Eucalyptus-based lipid nanoparticles as a green biocide against weeds and phytopathogens

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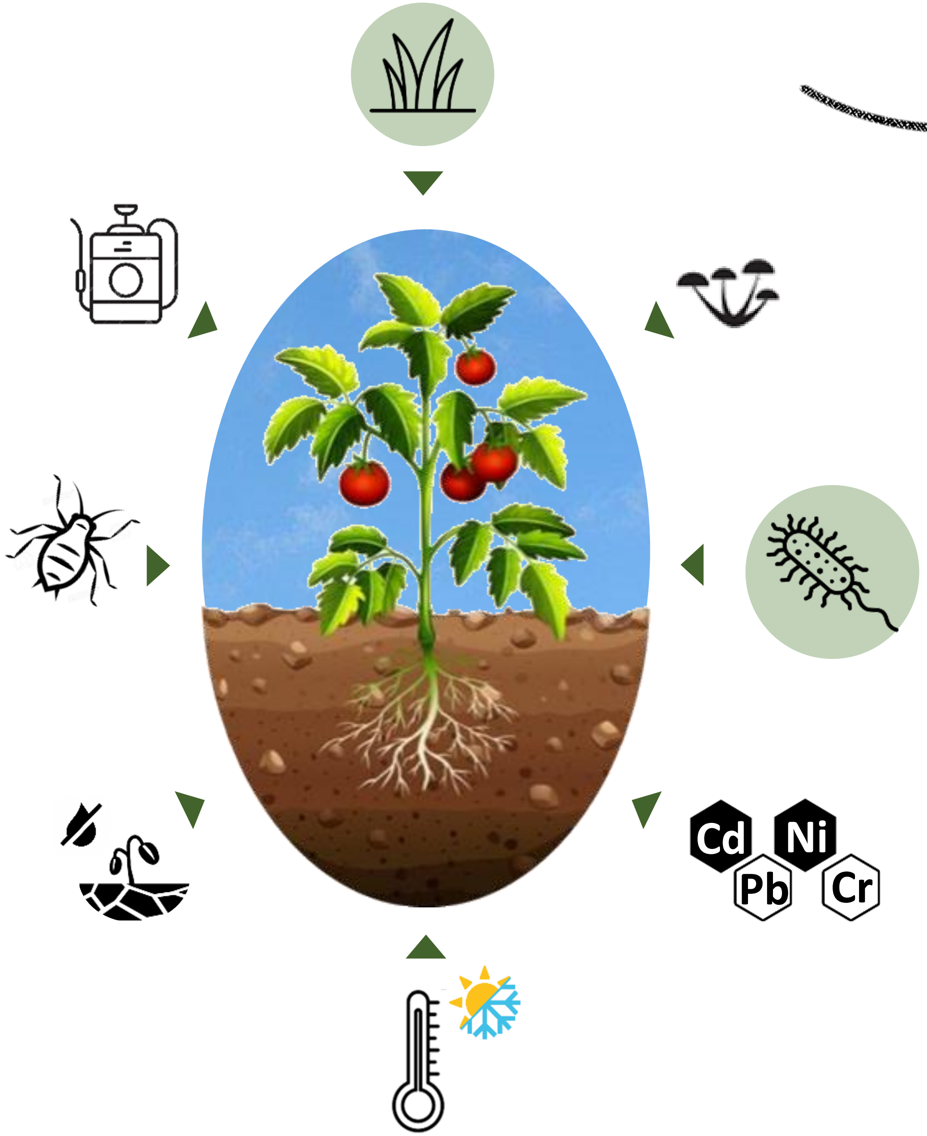
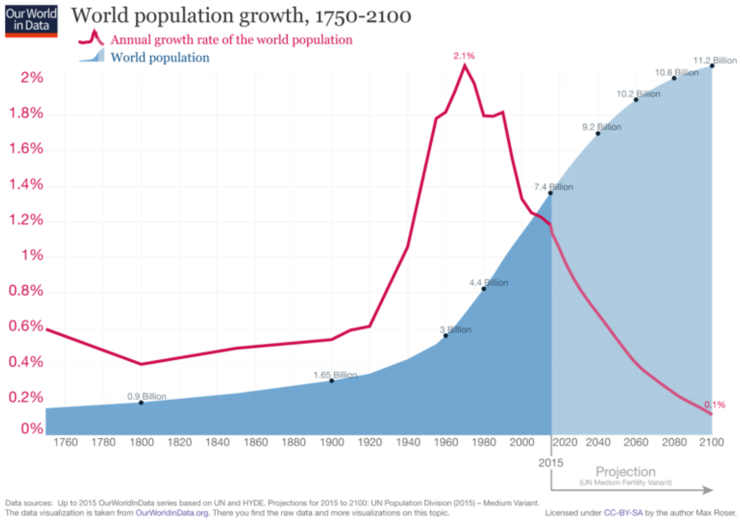
Background



Weed interference and **phytopathogen attacks** considerably **impair crop production**



Negatively affecting the efforts of increasing the food supply to **feed an ever-growing population**

Background

Weed management

Synthetic herbicide application

The excessive use of synthetic herbicides is associated with negative impacts in all environmental matrices

Bacterial disease management

29
Cu
Copper
63.546

Appropriated cultural practices

- Elimination of infected plants or seeds;
- Disinfection of pruning tools;
- Removal of associated weeds that can serve as pathogen reservoirs;
- Adequate fertilization.

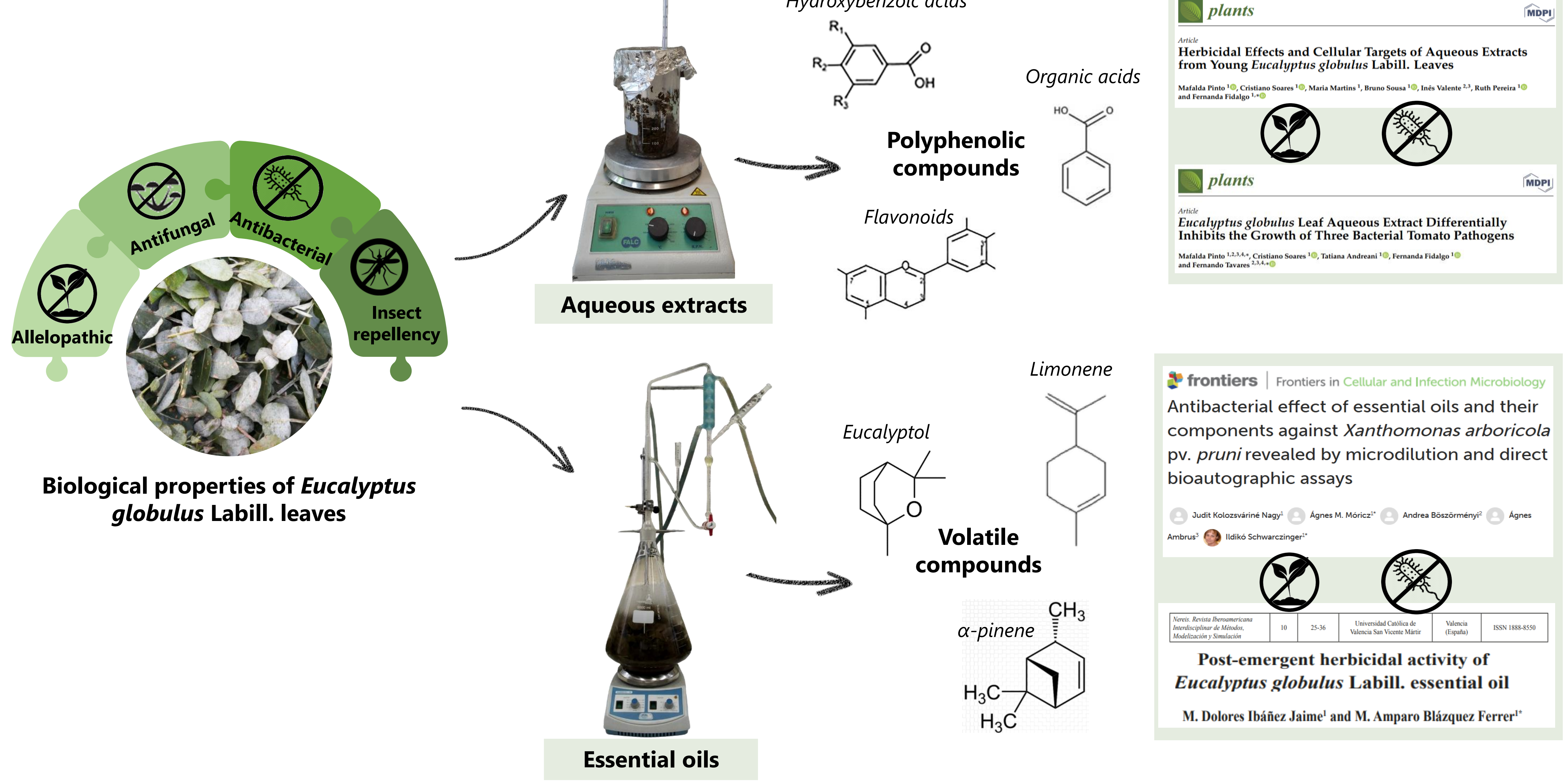
Application of copper-based plant protection products

- Due to tolerance to copper-based bactericides, the maximum amount applied is **restricted in European Union** (EU) to a maximum of 28 kg per ha over a period of 7 years [regulation (EU) 2018/1981]

Application of antibiotics forbidden in EU [Regulation (EU) 2019/6]

It is urgent to develop **eco-friendly strategies** to **control weeds** and **crop-related bacterial diseases** while improving **food production and quality**

Background



Antifungal
Antibacterial
Allelopathic
Insect repellency
Biological properties of Eucalyptus globulus Labill. leaves
Aqueous extracts
Essential oils
Hydroxybenzoic acids
OH
Organic acids
HO
Polyphenolic compounds
Flavonoids
Eucalyptol
Limonene
Volatile compounds
α-pinene
plants
MDPI
Article
Herbicidal Effects and Cellular Targets of Aqueous Extracts from Young Eucalyptus globulus Labill. Leaves
Mafalda Pinto, Cristiano Soares, Maria Martins, Bruno Sousa, Inês Valente, Ruth Pereira and Fernanda Fidalgo
plants
MDPI
Article
Eucalyptus globulus Leaf Aqueous Extract Differentially Inhibits the Growth of Three Bacterial Tomato Pathogens
Mafalda Pinto, Cristiano Soares, Tatiana Andreani, Fernanda Fidalgo and Fernando Tavares
frontiers | Frontiers in Cellular and Infection Microbiology
Antibacterial effect of essential oils and their components against Xanthomonas arboricola pv. pruni revealed by microdilution and direct bioautographic assays
Judit Kolozsváriné Nagy, Ágnes M. Móricz, Andrea Böszörményi, Ágnes Ambrus, Ildikó Schwarczinger
Nereis. Revista Iberoamericana Interdisciplinar de Métodos, Modelización y Simulación
10
25-36
Universidad Católica de Valencia San Vicente Mártir
Valencia (España)
ISSN 1888-8550
Post-emergent herbicidal activity of Eucalyptus globulus Labill. essential oil
M. Dolores Ibáñez Jaime and M. Amparo Blázquez Ferrer

Main goal

Enable their large-scale application

Enhance their bioactivities

To **develop a nanobiopesticide** through the **encapsulation of eucalyptus-based products** in **nanomaterials**, to be used in the **control of weeds** and **crop-associated bacterial diseases**

Pesticide large-scale application requires high product amount

↓

Unfeasibility of eucalyptus-based strategies

Nanoparticle synthesis

frontiers

Untargeted metabolomic profiling of fresh and dried leaf extracts of young and mature *Eucalyptus globulus* trees indicates differences in the presence of specialized metabolites

Mafalda Pinto[1], Cristiano Soares[1], Ruth Pereira[1], José António Rodrigues[2], Fernanda Fidalgo[1], Inês Maria Valente[2,3]

*Leaves from **young E. globulus** presented a **greater phytochemical diversity** and were used to produce:*

Aqueous extract ($250\ g\ L^{-1}$)

Essential oil

Incorporated into

Lipid nanoparticles (LN)

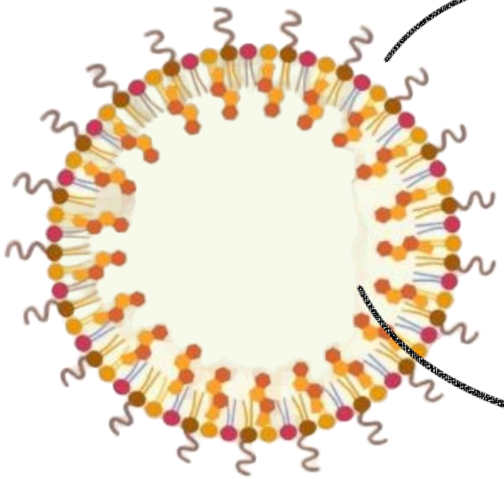
- Easily manufactured
- Produced in aqueous dispersions
- Green synthesis – without using organic solvents
- Synthesized with physiological and biodegradable lipids

- High loading capacity
- Increase the solubility of the active ingredient
- Great stability of the active ingredient during storage
- Slow and controlled release of the active ingredient

Nanoparticle synthesis

Aqueous extract

Aqueous phase



Lipid phase

Emulsion-based lipid nanoparticles

Essential oil

Two nanoformulations

- Lipid nanoparticle incorporating the **aqueous extract (LN-AE)**
- Lipid nanoparticle incorporating the **essential oil (LN-EO)**

Selection of the nanoformulations based on physico-chemical parameters:

Z-average: 0-200 nm

Polidispersity index: 0-0.25

Herbicidal activity of LN-AE

Lipid nanoparticle with the incorporation of the aqueous extract in the aqueous phase

LN-empty

Lipid nanoparticle with water incorporated in the aqueous phase **(control situation)**

Experimental design and results

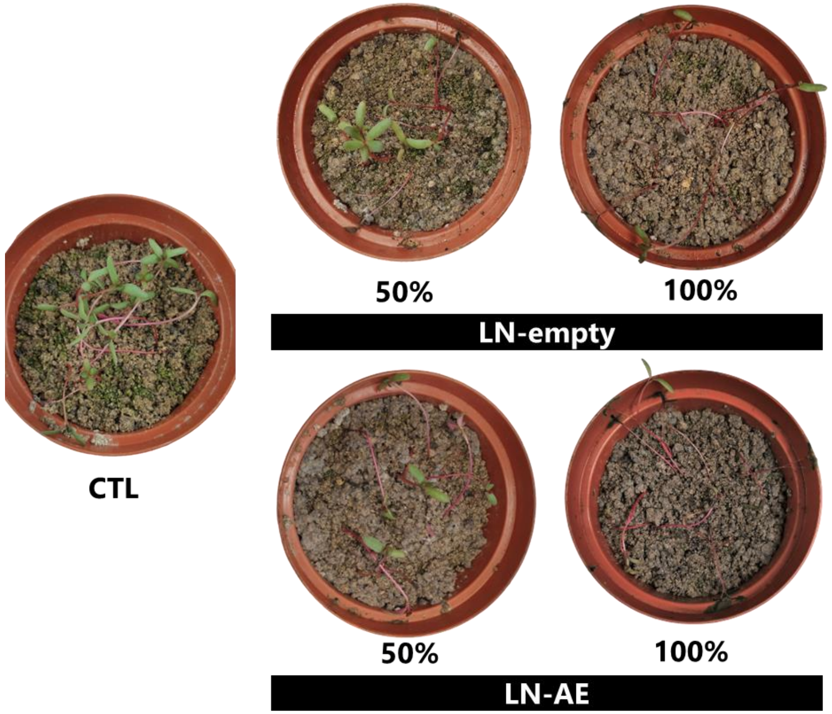
Two-weeks-old *Portulaca oleracea* L. (purslane) seedlings

Foliar-sprayed (single application) with:

- **Deionised water (dH_2O)**
- **LN-empty (50 and 100% v/v)**
- **LN-AE (50 and 100% v/v)**

Mortality percentage monitored after 1 week of treatment application

Macroscopic effects



Mortality percentage

CTL		0 ± 0% d
LN-empty	50%	71.3 ± 9.3% ab
	100%	83.6 ± 11.4% a
LN-AE	50%	50.4 ± 8.2% bc
	100%	91.9 ± 5.2% a

LN-AE had a **similar herbicidal activity** to **LN-empty**

Antibacterial activity of LN-AE

Experimental design and results

Xanthomonas euvesicatoria (Xeu)

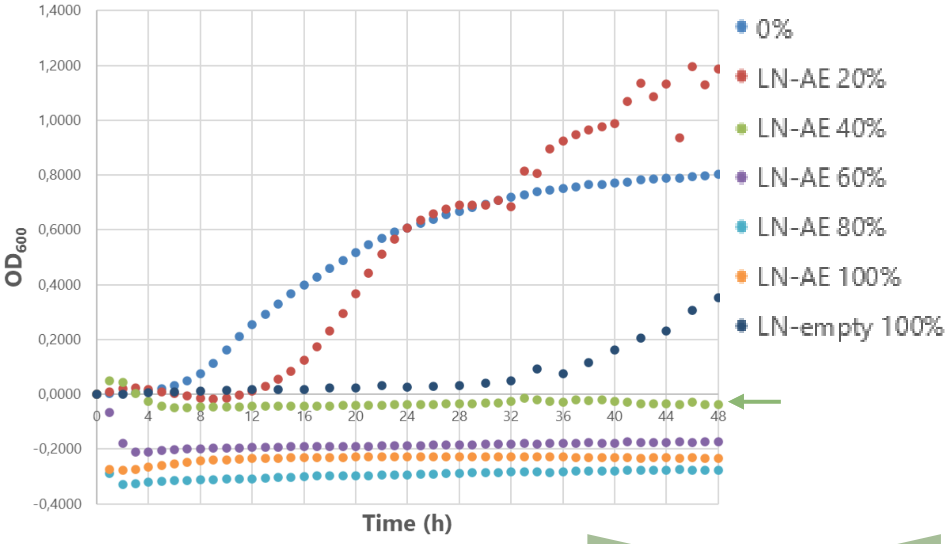
The causal agent of tomato bacterial leaf spot

Monitoring of *Xeu* growth for 48 h after the exposure to:

Deionised water (dH_2O)

LN-empty (100% v/v)

LN-AE (20-100% v/v)

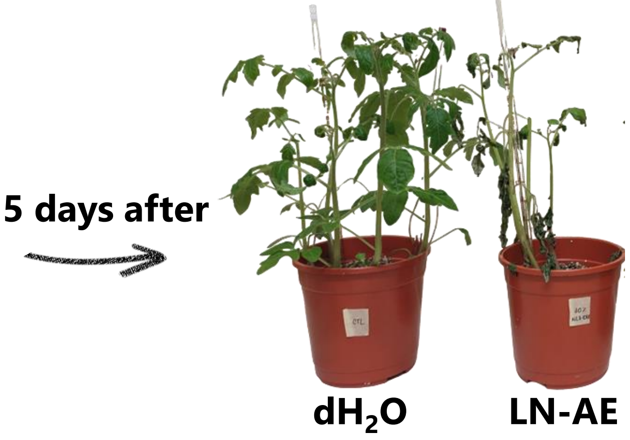


LN-AE at 40% was the **minimal inhibitory concentration (MIC)** **inhibiting *Xeu* growth** in a **stronger way** than **LN-empty**

Assessment of the safety of LN-AE MIC to tomato plants

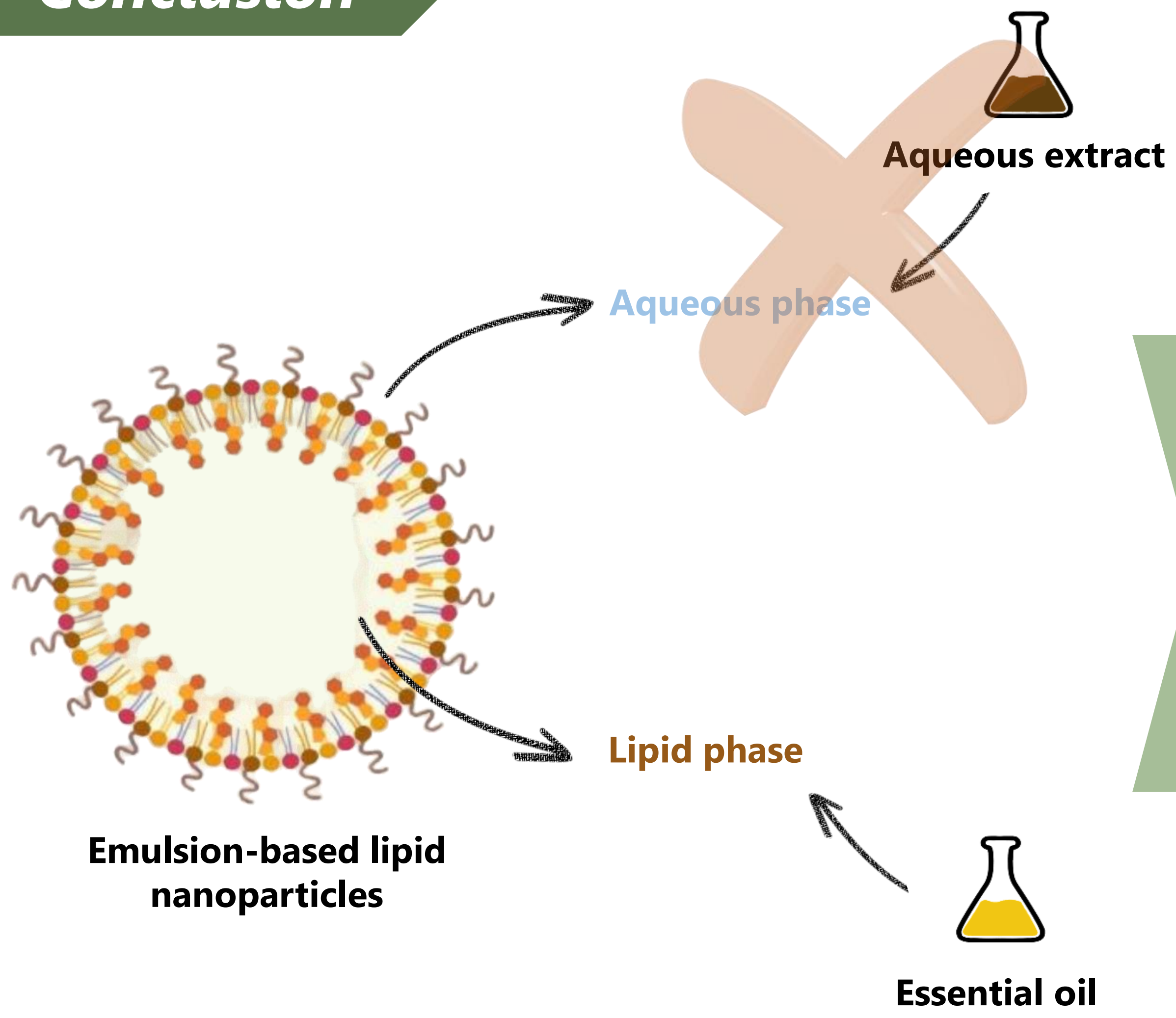
Healthy tomato plants (*Solanum lycopersicum* cv. cerasiforme) with one month were **foliar-sprayed** with **dH_2O and LN-AE at 40%**

5 days after



The application of **MIC of LN-AE strongly impaired the viability** of **tomato plants**

Conclusion



2 nanoformulations

LN-AE does not represent an effective strategy for weed control and for the treatment of bacterial leaf spot

Lipid nanoparticle incorporating the essential oil (LN-EO)

Selection of the nanoformulations based on physico-chemical parameters:

Z-average: 0-200 nm

Polidispersity index: 0-0.25

Herbicidal activity of LN-EO

Lipid nanoparticle with the essential oil in the lipid phase → ~~LN-empty~~

Experimental design and results

(preliminary assay)

Two-weeks-old *Portulaca oleracea* L. (purslane) seedlings

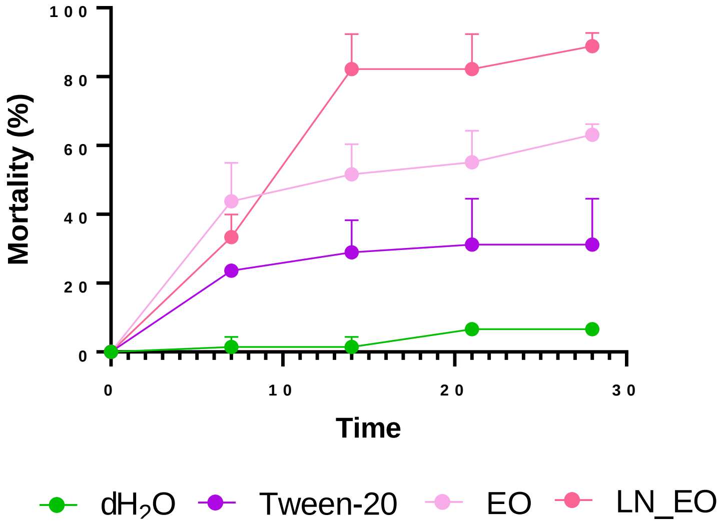
Foliar-sprayed (single application) with:

- **Deionised water (dH_2O)**
- **Tween-20**
- **Essential oil (EO)**
- **LN-EO**

Mortality percentage monitored for the following 4 weeks

Macroscopic effects

Mortality percentage



LN-EO induced a great weed mortality and enhanced EO herbicidal activity

Antibacterial activity of LN-EO

Experimental design and results

(preliminary assay)

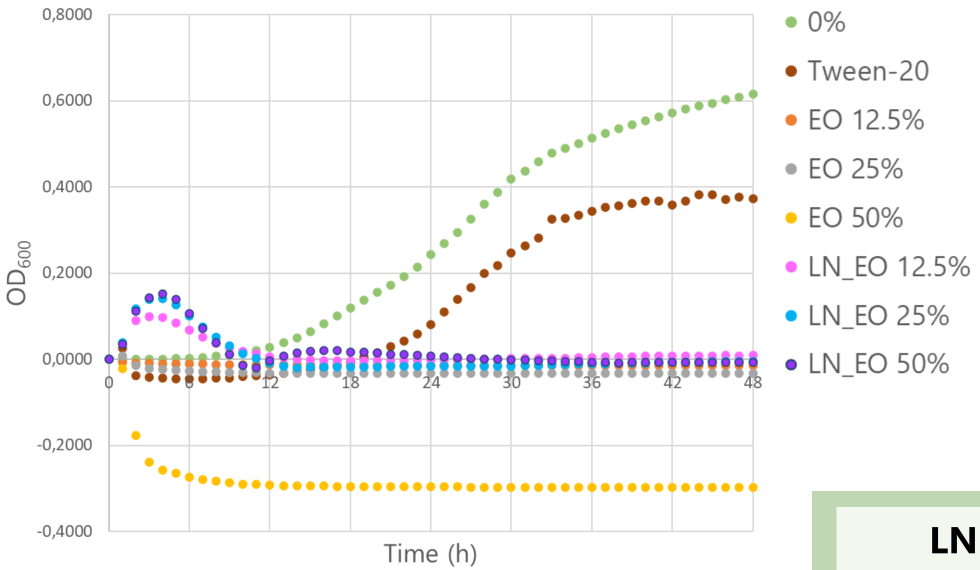
Monitoring of *Xeu* growth for 48 h after the exposure to:

- **Deionised water (dH_2O)**
- **Tween-20**
- **Essential oil (EO; 12.5-50%)**
- **LN-EO (12.5-50%)**

Determination of the number of CFU mL^{-1} after the 48-h incubation

Results

Growth curves

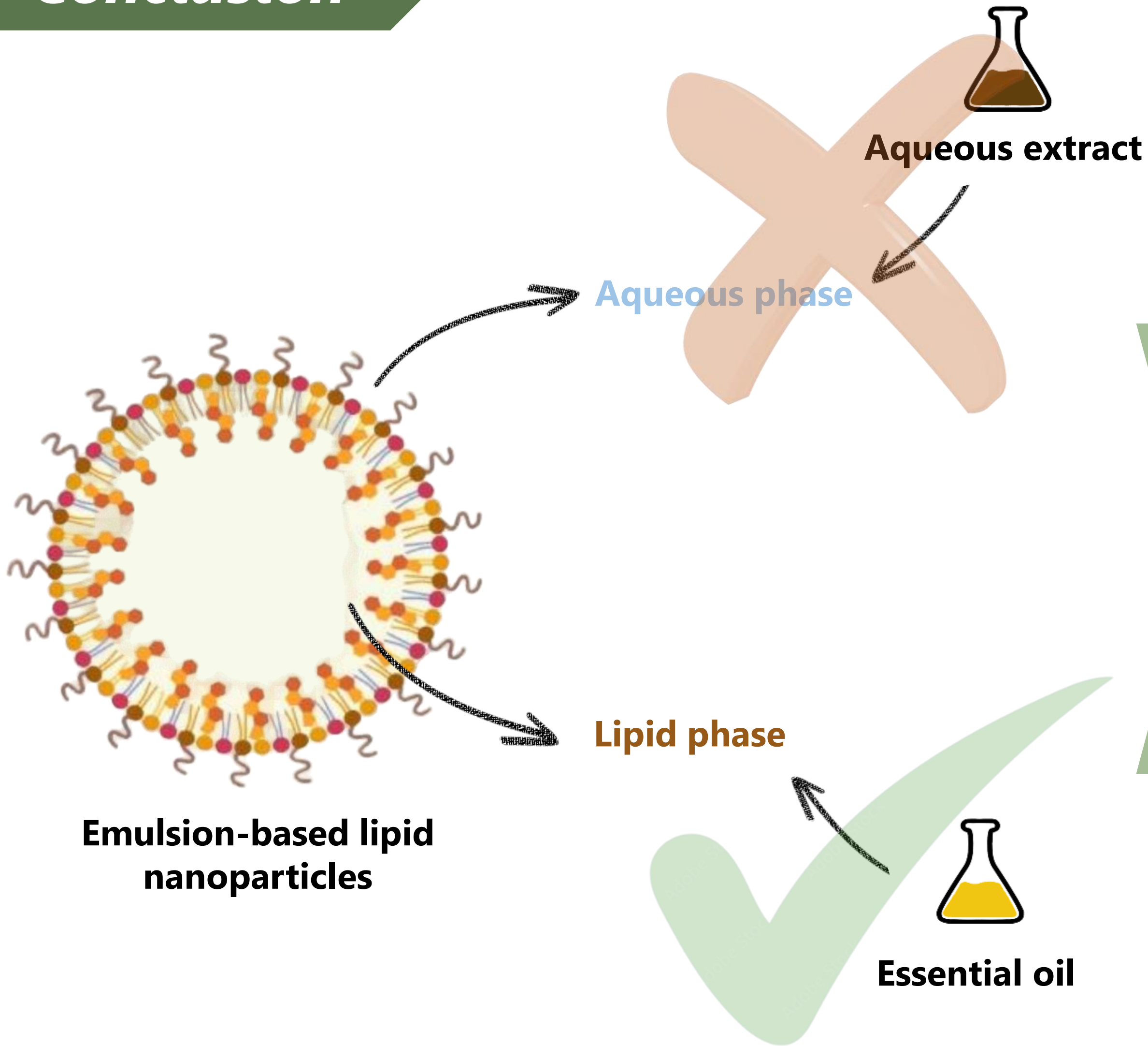


Number of CFU mL^{-1}

CTL		$(6.4 \pm 1.1) \times 10^9 \pm 0$ a
EO	12.5%	$(9.1 \pm 1.7) \times 10^3$ b
	25%	0 ± 0 c
	50%	0 ± 0 c
LN-EO	12.5%	0 ± 0 c
	25%	0 ± 0 c
	50%	0 ± 0 c

LN-EO had a **strong bactericidal effect against *Xeu*, enhancing EO antibacterial properties**

Conclusion



2 nanoformulations

LN-EO seems to have promising properties to be used in weed control and bacterial disease management

Lipid nanoparticle incorporating the essential oil (LN-EO)

Selection of the nanoformulations based on physico-chemical parameters:

Z-average: 0-200 nm

Polidispersity index: 0-0.25

Next steps

- Ascertain the most effective concentration of LN-EO against *P. oleracea*
- Evaluate LN-EO herbicidal activity against other weed species, particularly monocot species
- Assess the safety of LN-EO most effective dose against non-target plants

- Characterize the *in vitro* antibacterial activity of LN-EO against *Xeu*
- Study the efficacy of LN-EO in the treatment of infected tomato seeds

Seeds are the main dissemination vehicle of Xeu

- Evaluate the potential of LN-EO to prevent and/or treat bacterial leaf spot in tomato plants

Acknowledgements

2022 Christmas dinner with current and former members

@plantstresslab

Plant Stress lab

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PEST(bio)CIDE

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