Phytotoxicity of essential oils and extracts major constituents from MAP species against common sow thistle (*Sonchus oleraceus*)

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CENTRO DE INVESTIGACIÓN Y TECNOLOGÍA AGROALIMENTARIA DE ARAGÓN

* In collaboration with **NATIONAL CENTER FOR FOOD TECHNOLOGY AND SAFETY**

Domestication and valorisation of medicinal aromatic plants and other forestry resources

MAPs promotion and cultivation in rural areas as an alternative to conventional crops

Working on weed control

Research projects related with weed control

Bloque 1	HL (S M) 1	LB (M S) 2	MU (S M) 3	HH (S M) 4
Bloque 2	HH 5	MU 6	LB 7	HL 8
Bloque 3	LB 9	HH 10	HL 11	MU 12

Factor 1: Control mala hierba

HL: Aplicación hidrolato lavandín

LB: Laboreo tradicional

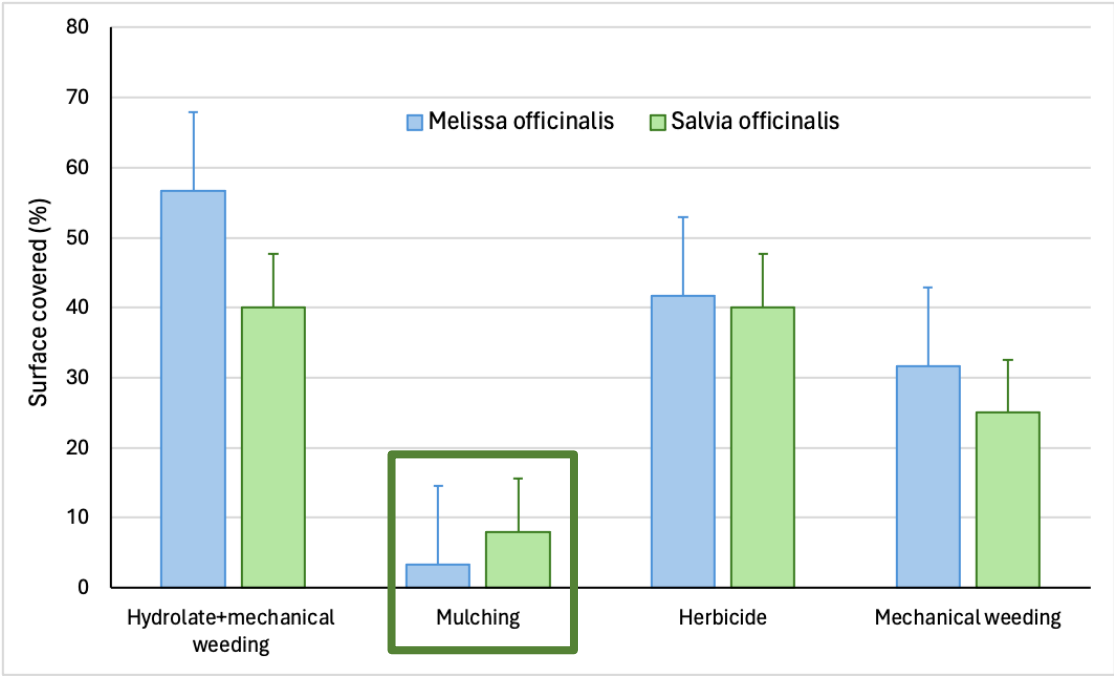
MU: Acolchado con restos destilación

HH: Aplicación herbicida

Factor 2: Especie de cultivo

S: Salvia

M: Melisa



Introduction

Annual species ***S. oleraeus* (common sow thistle)**

- Genus *Sonchus* are common weeds spread around the world
- Large genetic diversity, invasive potential and ecological adaptations (Khalsa et al., 2021)
- Grows in crops possess remarkable colonization capacity (Aizpuru et al., 1999; Ruiz-Rocamora et al., 2025)
- It contributes to ecosystem services, but in cultivated areas is regarded as weed due to competition with crops (Ruiz-Rocamora et al., 2025)

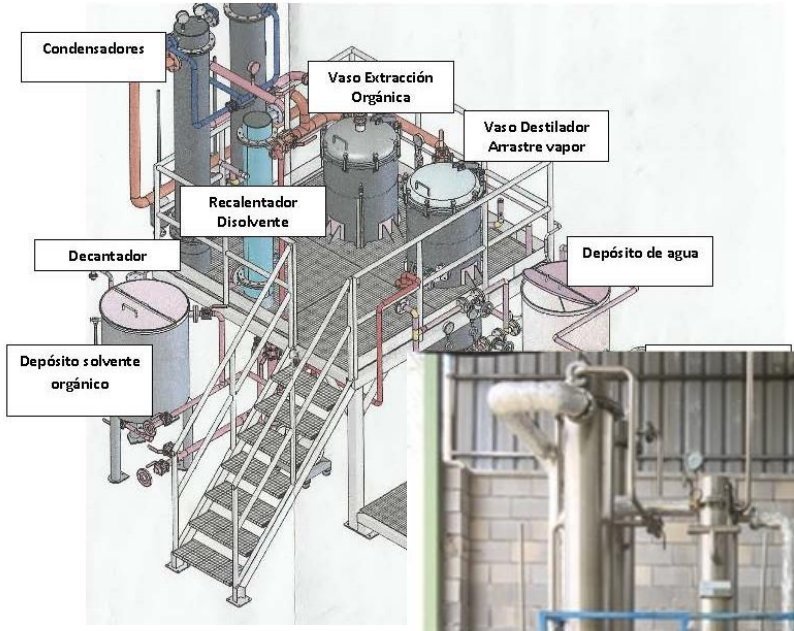
Reliance on chemical control has triggered herbicide resistance of biotypes of *S. oleraceus* (Khalsa et al., 2021)

Importance of integrated weed management and alternative strategies beyond chemical weed control

Evaluate the phytotoxic effect of different essential oils (EO), extracts (EXT) and their major constituents during *in vitro* germination of *S. oleraceus* seeds.

Obtention of essentil oils

Steam distillation in a semi industrial scale pilot plant



1 h

0.5 bar

Extracts obtention from distilled biomass

Hydroalcoholic extraction with Soxhlet apparartus

Ethanol 60%

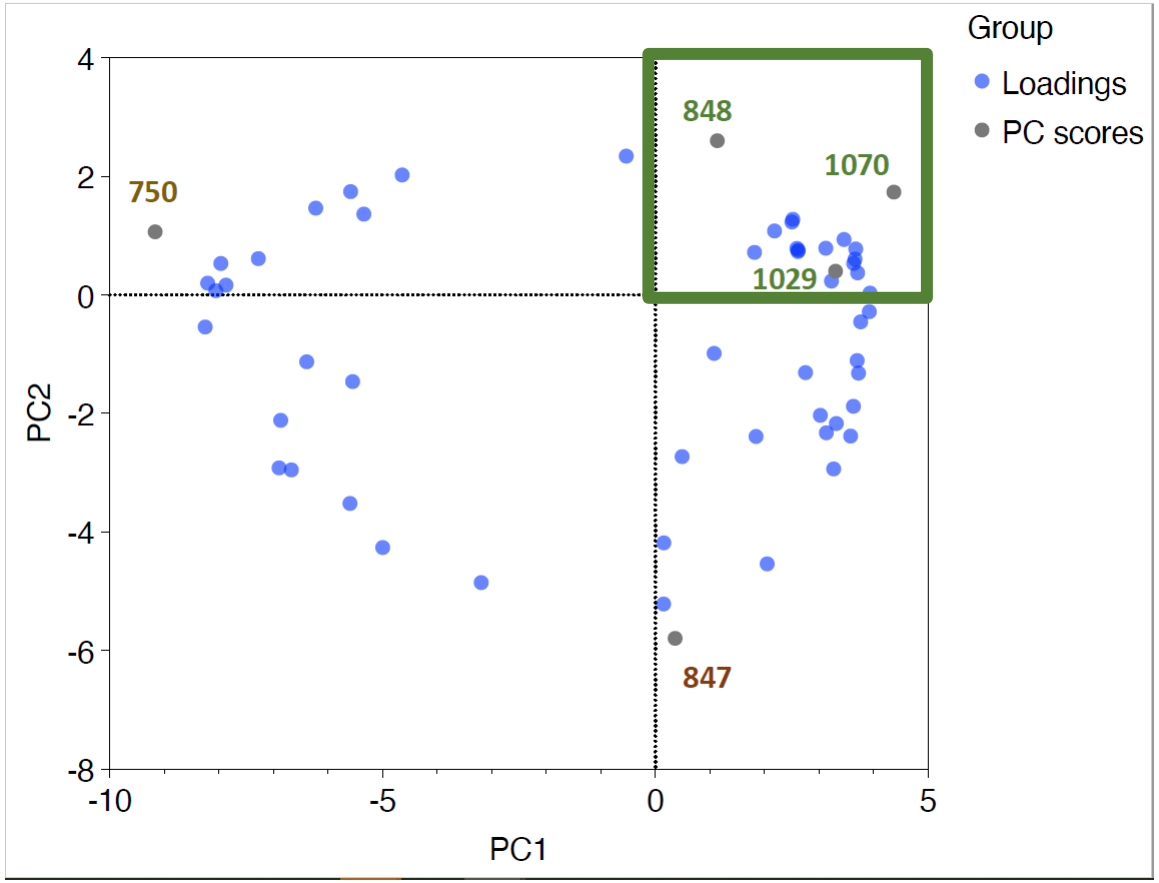
2.5 h

1:50 solvent to sample ratio

MAP species characterization

MAP species	Biomass production (kg/plant)	Essential oil yield (g/kg)
MAP 1	2022: 5870,8 kg/ha	2,70
	2023: 945,0 kg/ha	5,45
	2024: 793,75 kg/ha	4,54
MAP 2	-	2023: 3,59 2025: 8,04
MAP 3	2024: 1,23 (0,17)	2024: 2,89
MAP 4	-	2023: 4,51 2024: 3,55 2025: 2,57
MAP 5	-	2023: 22,24 2024: 9,61 2025: 13,23
MAP 6	2023: 0,85 (0,31)	6,34
	2024: -	4,04
MAP 7	2023: 0,49 (0,15)	5,51
	2024: 0,56 (0,11)	4,44

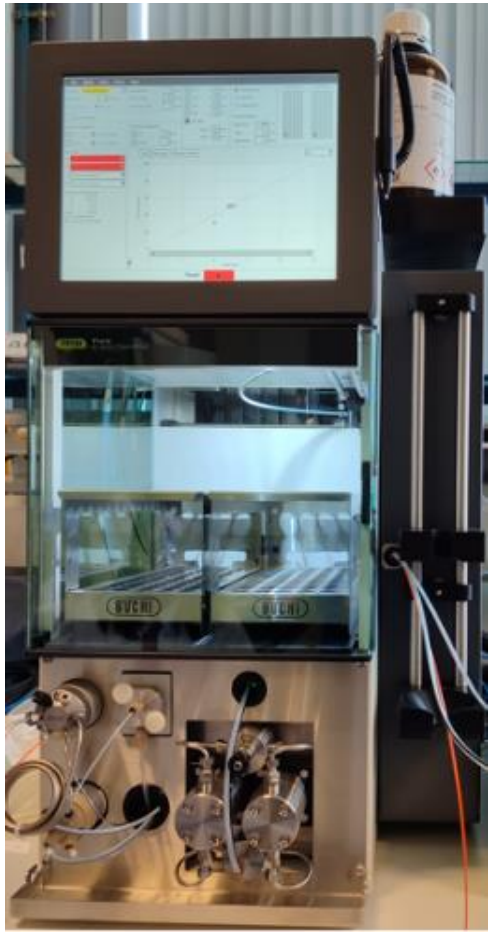
EO composition of wild collected MAP1 subjected to sustainable management of the population



Purification of extracts obtained from aromatic plants by Flash Chromatography

Equipment

BÜCHI equipped with ultraviolet (UV) and ELS (Evaporative Light Scattering) detectors.
The system allows operation in preparative mode, reaching up to 300 bar of pressure and a maximum flow rate of 150 mL/min. In flash mode, it operates up to 50 bar and 250 mL/min.



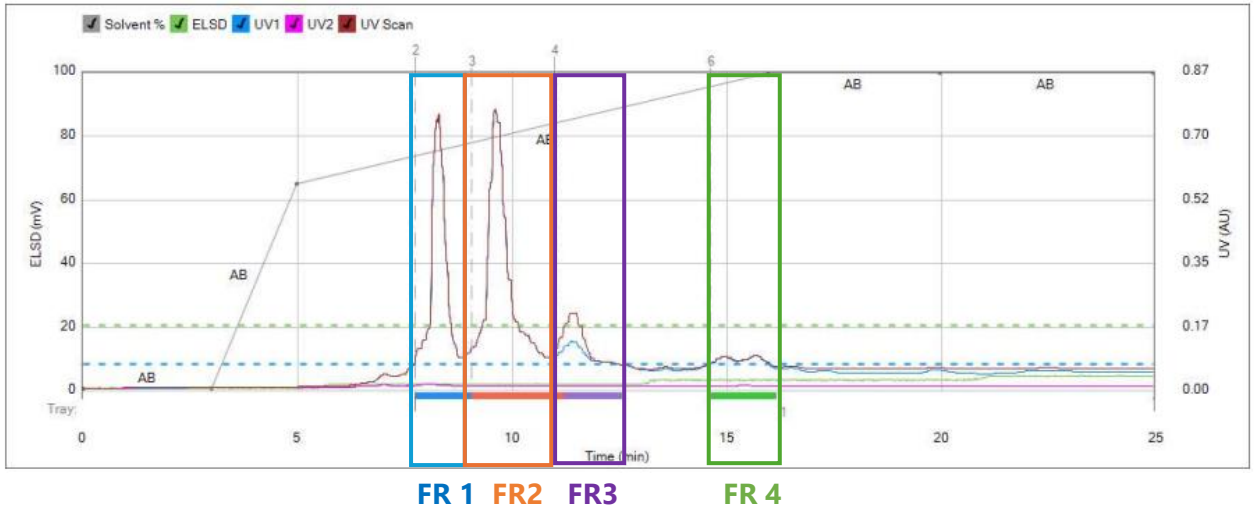
Chromatrographic Conditions

Flash chromatography conditions	Essential oils	Hydroalcoholic extracts
Stationary phase/loading capacity	FlashPure Select C18 / 120g	FlashPure Select C18 /120 g
Mobile pase (solvent A/solvent B)	water:methanol 70:30/ acetonitrile plus 0.5% formic acid	Water/acetonitrile plus 0.5% formic acid
Elution type	gradient	gradient
Detector	UV detector Evaporative light scattering detector (ELSD)	UV detector Evaporative light scattering detector (ELSD)

Purification of extracts obtained from aromatic plants by Flash Chromatography

Isolation of the main fractions

MAP species	Hydroalcoholic extracts	Essential oil
MAP 1	4	5
MAP 2	2	2
MAP 3	3	5
MAP 4	2	7
MAP 5	2	4
MAP 6	2	3
MAP 7	2	5
MAP 8	2	-



MAP species 4 EO fractions

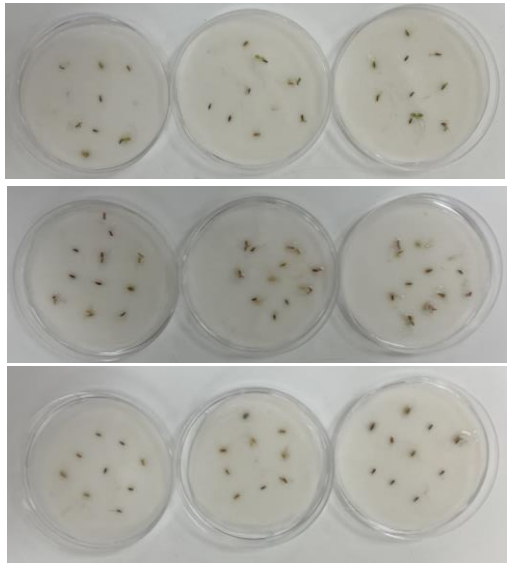
Preparation of solutions for phytotoxicity assay

1) Elution solvent evaporation

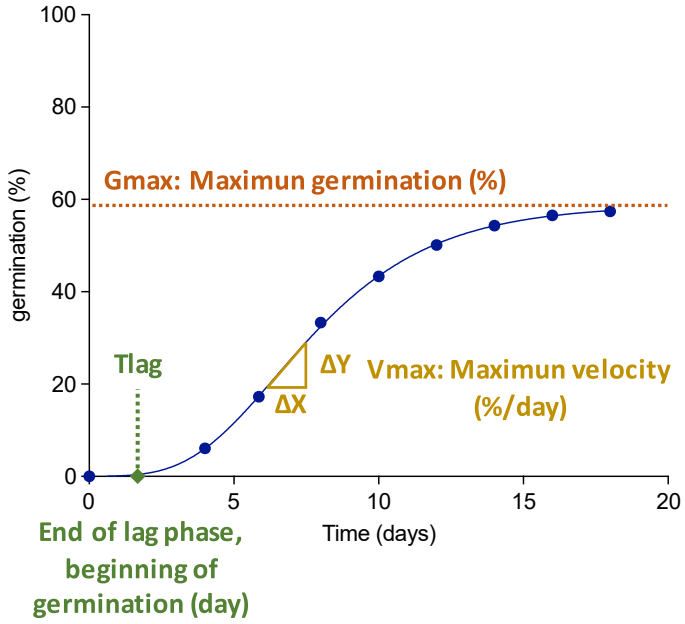
2) Fraction resuspension in 0,3% EtOH + 0,05% polysorbate (stock solution)

3) Petri dishes inoculation with 5 mL of AE and FRs at A, B and C ppm

4) Seed placing and monitoring each 2 days



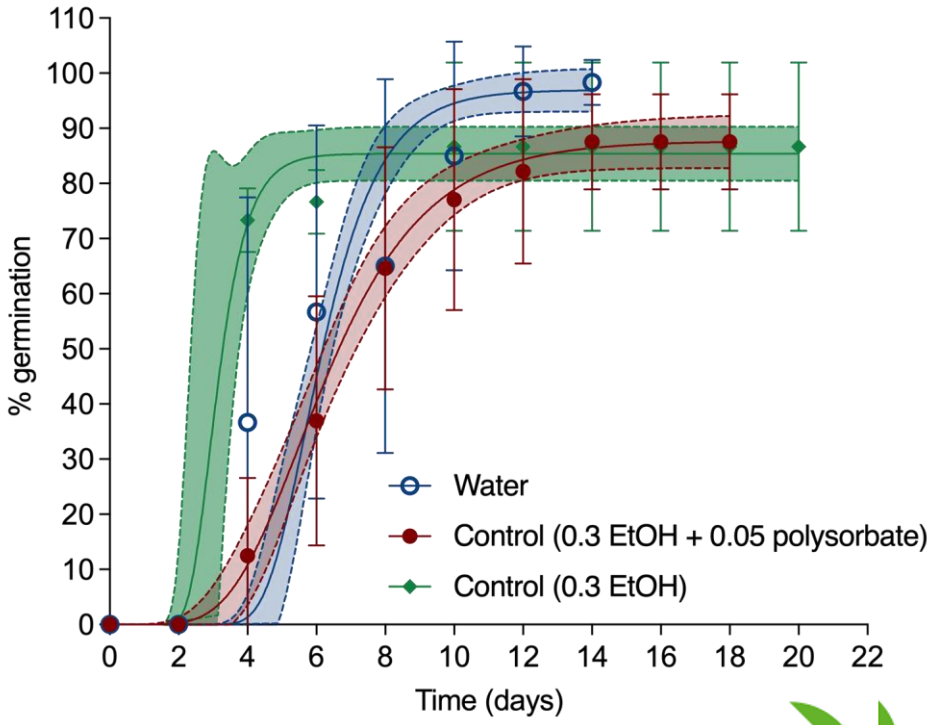
5) Regression of experimental data with Gompertz function modified by Scott et al., 1984



MGT: mean germination time (day)
Tlag + (1.577 * Gmax / (Vmax * e)

Inhibitory effectivity of control (0,3% EtOH + 0,05 polysorbate) against *S. oleraceus* and kinetic parameters

	Germination (%)	Inhibition (%)	Gmax (%)	Vmax (%/day)	Tlag (day)	MGT (day)
Water	98,33 (4,08)	-	96,95 (1,96)	30,50 (5,28)	4.53 (0.34)	6.38 (0.16)
Control (0.3 EtOH + 0.05 polysorb)	87,53 (8,62)	11,86 (10,17)	87,70 (2,48)	16,03 (2,20)	3.48 (0.38)	6.65 (0.28)
Control (0.3 EtOH)	86.67 (15.28)	13.00 (7.90)	85.40 (2.39)	52.23 (24.33)	2.30 (0.74)	3.25 (0.40)



Inhibitory effectivity of EOs and FRs against *S. oleraceus*

MAP species 1 (MAP1)

	Concentration (ppm)	Germination (%)	Inhibition (%)
EO	A	50,0 (26,5)	42,9 (30,2)
	B	73,3 (30,6)	16,2 (34,9)
	C	73,3 (15,3)	16,2 (17,5)
FR1	A	83,3 (20,8)	4,8 (23,8)
	B	-	-
	C	-	-
FR2	A	80,0 (0,0)	8,6 (0,0)
	B	64,8 (18,3)	26,0 (21,0)
	C	46,7 (15,3)	46,7 (17,5)
FR3	A	60,0 (17,3)	31,5 (19,8)
	B	73,3 (11,5)	16,2 (13,2)
	C	70,0 (10,0)	20,0 (11,4)
FR4	A	66,7 (15,3)	23,8 (17,5)
	B	70,0 (10,0)	20,0 (11,4)
	C	41,7 (17,6)	52,4 (20,1)
FR5	A	27,4 (20,5)	68,7 (23,4)
	B	0,0 (0,0)	100,0 (0,0)
	C	0,0 (0,0)	100,0 (0,0)

MAP species 2 (MAP2)

	Concentration (ppm)	Germination (%)	Inhibition (%)
EO	A	16,7 (11,5)	81,0 (13,2)
	B	0,0 (0,0)	100,0 (0,0)
	C	0,0 (0,0)	100,0 (0,0)
FR1	A	50,0 (26,5)	42,9 (30,2)
	B	56,7 (20,8)	35,3 (23,8)
	C	60,0 (20,0)	31,5 (22,8)
FR2	A	46,7 (5,8)	46,7 (6,6)
	B	43,3 (5,8)	50,5 (6,6)
	C	76,7 (11,5)	12,4 (13,2)

MAP species 3 (MAP3)

	Concentration (ppm)	Germination (%)	Inhibition (%)
EO	A	70,0 (10,0)	20,0 (11,4)
	B	66,7 (15,3)	23,8 (17,5)
	C	63,3 (20,8)	27,6 (23,8)
FR1	A	83,3 (28,9)	4,8 (33,0)
	B	64,2 (30,9)	26,6 (35,3)
	C	76,7 (25,2)	12,4 (28,8)
FR2	A	80,0 (10,0)	8,6 (11,4)
	B	80,0 (10,0)	8,6 (11,4)
	C	56,7 (25,2)	35,3 (28,8)
FR3	A	73,3 (11,5)	16,2 (13,2)
	B	73,3 (5,8)	16,2 (6,6)
	C	83,3 (15,3)	4,8 (17,5)
FR4	A	80,0 (17,3)	8,6 (19,8)
	B	73,3 (5,8)	16,2 (6,6)
	C	76,7 (15,3)	12,4 (17,5)
FR5	A	63,3 (15,3)	27,6 (17,5)
	B	63,3 (20,8)	27,6 (23,8)
	C	46,7 (20,8)	46,7 (23,8)

Inhibitory effectivity of EOs and FRs against *S. oleraceus*

MAP species 4 (MAP4)

	Concentration (ppm)	Germination (%)	Inhibition (%)
AE	A	73,3 (5,8)	16,2 (6,6)
	B	66,7 (11,5)	23,8 (13,2)
	C	40,0 (30,0)	54,3 (34,3)
FR1	A	96,7 (5,8)	-10,4 (6,6)
	B	83,0 (5,1)	5,2 (5,9)
	C	90,0 (10,0)	-2,8 (11,4)
FR2	A	66,7 (25,2)	23,8 (28,8)
	B	73,3 (15,3)	16,2 (17,5)
	C	83,3 (5,8)	4,8 (6,6)
FR3	A	89,6 (10,0)	-2,4 (11,4)
	B	83,3 (15,3)	4,8 (17,5)
	C	90,0 (0,0)	-2,8 (0,0)
FR4	A	80,0 (10,0)	8,6 (11,4)
	B	83,3 (11,5)	4,8 (13,2)
	C	66,7 (20,8)	23,8 (23,8)
FR5	A	96,7 (5,8)	-10,4 (6,6)
	B	90,0 (10,0)	-2,8 (11,4)
	C	96,7 (5,8)	-10,4 (6,6)
FR6	A	70,0 (17,3)	20,0 (19,8)
	B	33,3 (25,2)	61,9 (28,8)
	C	0,0 (0,0)	100,0 (0,0)
FR7	A	86,7 (11,5)	1,0 (13,2)
	B	46,7 (20,8)	46,7 (23,8)
	C	3,3 (5,8)	96,2 (6,6)

MAP species 5 (MAP5)

	Concentration (ppm)	Germination (%)	Inhibition (%)
AE	A	80,0 (10,0)	8,6 (11,4)
	B	63,3 (15,3)	27,6 (17,5)
	C	60,0 (10,0)	31,5 (11,4)
FR1	A	63,0 (6,4)	28,1 (7,3)
	B	66,7 (22,2)	23,8 (25,4)
	C	51,8 (28,0)	40,8 (31,9)
FR2	A	86,7 (5,8)	1,0 (6,6)
	B	63,3 (5,8)	27,6 (6,6)
	C	66,7 (15,3)	23,8 (17,5)
FR3	A	50,0 (10,0)	42,9 (11,4)
	B	50,0 (20,0)	42,9 (22,8)
	C	80,0 (10,0)	8,6 (11,4)
FR4	A	93,3 (5,8)	-6,6 (6,6)
	B	53,3 (30,6)	39,1 (34,9)
	C	6,7 (11,5)	92,4 (13,2)

MAP species 6 (MAP6)

	Concentration (ppm)	Germination (%)	Inhibition (%)
AE	A	56,7 (15,3)	35,3 (17,5)
	B	23,3 (5,8)	73,3 (6,6)
	C	0,0 (0,0)	100,0 (0,0)
FR1	A	90,0 (0,0)	-2,8 (0,0)
	B	70,0 (10,0)	20,0 (11,4)
	C	66,7 (11,5)	23,8 (13,2)
FR2	A	50,0 (17,3)	42,9 (19,8)
	B	66,7 (11,5)	23,8 (13,2)
	C	60,0 (17,3)	31,5 (19,8)
FR3	A	73,3 (25,2)	16,2 (28,8)
	B	50,0 (10,0)	42,9 (11,4)
	C	56,7 (30,6)	35,3 (34,9)

Inhibitory effectivity of EOs and FRs against *S. oleraceus*

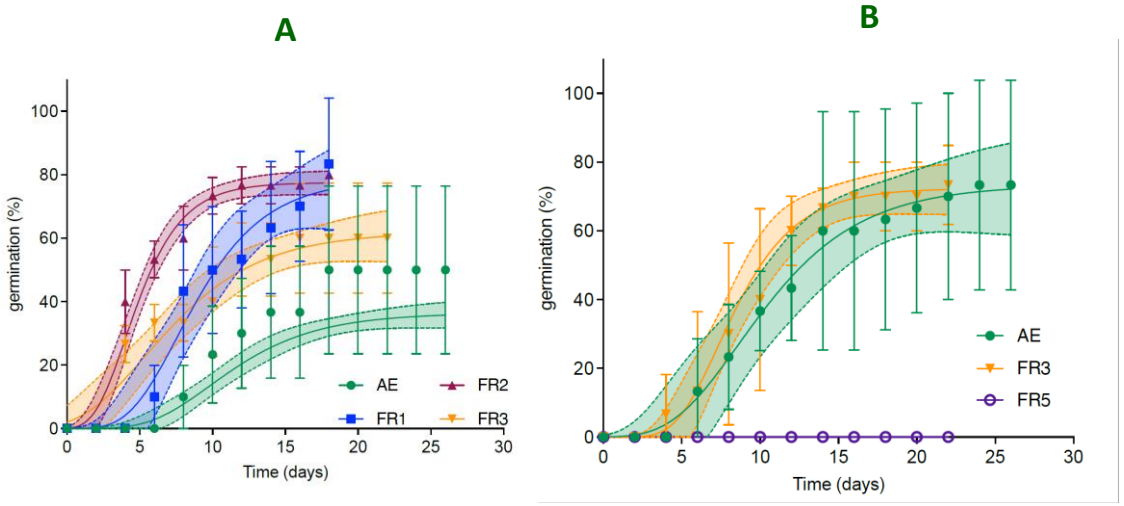
MAP species 7 (MAP7)

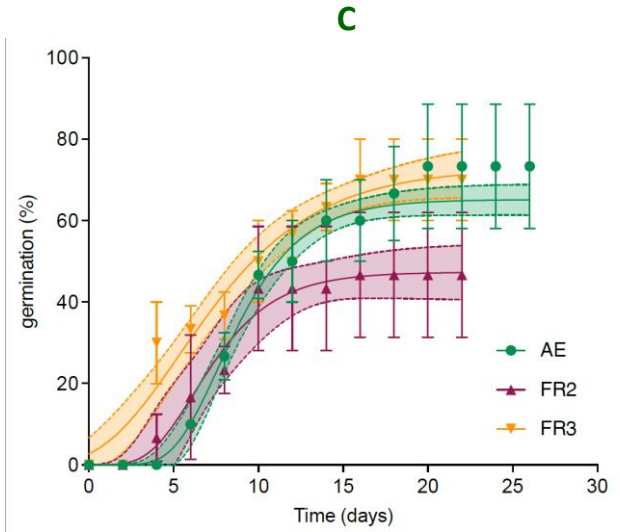
	Concentration (ppm)	Germination (%)	Inhibition (%)
AE	A	53,3 (20,8)	39,1 (23,8)
	B	0,0 (0,0)	100,0 (0,0)
	C	0,0 (0,0)	100,0 (0,0)
FR1	A	96,7 (5,8)	-10,4 (6,6)
	B	-	-
	C	70,0 (17,3)	20,0 (19,8)
FR2	A	90,0 (10,0)	-2,8 (11,4)
	B	90,0 (17,3)	-2,8 (19,8)
	C	100,0 (0,0)	-14,2 (0,0)
FR3	A	56,3 (20,5)	35,7 (23,5)
	B	54,4 (19,0)	37,8 (21,7)
	C	63,3 (5,8)	27,6 (6,6)
FR4	A	70,0 (0,0)	20,0 (0,0)
	B	-	-
	C	96,7 (5,8)	-10,4 (6,6)
FR5	A	68,5 (12,3)	21,7 (14,0)
	B	60,0 (17,3)	31,5 (19,8)
	C	60,0 (10,0)	31,5 (11,4)

Kinetic differences in the inhibition of *S. oleraceus* by MAP1 EO and FRs

	Main compounds (%)	Concentration (ppm)	Gmax (%)	Vmax (%/day)	Tlag (day)	MGT (day)
EO	Camphor a-pinene 1,8-cineole borneol Limonene Caryophyllene	A	36,40 (2,50)	3,30 (0,61)	6 (1)	12 (1)
		B	73,37 (8,31)	6,34 (2,14)	4 (2)	11 (2)
		C	65,18 (1,89)	9,05 (1,19)	5 (0)	9 (0)
FR1	-	A	78,61 (9,72)	8,69 (2,37)	4 (1)	10 (1)
		B	-	-	-	-
		C	-	-	-	-
FR2	-	A	77,49 (1,86)	13,31 (1,58)	2 (0)	5 (0)
		B	69,60 (4,96)	6,05 (1,16)	1 (1)	8 (1)
		C	47,35 (3,42)	6,65 (2,19)	4 (1)	8 (1)
FR3	-	A	61,95 (5,25)	5,23 (1,22)	1 (1)	8 (1)
		B	72,35 (3,90)	9,84 (2,25)	5 (1)	9 (1)
		C	72,96 (3,79)	6,01 (0,84)	2 (1)	8 (1)
FR4	-	A	71,20 (5,59)	5,75 (1,10)	2 (1)	9 (1)
		B	75,05 (7,07)	5,54 (0,98)	1 (1)	9 (1)
		C	42,76 (3,95)	7,45 (3,42)	6 (1)	9 (1)
FR5	-	A	10,30 (1,35)	0,000	5	5
		B	n.d.	n.d.	n.d.	n.d.
		C	n.d.	n.d.	n.d.	n.d.

* Unreliable value, model not fitted propperly , n.d.: not determined by the model. Germination is 0%
* -: concentration not tested

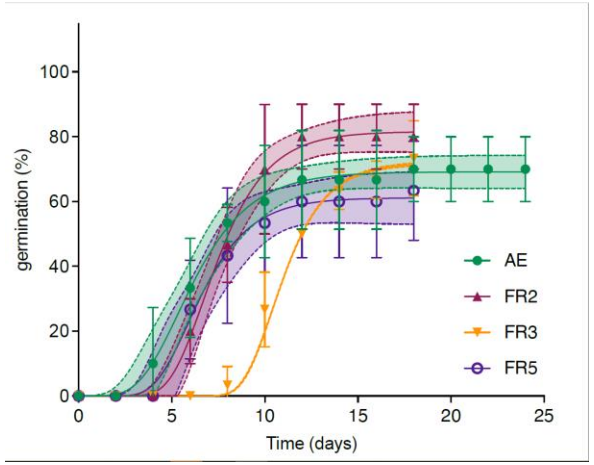




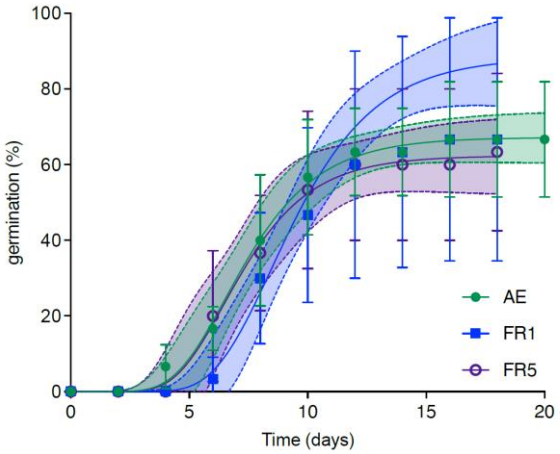
Kinetic differences in the inhibition of *S. oleraceus* by MAP3 EO and FRs

	Main compounds (%)	Concentration (ppm)	Gmax (%)	Vmax (%/day)	Tlag (day)	MGT (day)
EO	Epoxicimenes Chrisantenile acetate	A	69,16 (2,52)	12,03 (2,70)	3 (1)	7 (0)
	Sabinene Caryophyllene	B	67,18 (3,36)	11,45 (2,73)	4 (1)	8 (1)
	Camphor Chamazulene	C	64,51 (4,13)	9,00 (2,96)	7 (1)	11 (1)
FR1	-	A	78,10 (5,35)	16,78 (5,86)	5 (1)	8(1)
		B	88,37 (6,82)	13,40 (2,83)	6 (1)	10 (1)
		C	78,65 (8,17)	13,29 (4,12)	7 (1)	11 (1)
FR2	-	A	81,60 (3,16)	16,30 (2,98)	5 (0)	8 (0)
		B	86,23 (10,62)	8,40 (1,60)	5 (1)	11 (1)
		C	61,31 (16,74)	5,43 (1,57)	6 (1)	13 (3)
FR3	-	A	71,96 (0,24)	16,82 (0,15)	9 (0)	11 (0)
		B	73,26 (2,13)	14,96 (1,82)	7 (0)	9 (0)
		C	83,38 (8,75)	15,82 (4,86)	9 (1)	12 (1)
FR4	-	A	69,18 (2,08)	19,46 (3,86)	7 (0)	9 (0)
		B	70,76 (1,65)	20,30 (2,93)	7 (0)	9 (0)
		C	77,82 (3,86)	20,48 (5,91)	6 (1)	8 (0)
FR5	-	A	61,16 (4,05)	12,42 (4,21)	4 (1)	7 (1)
		B	62,33 (5,13)	11,29 (4,12)	4 (1)	8 (1)
		C	34,59 (2,13)	7,58 (2,34)	5 (1)	7 (0)

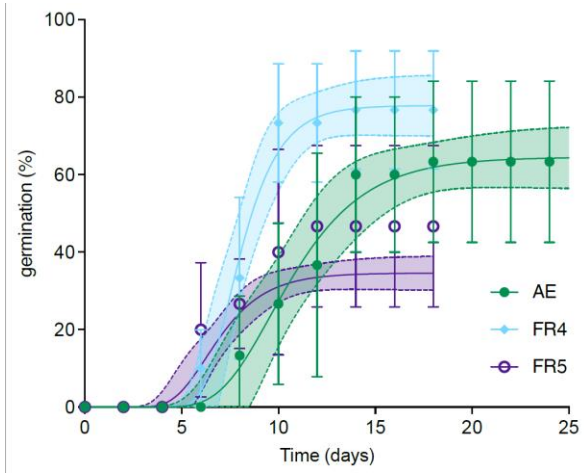
A



B



C

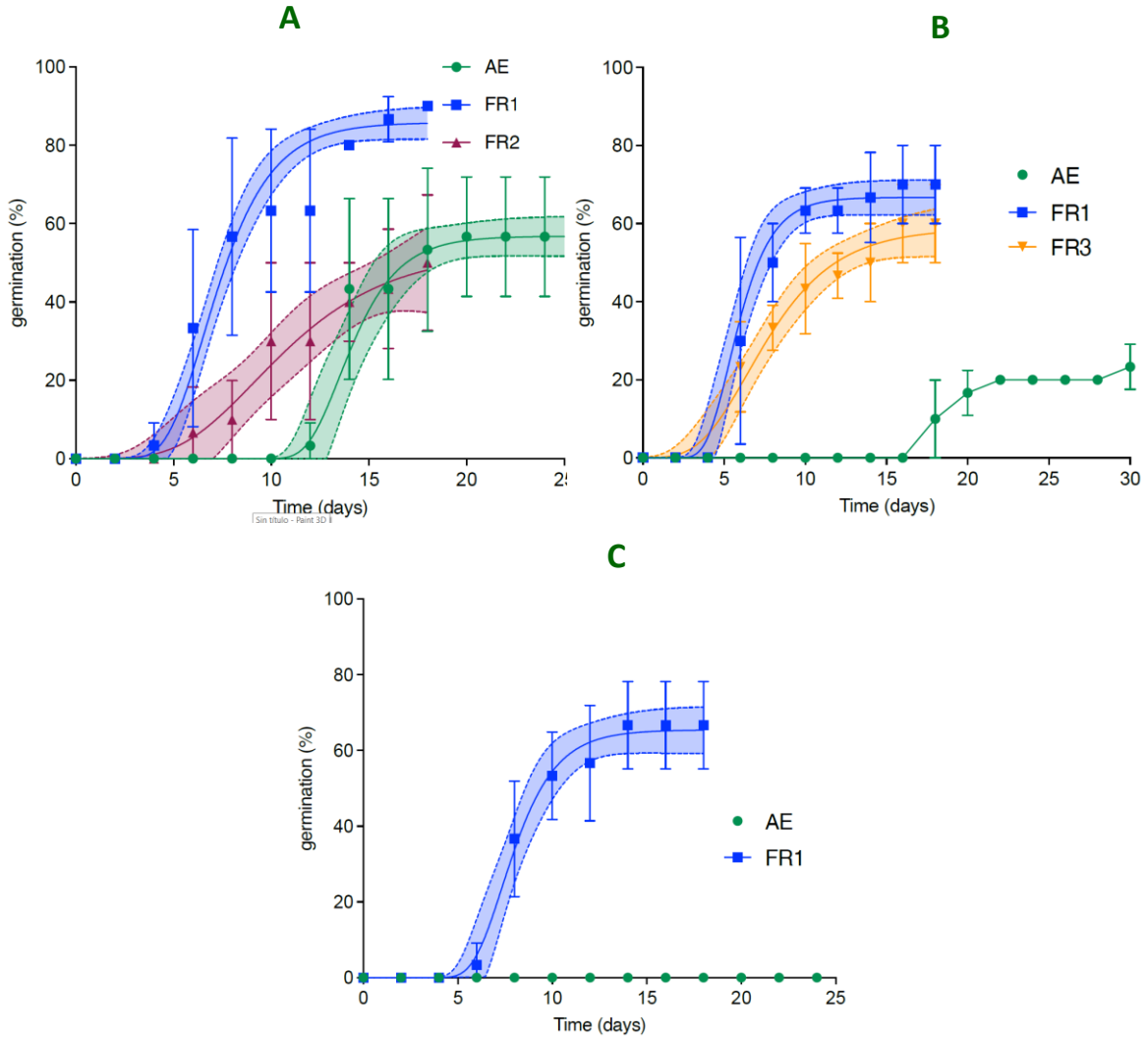


Kinetic differences in the inhibition of *S. oleraceus* by MAP6 EO and FRs

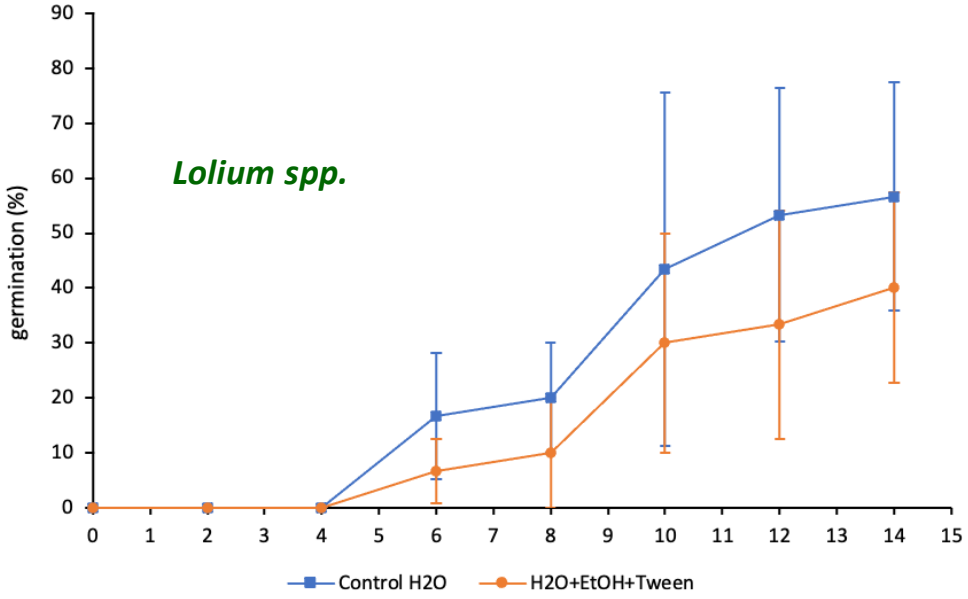
	Main compounds (%)	Concentration (ppm)	Gmax (%)	Vmax (%/day)	Tlag (day)	MGT (day)
AE	Piperitenone oxide Piperitenone Germacrene D Limonene Caryophyllene Cadinol	A	56,79 (2,56)	12,61 (3,80)	12 (1)	14 (1)
		B	20	34,97	18	18
		C	n.d.	n.d.	n.d.	n.d.
FR1	-	A	85,81 (2,08)	16,58 (2,24)	5 (0)	8 (0)
		B	66,70 (2,20)	17,15 (3,49)	4 (0)	6 (0)
		C	65,42 (3,07)	15,45 (3,67)	6 (1)	8 (0)
FR2	-	A	53,08 (12,10)	5,13 (1,70)	5 (1)	11 (2)
		B	71,28 (10,69)	5,58 (1,01)	3 (1)	11 (2)
		C	59,25 (7,19)	6,95 (2,14)	4 (1)	9 (1)
FR3	-	A	77,68 (11,62)	13,04 (5,52)	8 (1)	11 (1)
		B	58,78 (3,91)	7,26 (1,40)	3 (1)	8 (1)
		C	56,08 (6,33)	12,65 (7,95)	4 (1)	6 (1)

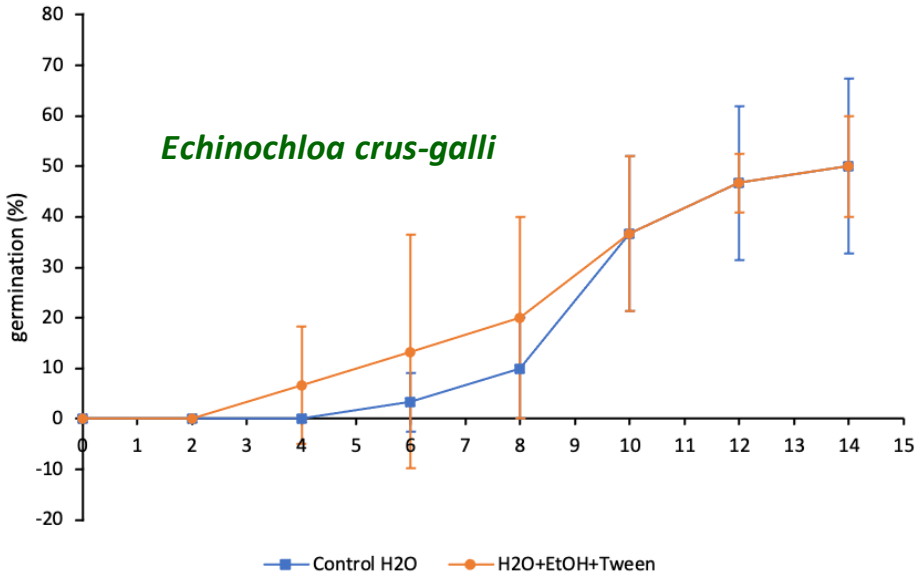
* Unreliable value, model non fitted propperly

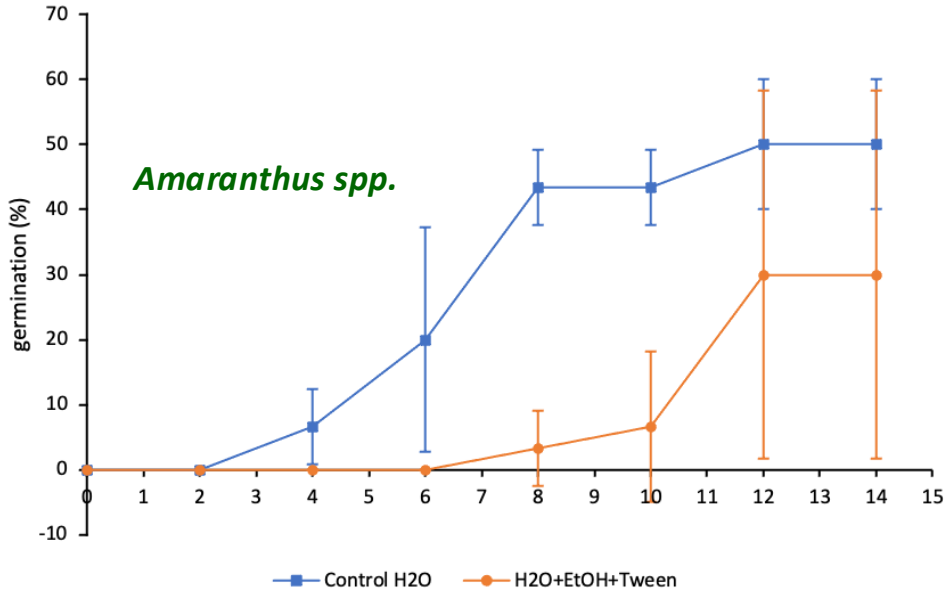
* n.d.: not determined by the model. AE germination is 0%

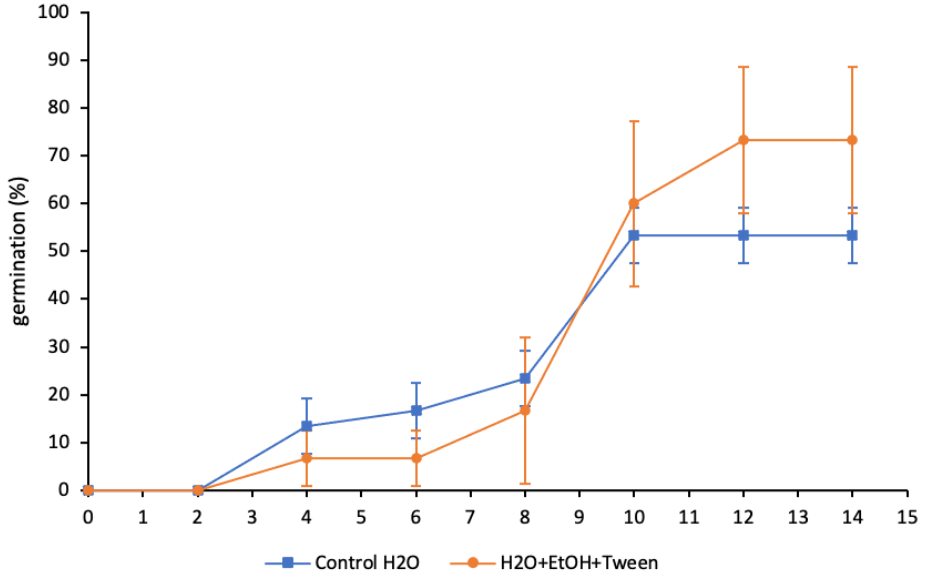


Inhibition of the germination of other weed species



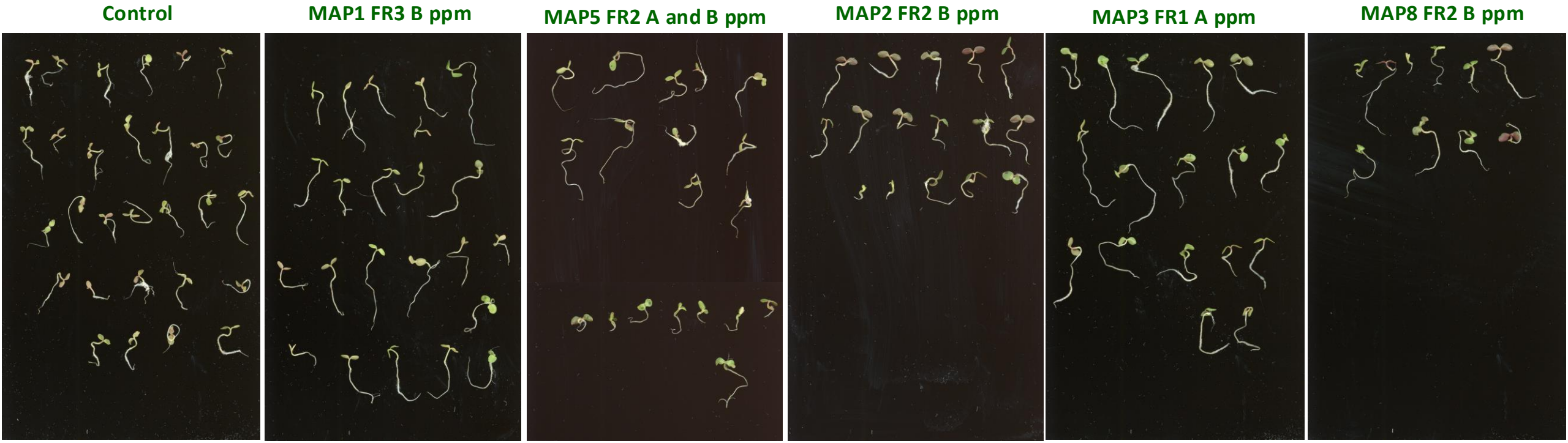






Inhibitory effectivity of EXTs and FRs against *S. oleraceus*

Major constituents of the plant extracts have not shown any potential to inhibit *S. oleraceus* germination



Conclusions

EO and FR delayed radicle emergence of *S. oleraceus* extending the lag phase of the germination

Fractions of MAP1 , MAP3, MAP5, and MAP4 exerted similar effectivity than the corresponding EO at the three concentrations tested

MAP2, MAP6 and MAP7 EO highly inhibited the germination of S. oleraceus at A and B ppm, losing some effectiveness when decreasing to C ppm

Future prospects

- Finishing the *in vitro* assay with the whole extracts and fractions to determine their phytotoxicity against *S. oleraceus*
- Determining the composition of the essential oil and extract fractions of interest considering their effectivity to avoid *S. oleraceus* germination and determining the correlation between individual compounds and the inhibition of the germination.

Future prospects

- Upscaling the assay in pots with a pre emergence application after planting a selected crop as a model (e.g. *Lactuca sativa*).

- Studying the persistence of these natural products and its toxicity to soil and water organisms.

- Explore seed germination and inhibition mechanisms to understand the large number of abnormal germs and seedlings observed with EO and FR.

Thank you for your attention

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weeding MAPs

GOBIERNO DE ARAGON

Phytotoxicity of essential oils and extracts major constituents from MAP species against common sow thistle (*Sonchus oleraceus*)

David Gimeno, Anabel Yetano-Cunchillos*, Carolina Gónzalez*, Alicia Cirujeda, Gabriel Pardo, Juliana Navarro-Rocha

CENTRO DE INVESTIGACIÓN Y TECNOLOGÍA AGROALIMENTARIA DE ARAGÓN

* In collaboration with **NATIONAL CENTER FOR FOOD TECHNOLOGY AND SAFETY**