Assessment of six essential oils for their antifungal activity against the wheat pathogen *Zymoseptoria tritici*

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Team : UMRt BioEcoAgro, team specialized metabolites

GxABT PhD Symposium

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JUNIA Grande école d'ingénieurs

LIÈGE université Gembloux Agro-Bio Tech

BioEcoAgro

(Credit: Kallerna)

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Introduction

Context

- Wheat is one of the most cultivated cereals
  - ➢ 799 million tons in 2023 (FAOSTAT., 2023)

- *Poaceae* family
  - ➢ *Triticum aestivum* (6n), 21 chromosomes

- Crop threatened by biotic stressors
  - ➢ **Septoria tritici blotch**

(Credit: ©SCHANKZ - STOCK.ADOBE.COM

Septoria tritici blotch

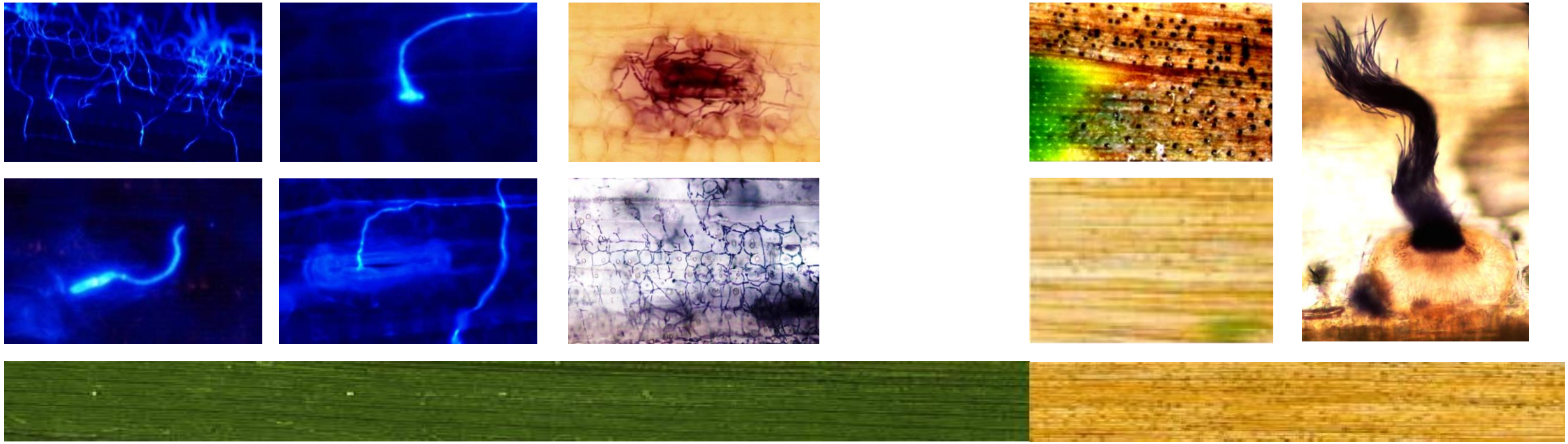
- *Zymoseptoria tritici*
  - Ascomycete
  - Hemibiotroph

- 21 chromosomes (Goodwin *et al*. 2011)
  - 13 core chromosomes
  - 8 dispensable chromosomes
  - Rich in transposable elements

- Asexual multiplication
  - Pycnidiospores
- Sexual reproduction
  - Ascospores

(Credit: Steinberg 2015)

Disease cycle



1st day

14th day

21st day

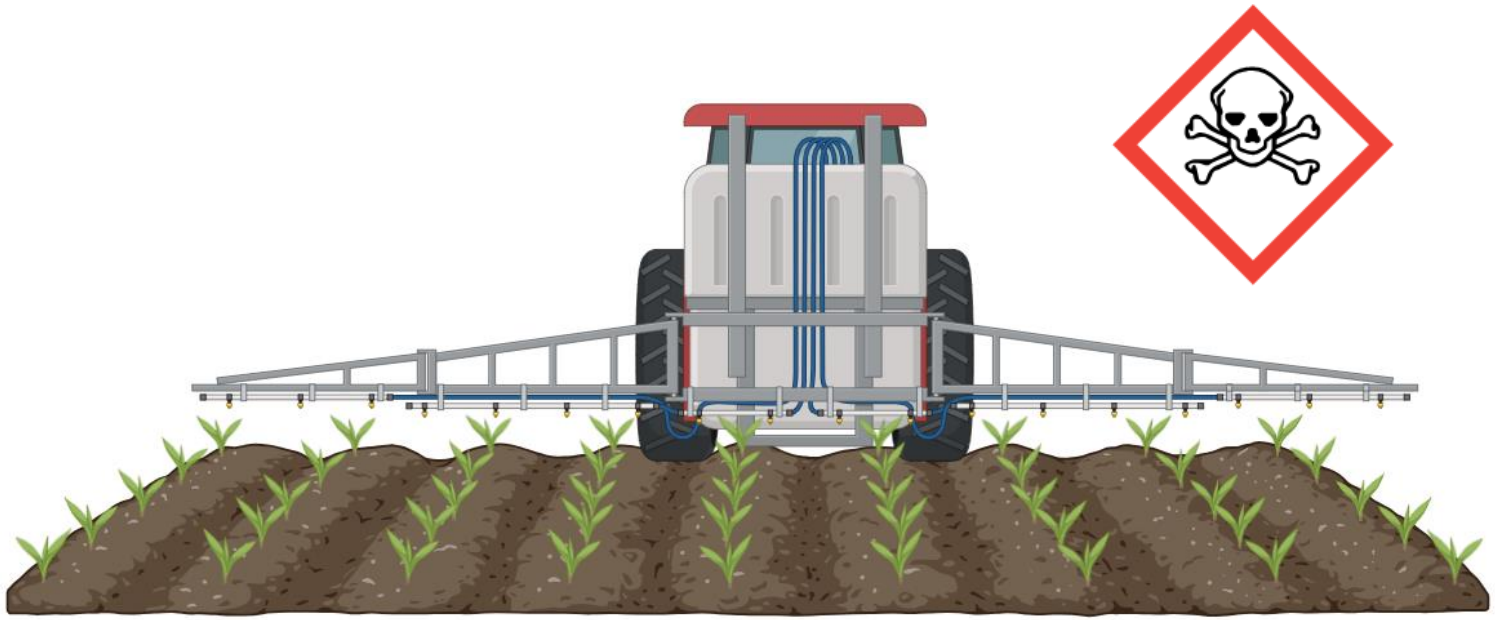
Biotroph phase
(≈ 2 weeks)

Necrotroph phase
(≈ 1 weeks)

Siah *et al.* (2010)
Siah et al. (unpublished)

Disease management

Conventional fungicides



- Harmful for the environment
- Fungicide resistance

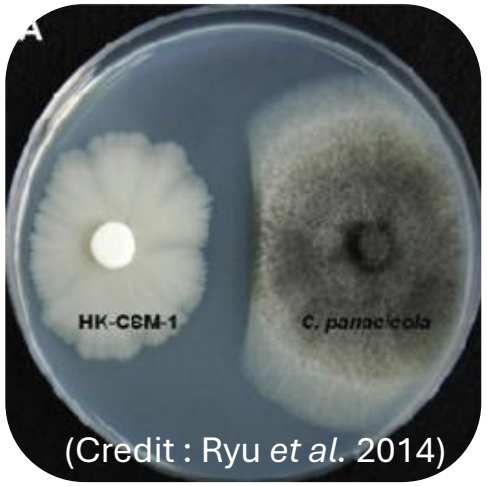
Biocontrol

Biocontrol

Macroorganisms

(Credit : Sciences et avenir.fr)

Microorganisms



(Credit : Ryu *et al.* 2014)

Pheromones and kairomones

(Credit : BASF.fr)

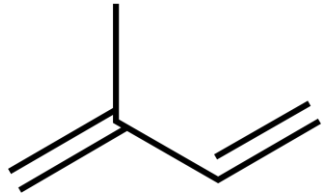
Natural substances

(Credit : Insphy.com)

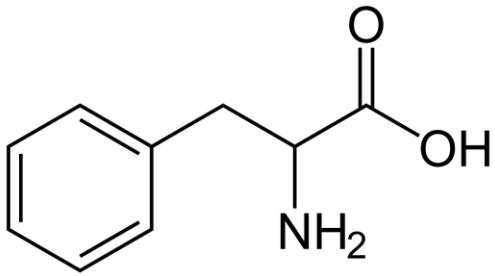
Essential oils

Essential oils

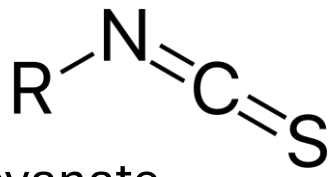
- Specialized metabolites
  - ➢ Interaction between the plant and the environment
- Rich in antimicrobial compounds (Sadgrove et al. 2022)
  - ➢ **Terpenes**
    - ➢ Involved in symbiotic relationships and the defense against bioagressors (Gershenzon & Dudareva 2007)
    - Synthesized through the mevalonate and MEP pathways
  - ➢ **Phenylpropanoids**
    - ➢ Involved in symbiotic relationships as well as the structure and reproduction of plants (Dong & Lin 2020)
    - Synthesized through the shikimate pathway
  - ➢ Isothiocyanates
    - 3 synthesis pathways
- ➢ **Potential biocontrol products (Raveau *et al.* 2020)**



Isoprene



Phenylalanine



Isothiocyanate

Reseach objectives

Research progress

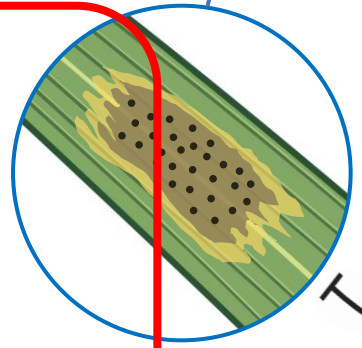
Direct effect

Growth inhibition

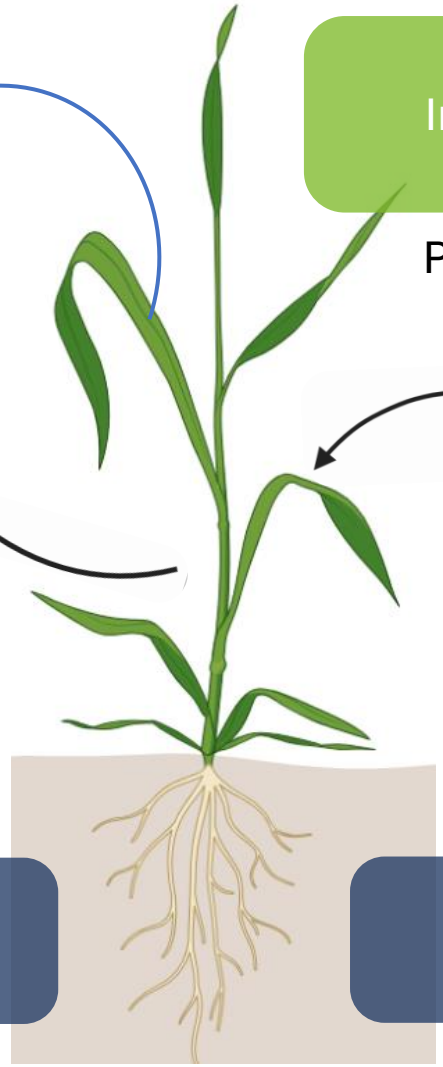
Indirect effect

Plant defense induction

Essential oils



Septoria tritici blotch



In vitro

In planta

In planta

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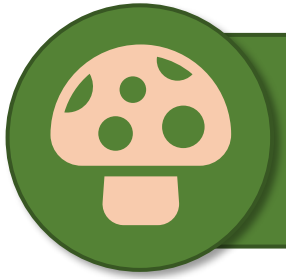
Methodology & Results

Experimental material



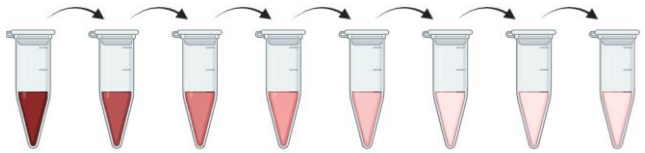
Zymoseptoria tritici strain T02596

Triticum aestivum
Susceptible variety : Alixan

Six different essential oils
EO1, EO2, EO3, EO4, EO5, EO6

Antifungal activity : Methodology

- In liquid medium
  - ➢ Glucose peptone



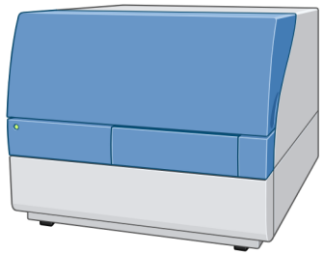
8 EO concentrations (mg.L^{-1})
(DMSO 0,5%)

140µL EO emulsion per well

+

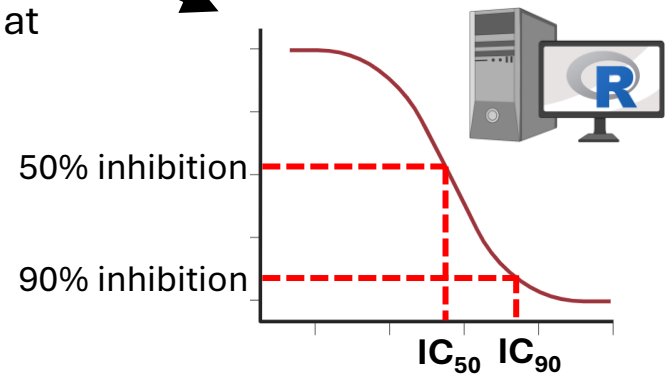
60µL *Z. tritici* spore suspension
(5.10^4 spores.L^{-1}) per well

Incubation of the plates for 10 days in darkness



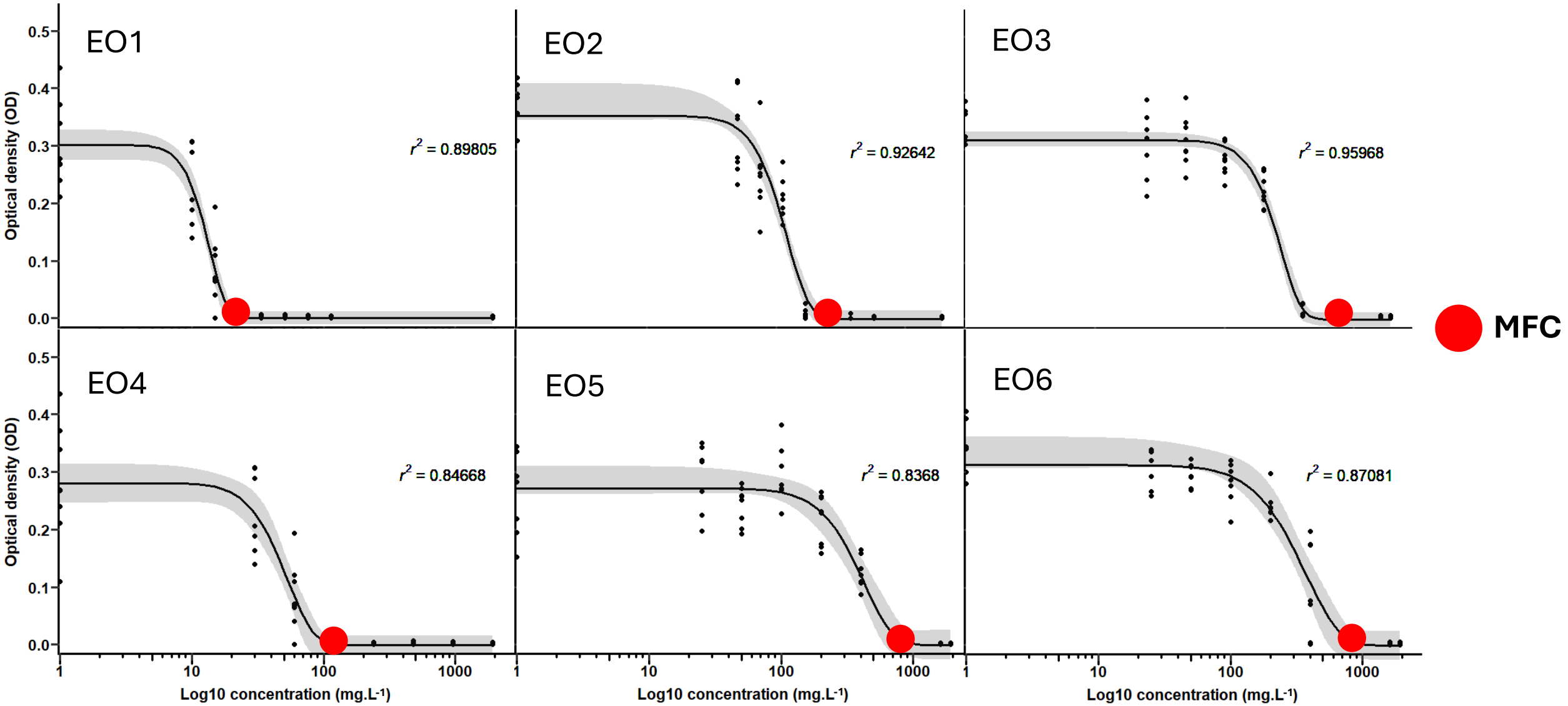
Optical density (OD) read at 405nm

Mycelium morphology
Optical microscopy
cotton blue dye

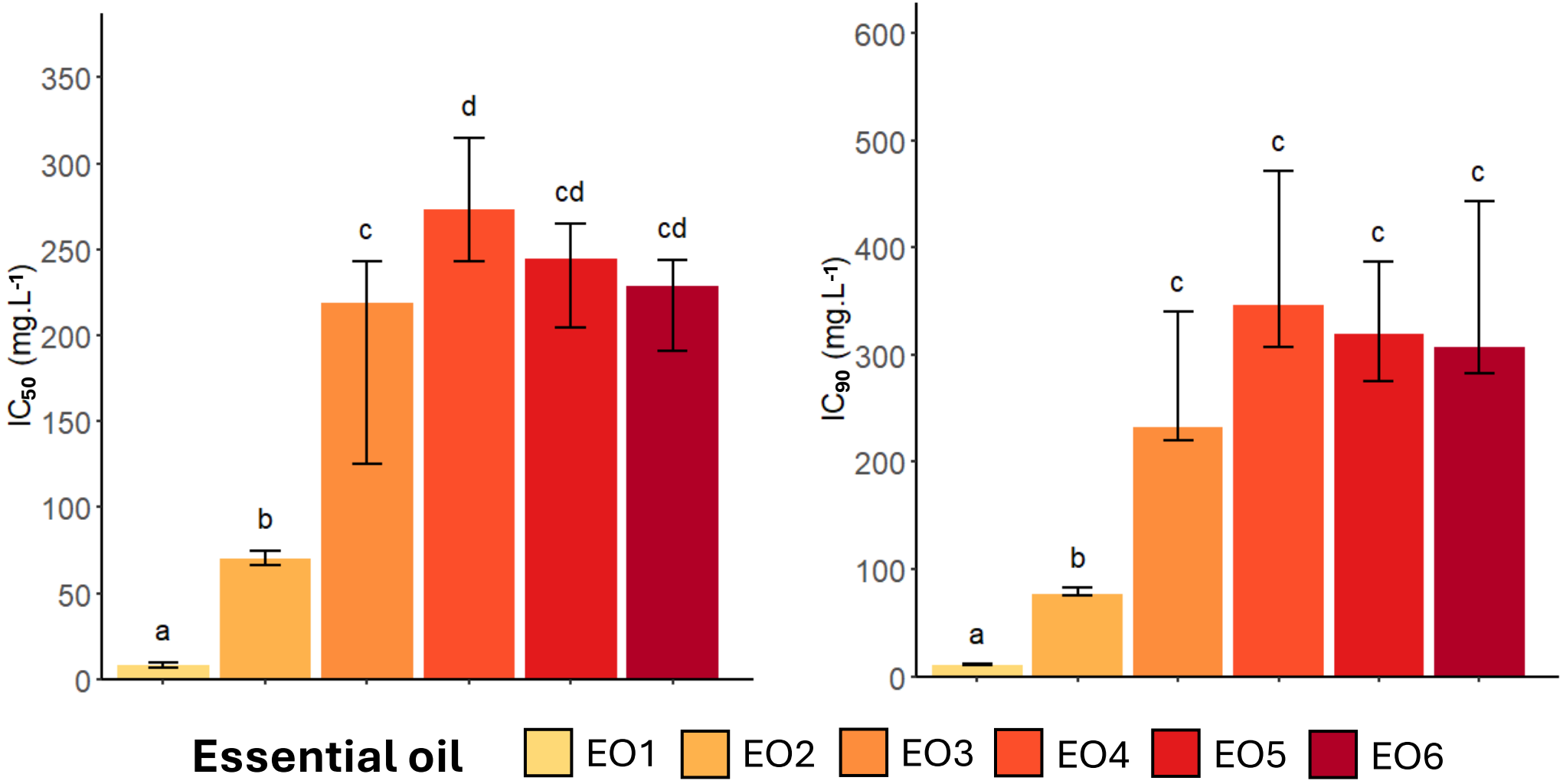


Dose response curves
(Ritz *et al.* 2015)

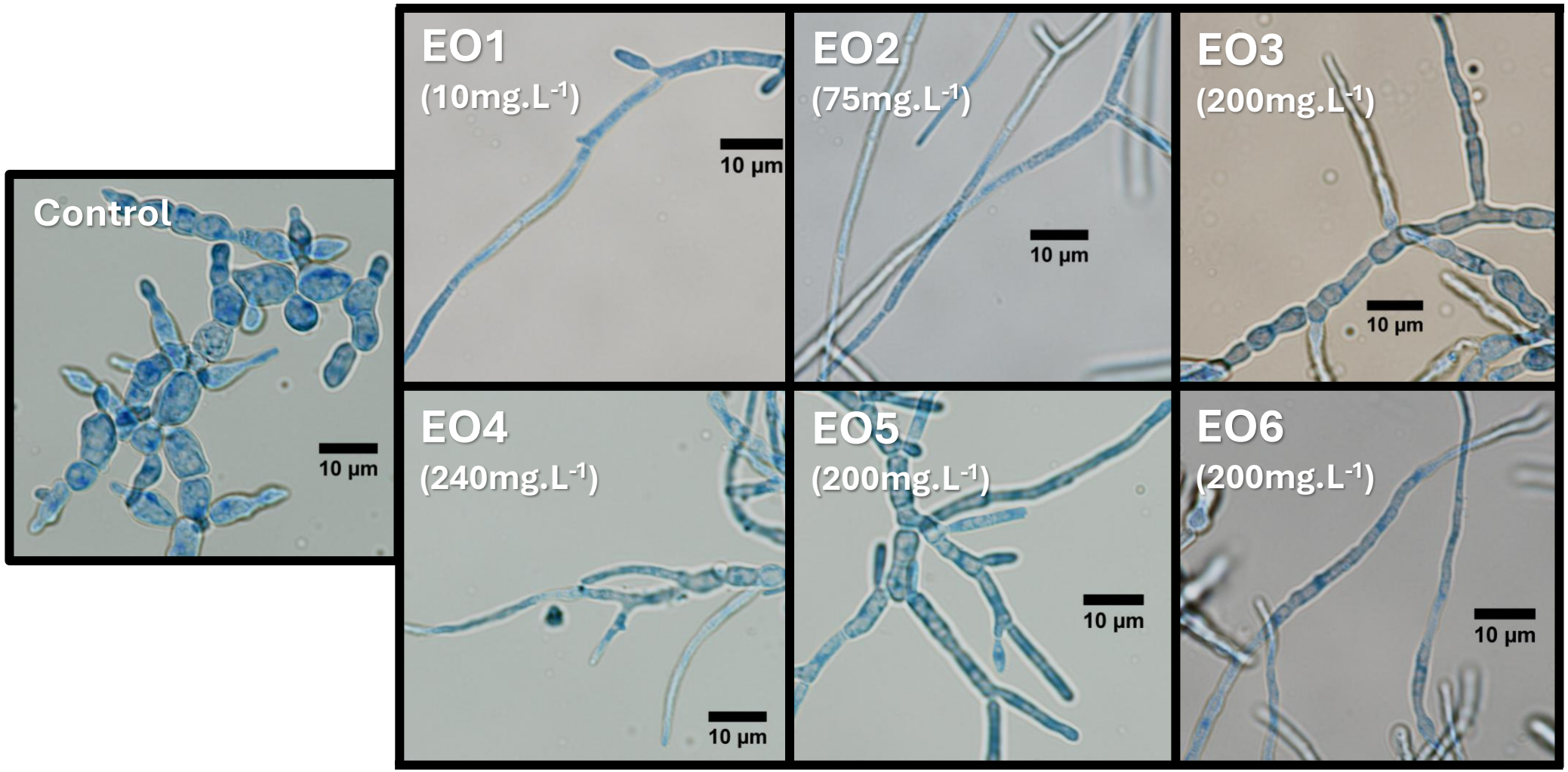
Antifungal activity : Dose response curves



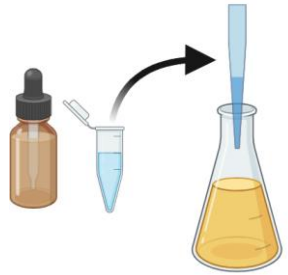
Antifungal activity : Results



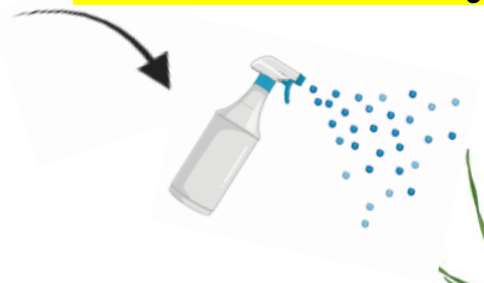
Antifungal activity : Results

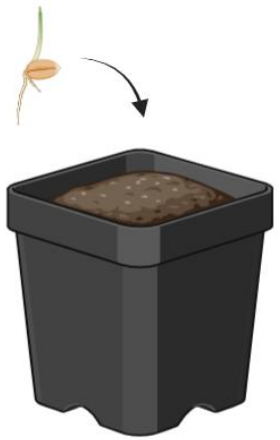


Phytotoxicity assessement : Methodology



Preparation of essential oil emulsions in Tween 20 (0,1%) at 4 concentrations **(1x, 5x, 10x, 20x IC_{90})**





Sowing of 12 pre-germinated seeds

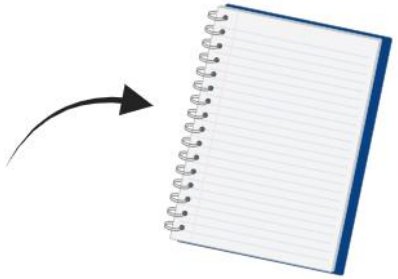
3 weeks of growth in semi-controlled conditions

EO treatment

1 week of growth in semi-controlled conditions

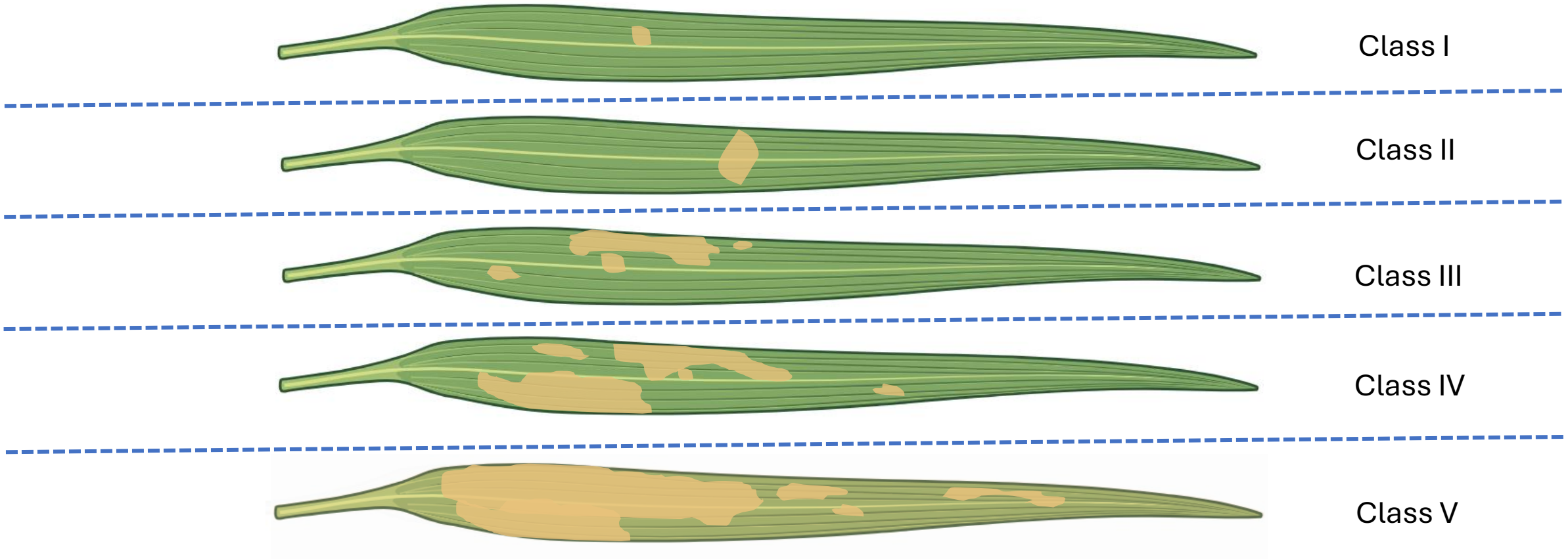
Results

-Number of plants showing phytotoxicity (incidence)
-Percentage of leaf area with phytotoxicity (intensity)

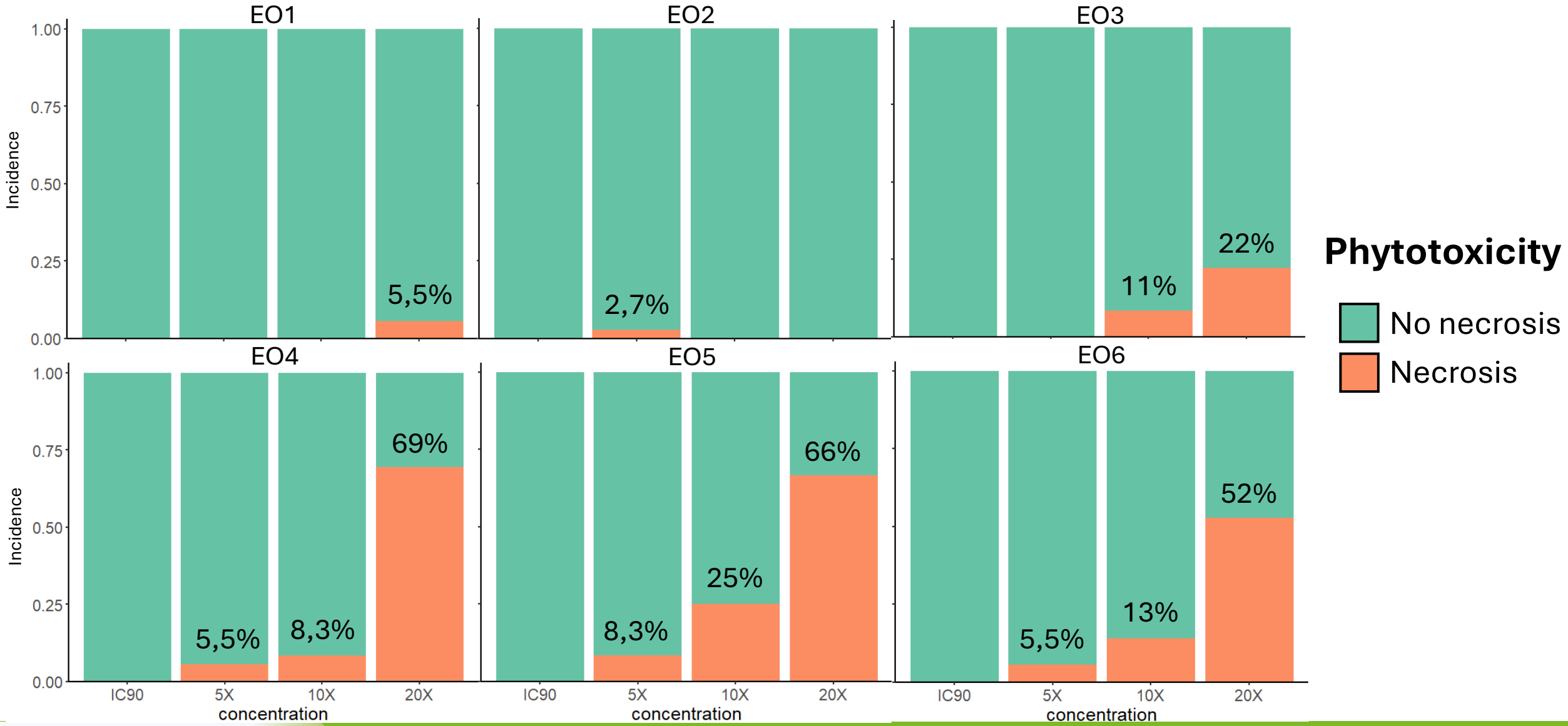


Phytotoxicity assessement : Methodology

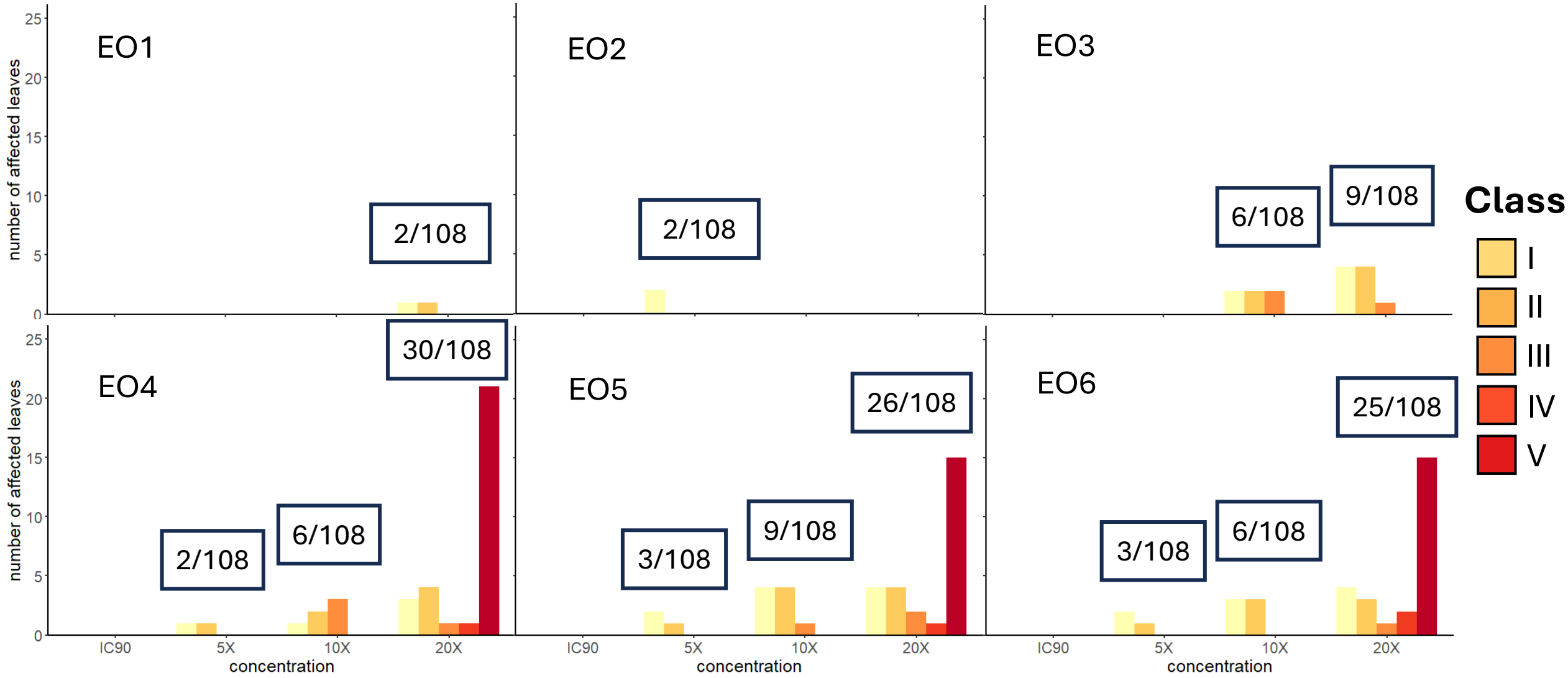
- Phytotoxicity index



Phytotoxicity assessement : Incidence results



Phytotoxicity assessement : Intensity results



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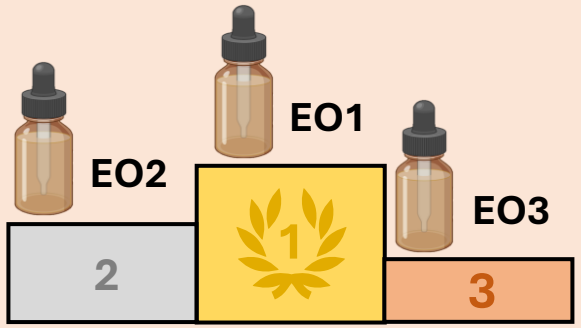
Conclusion and perspectives

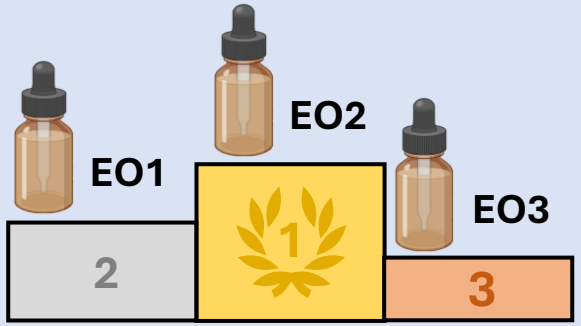
Conclusion

In vitro



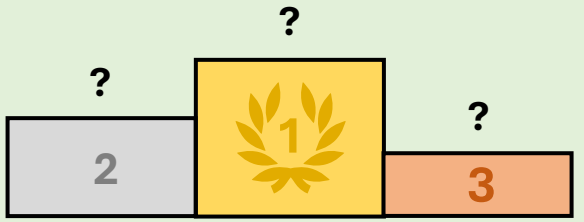
Identification of EOs with most promising antifungal activities

In planta phytotoxicity



Determination of non-phytotoxic concentration for each EO

In planta protection

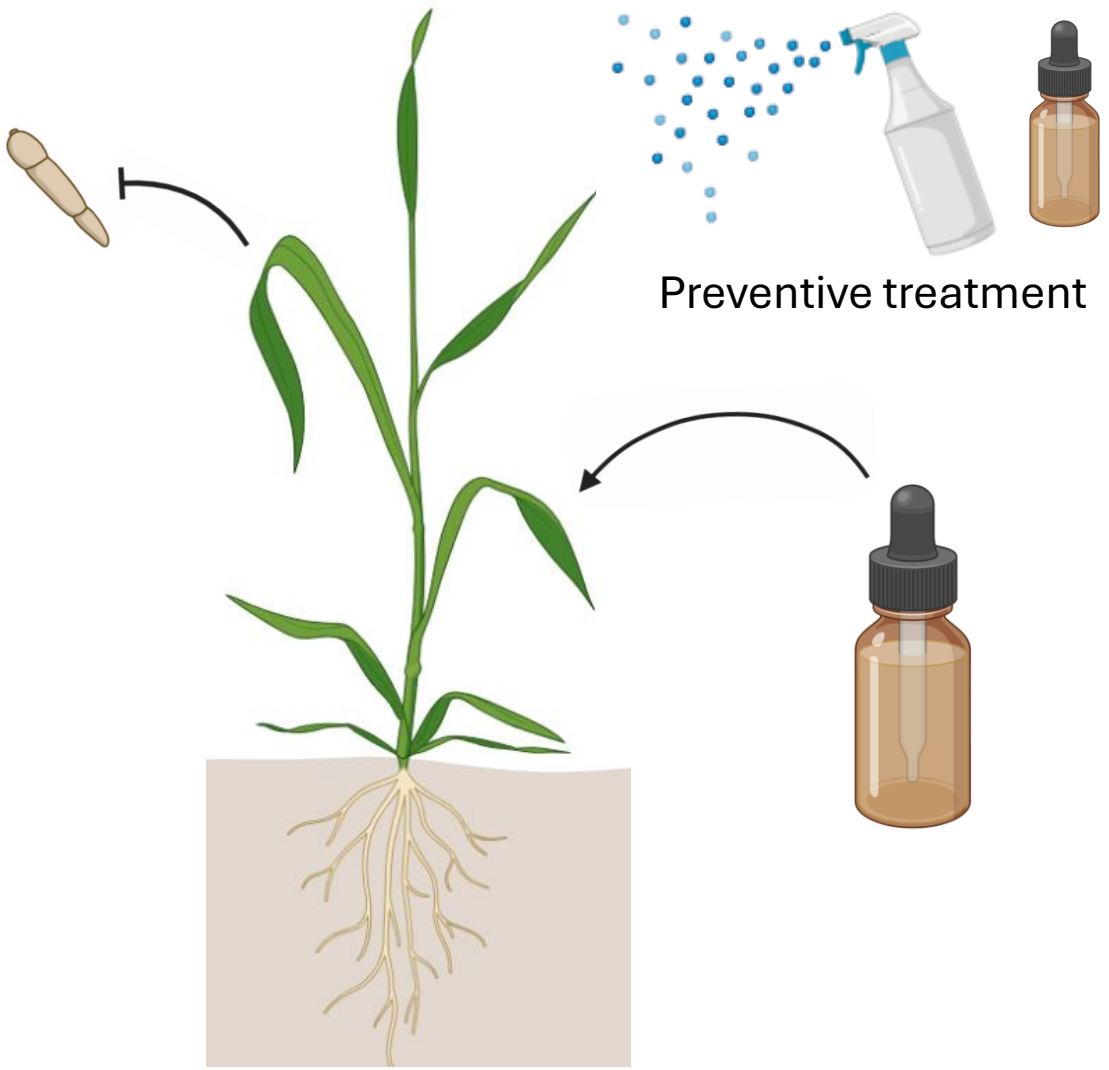


Protection bioassays in the greenhouse are **in progress** to confirm the *in vitro* results

Perspectives

- Characterization of the direct mode of action
  - Effect on fungal cytology
  - Effect on fungal transcriptome and metabolome
  - Effect on fungal cytoplasmic membranes

➢ Characterization of plant defense responses
  - ➢ Defense gene expression
  - ➢ Metabolome analysis



Thank you for your attention !

Credits :
Figures : R, Rstudio
Schemes : Biorender

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References

-Amezrou et al. 2023 A secreted protease-like protein in Zymoseptoria tritici is responsible for avirulence on Stb9 resistance gene in wheat **PLOS Pathogens** https://doi.org/ 10.1371/journal.ppat.1011376

-BASF RAK 1+2 MIX https://www.agro.basf.fr/fr/produits/catalogue_produits/Insecticides/RAK-1-2-MIX.html

-Brown et al. 2015 Genetics of resistance to Zymoseptoria tritici and applications to wheat breeding **Fungal genetics and biology** https://doi.org/10.1016/j.fgb.2015.04.017

-Biorender https://www.biorender.com

-Cools et al. 2011 Impact of Recently Emerged Sterol 14α-Demethylase (CYP51) Variants of Mycosphaerella graminicola on Azole Fungicide Sensitivity **Applied and Environmental Microbiology** https://doi.org/ 10.1128/AEM.00027-11

-Dong, N.Q., and Lin, H.X. 2021. Contribution of phenylpropanoid metabolism to plant development and plant-environment interactions. **J. Integr. Plant Biol.** 63: 180–209.

-Fones H, Gurr S. The impact of Septoria tritici Blotch disease on wheat: An EU perspective. **Fungal Genet Biol**. 2015 Jun;79:3-7

-Gershenzon, J., Dudareva, N. The function of terpene natural products in the natural world. **Nat Chem Biol** 3, 408–414 (2007). https://doi.org/10.1038/nchembio.2007.5

-Goodwin et al. 2011 Finished Genome of the Fungal Wheat Pathogen Mycosphaerella graminicola Reveals Dispensome Structure, Chromosome Plasticity, and Stealth Pathogenesis **PLOS Genetics**

-Insphy 2025 Huiles essentielles : comment reconnaître la vraie qualité ? https://www.insphy.com/blog/2-huiles-essentielles-comment-reconnaitre-la-vraie-qualite-

-Kallerna 2022 Wheat field in Vampula, Finland https://commons.wikimedia.org/wiki/File:Vehnäpelto_6.jpg

-Omrane et al. 2017 Plasticity of the MFS1 Promoter Leads to Multidrug Resistance in the Wheat Pathogen Zymoseptoria tritici **mSphere** https://doi.org/10.1128/mSphere.00393-17

-R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

-Raveau et al. 2020 Essential Oils as Potential Alternative Biocontrol Products against Plant Pathogens and Weeds: A Review **Foods** https://doi.org/10.3390/foods9030365

-Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-Response Analysis Using R. **PLoS ONE** 10(12): e0146021

-Ryu et al. 2014 Biological control of Colletotrichum panacicola on Panax ginseng by Bacillus subtilis HK-CSM-1 **Journal of Ginseng Research** https://doi.org/10.1016/j.jgr.2014.05.001

-Sadgrove et al. 2022 Fundamental Chemistry of Essential Oils and Volatile Organic Compounds, Methods of Analysis and Authentication **Plants**. https://doi.org/10.3390/plants11060789

-Sciences et Avenir. 2014100 pucerons par jour, un festin de coccinelle https://www.sciencesetavenir.fr/nature-environnement/100-pucerons-par-jour-un-festin-de-coccinelle_13722

-Siah et al. 2010 Correlation of in planta endo-beta-1,4-xylanase activity with the necrotrophic phase of the hemibiotrophic fungus Mycosphaerella graminicola **Plant Pathology** https://doi.org/ 10.1111/j.1365-3059.2010.02303.x

-Steinberg et al. 2015 Cell biology of Zymoseptoria tritici: Pathogen cell organization and wheat infection **Fungal Genetics and Biology** https://doi.org/10.1016/j.fgb.2015.04.002