Cristina Abbate, Aurelio Scavo, Stefania Fontanazza, Alessia Restuccia, Giovanni Mauromicale

Department of Agriculture, Food and Environment (Di3A) University of Catania Italy

EFFECT OF CYNARA CARDUNCULUS ALLELOPATHIC ACTIVITY ON SOIL WEED SEED BANK AND EUBACTERIAL COMMUNITIES



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10th European Conference on Pesticides and Related Organic Micropollutants in the Environment

16th Symposium on Chemistry and Fate of Modern Pesticides

10th MGPR International Symposium of Pesticides in Food and the Environment in Mediterranean Countries

ALLELOPATHY

Rice EL (1984): "Any direct or indirect harmful or beneficial effect by one plant (inclusing microorganisms) on another through production of chemical compounds that escape into the environment"

ALLELOCHEMICALS

Acetate pathway

Shikimate pathway

"Non-nutritional chemicals produced by one organism (plants, microorganisms, viruses and fungi) that affects the growth, health, behaviour or population biology of other species" (**Reese, 1979**).



Acetate and shikimate pathway, the biosynthetic pathways of major allelopathic substances - Scavo, Restuccia and Mauromicale (2018)

ALLELOPATHY

1) Volatilization

2) Leaching

4)

- **3)** Decomposition of plant residues
 - Root exudation



Mechanisms of action of allelochemicals in plants - Scavo, Restuccia and Mauromicale (2018)



Major pathways of release of allelochemicals into the environment - Scavo, Restuccia and Mauromicale (2018)

- O Cell division, elongation and structure
- S Cell membrane stability and permeability
- Synthesis of plant endogenous hormones
- Plant photosynthesis
- Sermination and growth of target plants
- Plant respiration
- Activity of various enzymes
- Synthesis of proteins and nucleic acids

MAIN IMPORTANT PLANT SPECIES WITH ALLELOPATHIC PROPERTIES



CYNARA CARDUNCULUS L.



CYNARA CARDUNCULUS L.

Globe artichoke is a perennial rosette plant, grown throughout the world for its immature inflorescences (*capitula*), which are consumed both as fresh and processed. More than 70% of its production is generated from the Mediterranean basin, with the remainder grown in the Americas and in China.





Cultivated cardoon is prized for its leaf petiole and leaf midrib. Its area of cultivation is thought to extend over 2–3 kha, spread over Spain, Italy, France and Greece.

Wild cardoon is a robust thistle distributed over the Mediterranean basin. It currently provides a source of vegetarian rennet used for producing some cheeses in Portugal, Spain, Morocco and Italy



ALLELOCHEMICALS IN C. CARDUNCULUS



11,13-dihidro-desasylcynaropicrin



11,13-dihidroxi-8-desoxygroseimin



cynaratriol



desacylcynaropicrin



grosheimin



cynaropicrin

HO



aguerin B



caffeoylquinic acids

HO HO dicaffeoylquinic acids





apigenin R = H luteolin R = OH

EXPERIMENTAL TRIALS



EXPERIMENT 1:

Allelopathic effects of *C. cardunculus* L. leaf aqueous extracts on seed germination of some Mediterranean weed species

EXPERIMENT 2:

Field allelopathic activity of *C. cardunculus* on weed seed bank size and composition



EXPERIMENT 3:

DGGE of soil eubacterial communities

EXPERIMENT 4:

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In vitro antagonistic activity of *C. cardunculus* leaf extracts against N cycle bacteria.

EXPERIMENT 1 PREPARATION OF LEAF AQUEOUS EXTRACTS



PREPARATION OF LEAF AQUEOUS EXTRACTS



EFFECT OF C. CARDUNCULUS EXTRACTS ON WEED GERMINATION

SCIENTIFIC NAME	FAMILY	COMMON NAME
Amaranthus retroflexus L.	Amaranthaceae	Redroot pigwed
Portulaca oleracea L.	Portulacaceae	Purslane
C. cardunculus var. sylvestris	Asteraceae	Wild cardoon
Diplotaxis erucoides L.	Brassicaceae	White wall rocket
Lavatera arborea L.	Malvaceae	Tree mallow
Brassica campestris L.	Brassicaceae	Field mustard
Solanum nigrum L.	Solanaceae	Black nightshade













EXPERIMENT 1 RESULTS

Seed germination percentage reduction (%) of different types of *C. cardunculus* extracts compared to the control (average of seven weed species).



EXPERIMENT 1 RESULTS

Effects of *C. cardunculus* leaf aqueous extracts of on seed Germination (G %) of six weed species.

Leaf aqueous extract	Amaranthus retroflexus	Portulaca oleracea	Diplotaxis erucoides
CONTROL	94.0 ± 2.37d	$61.0 \pm 4.88d$	66.3 ± 5.29d
GLOB ART 40	$73.0 \pm 4.44c$	$26.0 \pm 4.39 ab$	$35.0 \pm 5.33c$
WILD CARD 40	$78.0 \pm 4.14c$	$16.0 \pm 3.67a$	37.5 ± 5.41c
CULT CARD 40	$83.0 \pm 3.76c$	$30.0 \pm 4.58 bc$	18.8 ± 4.36ab
GLOB ART 80	$17.0 \pm 3.76a$	$31.0 \pm 4.62bc$	16.3 ± 4.12a
WILD CARD 80	$37.0 \pm 4.83b$	18.0 ± 3.84a	$40.0 \pm 5.48c$
CULT CARD 80	$40.0\pm4.90b$	$35.0 \pm 4.77c$	$30.0 \pm 5.12 bc$

Values are given as means \pm standard error. Different letters indicate statistical significance for $P \le 0.05$.

RESULTS

Autoallelopathic effect of leaf aqueous extract of *C. cardunculus* on Response Index (RI) and seed Germination (G %).

Leaf aqueous	Wild cardoon		
extract	RI	G %	
Control		$28.0 \pm 4.49^{\circ}$	
GLOB ART 40	0,07	$30.0\pm4.58^{\circ}$	
WILD CARD 40	-0,43	16.0 ± 3.67^{ab}	
CULT CARD 40	-0,29	20.0 ± 4.00^{bc}	
GLOB ART 80	-0,25	21.0 ± 4.07^{bc}	- 63%
WILD CARD 80	-0,21	$22.0 \pm 4.14^{\mathrm{bc}}$	
CUL CARD 80	-0,68	9.0 ± 2.86^{a}	

Values are given as means \pm standard error. Different letters indicate statistical significance for $P \le 0.05$.

ALLELOPATHIC ACTIVITY ON WEED SOIL SEED BANK





ALLELOPATHIC ACTIVITY ON WEED SOIL SEED BANK

Amounts of weed seeds in the soil seed bank in Agricultural Experimental Farm of Catania University (A) and Passitti (B).



Values are given as means \pm standard error. Different letters indicate statistical significance for $P \le 0.05$.

EXPERIMENT 3 SOIL MOLECULAR ANALYSES

✓ DNA was extracted directly from 250 mg of soil according to Martin-Laurent method (2001)

✓ PCR of 16S rDNA fragments was performed using the universal eubacterial primers GC968f and 1401r (Felske et al., 1997)

EXPERIMENT 3 SOIL MOLECULAR ANALYSES

DGGE

(Denaturing gradient gel electrophoresis)

✓ 16S rDNA amplicons were loaded on 6% polyacrylamide gel containing a denaturant gradient of 46-56% parallel to the electrophoresis direction made of urea and formamide.

✓ Gels were electrophoresed at a constant temperature (60°C) and voltage (75 V) for 16 hours, followed by 2 hours coloration using SYBR Green I.

RESULTS





DGGE bands were identified by DNA sequencing:

1 as Bacillus subtilis

> 2 as Pseudomonas putida

> 3 as Azospirillum brasilense

SUSTAINABILITY IN AGROECOSYSTEMS



INVITRO ANTIMICROBIAL ACTIVITY



BACTERIA
Sinorhizobium meliloti

Bacillus licheniformis

EXPERIMENT 4 RESULTS

In vitro antagonistic activity of Cynara cardunculus var altilis DC. leaf extracts against bacteria.

	zones of inhibition (mm)*, **, ***								
Bacteria	WE 0.3336 (mg TP ml ⁻¹)	ME 1.7676 (mg TP ml ⁻¹)	EE 1.2684 (mg TP ml ⁻¹)	WE 0.278 (mg TP ml ⁻¹)	ME 1.473 (mg TP ml ⁻¹)	EE 1.057 (mg TP ml ⁻¹)	WE 0.139 (mg TP ml ⁻¹)	ME 0.7365 (mg TP ml ⁻¹)	EE 0.5285 (mg TP ml ⁻¹)
Rhizobium leguminosarum	-	_	_	_	_	_	_	_	-
Sinorhizobium meliloti	_	-	-	_	-	-	-	-	-
Bacillus licheniformis	_	23.5	17.7	_	22	18	<u>- C</u>	16	21

* Values are given as means of 3 replicates with pooled standard error of a mean (SEM) =0.04 **100 μ L of extract was applied to each well

***WE: water extracts; ME: methanolic extracts; EE: ethanolic extracts; –no inhibition zone TP: total polyphenols

Inhibition growth was calculated in mm after 2 days of incubation

EXPERIMENT 4 RESULTS

In vitro antagonistic activity of *C. cardunculus* ethanolic and methanolic extracts against *Bacillus licheniformis*



Cultivated cardoon ethanolic extract 1.057 mg TP ml

Cultivated cardoon methanolic extract 1.473 mg TP ml

CONCLUSIONS

In vitro weed seed germination



Effects of *C. cardunculus* extracts varies with the <u>botanical variety</u>, the <u>weed species</u> and the <u>concentrations of the extracts</u>.

The rate of germination decreased with increasing extract concentration.

- 2 On average of six weed species, the best extract was **cultivated cardoon 80%**.
- The most sensitive weed species were *A. retroflexus* (-58.1%), *D. erucoides* (-43.9%) and *P. oleracea* (-42.5%).

Field allelopathic activity on weed soil seed bank



The presence of globe artichoke, cultivated and wild cardoon for three consecutive years significantly reduced the amount of weed seeds in the soil seed bank of two different areas. Moreover, cultivated cardoon decreased the number of weed species compared to globe artichoke and a biennial rotation wheat/faba bean.

CONCLUSIONS

DGGE showed that cultivated cardoon had:

- negative influence towards B. subtilis
- positive effects towards P. putida and A. brasilense



In vitro antibacterial activity showed that cultivated cardoon leaf extracts:

- did not exert negative effects on R. leguminosarum and S. meliloti
- its methanolic and ethanolic extracts inhibited *B. licheniformis* at all concentrations tested



Our results suggest **the advantages to introduce** *C. cardunculus* within a crop rotation system in Mediterranean agroecosystem as indirect method to reduce the weed soil seed bank pressure without the adoption of chemical-synthesis herbicides and with respect of soil eubacterial communities, making possible eco-friendly managements.

THANKS FOR YOUR ATTENTION

