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Germination Index as a tool to assess phytotoxicity of olive mill solid wastes

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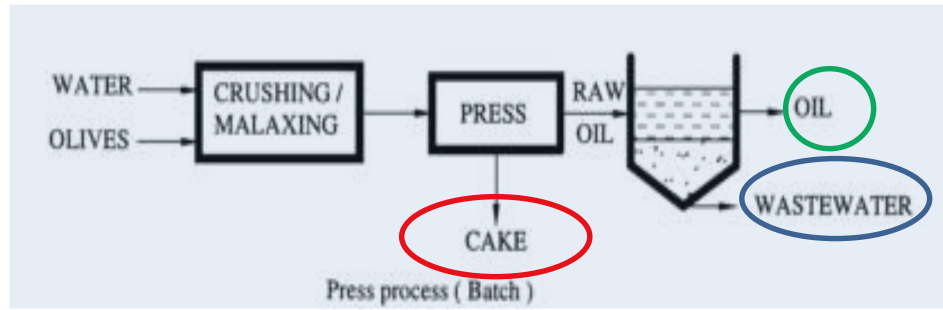
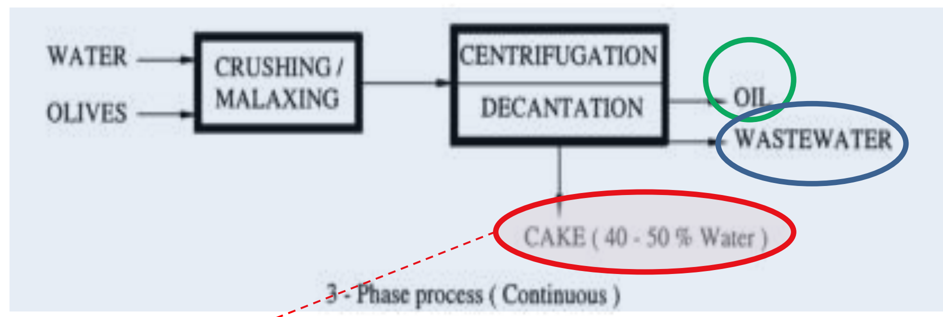
1. Introduction

Olive oil production processes:

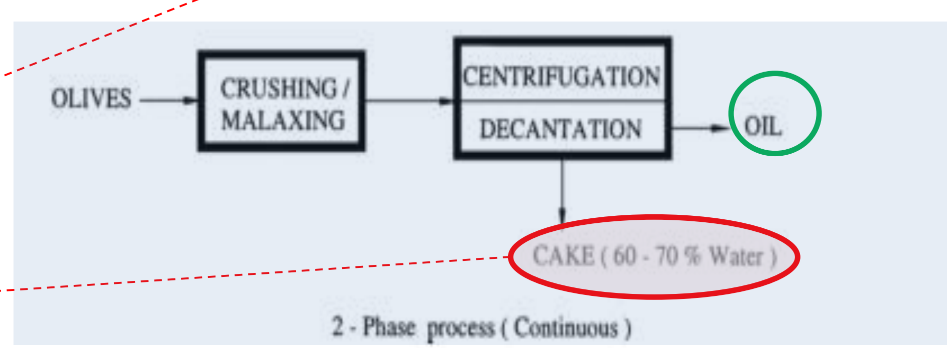
- Traditional press



- **3-phase centrifugation**



- **2-phase centrifugation**



Olive mill solid wastes :

3P-OMSW

2P-OMSW

(Azbar et al., 2004)

1. Introduction

3-phase olive oil production

- Olive husk/ olive pomace
- Wastewater

High energy and water demands

High wastewater production

2-phase olive oil production

- **Water-solid mixture/** alperujo/ wet olive husk …

Process called "**ecological**":

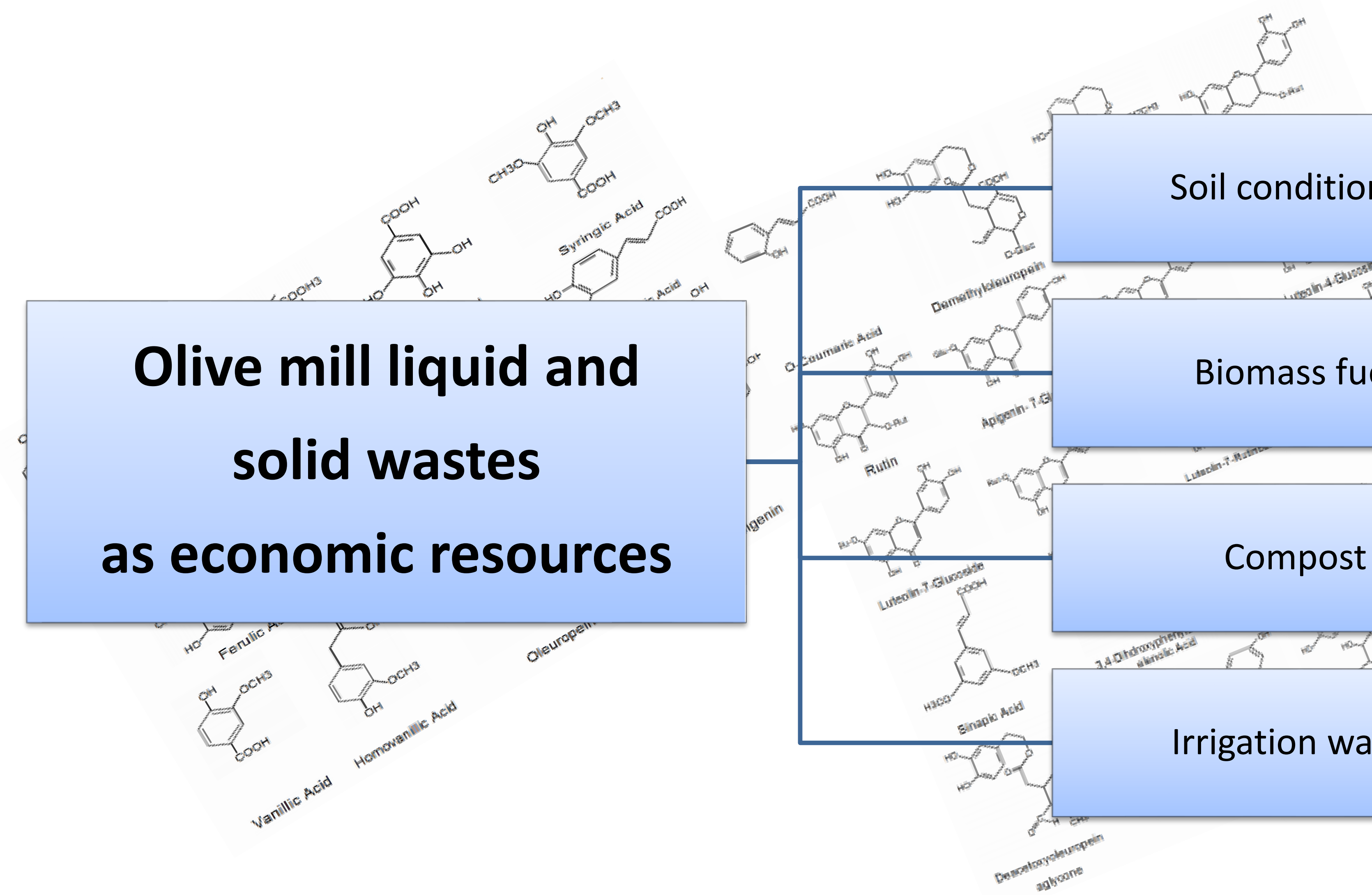
Less wastewater with lower pollutant load

BUT

Water-solid waste has high moisture and organic load

OMSW constitute an important environmental concern

1. Introduction



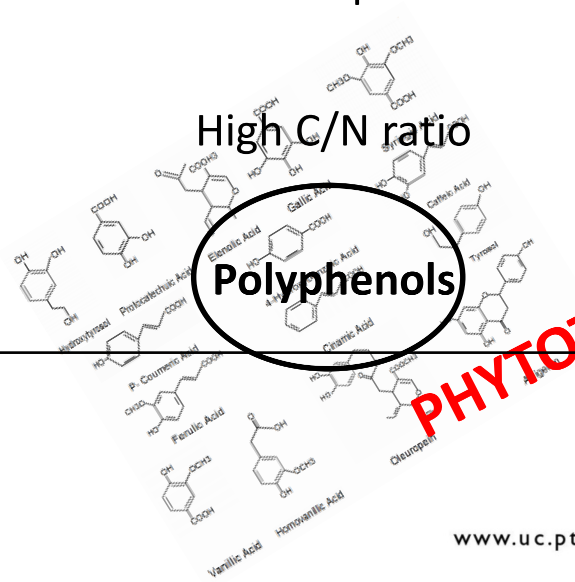
Olive mill liquid and solid wastes as economic resources

- Soil conditioner
- Biomass fuel
- Compost
- Irrigation water

1. Introduction

Soil applications of olive mill wastes

Advantages	Disadvantages
High nutrients concentration (K, P)	High mineral salt content
High antimicrobial capacity	Low pH
	High C/N ratio



PHYTOTOXICITY

1. Introduction

Main objective of the study:

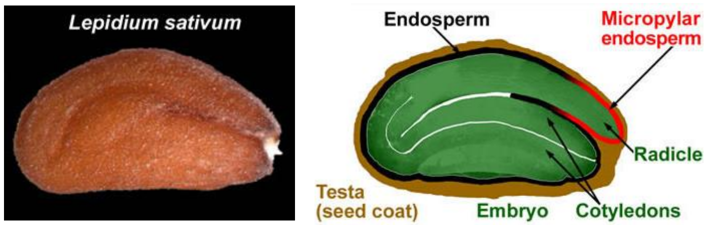
Investigate the main compounds responsible for phytotoxicity of OMSW

Specific objectives:

1. Characterization of two olive mill solid wastes (OMSW):
   - 2P-OMSW
   - 3P-OMSW

2. Assess phytotoxicity of both wastes: germination assays with *Lepidium Sativum* (garden cress):
   - Rate of seed germination
   - Root length
   - Germination Index

   Influence of :
   - L/S ratio
   - Phenolic compounds concentration



Müller et al. (2006) - © Plant Cell Physiology, Oxford University Press, http://pcp.oxfordjournals.org

2. Experimental methodology

1) Sampling of both wastes

2P-OMSW

Collected from a **2-phase** olive mill in the Spanish region of Estremadura.

Wet appearance (**H ≈70%**)

3P-OMSW

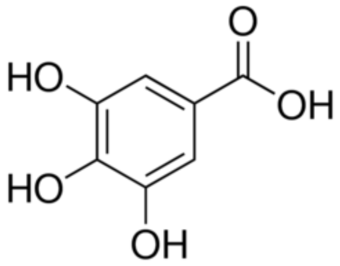
Collected from a **3-phase** olive mill in the center of Portugal.

Dry appearance (**H ≈20%**)

2. Experimental methodology

2) Characterization of 2P-OMSW and 3P-OMSW

- ✓ Moisture (H) and Total Solids (TS)
- ✓ Volatile Solids (VS) and Total Organic Carbon (TOC)
- ✓ Water Holding Capacity (WHC)
- ✓ pH
- ✓ Electrical Condutivity (EC)
- ✓ Chemical Oxygen Demand (COD)
- ✓ Total Phenolic Content (TPH)
- ✓ Total Kjeldahl Nitrogen (TKN)
- ✓ Total Nitrogen (TN)
- ✓ Phosphorous Concentration (P)

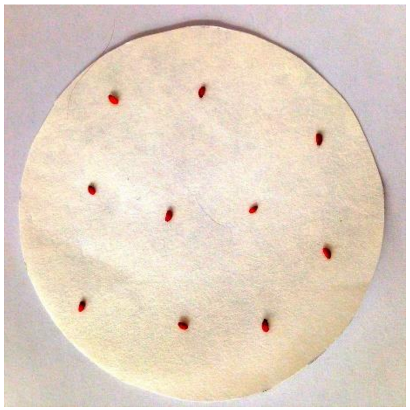


Gallic acid

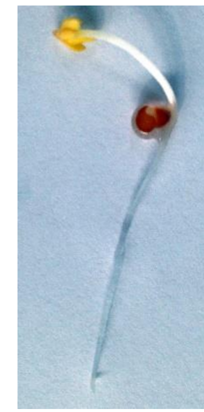
2. Experimental methodology

3) Germination Index assays

Germination assays were performed using the method discribed by Trautmann and Krasny (1977)



48 h
25 °C
darkness



Petri dishes with 9 cm diameter
10 seeds of *Lepidum Sativum*
5 mL of waste extract/ phenolic solution

- Number of germinated seeds (N_{SG})
- Root length (L_R)

Determination of:
- Relative Seed Germination (RSG)
- Relative Root Growth (RRG)

(by comparison with the blank)

$$GI\ (\%) = RSG \times RRG \times 100$$

GI (%)	
> 100	The material maximizes plants germination and root growth
80 – 100	No phytotoxic
60 – 80	Moderately phytotoxic
60 – 40	Phytotoxic
< 40	Highly phytotoxic

2. Experimental methodology

3) Germination Index assays

i. L/S ratio assays: 10, 50, 100, 250 and 500 L/kg

ii. Phenolic compounds assays: concentration range 5–500 ppm

- ✓ 3,4,5-Trimethoxybenzoic acid
- ✓ 4-Hydroxybenzoic acid
- ✓ Caffeic acid
- ✓ Cinnamic acid
- ✓ Gallic acid
- ✓ p-Coumaric acid
- ✓ Phenol
- ✓ Protocatechuic acid
- ✓ Syring acid
- ✓ Vanillic acid

iii. Synthetic effluent assay: mixture of six phenolic acids tested at 100, 50 and 25 ppm (Martins, Rossi, & Quinta-ferreira, 2010)

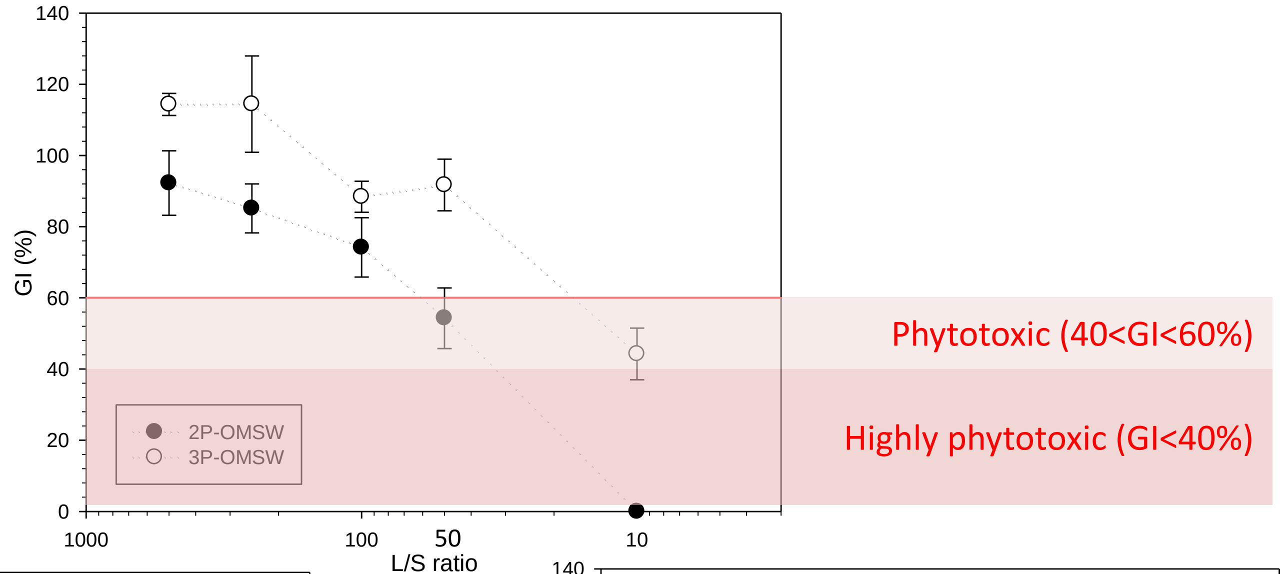
- ✓ 3,4,5-Trimethoxybenzoic acid
- ✓ 4-Hydroxybenzoic acid
- ✓ Protocatechuic acid
- ✓ Syringic acid
- ✓ Vanillic acid
- ✓ Veratric acid

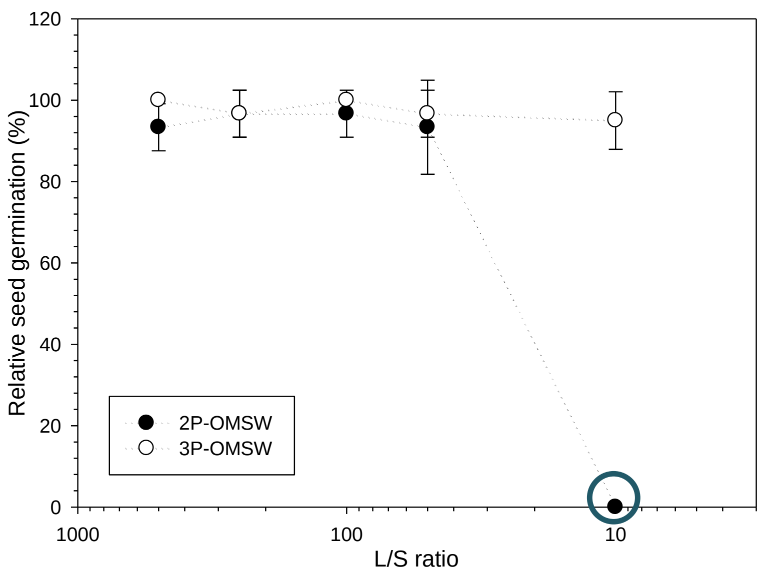
3.1 – Characterization of 2P and 3P-OMSW

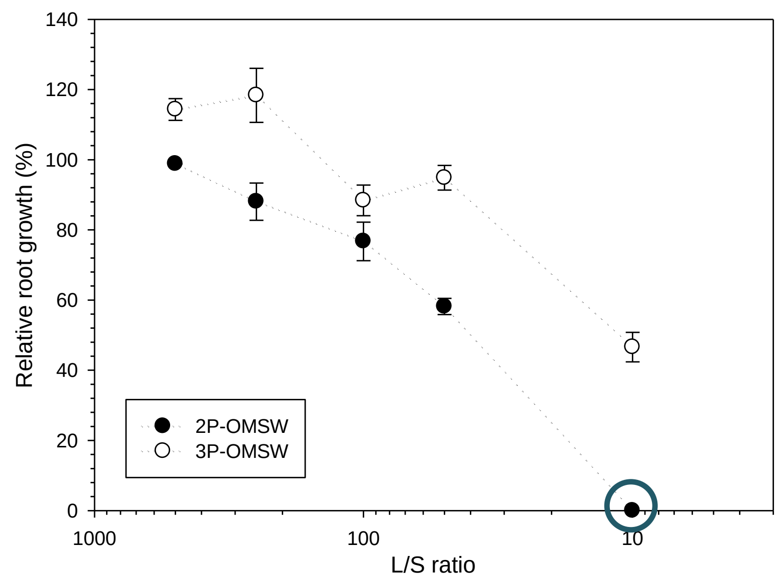
	2P-OMSW	3P-OMSW
H (%)	67,1 ± 0,5	17,6 ± 0,6
TS (%)	32,9 ± 0,5	82,4 ± 0,56
VS (%)	95,2 ± 0,2	94,2 ± 1,8
TOC (%)	52,9 ± 0,1	54,4 ± 1,0
WHC (%)	97,8 ± 6,9	91,4 ± 16,8
pH	4,82 ± 0,00	4,96 ± 0,02
EC (mS/cm)	2,77 ± 0,03	0,92 ± 0,05
COD (g O2/ g dm)	2,40 ± 0,19	2,48 ± 0,05
TPH (mg GAE/ g dm)	0,99 ± 0,03	0,93 ± 0,03
TKN (mg/ g dm)	10,5 ± 4,7	11,1 ± 1,8
TN (mg/ g dm)	11,7 ± 0,05	14,0 ± 0,9

mean ± std
dm – dry matter
GAE – gallic acid equivalentes

3.2 Germination assays with olive mill wastes extracts

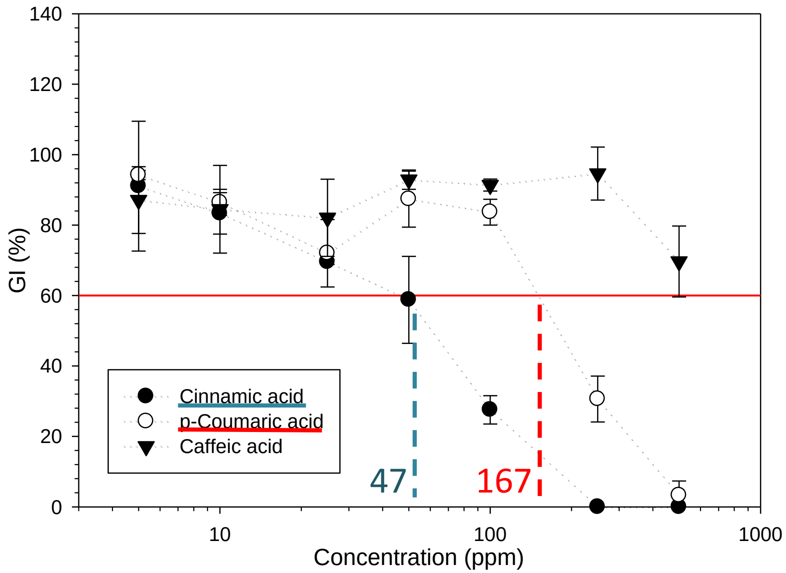


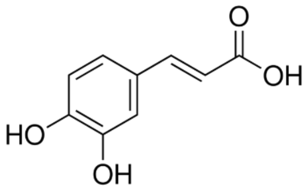




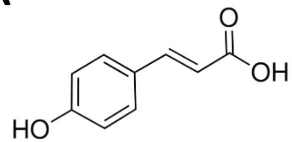
3. Results and discussion

3.3 Germination assays with phenolic compounds (Cinnamic Acids)

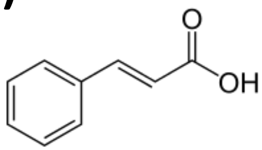




Caffeic



p-Coumaric

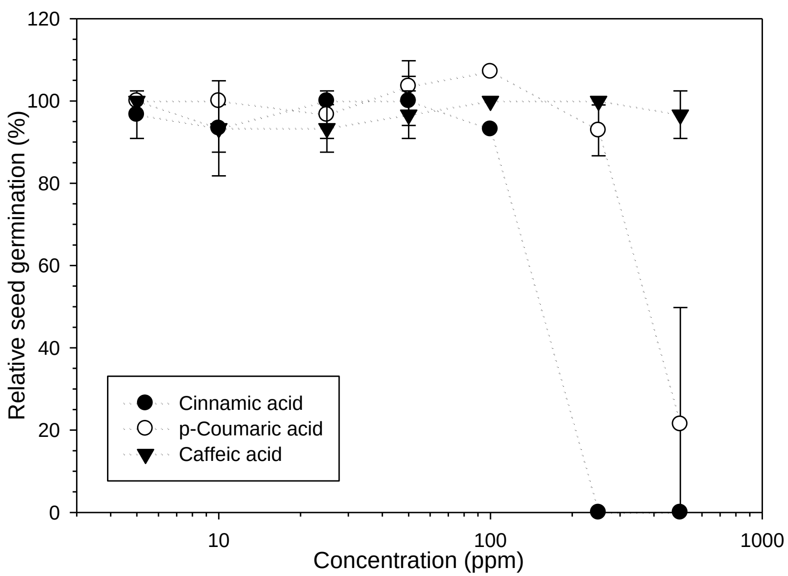


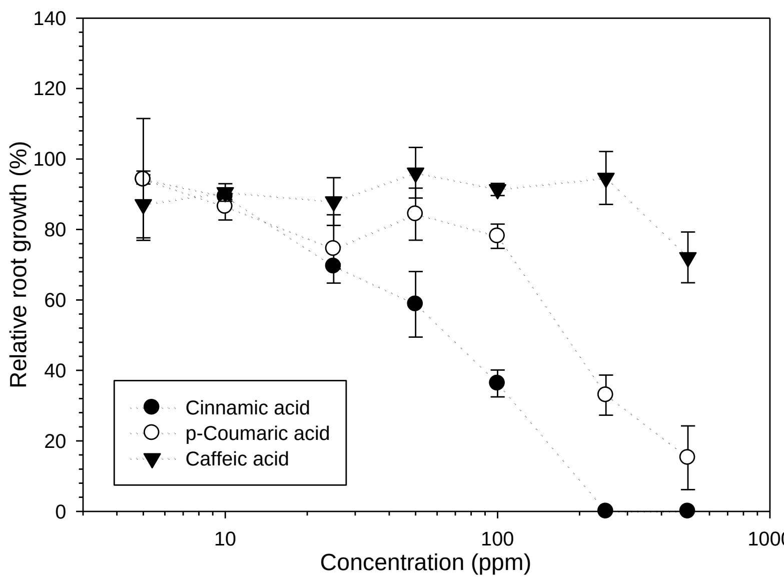
Cinnamic

Less –OH groups → Higher phytotoxicity

→ Less germinated seeds

→ Less root growth





3. Results and discussion

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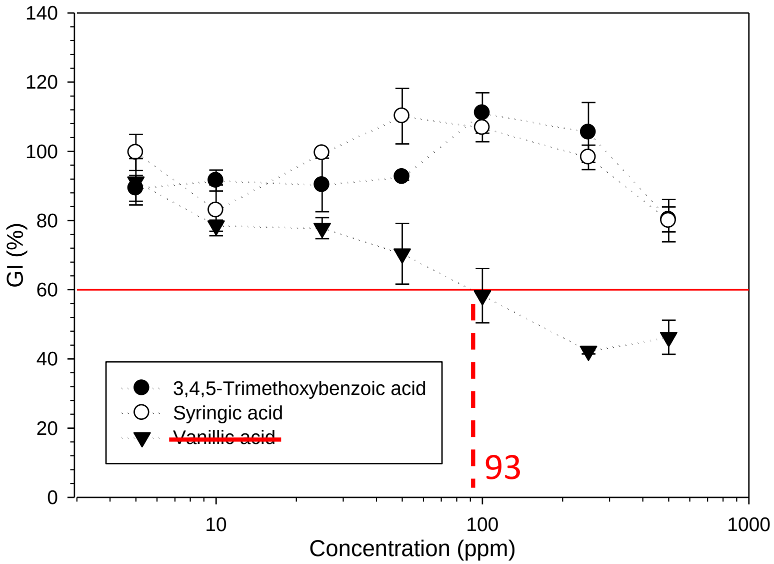
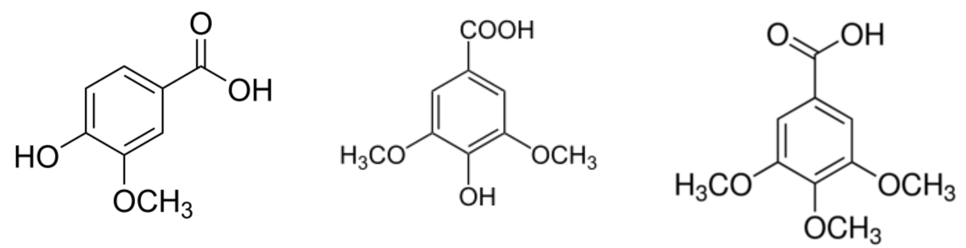
3.3 Germination assays with phenolic compounds (– OCH_3 –OH acids)

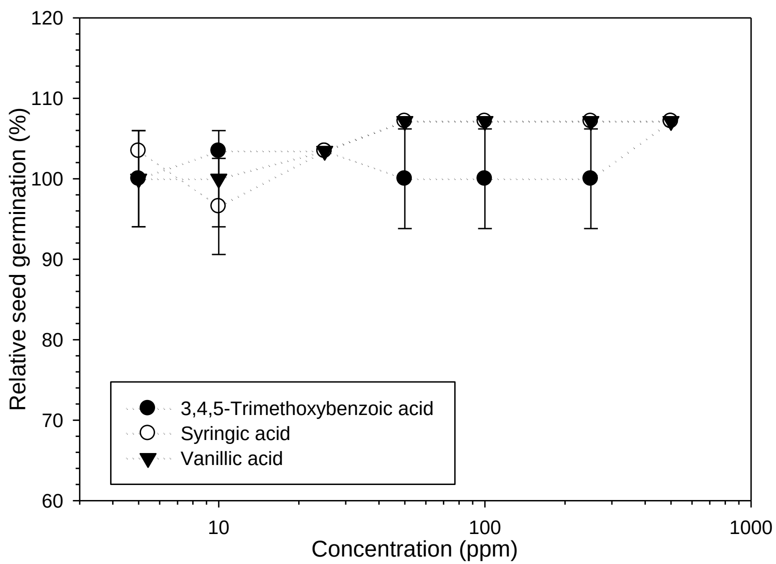


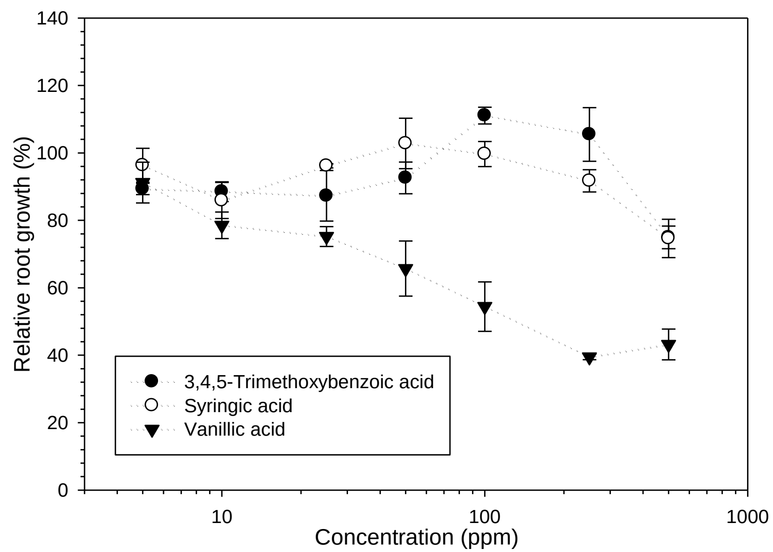


Vanillic Syringic 3,4,5-Trimethoxybenzoic

Less –OCH_3 groups → Higher phytotoxicity

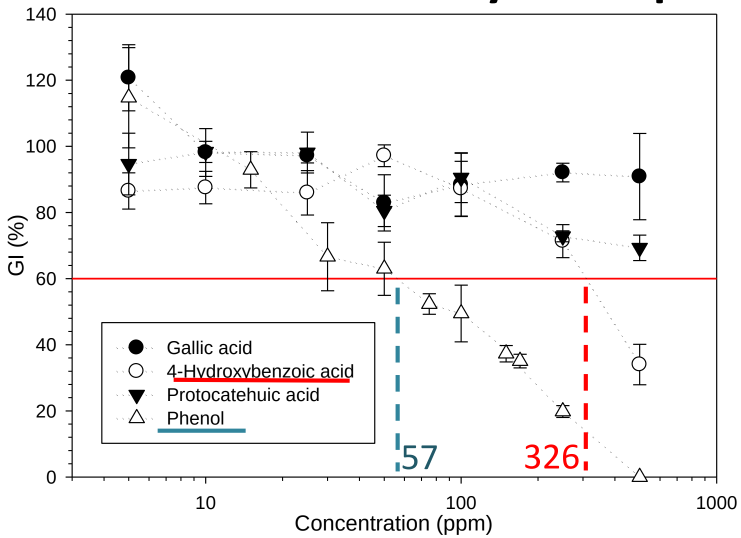
→ Less root growth

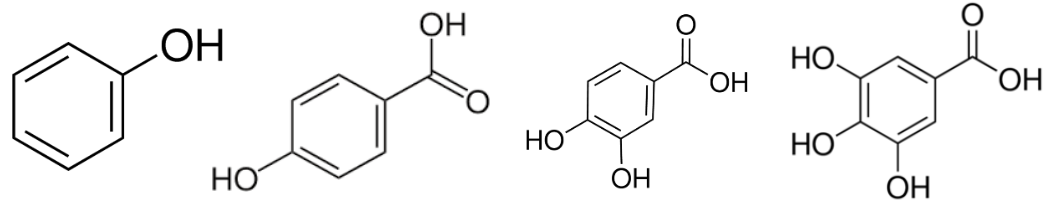




3. Results and discussion

3.3 Germination assays with phenolic compounds (benzoic acids and phenol)

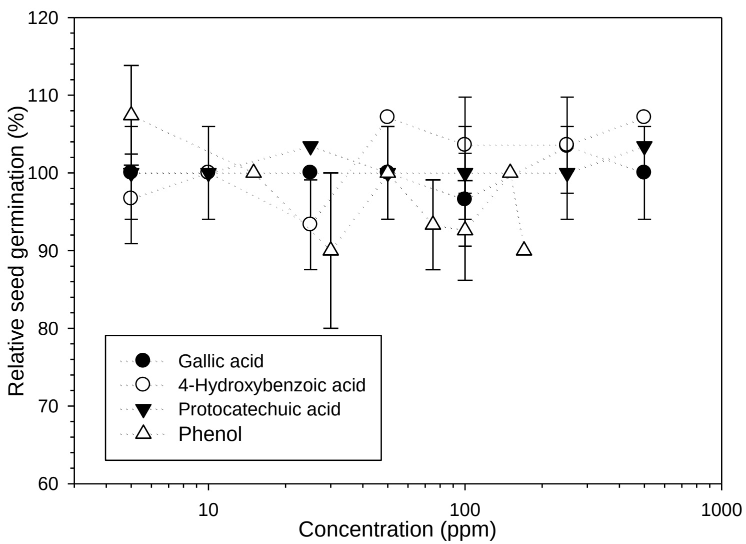


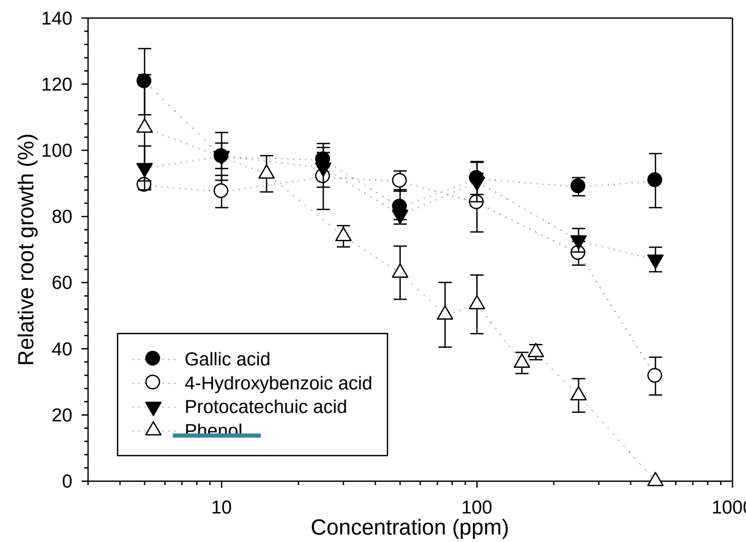


Phenol 4-Hydroxybenzoic Protocatechuic Gallic

Less –OH groups → Higher phytotoxicity

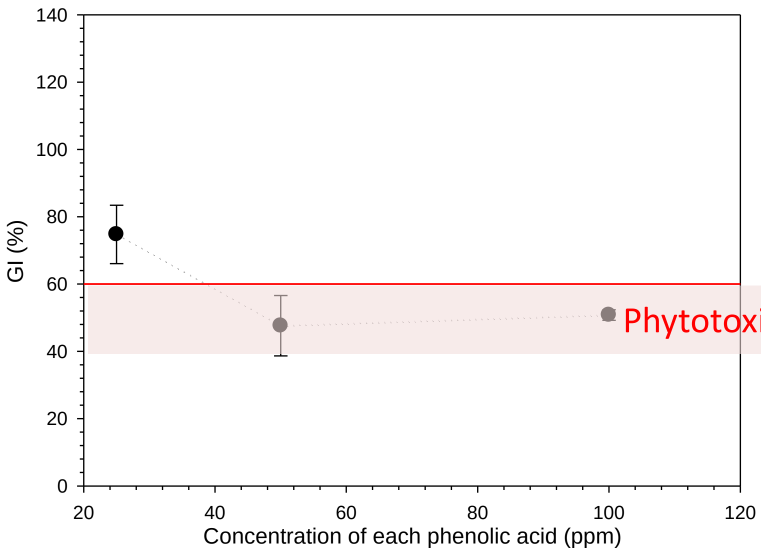
→ Less root growth

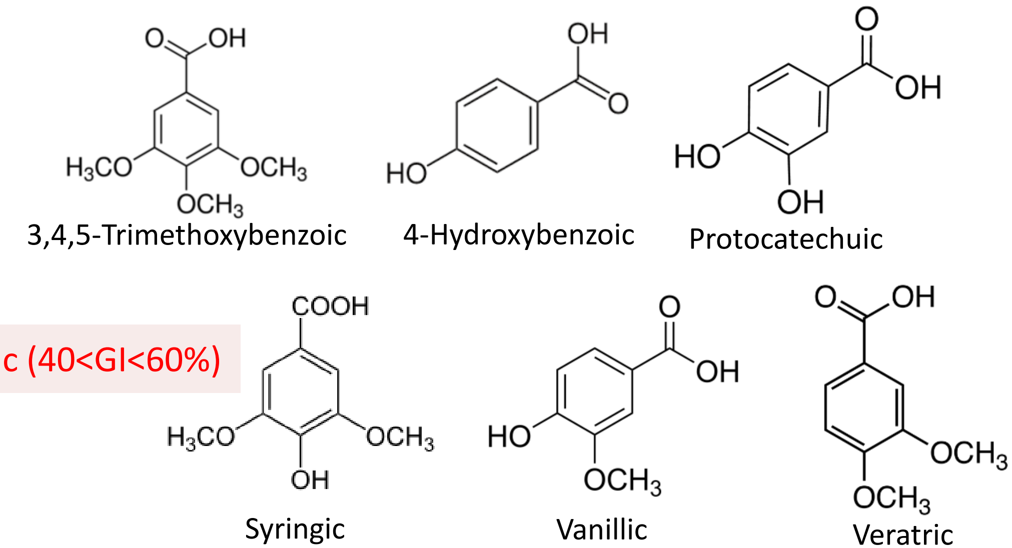


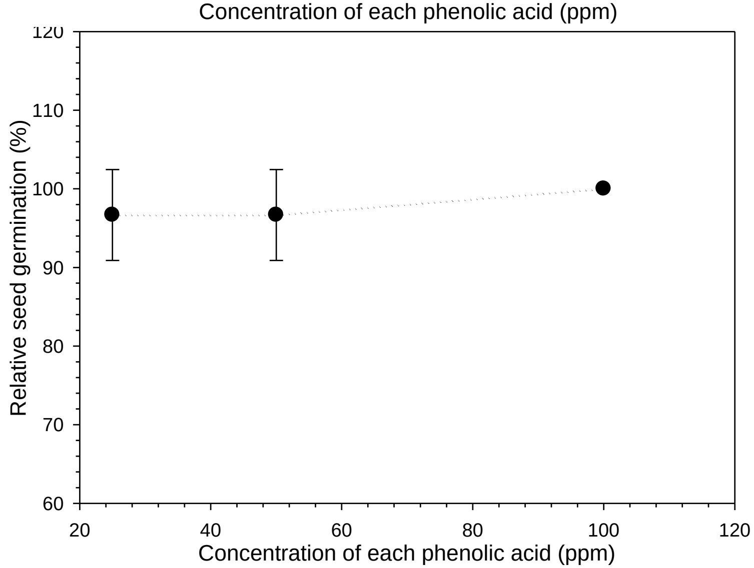


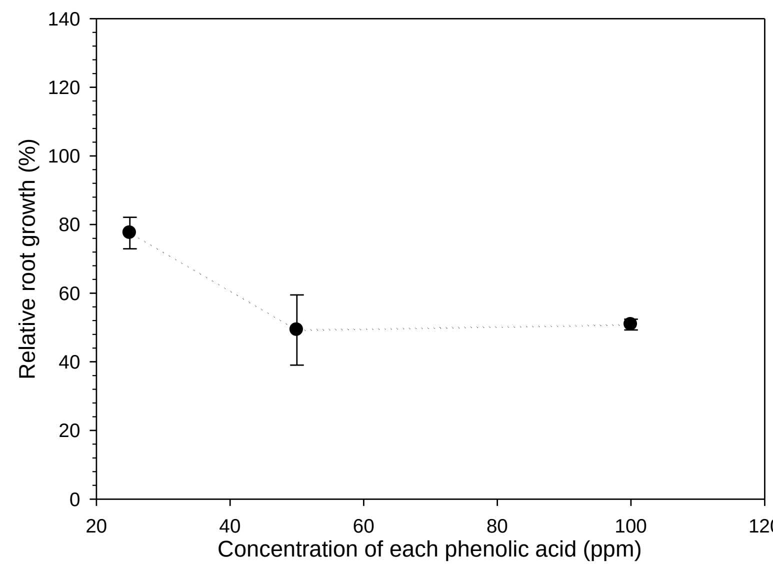
3. Results and discussion

3.4 Germination assays with a synthetic effluent









4. Conclusions

- ✓ 2P-OMSW is more phytotoxic than 3P-OMSW for *Lepidium Sativum;*
- ✓ For cinnamic acids, molecules with less –OH groups seem to have higher phytotoxicity, less root growth and less germinated seeds;
- ✓ For –OCH3 acids, less –OCH3 groups reveal higher phytotoxicity and less root growth;
- ✓ For benzoic acids, less –OH groups also causes higher phytotoxicity and less root growth.

Thank you for your attention

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