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Fighting Cercospora leaf spot in sugar beet with essential oils

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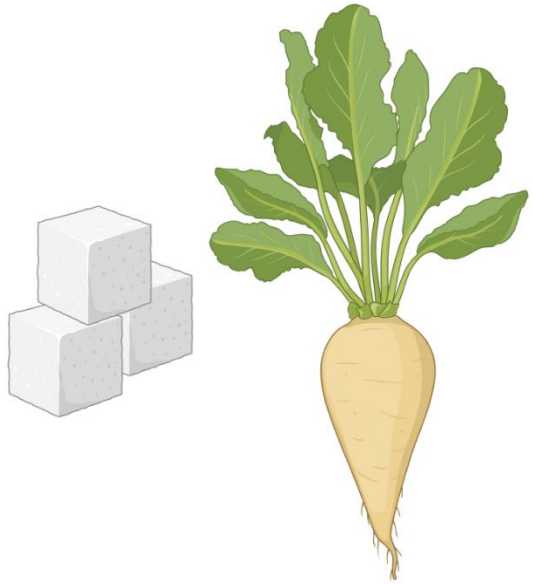
Agro-Bio Tech

Program ERDF 2021-2027 Project PHENIX Biocontrol ULiège-SPW (ID 428)

Sugar beet



- Sugar beet, *Beta vulgaris* ssp. *vulgaris :*
  - 20% of total sugar production - second largest global source of sugar
  - 46% of total sugar beet comes from Europe (Muir & Anderson, 2022)
- Subject to several attacks:

Virus: Beet yellows

Bacteria: Syndrome ‘Basses Richesses’

And of course, fungi !

Ascomycete fungus *Cercospora beticola*

- Causal agent of Cercospora leaf spot (CLS) disease
- One of the most destructive foliar diseases in sugar beet !
- Yield losses reaching 40% and reduced sugar content up to 50% (Gouda et al., 2022)
- Polycyclic pathogen = particularly difficult to control!
- **How can we limit its expansion?**

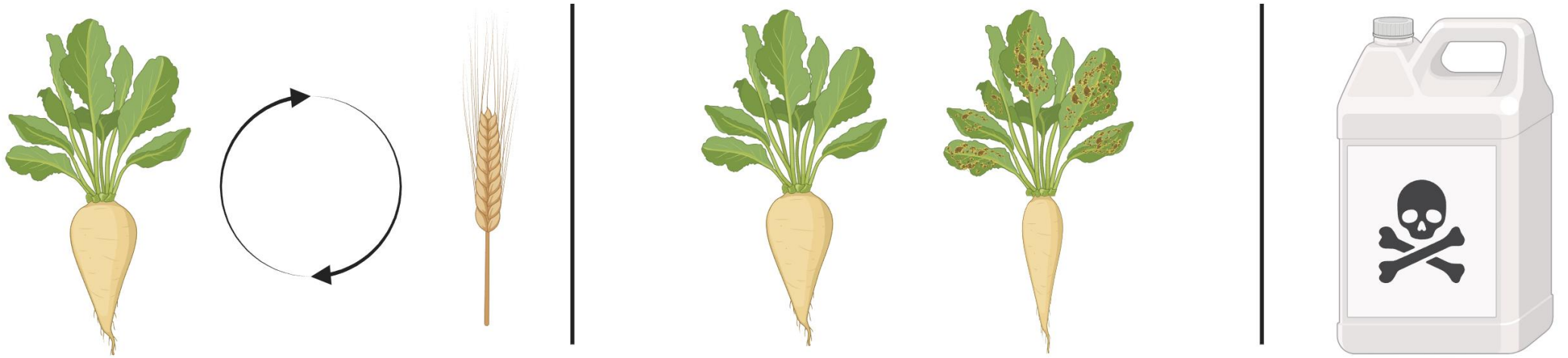

(Burgeon, 2025)

Fighting cercospora leaf spot disease

Agronomical practices

Use of resistant varieties

Use of pesticides



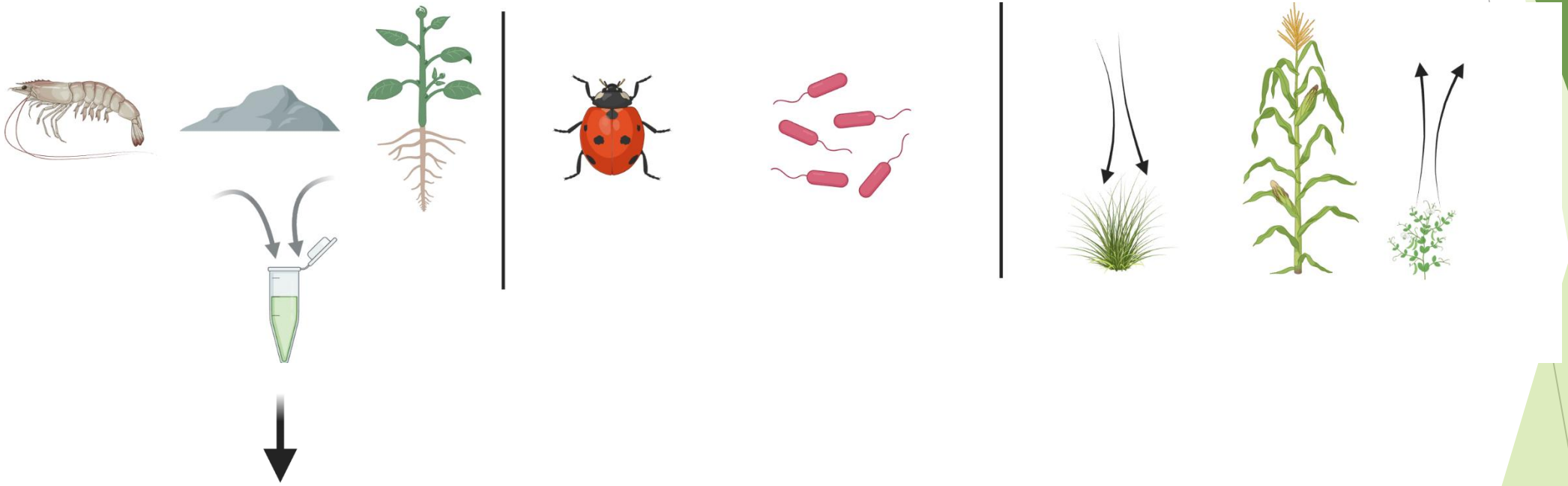
- Conventional pesticides: Increasing resistance of *C. beticola* strains to synthetic active ingredients (Rangel et al., 2020)
- **Alternative? Biocontrol solutions!**

Biocontrol solutions

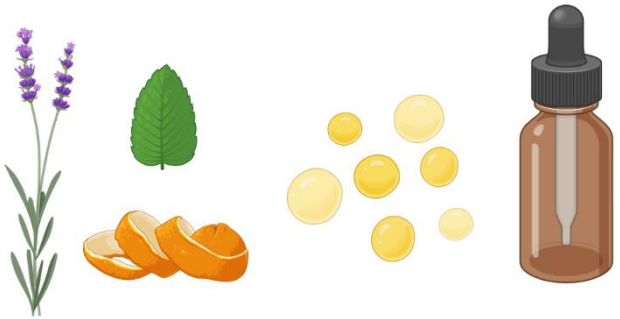
Natural substances

Living organisms

Semiochemicals



Essential oils



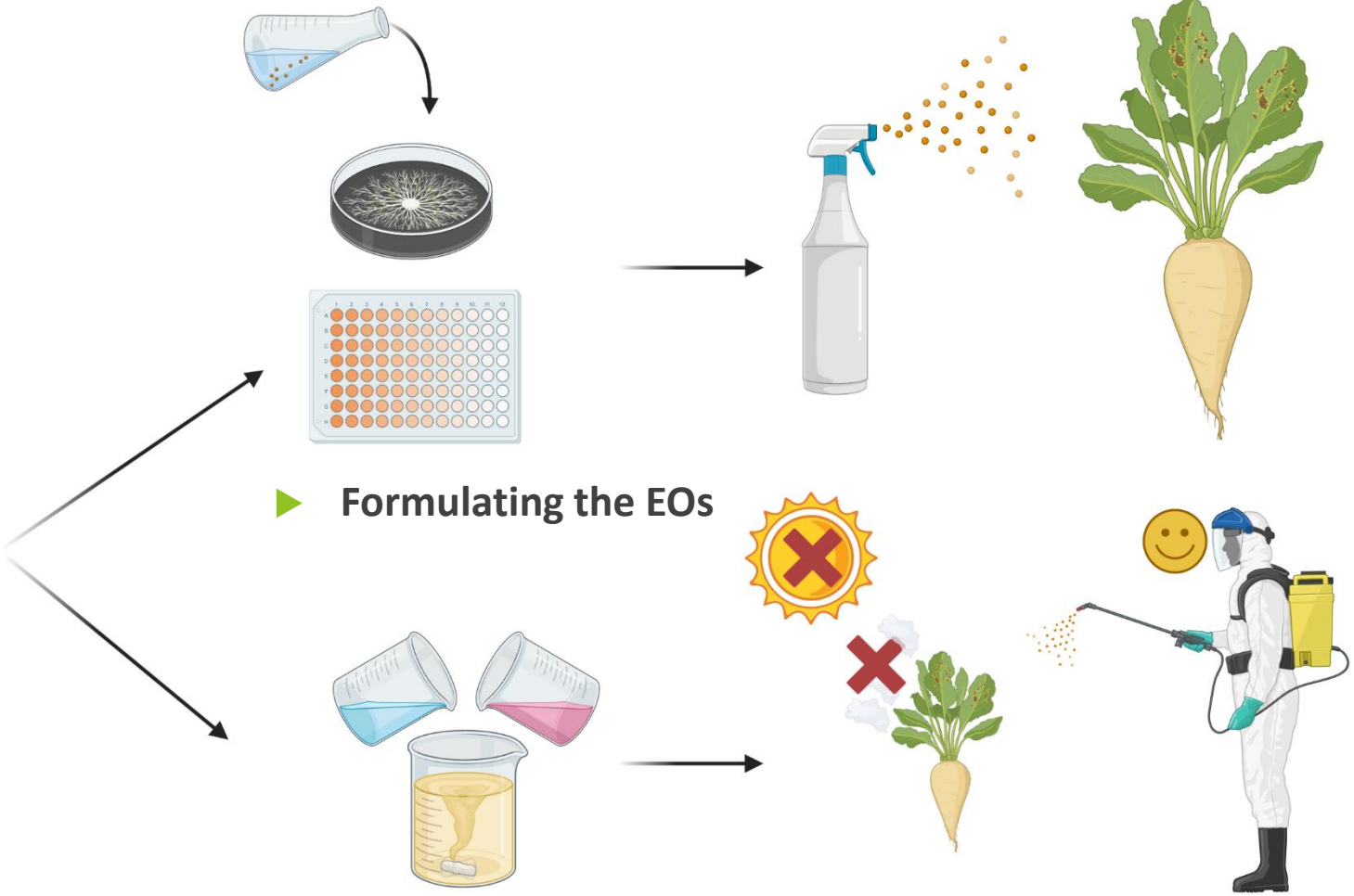
- Complex chemical composition :
  - Large array of biological activities
  - Less prone to induce resistance in pathogens (Deresa & Diriba, 2023)

5

Development of an essential oil based biofungicide against *C. beticola*

- **Understanding which EO is the most promising to control *C. beticola***

4 EOs selected:
1 *Cinnamomum sp.*
2 *Cymbopogon sp.*
1 T*hymus sp.*
(De Clerck et al., 2020; Kiniec et al., 2024)



- **Formulating the EOs**

Which EO is the most promising?
In vitro testing

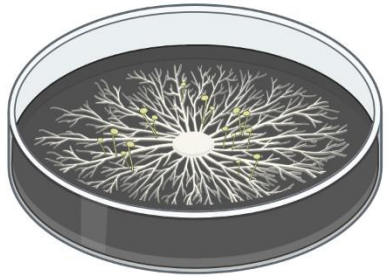
6 different modalities tested:

- *Cinnamomum*
- *Cymbopogon 1*
- *Cymbopogon 2*
- *Thymus*
- Water and tween (Negative control)
- Spyrale (Positive control)

EO emulsion = EO + Tween 20 in water, high speed homogenization

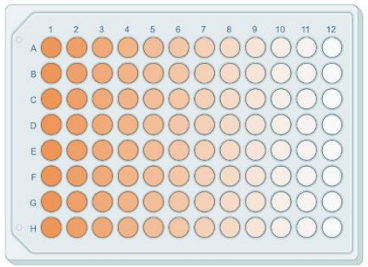
Tested on 1 *C. beticola* strain sampled in France in 2020

Impact on mycellial growth



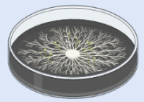
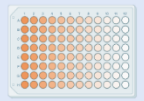
Minimum inhibitory concentration (MIC)

Impact on spore germination



Median inhibitory concentration (IC50)

Which EO is the most promising?
In vitro testing

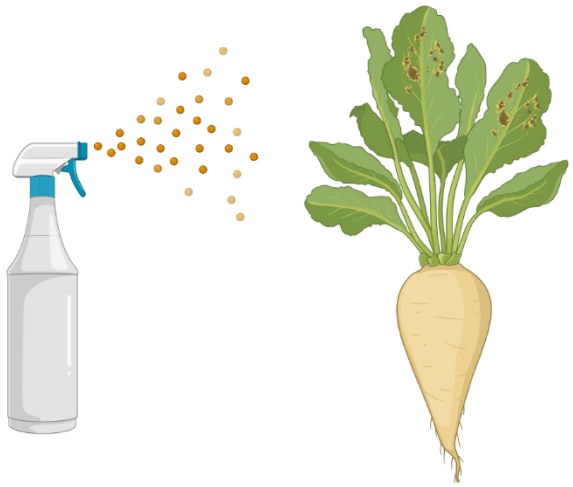
		Cinnamomum	*Cymbopogon* 1	*Cymbopogon 2*	*Thymus*	Spyrale (T+)
Mycellium 	**MIC**	281.25	375.00	562.50	140.63	6.25
Spore 	**IC_{50}**	32.57 ± 3.09	276.13 ± 22.27	256.15 ± 22.27	272.27 ± 17.37	27.92 ± 3.48

MIC (in µL/L), IC_{50} (in µL/L , mean ± S.E.)

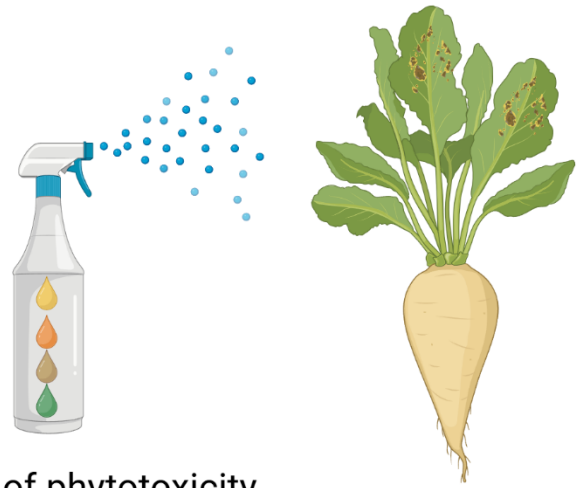
- **Which EO seems to be the most promising? Depends which fungal structure is targeted**
  - *Thymus sp.* better against mycellial growth
  - *Cinnamomum sp.* better against spore germination

Which EO is the most promising?
In vivo efficacy testing

Inoculation of *C. beticola* spores
Susceptible variety of sugar beet



Spraying of EO emulsions and controls
(72 hours post inoculation)

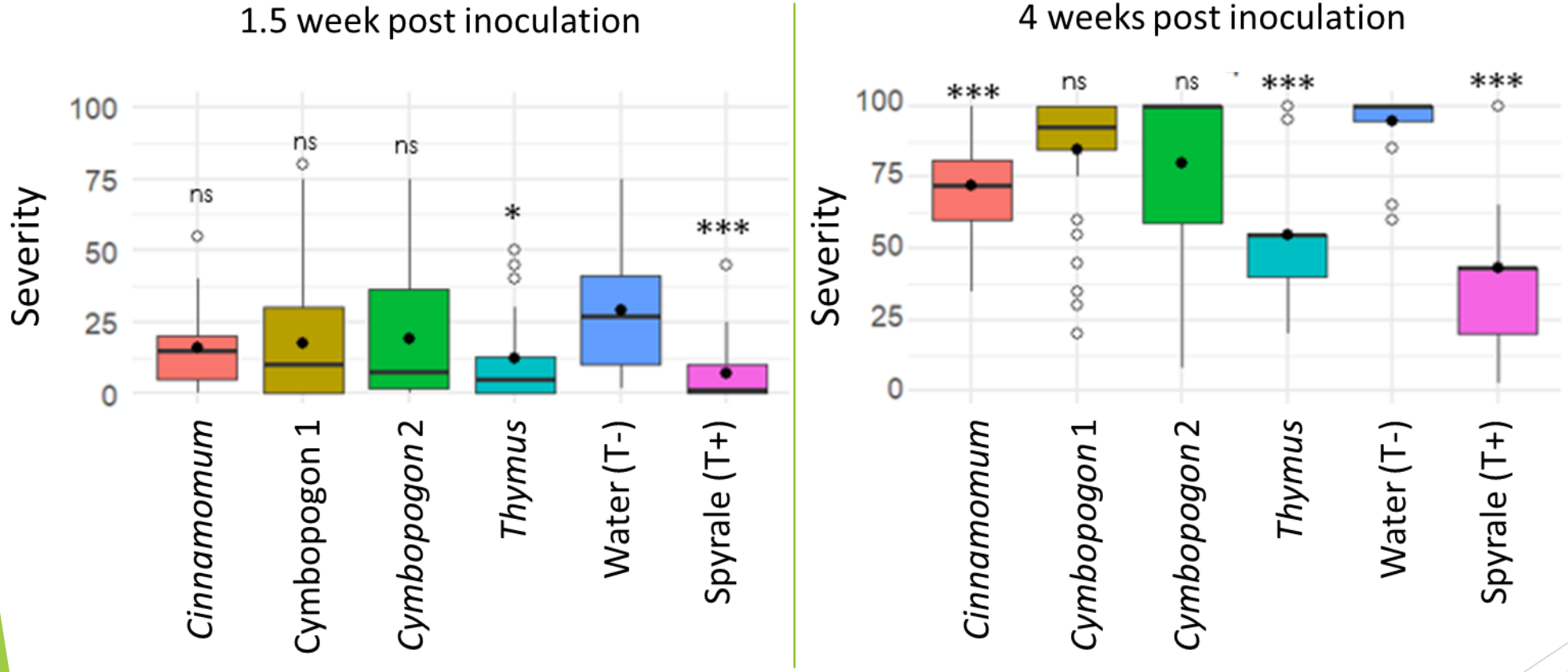


Abscence of phytotoxicity
verified prior to efficacy testing

Disease severity
evaluation
1.5, 2 , 2.5, 3, 3.5 and 4
weeks post inoculation

- *In vitro* trends confirmed?

Which EO is the most promising?
In vivo efficacy testing

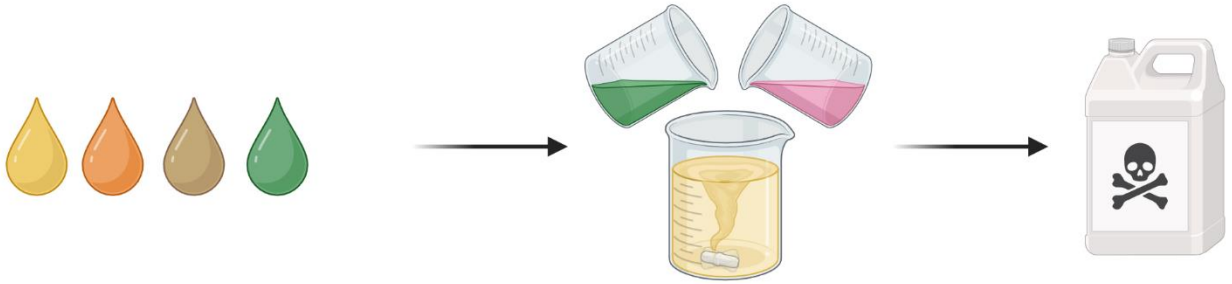


*, **,*** represent significant different with negative control (T-) following pairwise Wilcoxon signed-rank tests

- **Are the *in vitro* trends found *in vivo* as well?** Yes !
  - *Thymus sp.* first to control CLS
  - Joined by *Cinnamomum sp.* four weeks post inoculation

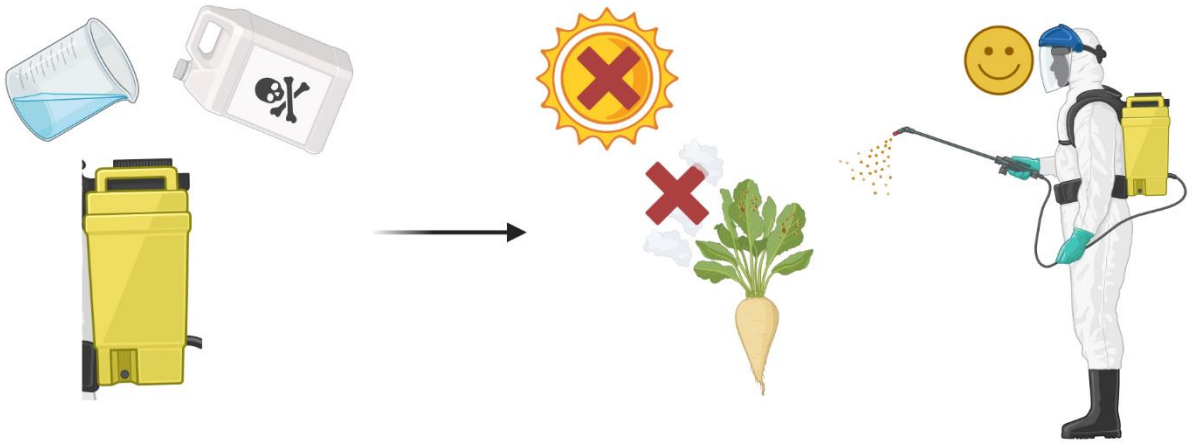
Formulating the EOs

At the lab



Concentrated product
Stable over time

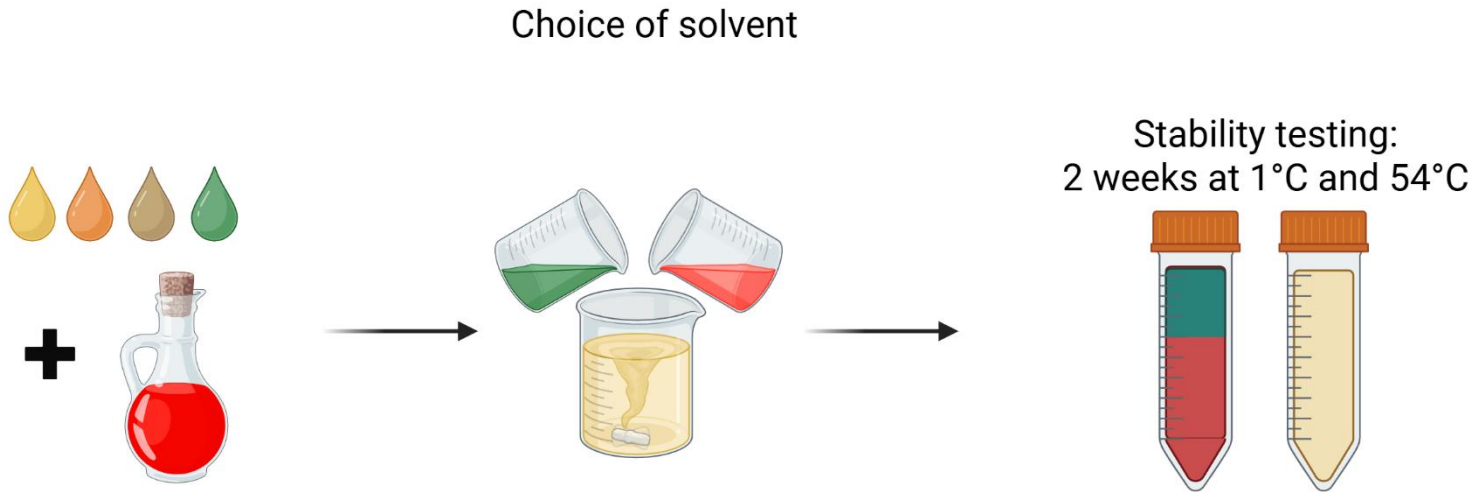
At the farm

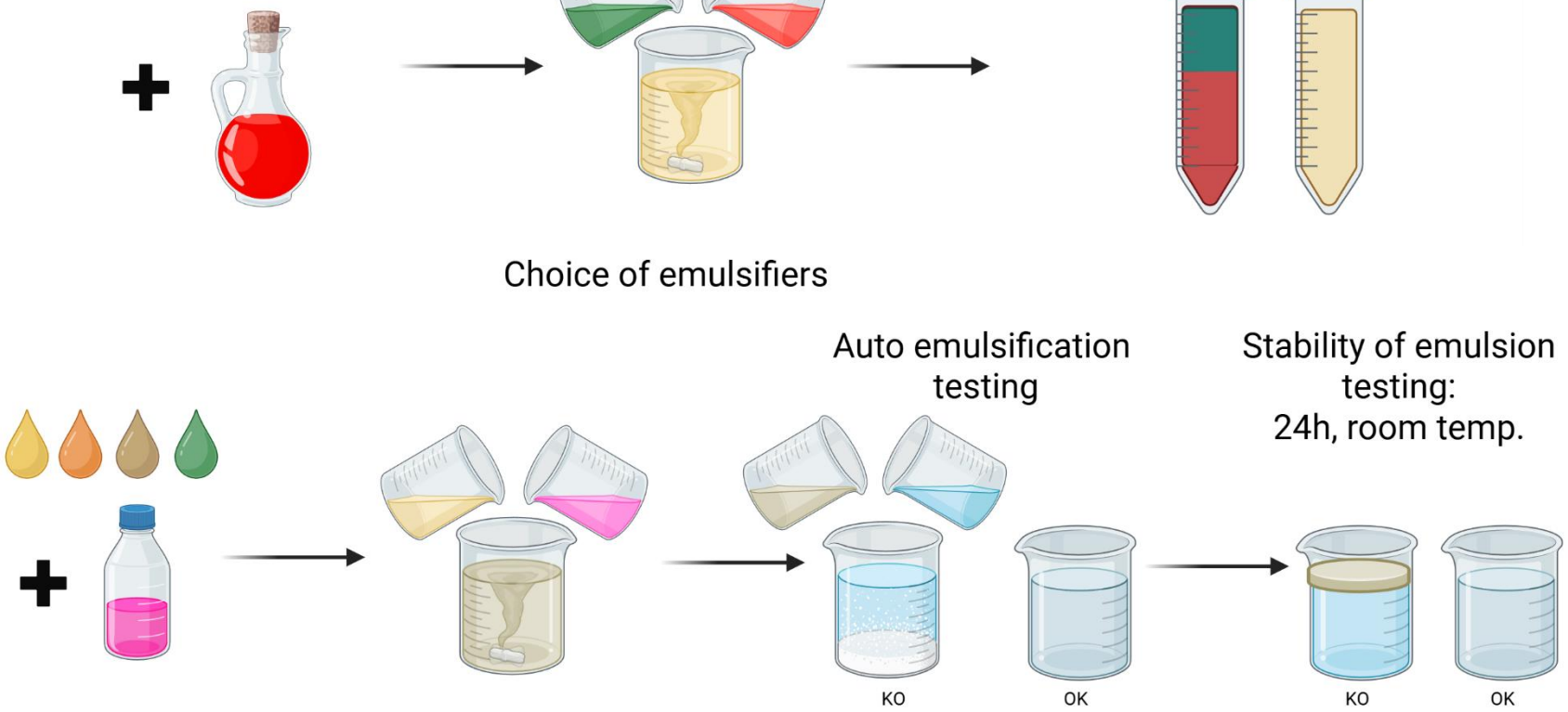


Easy to prepare
Easy to disperse
Maximal efficacy

Formulating the EOs

Essential oil based concentrated product = Emulsifiable concentrate (EC)





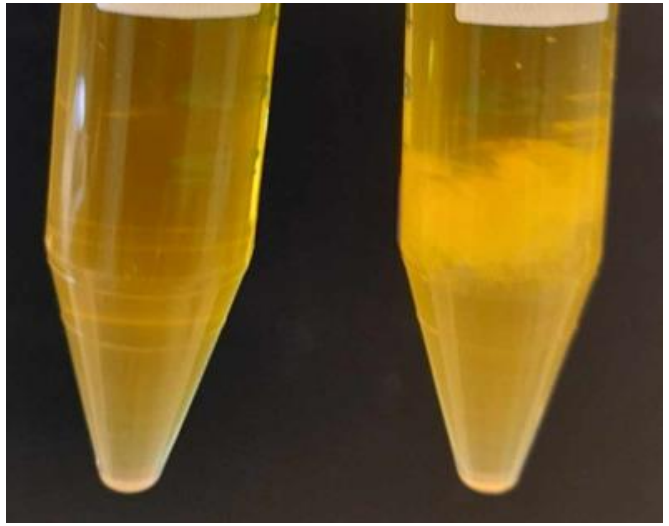
EO formulation is very time consuming! But promising results are obtained

Formulating the EOs

- **Choice of solvent:** Stability analysis after 2 weeks

EO + Solvent 1

54°C 1°C



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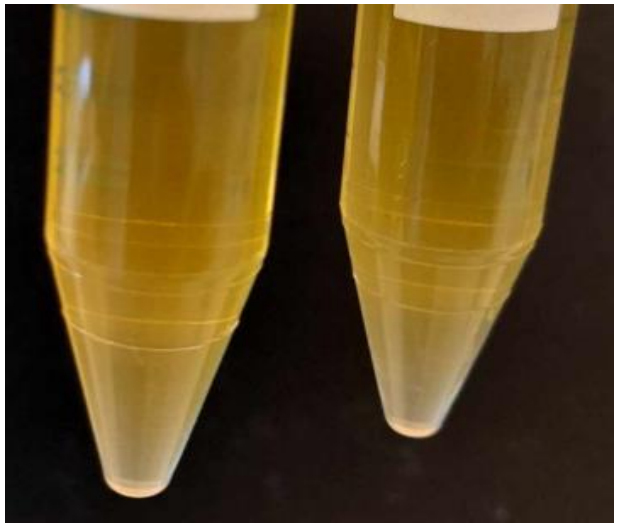
EO + Solvent 2

54°C 1°C



OK

- Selection of solvent 2 → Moving on to the **choice of emulsifiers**

Formulating the EOs

- **Choice of emulsifiers**

Auto emulsification testing

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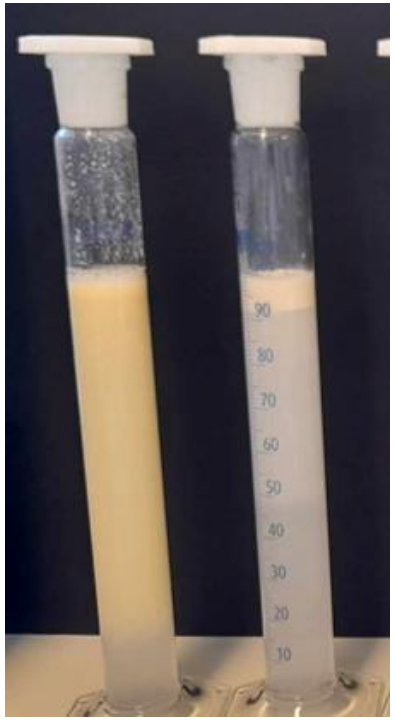
OK

Stability over time



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OK

Conclusions and perspectives

- Proven potential of EOs to control *C. beticola*
  - *Cinnamomum sp. , Thymus sp.* still demonstrate an efficacy1 month post-pulverisation of basic formulations
  - Prototype formulations are under development

- But still a long way to go before considering homologation and commercialization
  - Mode of actions remain to be studied
  - Formulation must still be improved to ensure maximal efficacy
  - Fields trials must be performed to:
    - determine the correct positioning of the product
    - ensure that greenhouse *in vivo* trends are still observed in real life conditions

Thank you for your attention

- Please feel free to ask any questions !

- Clément Burgeon – cburgeon@uliege.be

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