of the Growth of *Fusarium avenaceum* and Its Enniatin Production

Yasmine Chakroun^{1,2}, Souheib Oueslati², Vessela Atanasova¹, Florence Richard-Forget¹, Manef Abderrabba² and Jean-Michel Savoie^{1,*}



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

Valentin Leanne-Rialland¹, Vessela Atanasova², Sylvain Chereau², 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)	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

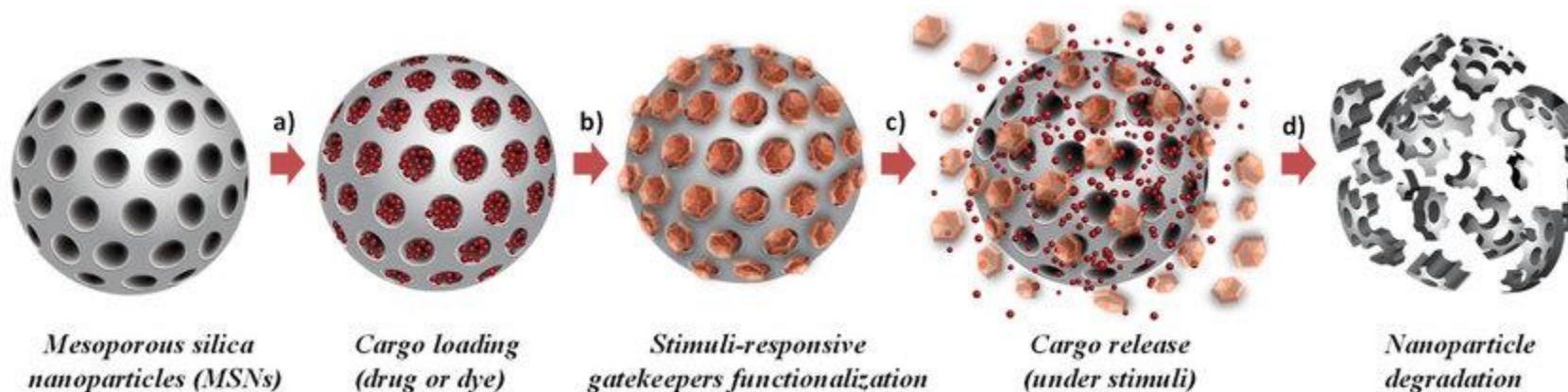
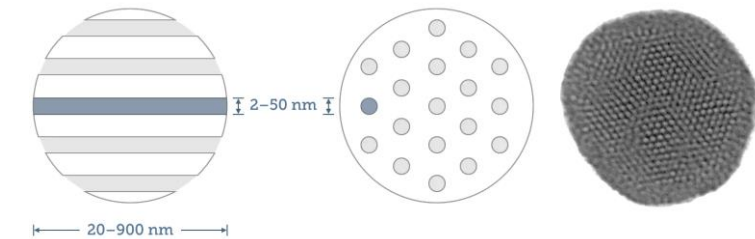
 *molecules*



Article

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

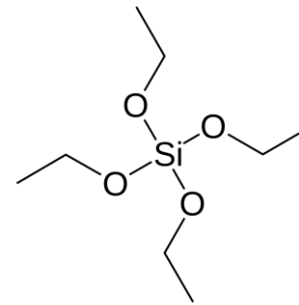
Yasmine Chakroun ^{1,2} , Youssef Snoussi ^{2,3}, Mohamed M. Chehimi ^{3,4} , Manef Abderrabba ², Jean-Michel Savoie ^{1,*}  and Souheib Oueslati ^{2,*} 



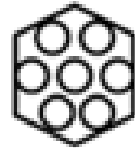
Poscher & Salinas 20200

➤ Synthesis of Mesoporous Silica Particles (MSP)

- Precursor : tetraethyl orthosilicate TEOS ($\text{Si}(\text{OCH}_2\text{CH}_3)_4$)
- 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 m² / g

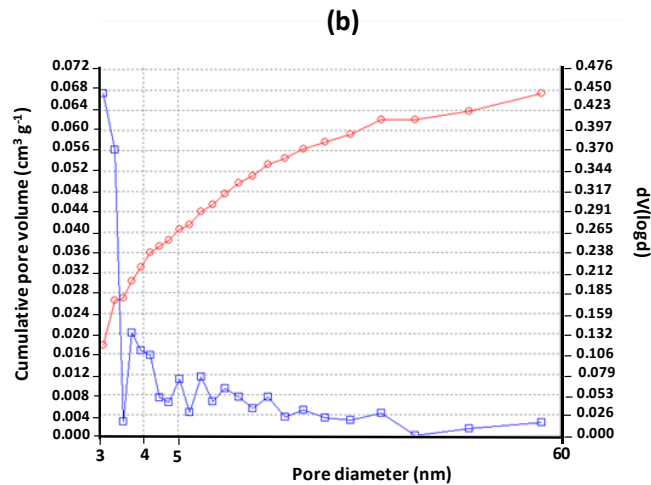


Figure 1: Pore size distribution of empty mesoporous silica particles (MSPs) (blue line) and cumulative pore volume (red line).

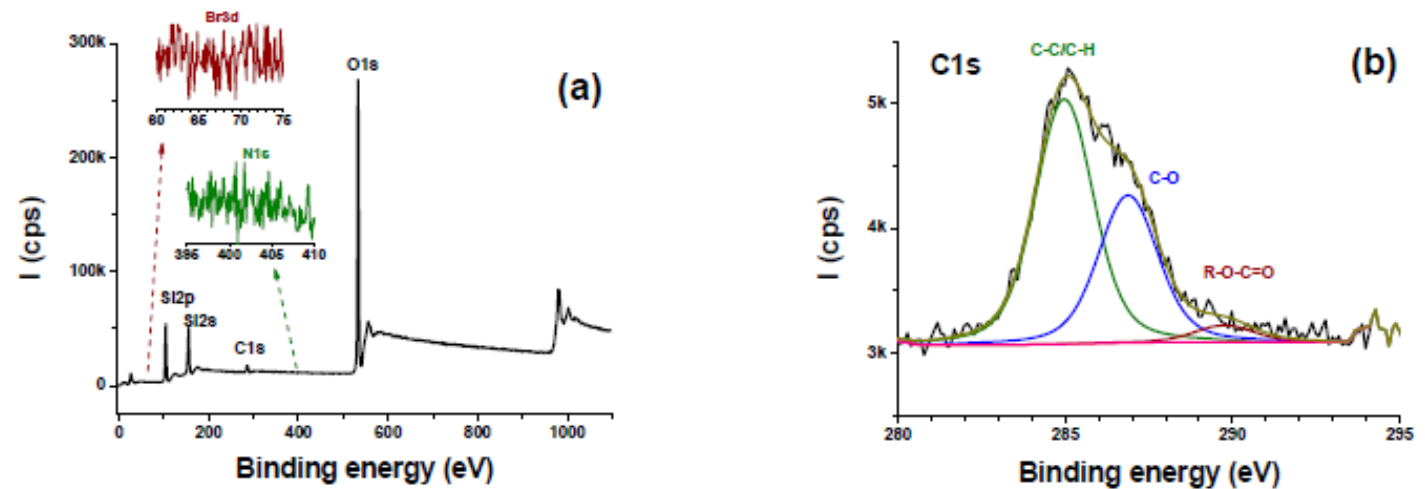
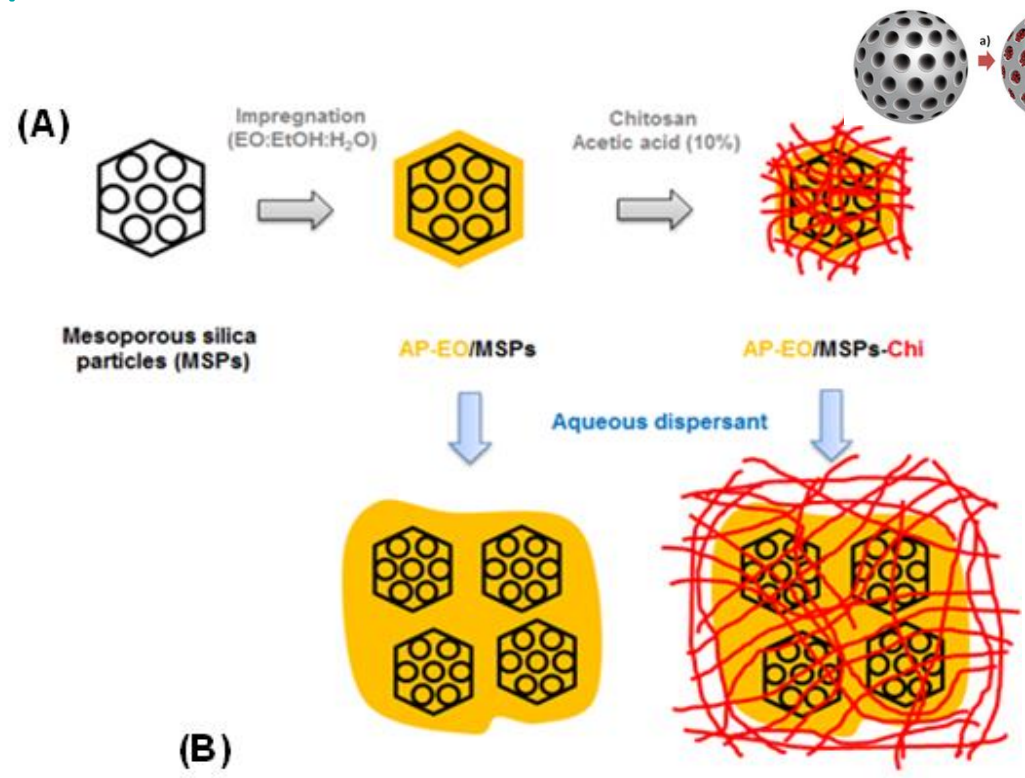


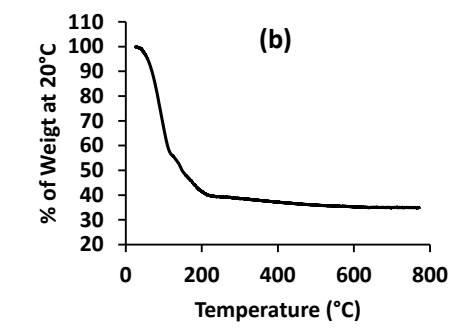
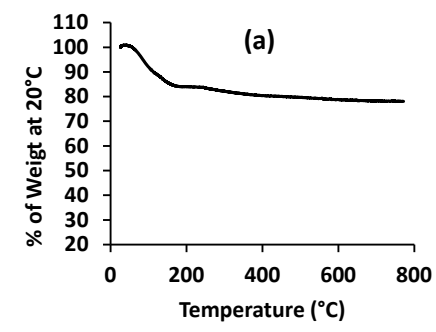
Figure 2. Survey (a), and peak-fitted C1s (b) spectra of MSPs. N1s (green) and Br3d (red) narrow regions are shown in inset of (a).

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

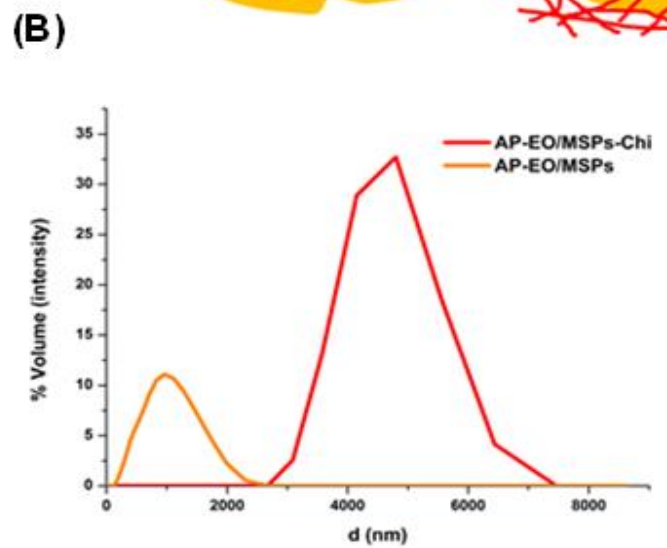
Encapsulation of the essential oil in mesoporous silica and coating with chitosan



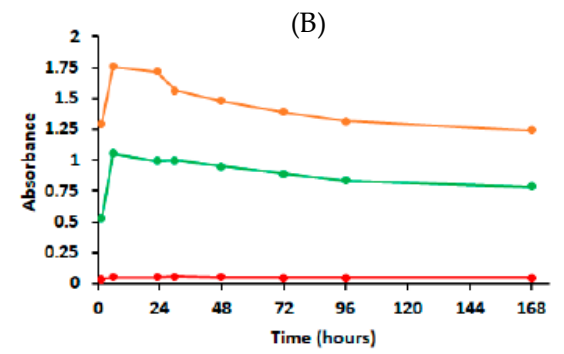
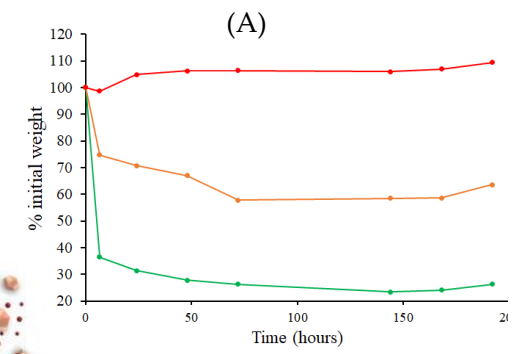
Yield of encapsulation of the EO = 45%



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

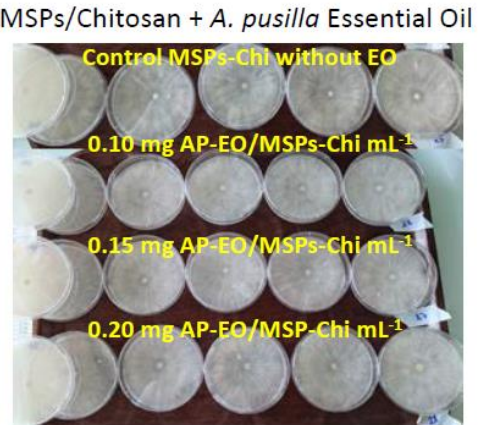
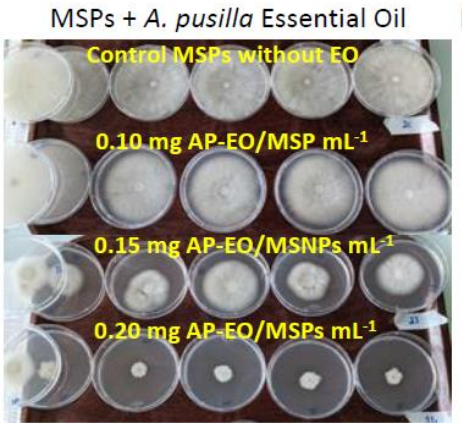
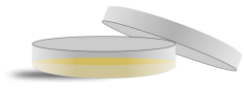


Release of *A. pusilla* EO in air (A) and in PBS buffer (B)

Encapsulation in MSP => slower release of EO

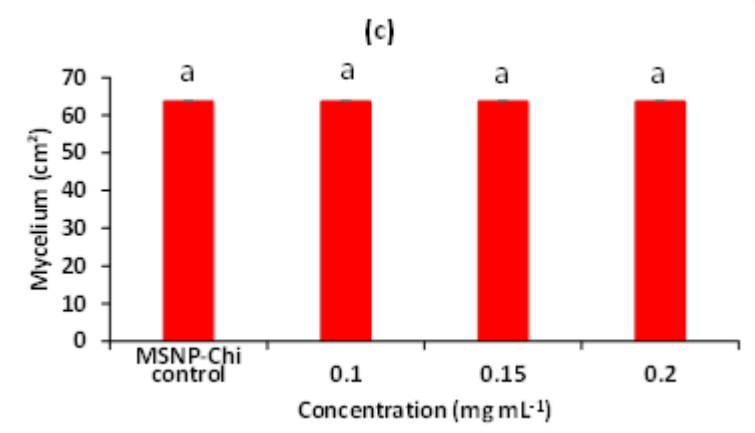
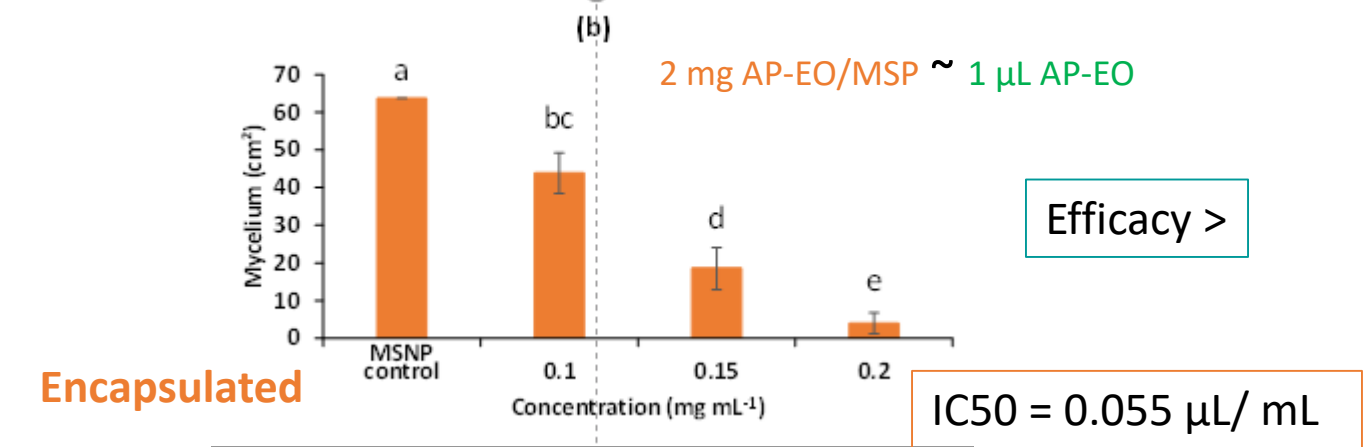
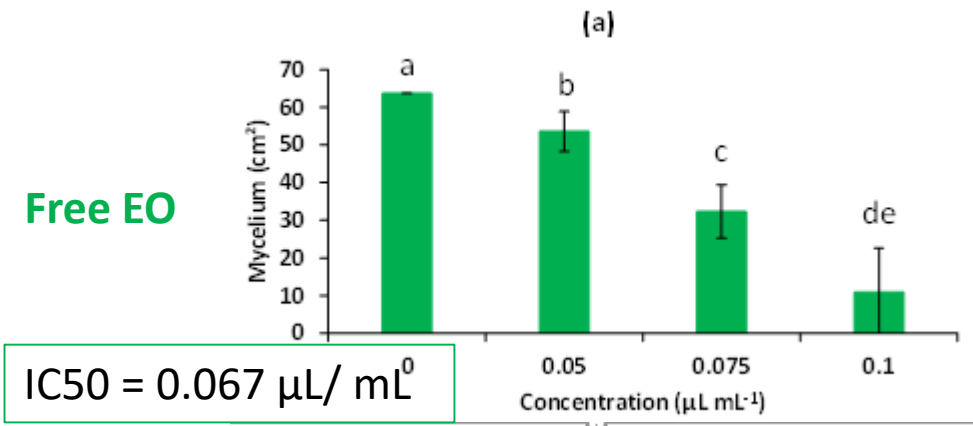
Coating with chitosan => protect from release of EO

Antifungal and antimycotoxin activities

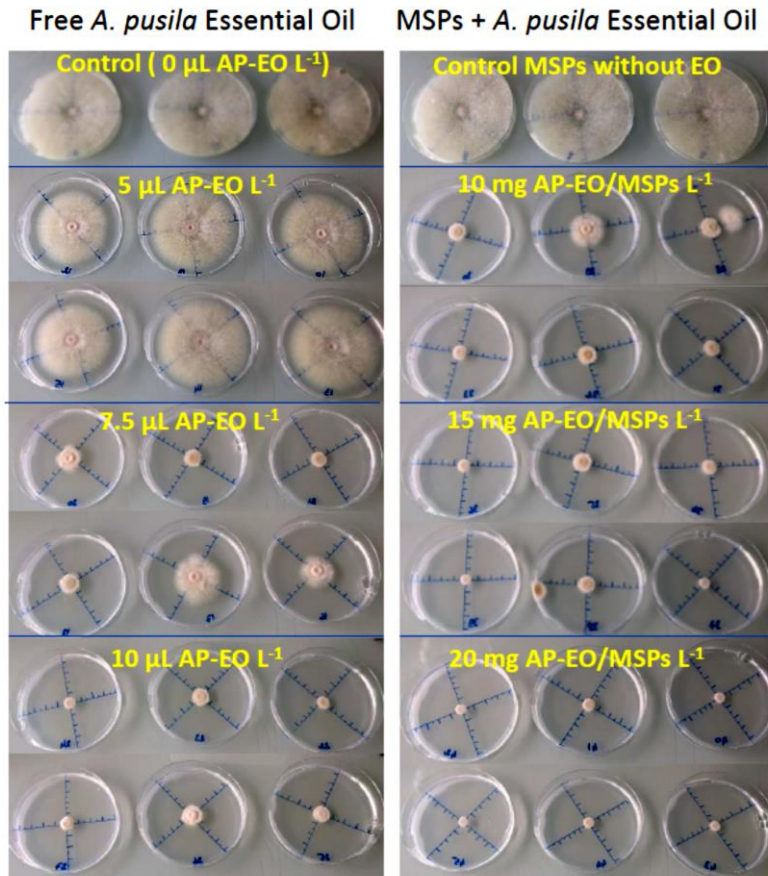
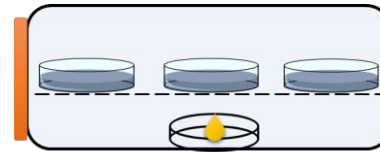


Effects of MSPs and AP-EO introduced into the culture medium on the mycelial growth of *Fusarium avenaceum*, incubated for 10 days at 25 °C.

Coated with Chitosan

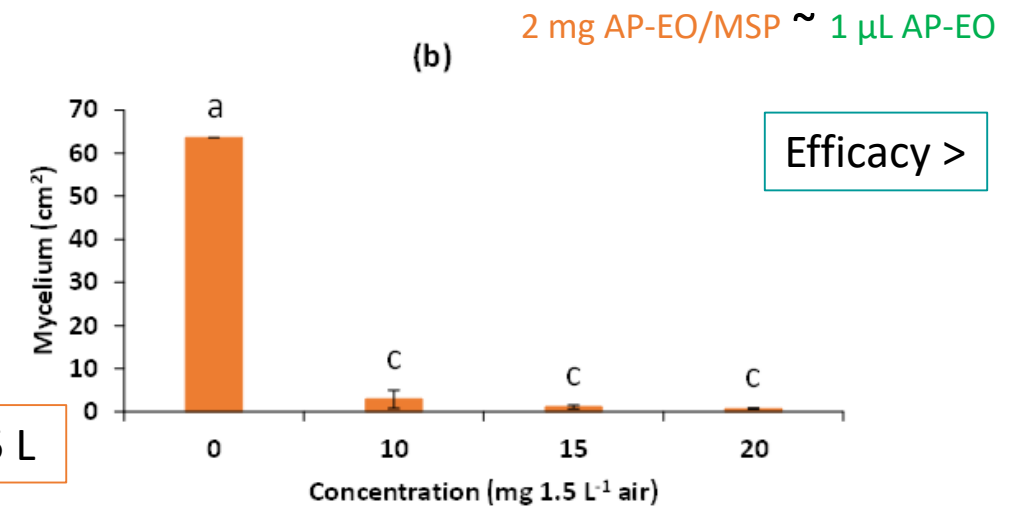
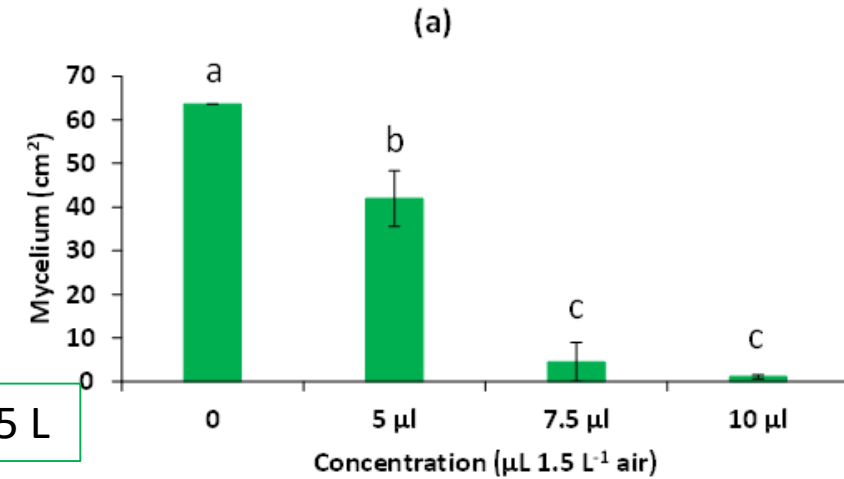


Antifungal and antimycotoxin activities



IC50 = 4.86 μL / flask 1.5 L

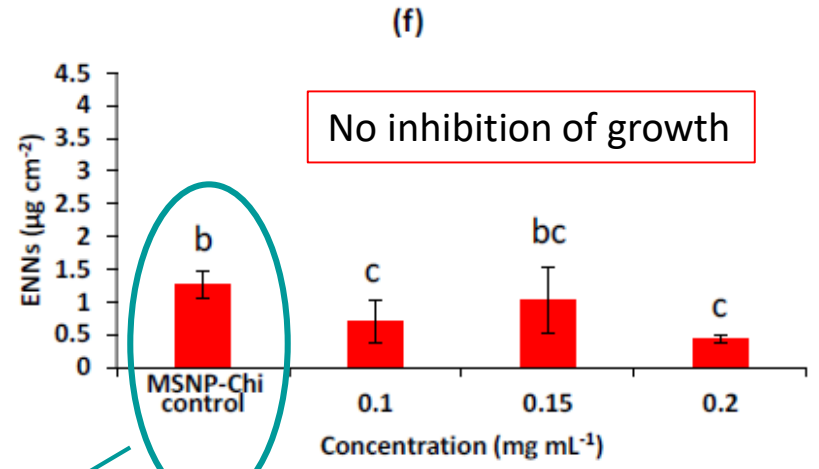
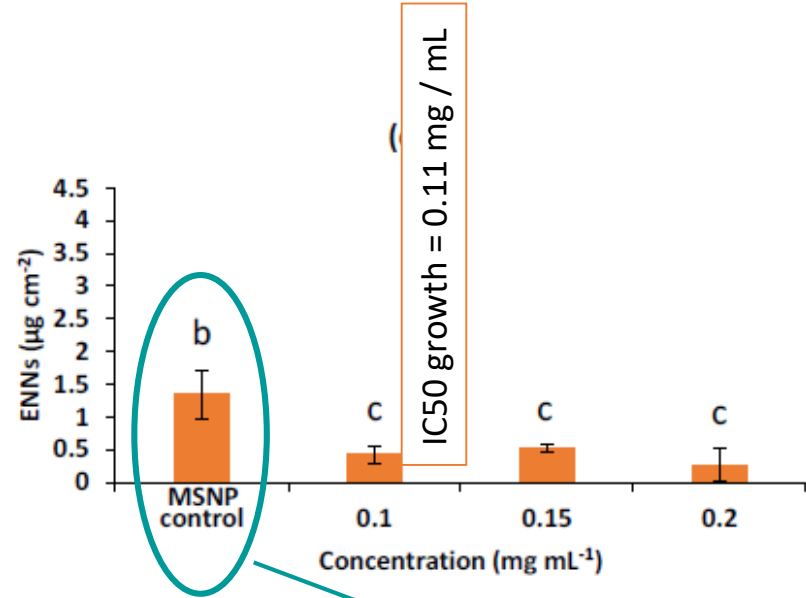
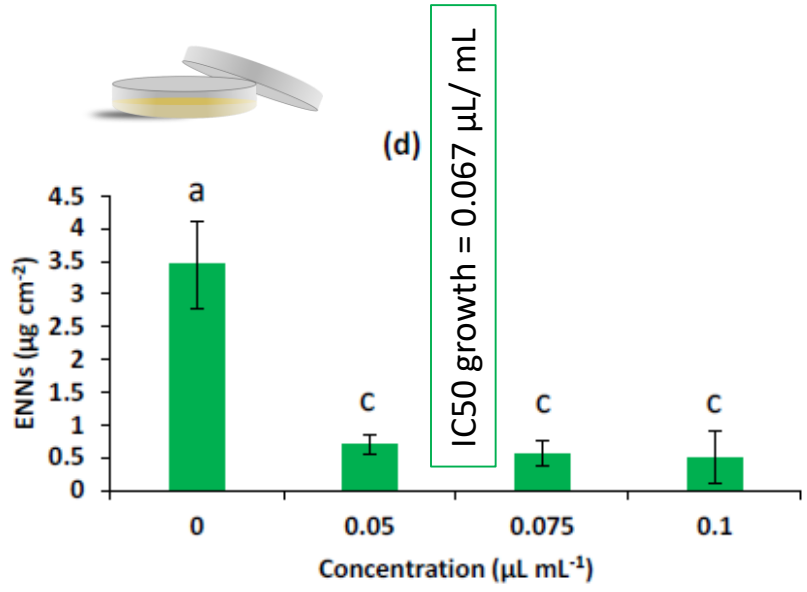
IC50 = 3.15 μL / flask 1.5 L



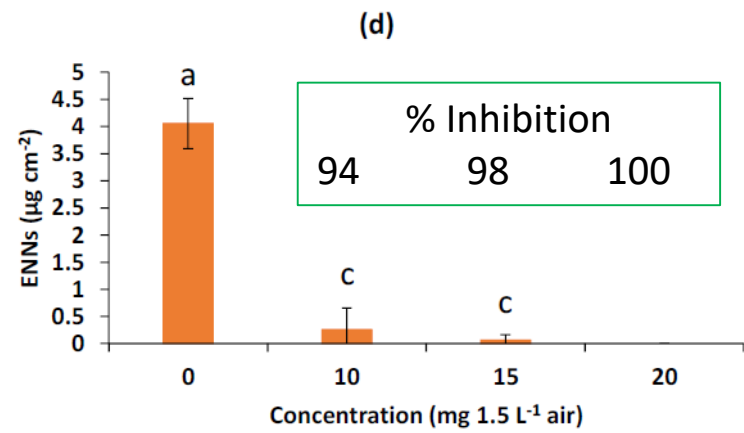
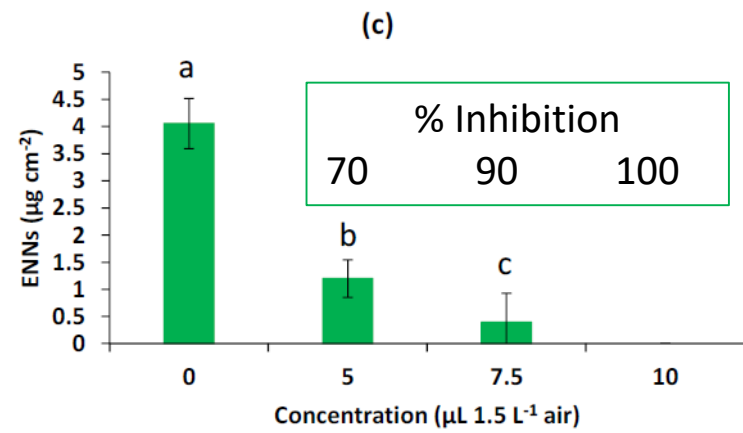
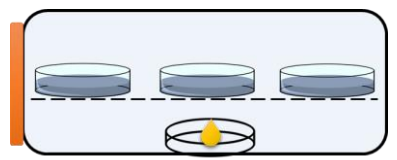
Effects of the **volatile compounds** released from AP-EO and AP-EO/MSPs on the mycelial growth of *Fusarium avenaceum* incubated for 10 days at 25 °C.

Antifungal and antimycotoxin activities

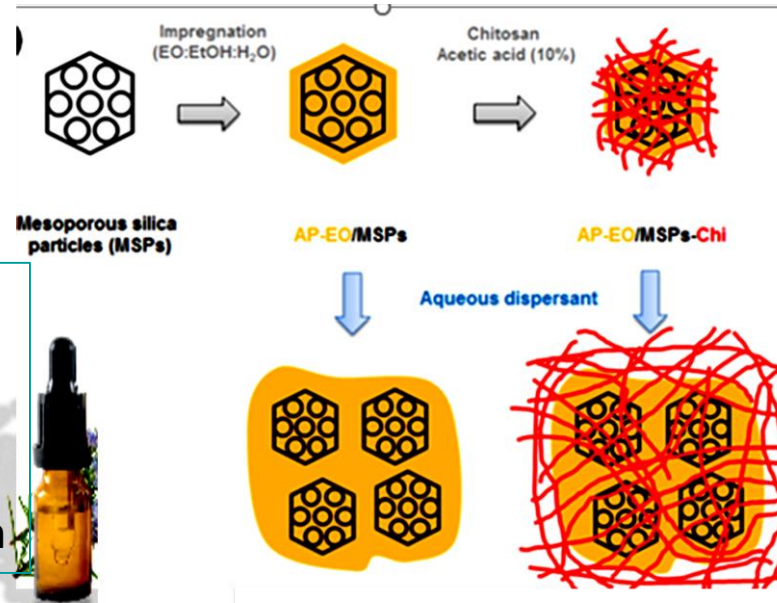
Enniatins concentration / Mycelial Biomass unit
(decreases = inhibition of the synthesis)



> 60 % inhibition



➤ Conclusion



Ammoides pusilla essential oil:

- Antifungal activity
- +
- Inhibition of Mycotoxin production



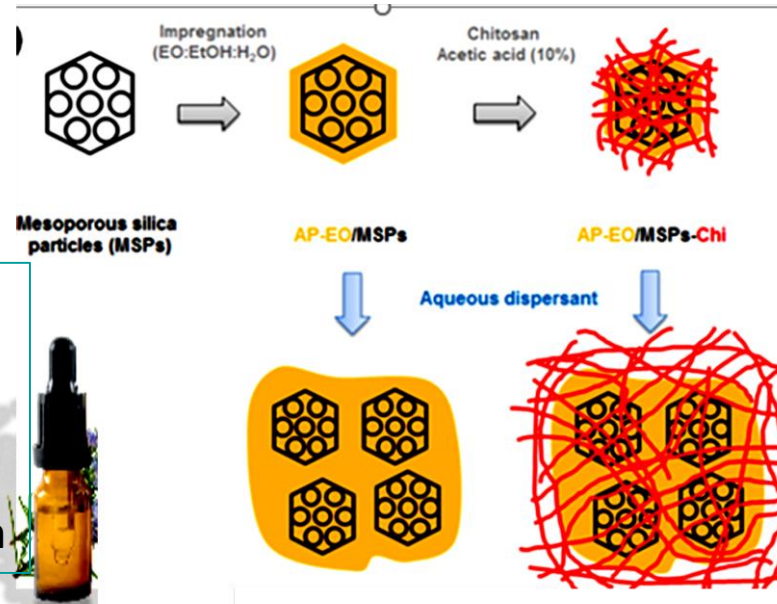
Encapsulation in MSPs

- 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

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

➤ Conclusion



Ammoides pusilla essential oil:

- Antifungal activity
- +
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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
FRANCE



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- Anne Goubet**
- Corine Grimaldi**
- Christophe Billette**

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