

2024 | DANI DOKTORATA
BIOTEHNIČKOG PODRUČJA



Potential of phenolic acids in *Ambrosia artemisiifolia* L. control

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Why *Ambrosia artemisiifolia* L. ?



Source: Buters, J., Alberternst, B., Nawrath, S., Wimmer, M., Traidl-Hoffmann, C., Starfinger, U., ... & Bergmann, K. C. (2015). *Ambrosia artemisiifolia* (ragweed) in Germany—current presence, allergological relevance and containment procedures. *Allergo Journal International*, 24, 108-120.

European Green Deal

„From Farm to Fork”



Integrated pest
management

Cover crops

Bioactive compounds



Why phenolic acids?

Previous research:

Phenolic compounds found in dry matter of
aboveground biomass of cover crops

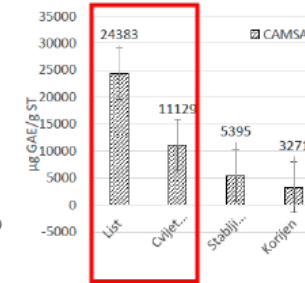


Brassicaceae CC (*Sinapis alba*,
Raphanus sativus, *Camelina sativa*)

LCMS



spojevi	Fenoli (µg/g)					
	SINAL	FAGES	RAPSA	GUIAB	CS	CCMIX
Gallie acid	65.5	32.3	18.5	17.5	17.5	26.3
Protocatechuic acid	55.5	386.3	63.5	38.3	100.5	113.5
p-Hydroxybenzoic acid	222.3	38.5	154.3	35.8	36.8	27.0
Chlorogenic acid	n.d.	87.8	n.d.	37.5	1057.0	587.8
Vanillic acid	31.8	n.d.	37.0	n.d.	79.3	n.d.
Caffeic acid	14.5	46.3	26.8	35.0	102.5	134.8
Epicatechin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Catechin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Syringic acid	7.0	12.3	6.8	13.0	27.3	n.d.
Vanillin	19.0	13.3	19.0	13.8	44.8	n.d.
p-coumaric acid	25.3	26.5	84.5	16.3	74.8	29.5
Sinapinic acid	4.0	n.d.	n.d.	n.d.	n.d.	11.8
Ferulic acid	39.8	11.8	552.0	2.5	35.5	1143.0
Rutin	n.d.	1844.3	6.0	28.8	413.5	416.5
Quercetin	71.6E+03	895.6	189.9	980.5	68.8	88.8
Resveratrol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ellagic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Myricetin acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercitin	40.8	135.8	8.3	7.0	33.3	59.5
Naringenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Kaempferol	46.5	n.d.	n.d.	40.0	n.d.	n.d.

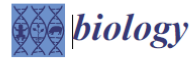


Ščepanović, M., Sarić-Krsmanović, M., Šoštarić, V., Brijaćak, E., Lakić, J., Trifunović, B. Š., Umiljendić, J. G., Radivojević, L. (2021). Inhibitory effects of brassicaceae cover crop on ambrosia artemisiifolia germination and early growth. *Plants*, 10(4).
<https://doi.org/10.3390/plants10040794>

Which phenolic acids?

- *p*-coumaric acid
- chlorogenic acid
- caffeic acid
- ferulic acid
- gallic acid
- protocatechuic acid
- *p*-hydroxybenzoic acid
- syringic acid
- vanillic acid

5 doses (10^{-7} mol)
D1-D5



Communication

Selected Phenolic Acids Inhibit the Initial Growth of *Ambrosia artemisiifolia* L.

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Simple Summary: In the context of international commitments to reduce the environmental impact of herbicides, ecologically more favorable control method approaches must be explored. This is particularly important for allergenic *Ambrosia artemisiifolia* L., one of the most harmful species in the world. Secondary plant metabolites and, in particular, some phenolic compounds are known to have a strong allelopathic effect on weed growth. In this study we investigated whether phenolic acids (chlorogenic acid, caffeic acid, ferulic acid, gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, syringic acid, vanillic acid, and *p*-coumaric acid) can inhibit the early growth of *A. artemisiifolia*. Phenolic acids were tested at five different dose levels that were up to 16 times than those naturally occurring in plants. The results show that the suppression of the early growth of *A. artemisiifolia* is strongly dependent on phenolic acid and its dose. Treating seeds with ferulic acid, vanillic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, or a mixture of all phenolic acids resulted in significantly better inhibition of early growth parameters than other phenolic acids. However, none of the phenolic acids tested were effective as bioherbicides at their naturally occurring doses in plants. Therefore, selected doses of phenolic acids with significantly reduced herbicide doses should be further explored to effectively control *A. artemisiifolia*.

Abstract: This study aimed to investigate whether different doses of specific phenolic acids (chlorogenic acid, caffeic acid, ferulic acid, gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, syringic acid, vanillic acid, and *p*-coumaric acid), alone or in combination, can inhibit the early growth of the common ragweed (*Ambrosia artemisiifolia* L., Asterales: Asteraceae). A seed bioassay was performed in Petri dishes and placed in a climate chamber to assess the effects of five dose levels of phenolic acids to radicle and shoot length, as well seedling biomass of *A. artemisiifolia*. The lowest dose of phenolic acid corresponded to the natural phenolic acid concentration previously reported in dry plant tissue samples from Brassicaceae cover crop plants. Results show that the inhibition of the early growth of *A. artemisiifolia* depends strongly on phenolic acid. Across different treatments, high doses of phenolic



Citation: Šćepanović, M.; Koščak, L.; Šoštarčić, V.; Pismarović, L.; Milanović-Litre, A.; Kljak, K. Selected Phenolic Acids Inhibit the Initial Growth of *Ambrosia artemisiifolia* L. *Biology* **2022**, *11*, 482. <https://doi.org/10.3390/biology11040482>

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The aims of the research:

- to investigate the inhibitory and selective potential of three phenolic acids to *A. artemisiifolia* and *Z. mays*, respectively (*in vitro*);
- to evaluate the effect of foliar application of phenolic acids on *A. artemisiifolia* and *Z. mays* - chlorophyll fluorescence and multispectral imaging (*in vivo*).



Materials and methods – *in vitro*

- phenolic acids: *p*-coumaric acid (PCA),
ferulic acid (FA),
vanillic acid (VA).
- control – distilled water
- phenolic acids differ in their solubility in water – different range of doses

PCA	FA	VA
200	200	200
-	400	400
-	-	600

Dose ($\cdot 10^{-7}$ mol)




Materials and methods – *in vitro*

- common germination tests
- 25 °C day/15 °C night, 70% RH
- sensitivity:
 - germination %
 - hypocotyl/coleoptile and radicle length
 - fresh/dry seedling biomass
- ANOVA, Tukey HSD,
 - R environment and software

Specie	AMBAR	ZEAMA
No.seeds/P.dish	50	25
Rep/PHA	4	4
Duration(days)	10	7



Materials and methods – *in vivo*

- chlorophyll fluorescence and multispectral analysis
- CropReporter: 
 - A. artemisiifolia* - images taken for 7 consecutive days after application of vanillic acid
 - Z. mays* – image taken on 7th day after application
- repeated measures ANOVA, cluster analysis



RGB

NPQ

NDVI

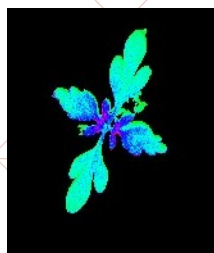
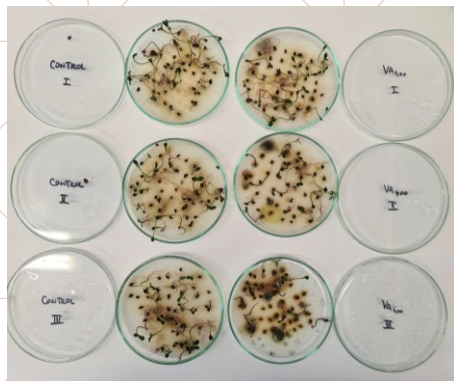
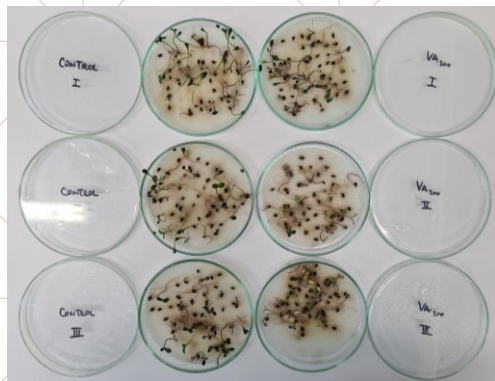
Fv/Fm

Fq'/Fm'

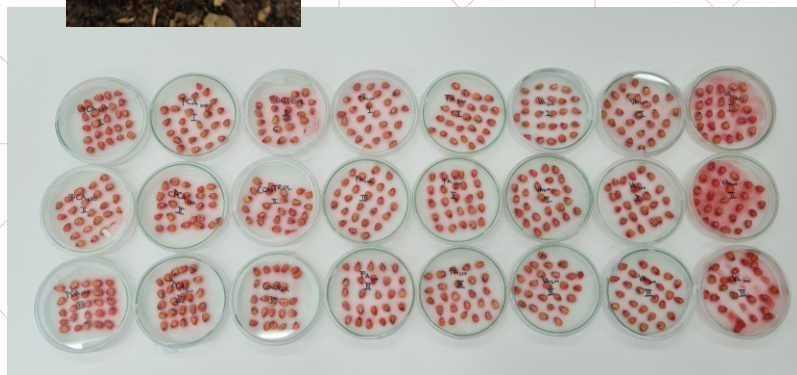
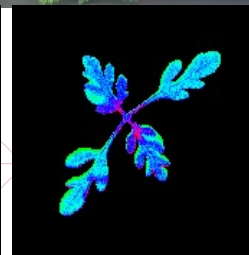
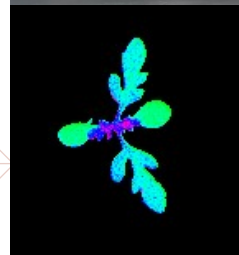
Chl

Ant





RESULTS



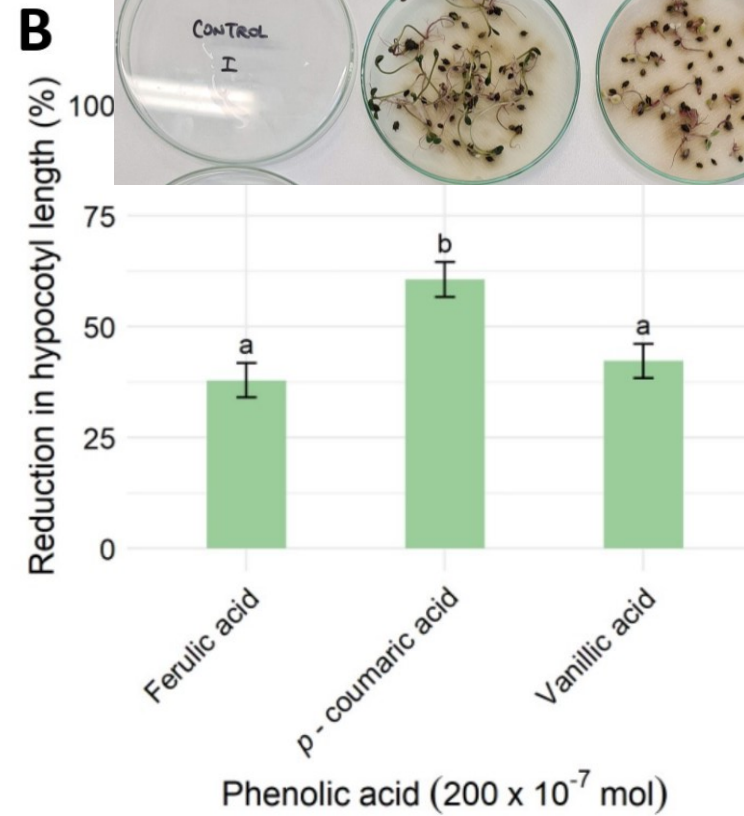
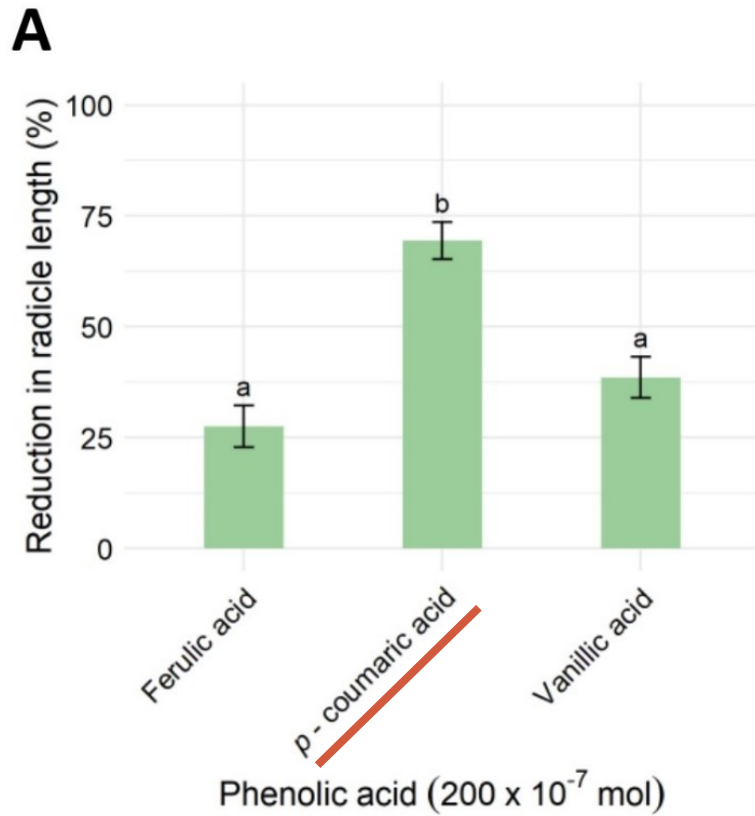


Figure 1. The effect of phenolic acids on *A. artemisiifolia* L. early germination parameters at dose 200×10^{-7} mol.

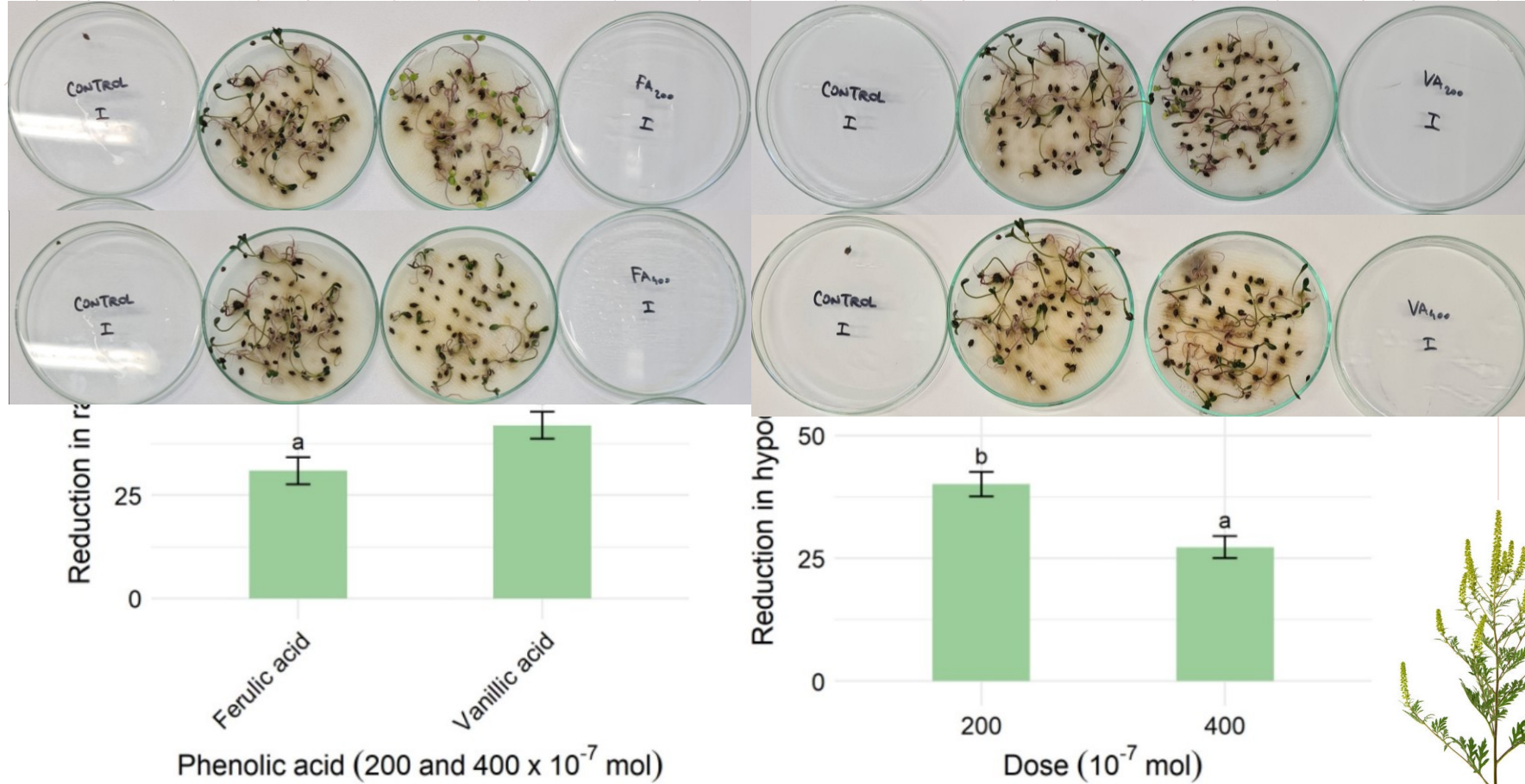


Figure 2. The effect of ferulic and vanillic acid on *A. artemisiifolia* L. early germination parameters at dose 200 and 400 *10⁻⁷ mol.

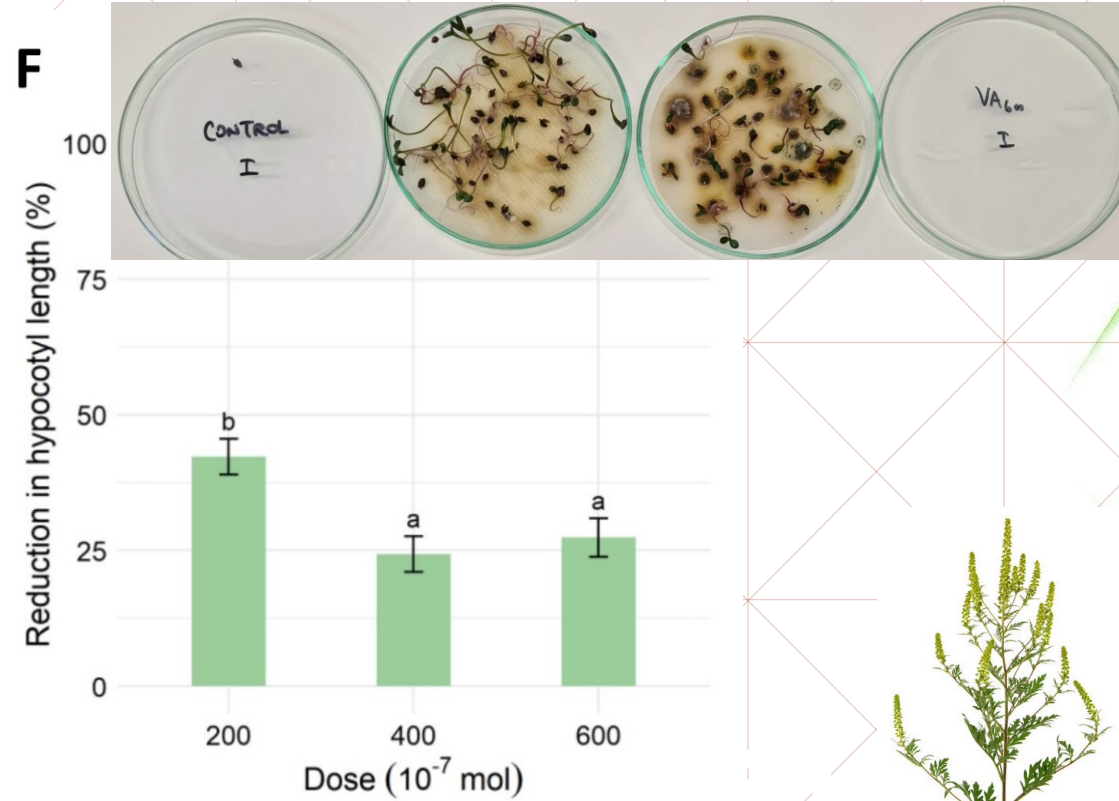
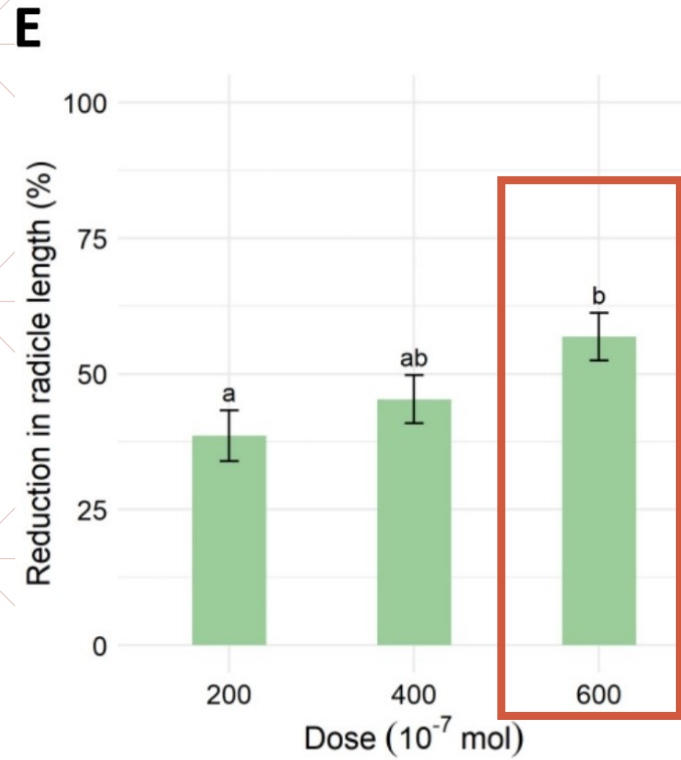
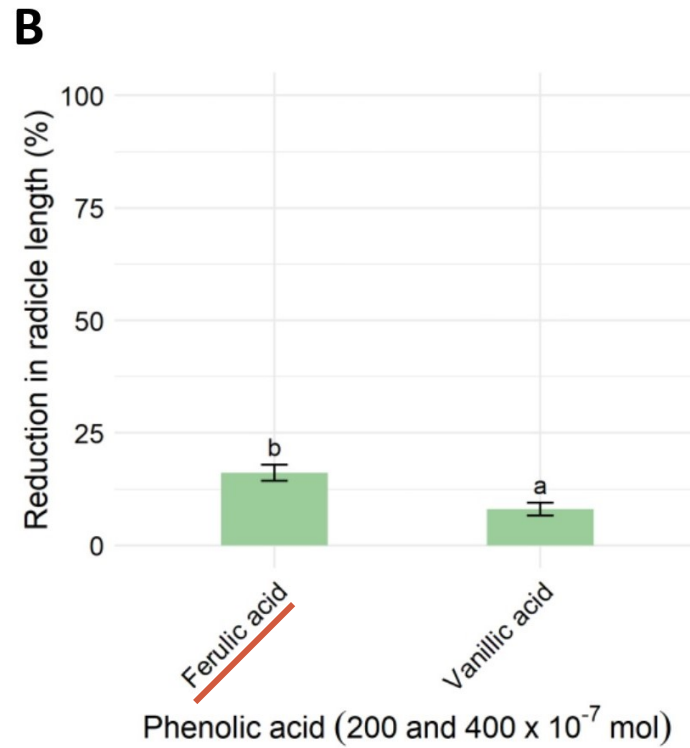
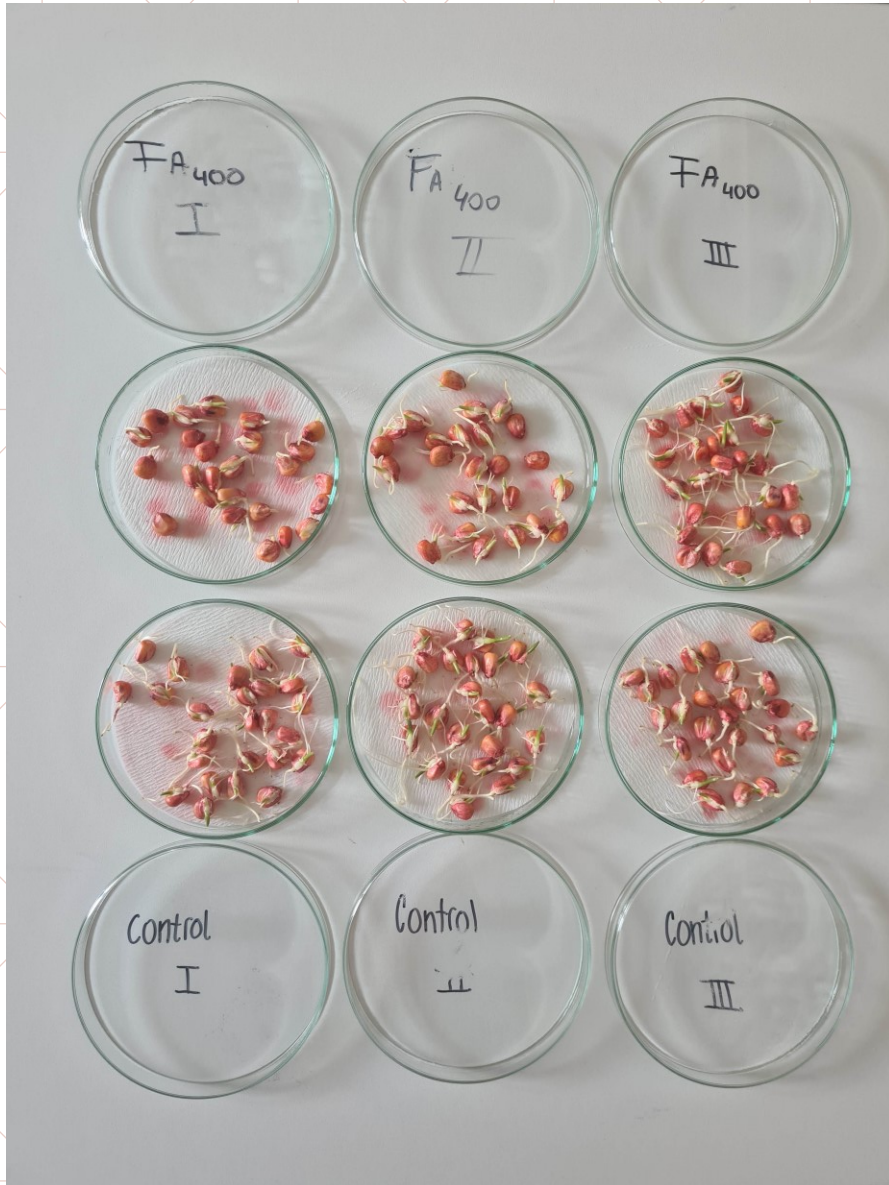
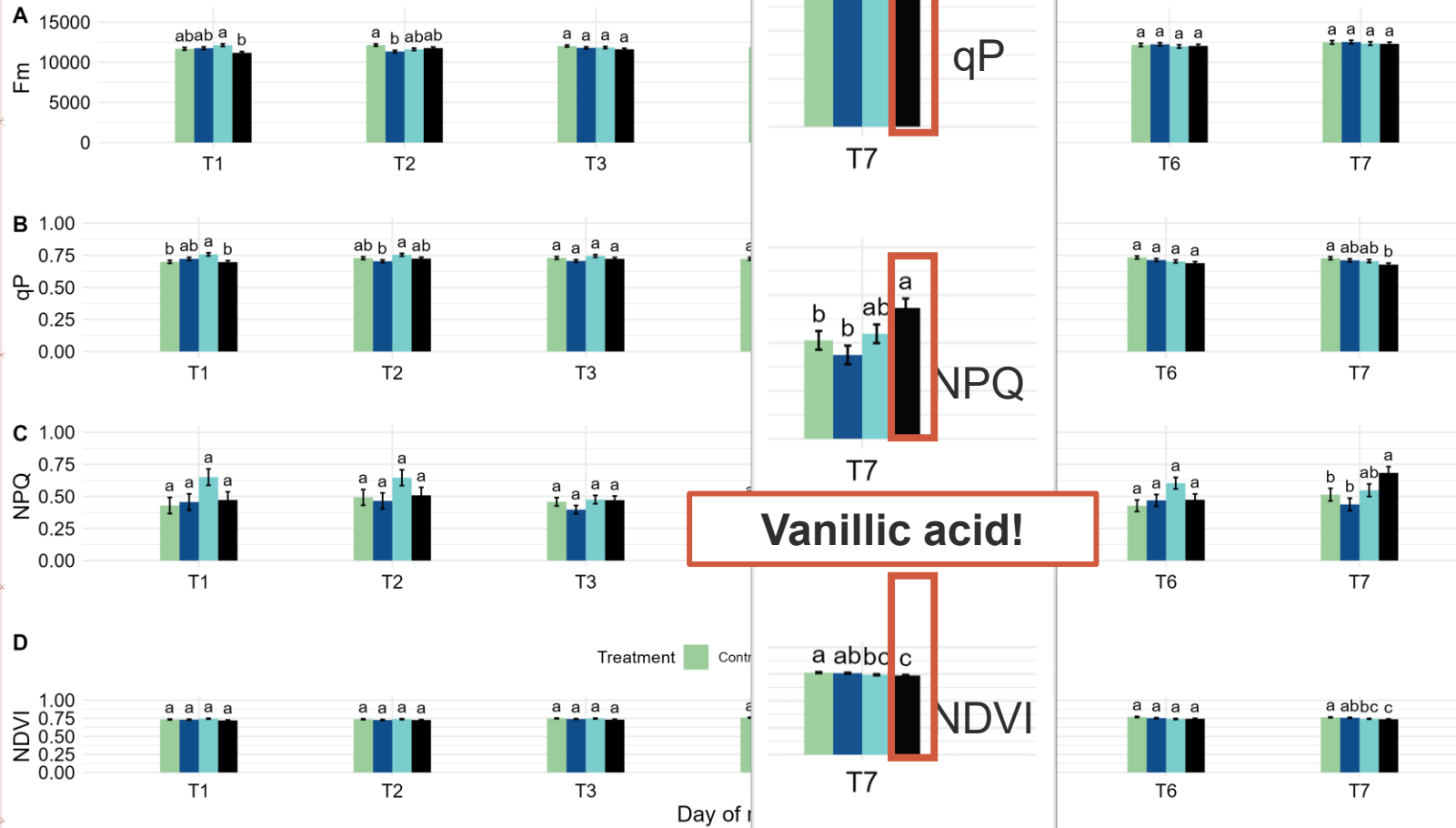


Figure 3. The effect of vanillic acid on *A. artemisiifolia* L. early germination parameters at doses 200/400/600 $\cdot 10^{-7}$ mol.



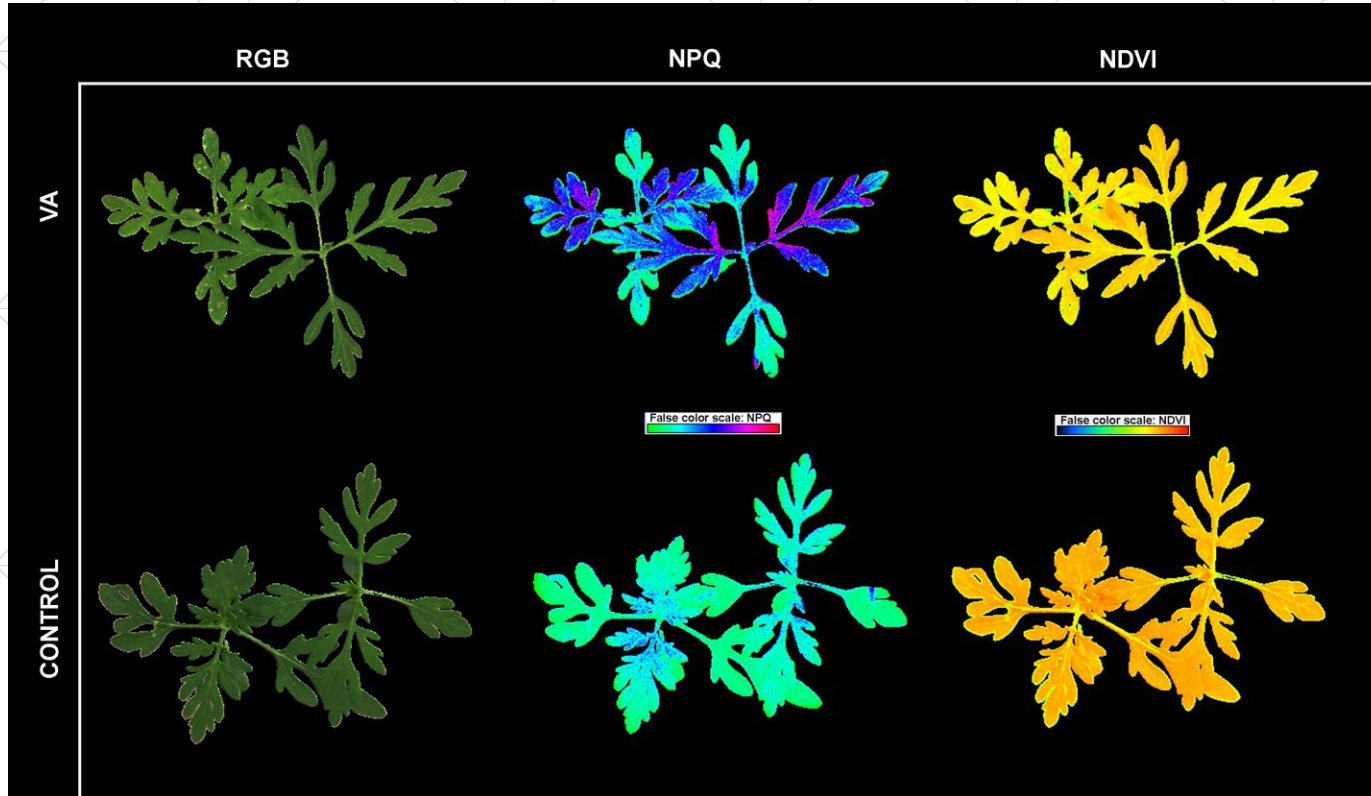
acids on *Z. mays* early germination parameters at
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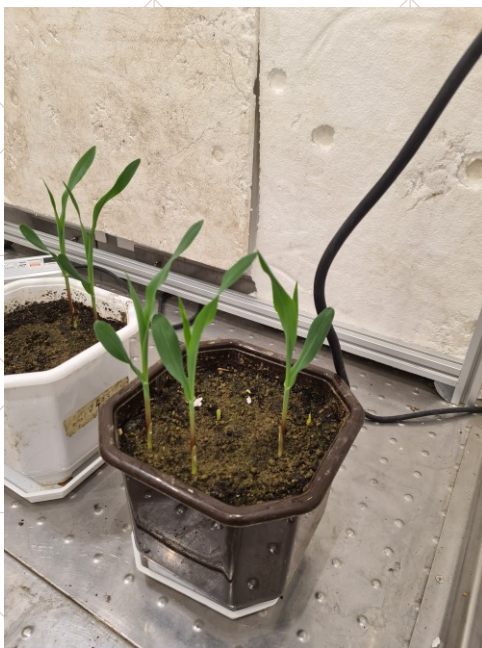


- Figure 5. The effect of phenolic acids on *A. artemisiifolia* L. chlorophyll fluorescence and multispectral traits.





- Figure 6. The effect of vanillic acid on *A. artemisiifolia* chlorophyll fluorescence and multispectral traits at dose $600 \cdot 10^{-7}$ mol.



- Figure 7. The effect of ferulic acid foliar applied on *Z. mays* at dose $400 \cdot 10^{-7}$ mol.

Conclusions:

- inhibitory potential of *p*-coumaric acid against *A. artemisiifolia* (*in vitro*)
- *Z. mays* selectivity to phenolic acids (*in vitro* and *in vivo*)
- effect of phenolic acids depends on:
 - the tested species,
 - the compound,
 - dose,
 - uptake pathway (*in vitro* vs. *in vivo*).



Thank you for your
time!

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