ANTIOXIDANT PROPERTIES OF RAPESEED CAN BE MODIFIED BY CULTIVATION AND BIOLOGICAL STRESS

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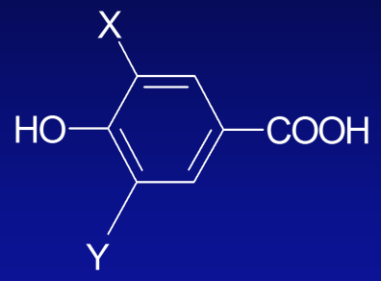




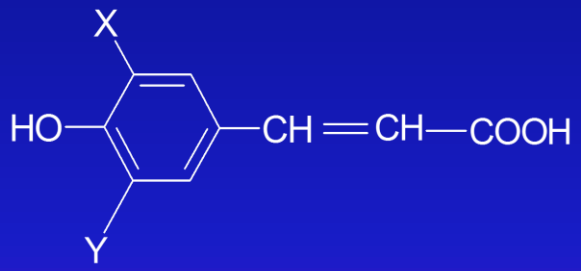
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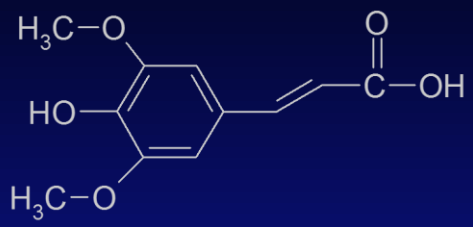
Chemical structure of phenolic acids



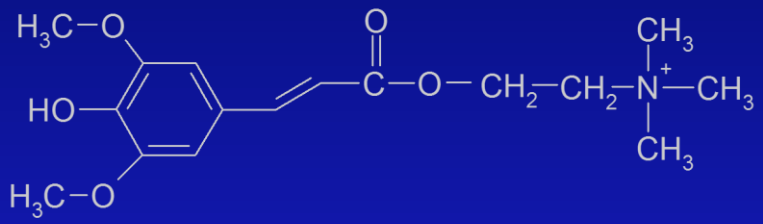
Acid	X	Y
Protocatechuic	H	OH
Vanillic	OCH_3	H
Syringic	OCH_3	OCH_3
Gallic	OH	OH
p -Hydroxybenzoic	H	H



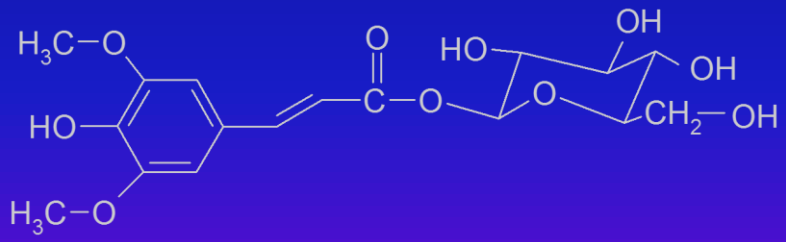
Acid	X	Y
p -Coumaric	H	H
Caffeic	H	OH
Ferulic	H	OCH_3
Sinapic	OCH_3	OCH_3



Sinapic acid



Sinapine



Glucopyranosyl sinapate

The major objective of the present study was to investigate the effect of cultivation (different level of fertilization) and action of pathogen fangus on the rapessed phenolic compounds present in the extract and their antioxidant properties.

Cultivars: California, Castilla, Nelson F1

Characteristic of the cultivation conditions

Fertilization	Control	Intensive	Spare
Phosphorus (Autumn)	60 kg	80 kg	40 kg
Potassium (Autumn)	120 kg	150 kg	60 kg
Nitrogen			
-Autumn	30 kg	30 kg	30 kg
- Spring I	120 kg	120 kg	120 kg
-Spring II	60 kg	80 kg	40 kg
-Total	210 kg	230 kg	190 kg
Sulphur (Spring)	45 kg	60 kg	-

Effect of pathogen:

Cultivar: Hybryda 1

Green house of the University of Warmia and Mazury

Cultivation in the vason (volum of 9 l). During the phase of budding plant plantation was inoculated with spores of fungal *Alternaria brassica.*
The seeds of stage of full maturity were analysed.

Extraction:

Phenolic compounds were extracted from the defatted seeds with 80% (v/v) aqueous methanol at 80^{o} C for 15 min at a solid to solvent ratio of 1: 10 (w/v). Extraction was carried out in dark-colored flakes using a shaking water bath. The extraction was repeated twice more, supernatants combined and acetone evaporated under vacuum at 40 ^{o}C in a rotary evaporator. The remaining water solution was lyophilised.

Chemical analysis:

- Total phenolics (Folina – Ciocalteu'a phenol reagent)
- Antiradical activity against DPPH radical (Yen i Chen, 1995)
- Antiradical actiovity against ABTS cation radical (TEAC) (Re et al., 1999)
- FRAP (Prior et al., 2005)
- RP-HPLC

HPLC analysis of phenolic compounds

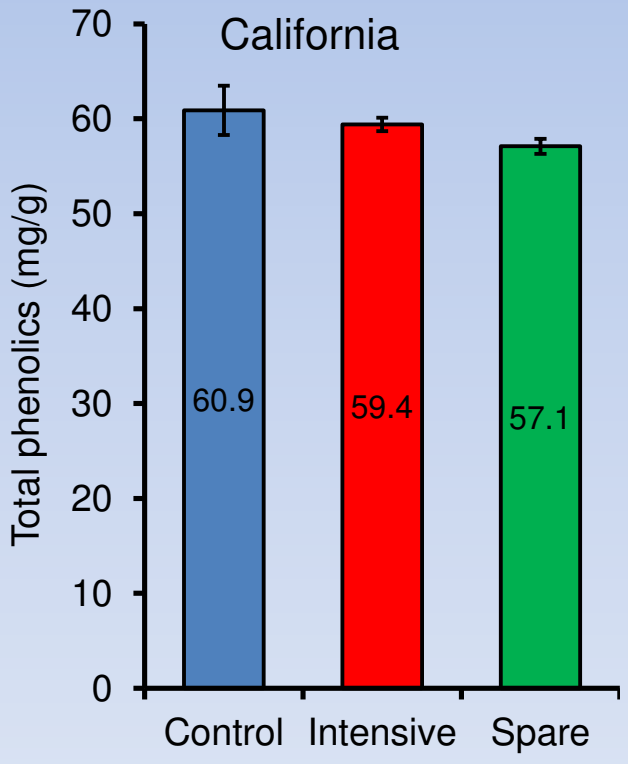
A Shimadzu HPLC system was employed:

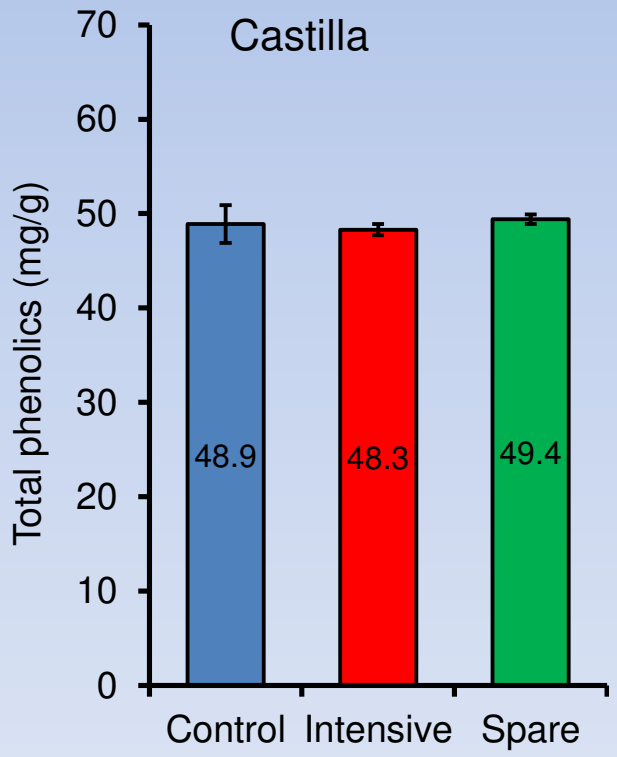
LC-10ADVP pump
Controler SCL-10AVP
Photodiode array detector UV-VIS SPD-M10AVP,
Controler SCL-10AVP

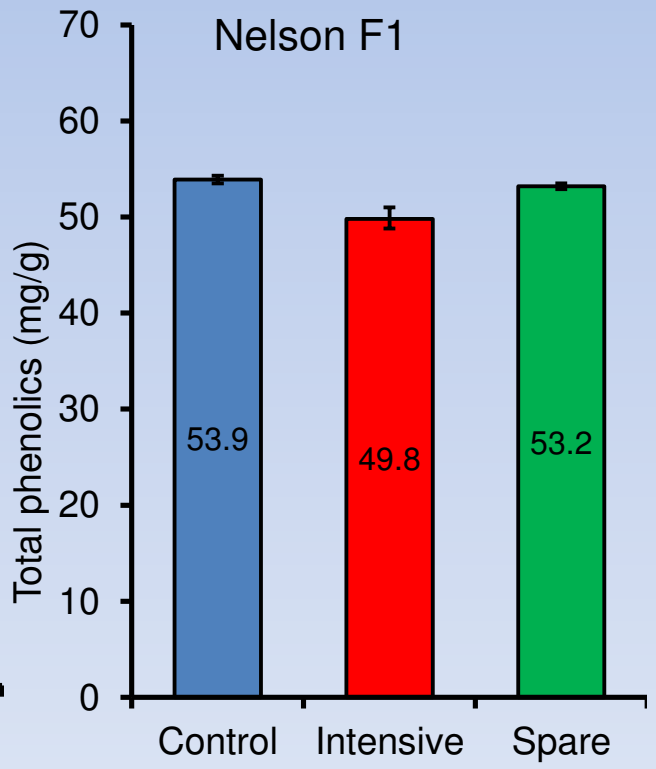
Conditiones of separations:

Prepacked LUNA C8 column (5µm, 4.6 x 250 mm; Phenomenex)
A gradient: A - water-methanol (90:10; v/v) with 1.25% *o*-phosphoric acid, B - methanol with 0.1% *o*-phosphoric acid; linear gradient from 0 to 60% B for 50 min.
Flow rate – 1 ml/min; injection volume 20 µl; the detector was set at 330 nm.

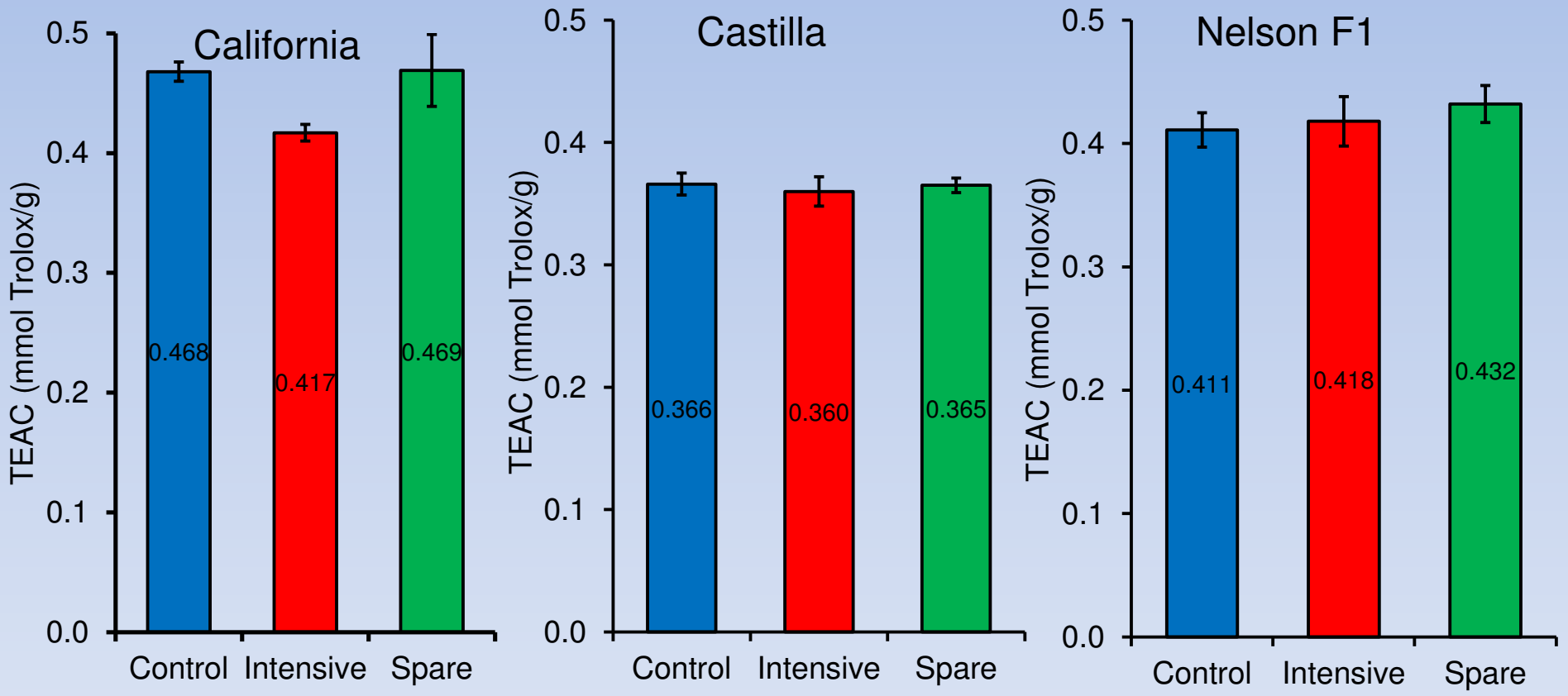
Content of total phenolics in the extracts (mg/g)



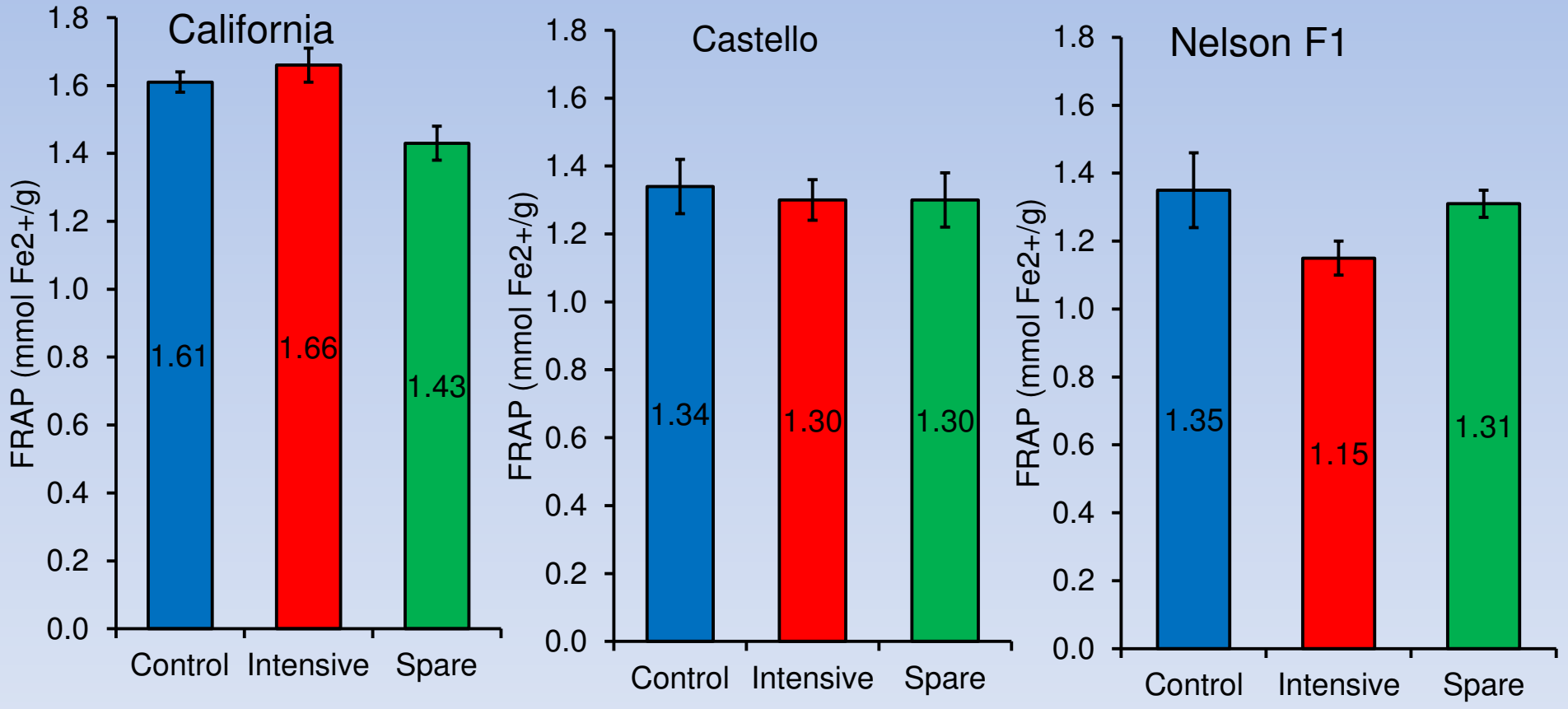




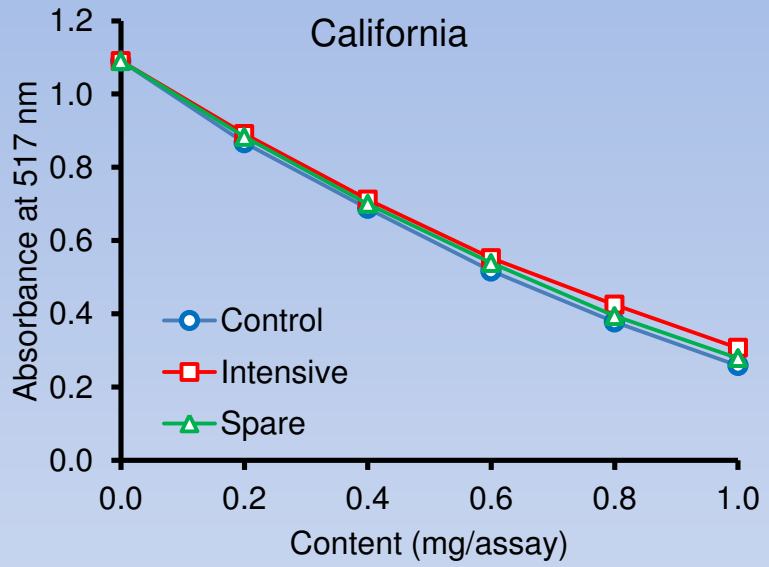
TEAC of the extracts (mmol Trolox/g)

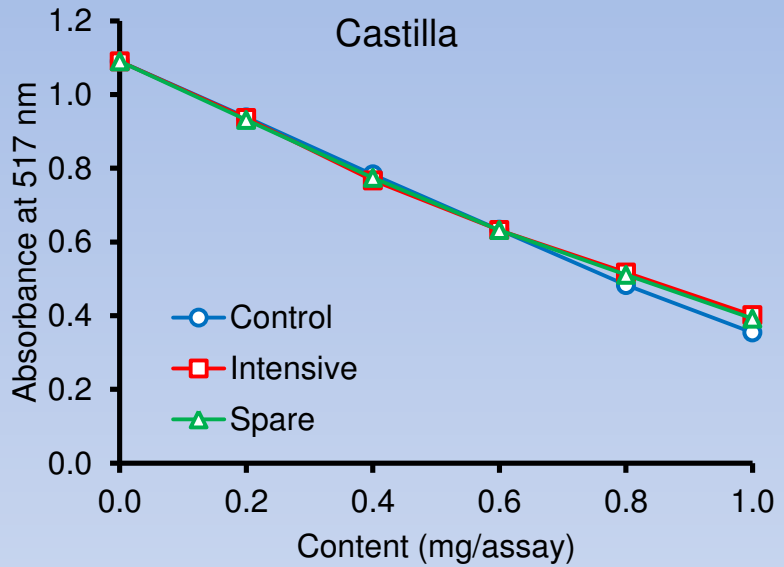


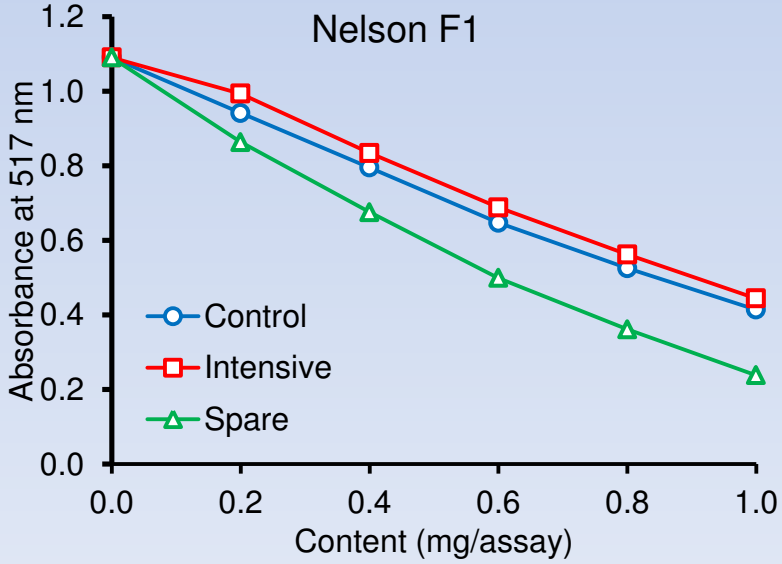
FRAP of the extracts (mmol Fe2+/g)



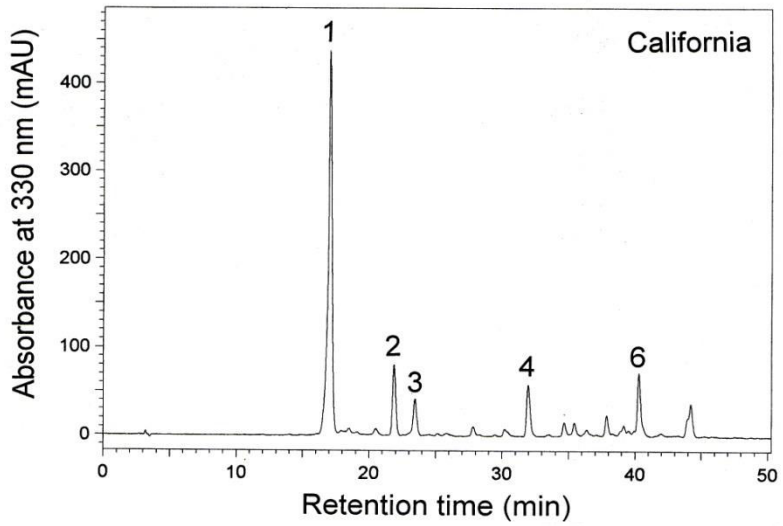
Antiradical activity of the extracts against DPPH radical

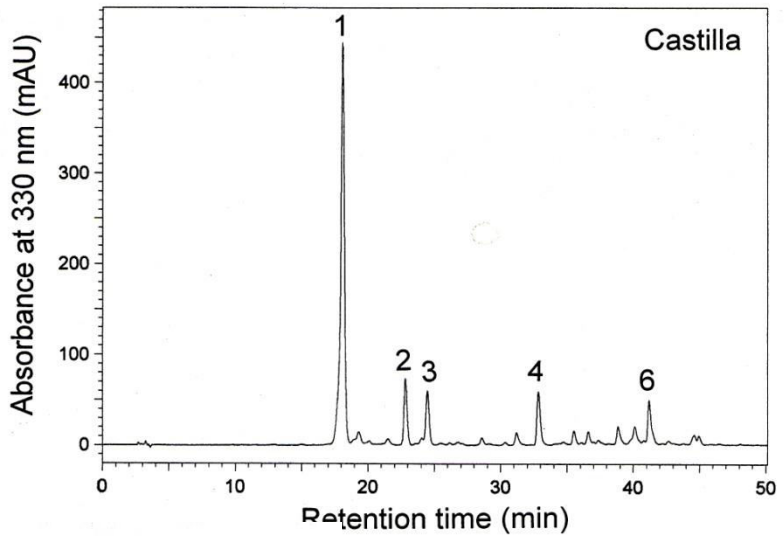


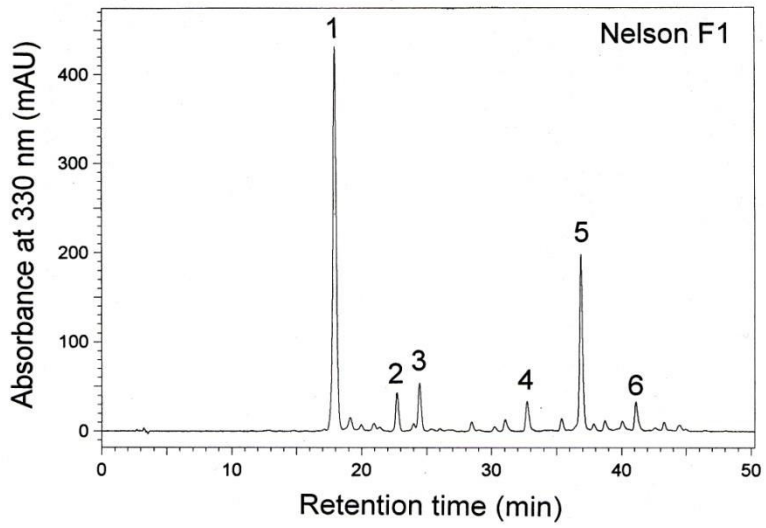




HPLC chromatograms of the extracts







Content of individual phenolic compounds in the extracts of California (mg/g)

Compound	Control	Intensive	Spare
1	80.1 ± 1.4	81.9 ± 5.1	76.2 ± 4.0
2	8.4 ± 0.3	7.7 ± 0.6	7.6 ± 0.6
3	5.6 ± 0.3	5.1 ± 0.2	5.1 ± 0.3
4	6.3 ± 0.4	6.2 ± 0.3	6.1 ± 0.3
5	-	-	-
6	8.5 ± 0.5	8.3 ± 0.2	7.6 ± 0.5

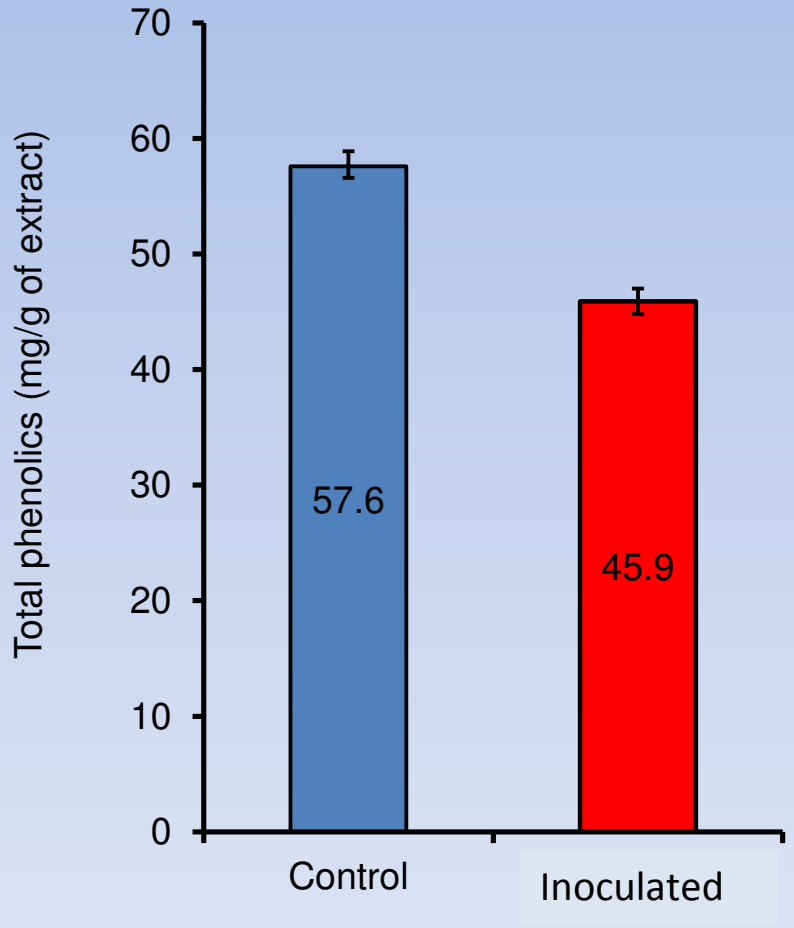
Content of individual phenolic compounds in the extracts of Castilla (mg/g)

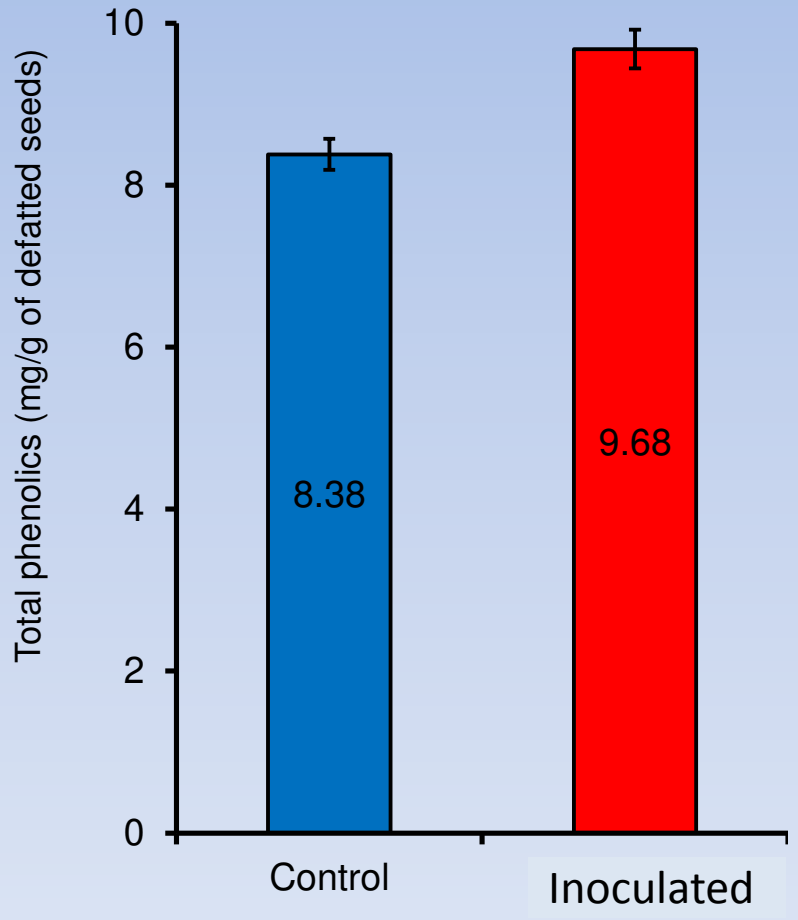
Compound	Control	Intensive	Spare
1	76.3 ± 3.4	73.0 ± 5.6	70.1 ± 1.4
2	7.1 ± 0.9	7.1 ± 1.2	6.4 ± 0.2
3	5.9 ± 0.5	5.9 ± 1.0	5.4 ± 0.5
4	5.9 ± 0.7	5.2 ± 0.6	5.5 ± 0.1
5	-	-	-
6	6.0 ± 0.7	5.7 ± 0.2	6.4 ± 1.5

Content of individual phenolic compounds in the extracts of Nelson F1 (mg/g)

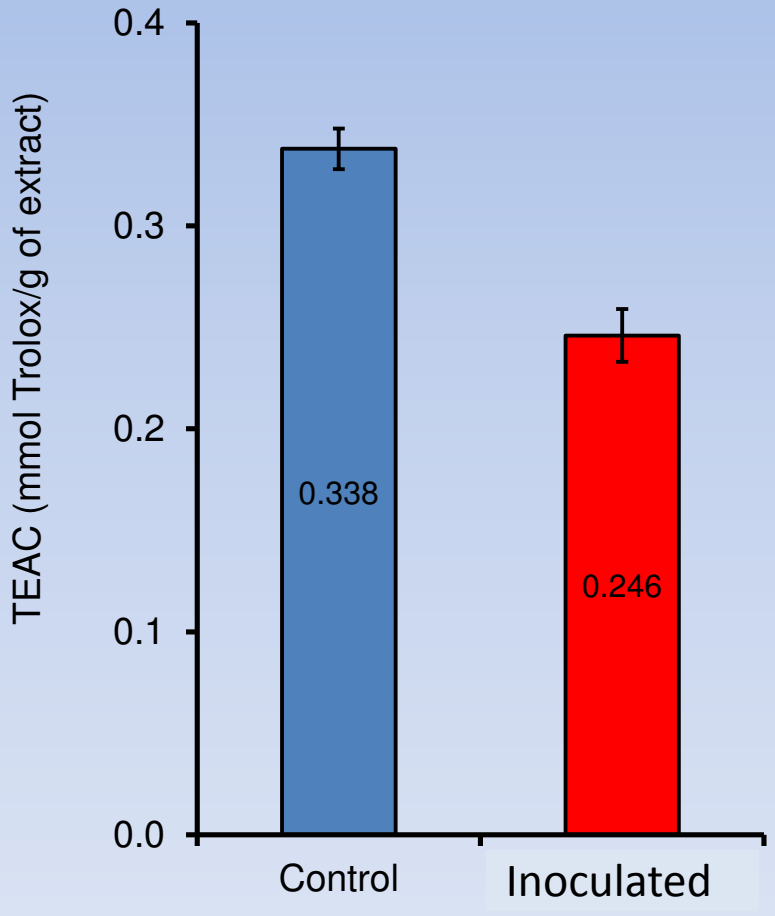
Compound	Control	Intensive	Spare
1	65.8 ± 3.0	64.5 ± 3.3	70.8 ± 5.3
2	4.0 ± 0.1	2.5 ± 0.2	3.1 ± 0.8
3	5.2 ± 0.1	4.9 ± 0.2	5.0 ± 0.6
4	3.8 ± 0.2	3.9 ± 0.5	4.4 ± 0.8
5	18.3 ± 0.5	16.9 ± 1.0	17.3 ± 0.9
6	3.8 ± 0.4	3.2 ± 0.4	3.5 ± 0.8

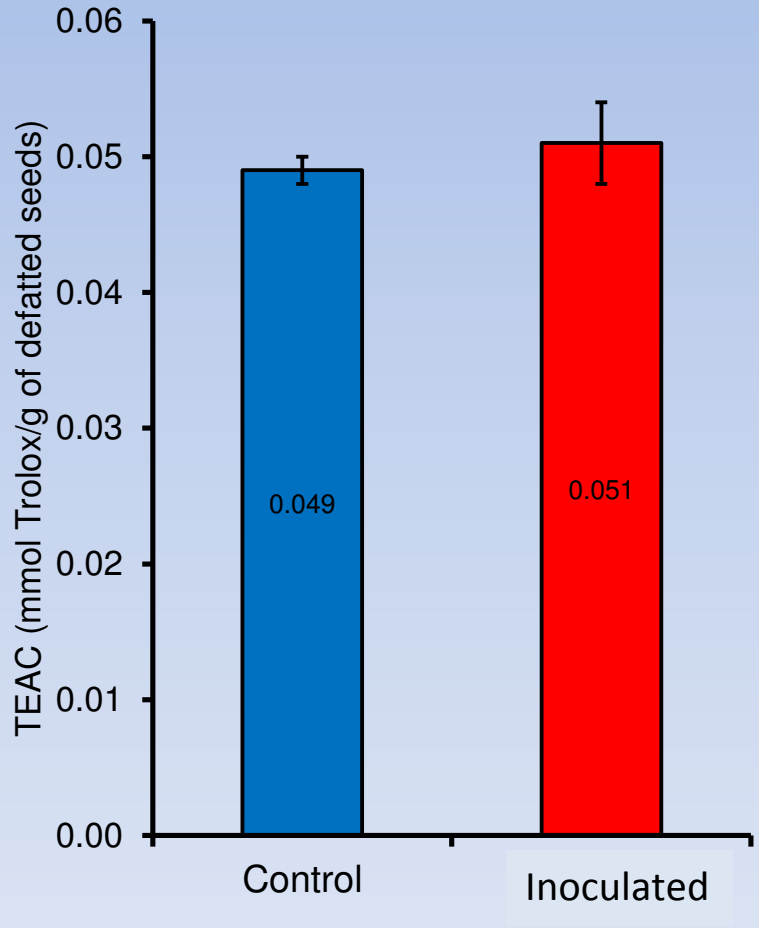
Content of total phenolics in the extracts and seeds



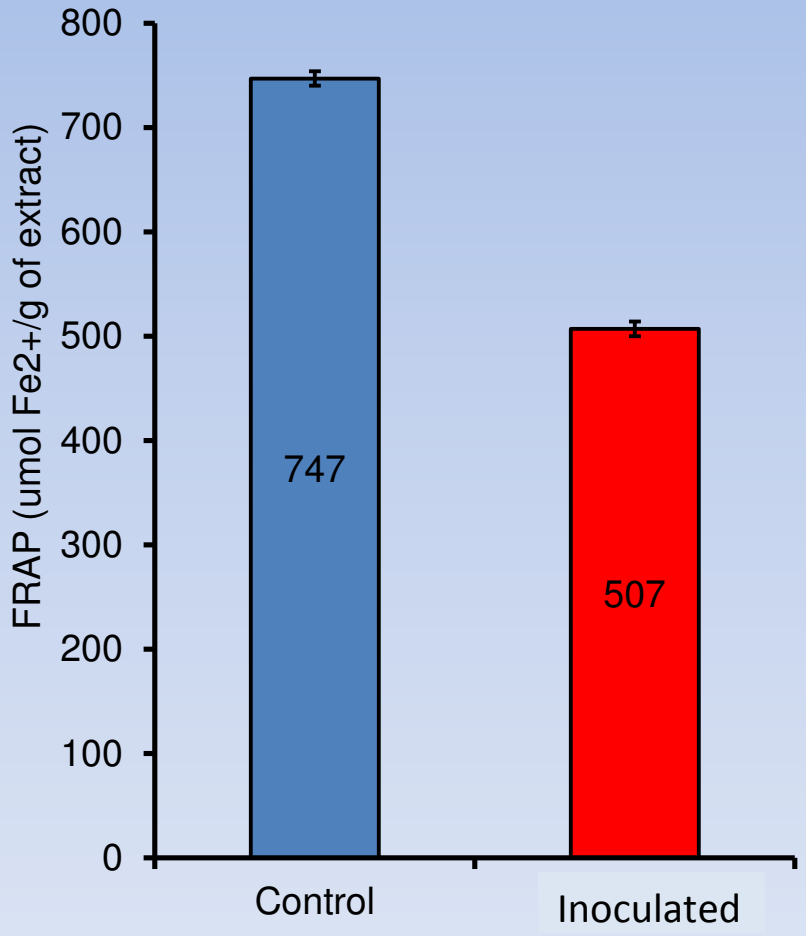


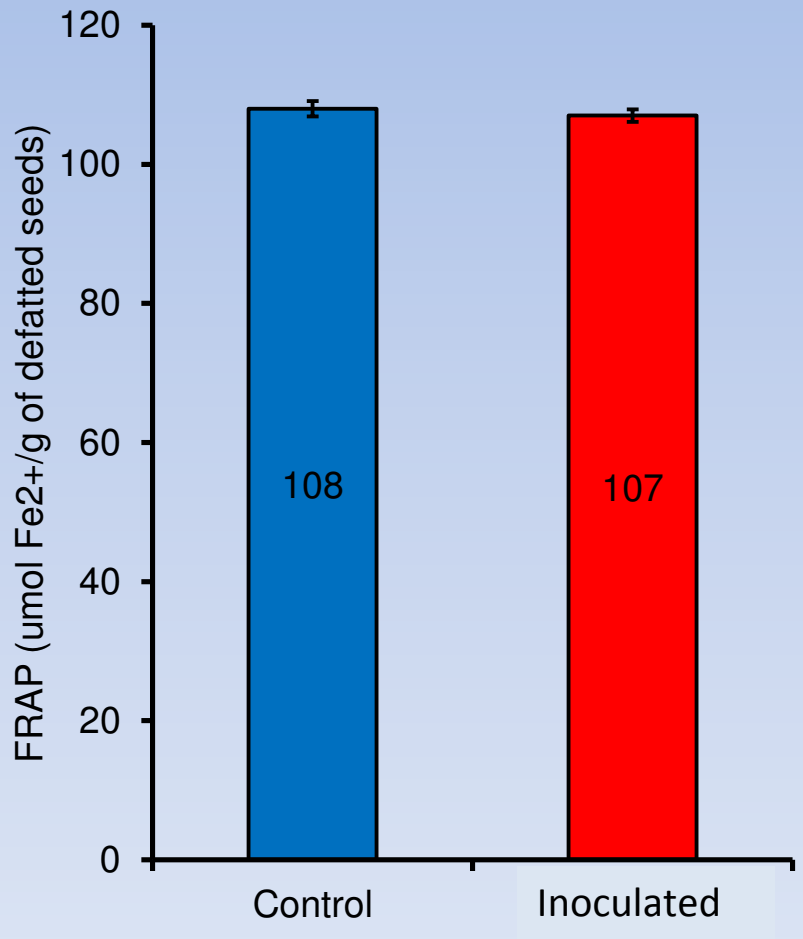
TEAC of the extracts and seeds



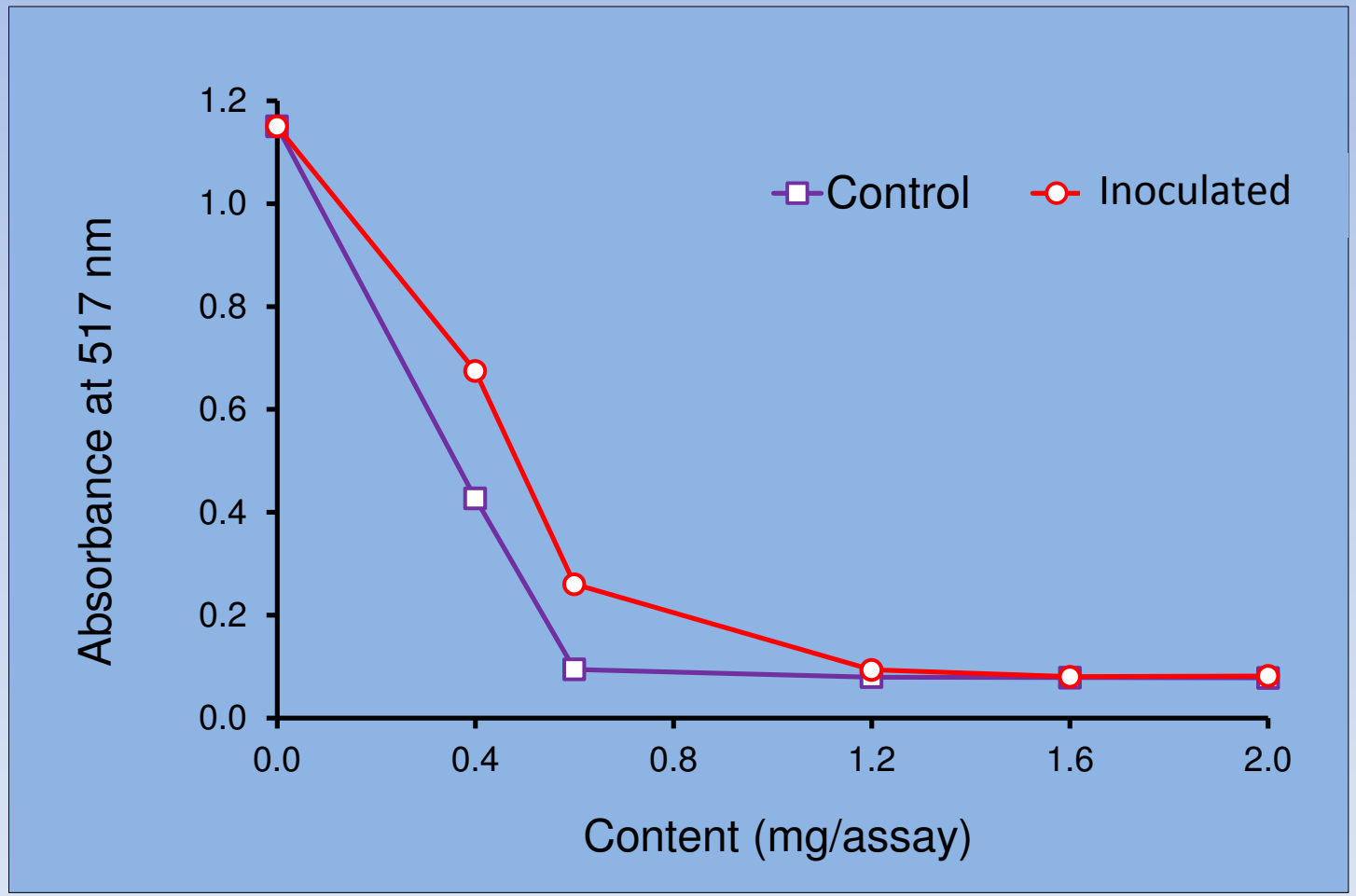


FRAP of the extracts and seeds

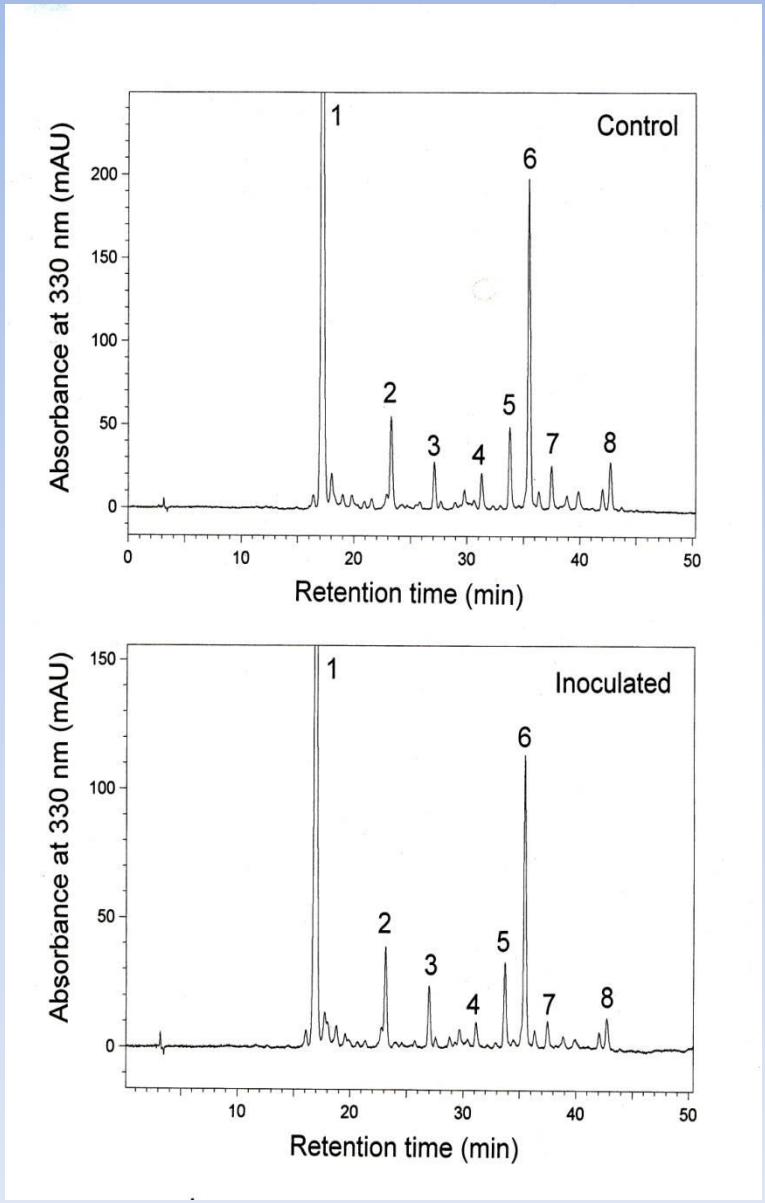




Antiradical activity of the extracts aginst DPPH radical



HPLC chromatograms of the extracts



Content of individual phenolic compounds in rapeseed extracts (mg/g of extract)

Compound	Control	Inoculated
1 (sinapine)	58.2 ± 2.2	37.9 ± 1.8
2	3.3 ± 0.2	2.3 ± 0.2
3	1.7 ± 0.2	1.4 ± 0.1
4	1.6 ± 0.2	0.7 ± 0.1
5	3.1 ± 0.2	2.2 ± 0.1
6	11.2 ± 0.8	7.0 ± 0.5
7	2.0 ± 0.1	1.0 ± 0.1
8	2.1 ± 0.1	1.1 ± 0.1

Content of individual phenolic compounds in rapeseeds (mg/g of defatted seeds)

Compound	Control	Inoculated
1 (sinapine)	8.49 ± 0.41	8.00 ± 0.39
2	0.48 ± 0.03	0.48 ± 0.03
3	0.25 ± 0.01	0.29 ± 0.01
4	0.24 ± 0.01	0.16 ± 0.01
5	0.45 ± 0.02	0.46 ± 0.02
6	1.63 ± 0.05	1.47 ± 0.05
7	0.29 ± 0.01	0.21 ± 0.01
8	0.30 ± 0.01	0.23 ± 0.01

Conclusions:

•All the rapeseed extracts were characterized by the high content of phenolic compound (phenolic acids).

•Strong antioxidant activities of the rapeseed extracts were observed and assayed using different chemical methods.

•In the seeds of Nelson F1 we found sinapic acid derivative which was absent in the seeds of California and Castilla.

•The weak effect of fertilization on the antioxidant properties was observed. However, it was different for the individual rapeseed cultivars and the chemical methods used for the measure the antioxidant activity.

•In the extract of the seeds treated by *Alternaria brassica* the content of phenolic compounds as well as antioxidant activity were lower than in the extracts of the untreated seeds.

Thank you

