APPORTS D'UN INOCULUM MYCORHIZIEN DANS LE CADRE D'UNE FILIÈRE DE PHYTOMANAGEMENT DES SOLS CONTAMINÉS PAR DES ÉLÉMENTS TRACE MÉTALLIQUES

RAVEAU Robin – PhD Student

Under the supervision of LOUNES-HADJ SAHRAOUI Anissa & FONTAINE Joël

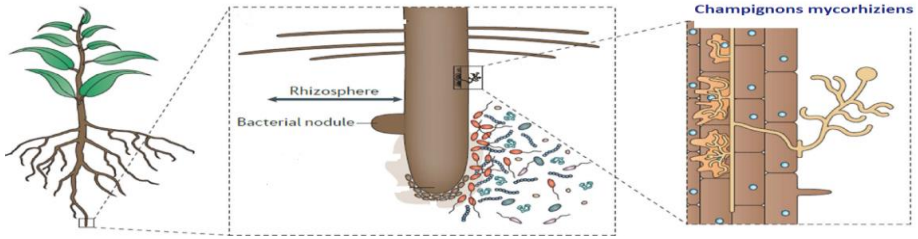
10/30/2019

Phytomanagement of contaminated areas

Aided phytostabilization channel at field scale

Use of natural plant ability and its associated soil microorganisms to stabilize inorganic pollutants

Biological Amendments **Arbuscular Mycorrhizal Fungi**



- Exploration of more important soil volume by the AMF
- Better water and mineral nutritions for the plant
- Better plant growth and stress tolerance
- Contribution to pollutant stabilization

+

Valorization of the biomass produced on polluted soils (essential oil production)

Chosen aromatic and medicinal plant species

Angelica (*Angelica archangelica*)

Clary Sage (*Salvia sclarea*)

Coriander (*Coriandrum sativum*)

Selection criteria

- Plant vegetation and growing cycle (annual / bisannual) – plant acclimatization – agricultural practices – harvested parts
- Phytostabilization potential
- Commercial potential in a non-food valorization channel (Seed prices and availability – EO selling price – market opportunities)

Project objectives

Valorization channel => non-food perspective - vegetable biomass produced on agricultural contaminated soils - combination of aided phytostabilization (aromatic plants) and essential oil production

Setting up of a large scale *in situ* assay

Growth monitoring : aromatic plant growth & development on contaminated soils

Amendment

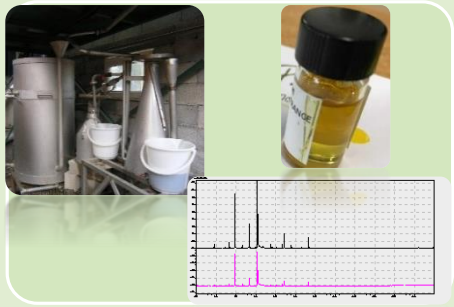
(biological)

contribution on :

- biomass and EO's yields

- MTE immobilisation

(INERIS collaboration)



- EO distillation

- EO chemical composition investigation

- EO quality, regarding MTE contamination (INERIS collaboration)

Biological properties assessment :

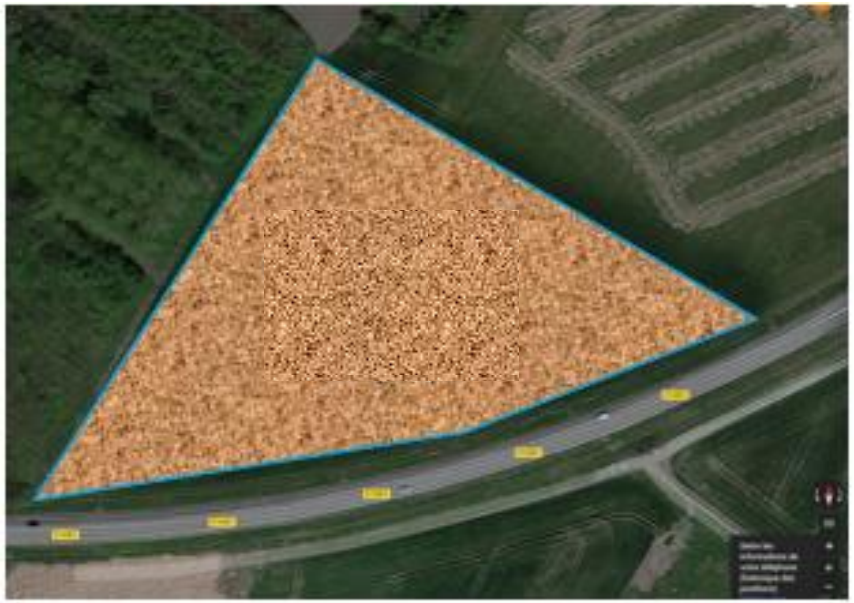
Antifungal, herbicidal anti-inflammatory, anti-oxydant

In situ experimental site : Metaleurop

Two experimental plots of 2 hectares :

Non-contaminated plot

TE-Contaminated plot



- **MTE contamination**

Pre-sowing sampling – Initial state

6th April 2017 on both experimental plots

- Characterisation of :
  - Physico-chemical parameters
  - Initial TE contamination assessment
    - ➢ Tracking of 9 MTE

Soil texture	Sandy clay loam
Cation-exchange capacity (me/kg)	100 - 200
pH	7,7 - 8
Organic matter (g/kg)	28 - 32,9
C/N ratio	7,3 - 10,7
CaCO3 total content (%)	0,3 - 0,9
Assimilable phosphorus (g/kg)	0,14 - 1
Exchangeable Potassium (g/kg)	0,26 - 0,27
Exchangeable Magnésium (g/kg)	0,13 - 0,31
Exchangeable Calcium (g/kg)	2,5 - 6,3
Exchangeable Sodium (g/kg)	0,02 - 0,03

High
Decent
Average
Low
Very low

- **Cd** 7,2 ppm – **Pb** 394 ppm – **Zn** 443 ppm

  Up to 10/15 times higher

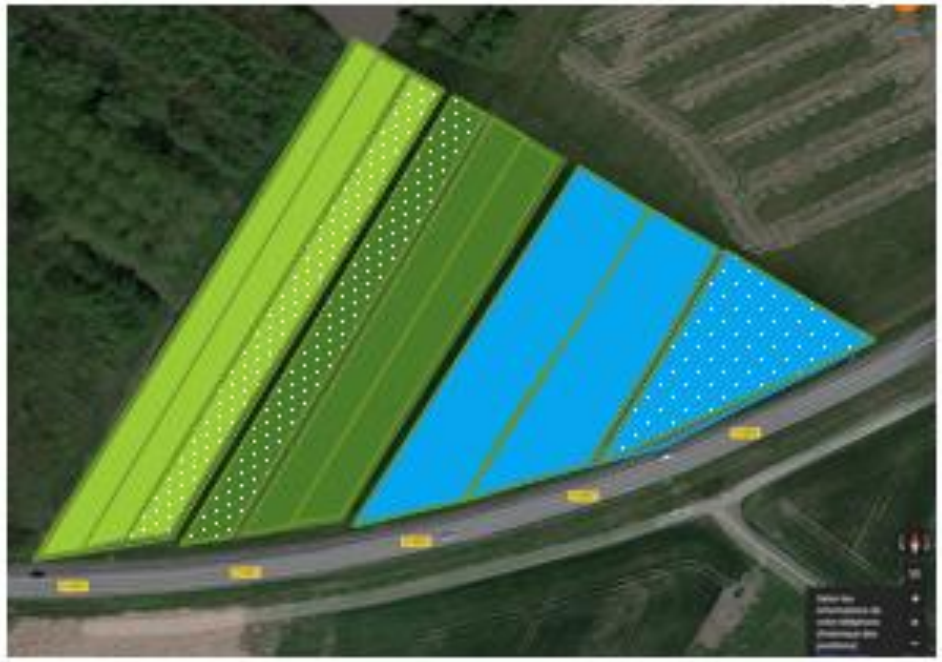
- Interpretation based on reference values given for a field crop scenario (Maize – Wheat)

In situ experimental design

2017 and 2018

Non-contaminated plot

Contaminated plot



Angelica Coriander Sage

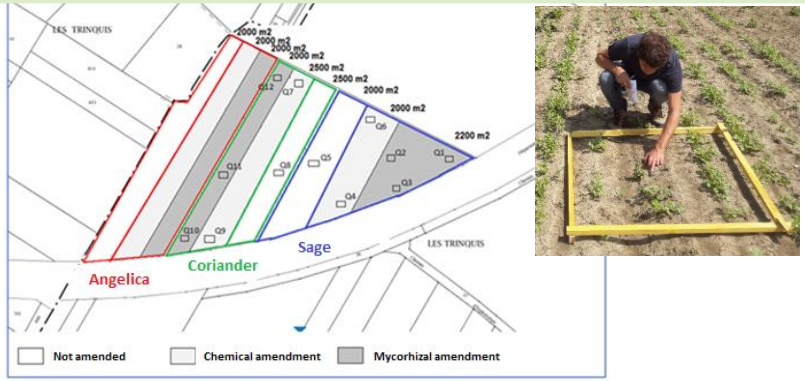
Not amended - control

Amended with a biological inoculum (mycorrhizal fungi)

Aromatic plants - sowing and growth monitoring

Plants sowing – April 2017 & 2018

Seedlings – emergence rates



Pre-harvest sampling (soil and plants)

Mycorrhizal inoculum

- AGTIV specialised crops, Premier Tech agriculture) – seed coating

- Inoculum decision criterion

  => tracking (molecular biology)

	Year	Plot	Amendment	Emergence rate (mean)	
Sage	2017	Non-contaminated (Rod)	Not amended	45 ± 6	b
			Amended	45 ± 10	ab
		Contaminated (Evin)	Not amended	71 ± 9	a
			Amended	65 ± 8	a

- Good emergence rates – higher ones on the contaminated plot
- No positive impact of the inoculation on measured ermergence rates

⇒ MTE analysis (soil & plant)

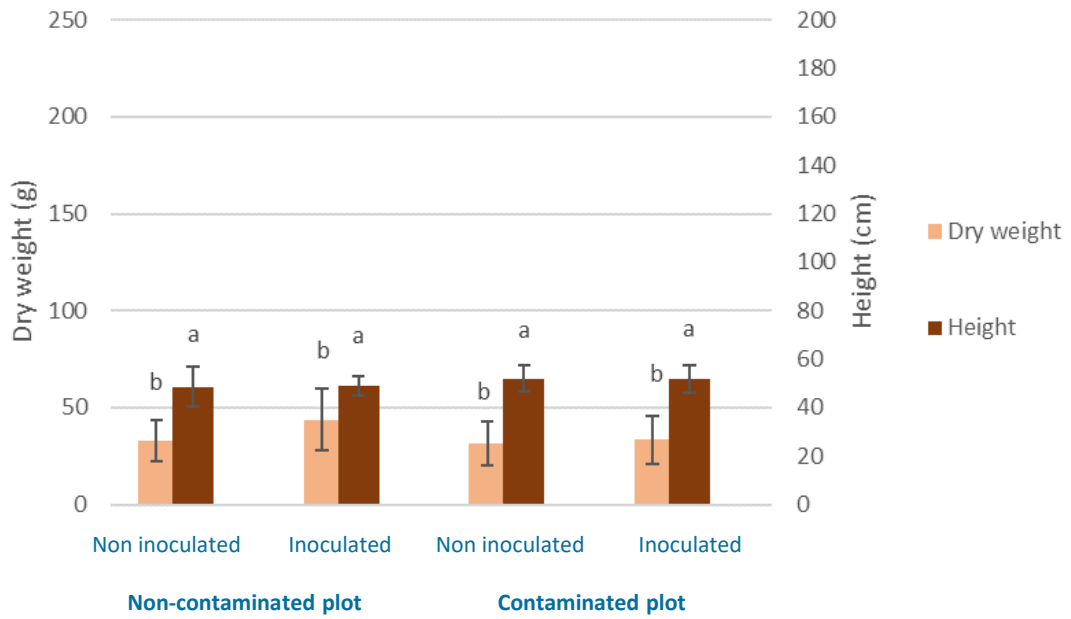
⇒ Biomass characterisation (dry weight – height)

⇒ Plant colonisation by AMF ?

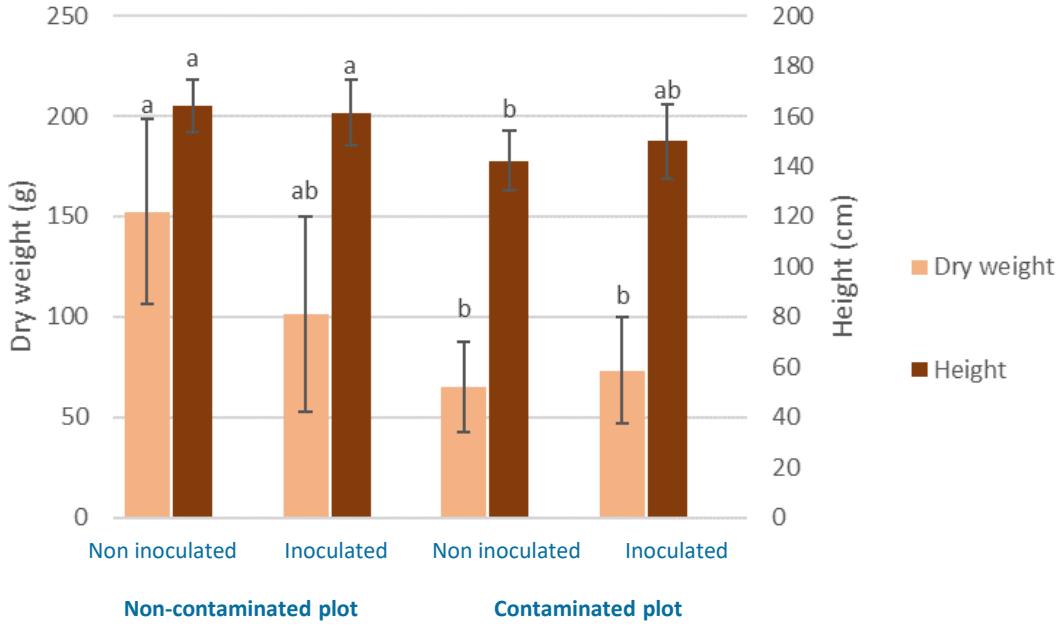
⇒ Inoculum tracking (soil and roots) - soil microbial communities monitoring

Mean height and dry weight of sage

2017



2018

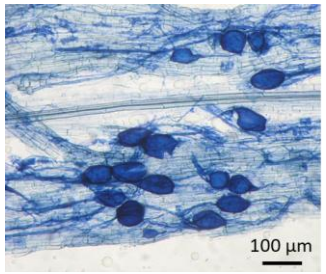


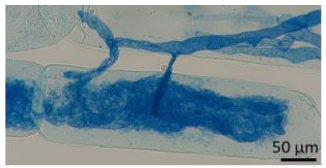
- Good plant growth

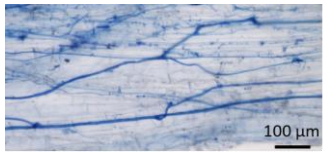
- No positive effect of the inoculation on sage's biomass

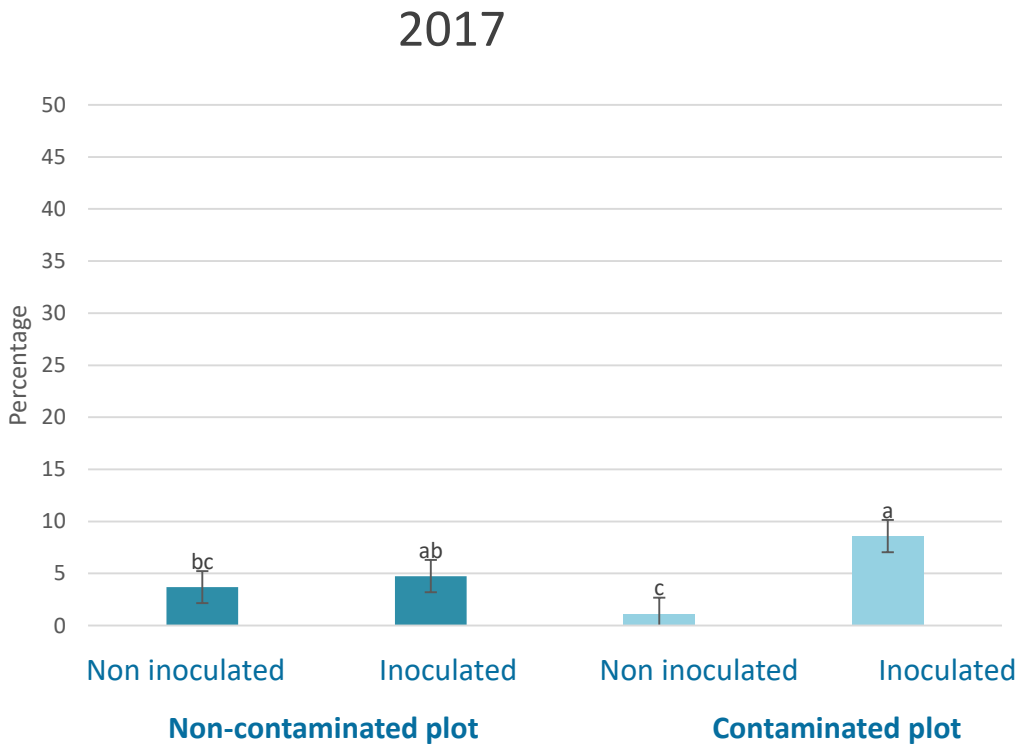
Statistical analysis : multiple comparison, Anova 1F with Bonferroni correction

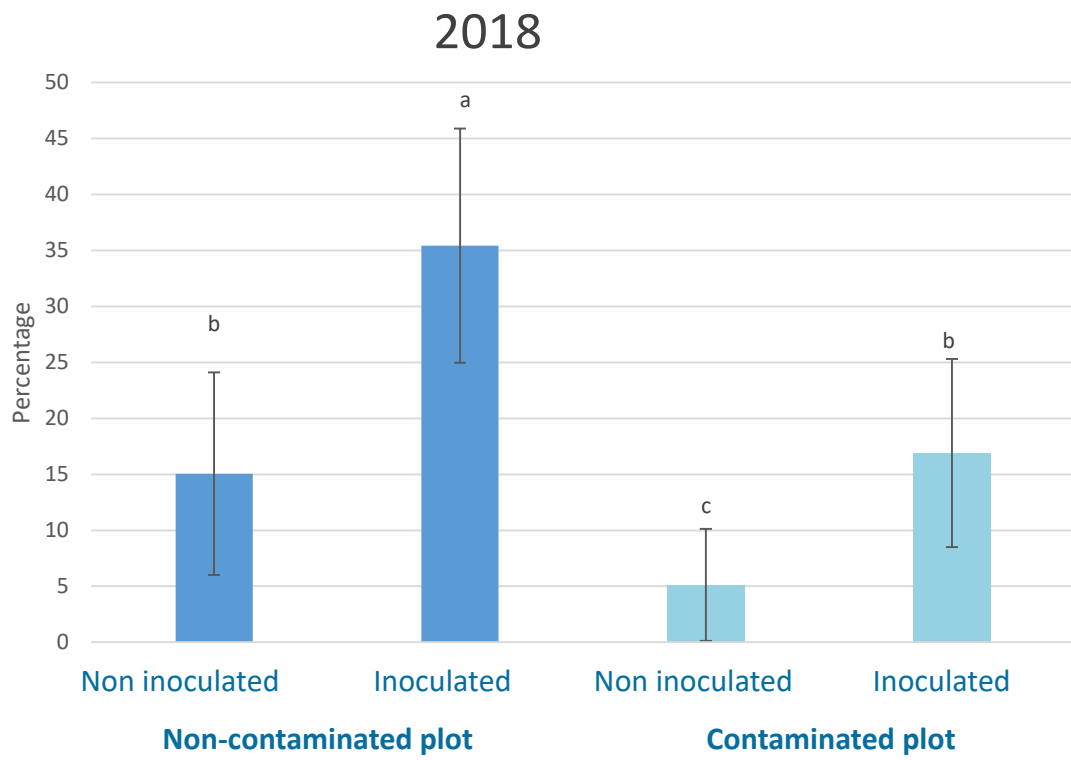
Sage mycorhizal rates











- No effect of soil contamination by MTE on sage mycorhization

- Significant effect of the inoculation on both plots

Statistical analysis : multiple comparison, Anova 1F with Bonferroni correction

Essential oil production - sage

		Non-contaminated (Rod)		Contaminated (Evin)	
		Non inoculated	Inoculated	Non inoculated	Inoculated
Sage 2018	Distillation yield (%)	0,022	0,020	0,036	0,021
	Essential oil amount (kg/ha)	12,9	20,3	33,7	24,2

- High yields on the second year of cultivation, satisfactory in comparison with reference yields obtained for a traditionnal sage cultivation (CRIEPPAM - ITEIPMAI)
  - ⇒ **Significant amount of biomass with mature flowered parts**
  - ⇒ **Appropriate date and harvesting method**

Metal Trace Elements monitoring

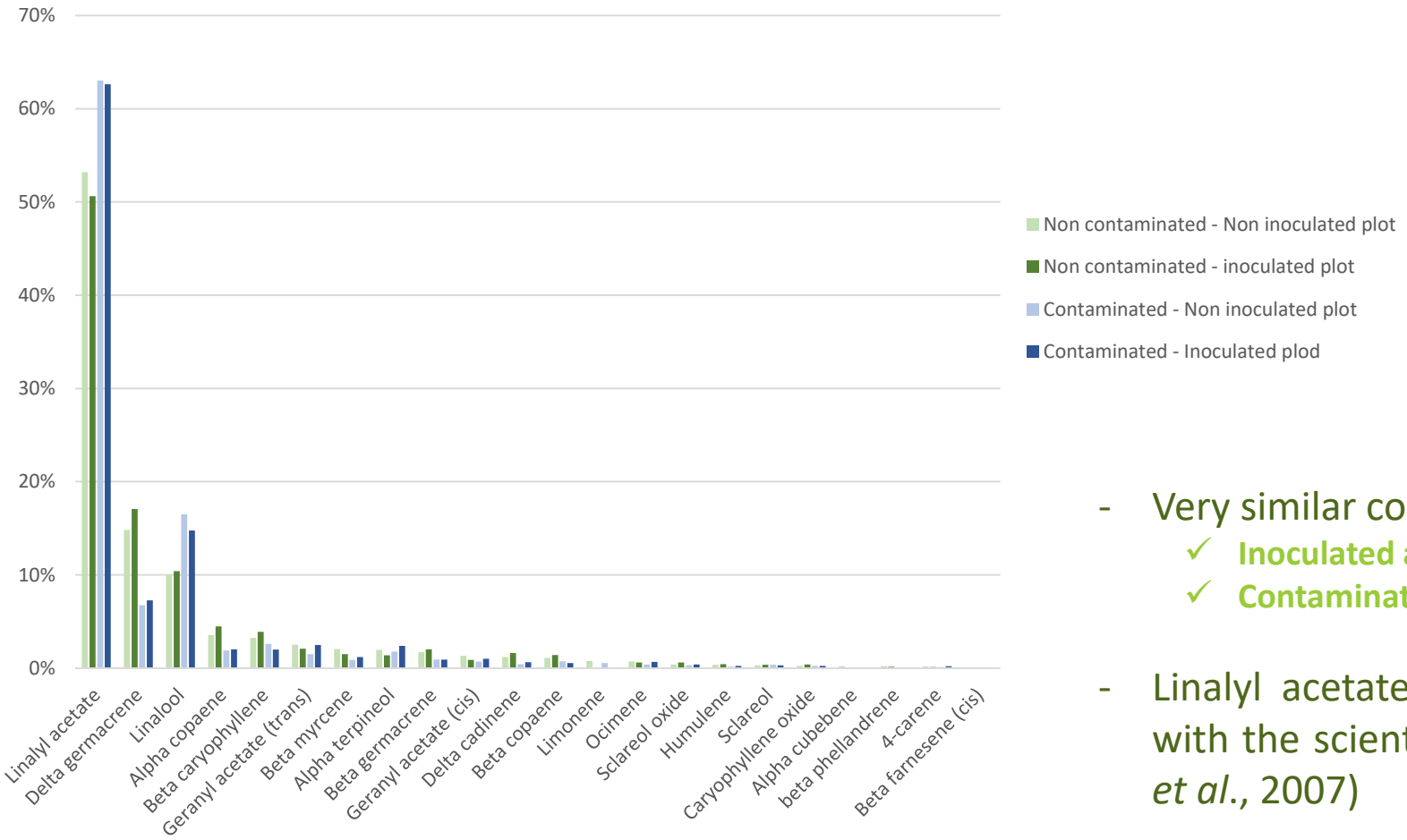
- MTE mobility - transfer in aerial parts ?
  - Significantly **higher** amounts of **lead** and **zinc** in comparison with the non-contaminated plot
  - Reduction of lead mobility on the sage inoculated plot
  - Significantly **smaller** amounts measured for the MTE detected in the biomass sampled on the inoculated plots

- Essential oils :
  - **No** MTE in essential oils

- Distillation residues :
  - Higher concentrations than in aerial parts

❑ **The efficiency of the phytostabilization channel is in process – MTE immobilization**

EO chemical composition analysis (in %) – sage 2018



- Very similar compositions between :
  - ✓ **Inoculated and non-inoculated conditions**
  - ✓ **Contaminated and non-contaminated plots**

- Linalyl acetate and linalool as major compounds, in line with the scientific literature (Aćimović *et al.*, 2018 ; Kuźma *et al.*, 2007)

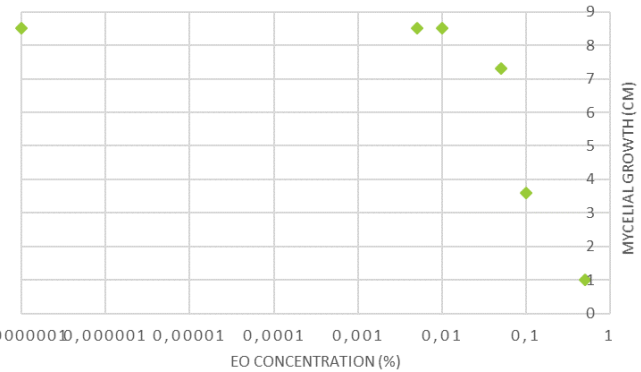
Antifungal assay

- **Phytopathogenic fungi :** *Fusarium culmorum* & *Zymoseptoria tritici*

- Antifungal effect investigated by direct contact or volatility assays (Chutia *et al.,* 2009 ; Huang *et al.,* 2010)
  - Concentrations ranging from 0,005 to 0,5% in the culture medium (PDA) or in liquid broth

⇒ Determination of an inhibition zone

⇒ IC_{50} value determination (graphical interpolation complemented with a statistical method - Sahmer *et al.,* 2014)



	IC_{50} ($g.L^{-1}$)	
Sage 2018	Non-contaminated plot, non inoculated	2.08 [a]
	Non-contaminated plot, inoculated	2.63 [a]
	Contaminated plot, non inoculated	1.47 [a]
	Contaminated plot, inoculated	1.54 [a]

Multiple comparison – ANOVA 1F

- Fungistatic effect demonstrated
- No significant difference in terms of IC_{50}
- Obtained IC_{50} are significantly higher than those commonly listed for other EO or chemicals (Ben Ghnaya *et al.,* 2016 ; Li *et al.,* 2017 ; Matusinski *et al.,* 2015 ; Terzi *et al.,* 2007)

Herbicidal assay

- **EO's effect evaluated on two plant species :** *Lactuca sativa* and *Lolium perenne*

- Herbicidal effect evaluated by direct contact, under controlled conditions
  - Day/night cycle (16h – 20°C / 8h – 16°C)
  - Non complemented agar medium

⇒ Determination of the inhibition of :
- Germination rate
- Root elongation

⇒ Estimation of IC_{50} values (graphical interpolation and statistical method – Sahmer *et al.,* 2014)

	IC_{50} ($g.L^{-1}$)	Rye-grass	Lettuce
Sage 2018	Non-contaminated plot, non inoculated	0.77 [a]	1.16 [a]
	Non-contaminated plot, inoculated	0.66 [a]	1.17 [a]
	Contaminated plot, non inoculated	0.50 [a]	0.54 [a]
	Contaminated plot, inoculated	0.34 [a]	0.82 [a]

- Herbicidal effect demonstrated
- No significant difference in terms of IC_{50}
- **References :** Judd *et al*., 2015; Lyu *et al*., 2018; Paul *et al*., 2001 ; Yazdanbakhsh and Fisahn, 2010

Conclusions

Plant development	• Very good development on contaminated soils • Satisfactory biomass yields & EO amounts
Inoculation benefits	• Improvement of the root colonisation by AMF • Reduction of the MTE mobility & transfer
EO composition and properties	• Faithful chemical composition • =>Linalyl acetate, germacrene and linalool • Fungistatic & herbicidal effects demonstrated
EO quality	• No essential oil contamination by MTE

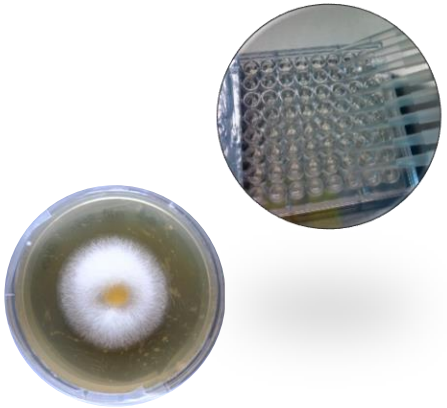
➢ Market potential for the EO, considering their biological properties and MTE absence

➢ Valorization opportunity in a phytomanagement approach

Perspectives

- Carry out studies, to evaluate :
  - The social acceptability of the channel
  - The techno-economical feasibility

- Investigate other biological properties :
  - Antifungal (against other phytopathogenic fungi), insecticidal, anti-oxydative and anti-inflammatory properties
  - Carry out *in planta* antifungal assays



- Consider the future valorization of distillation residues - identify and set up the potential recovery methods (anaerobic digestion, combustion, composting)

Thank you for your attention

UCEIV

Anissa LOUNES-HADJ SAHRAOUI, Joël FONTAINE, Anthony VERDIN, Sophie FOURMENTIN

Partners

Valérie BERT, Hervé FLANQUART , Patrice FERRANT

Funders

**Contact : Robin Raveau*

robin.raveau@univ-littoral.fr

Project Website: https://phyteo.univ-littoral.fr