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

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Abstracts

It is known that the presence of weeds causes serious losses to the agricultural production, both in quantitative and qualitative terms. The major problem in modern agriculture is the environmental impact of synthetic herbicides and the increase in herbicide-resistant weed species. Allelopathic compounds can be used to develop a sustainable weed management system based on natural products. The objective of this study was to evaluate the allelopathic potential of leaf aqueous extracts (40 and 80%) obtained from *Cynara cardunculus* L. plant species on seed germination and mean germination time of six common weeds in Mediterranean agroecosystems: *Amaranthus retroflexus* L., *Diplotaxis erucoides* (L.) DC., *Portulaca oleracea* L., *Lavatera arborea* L., *Brassica campestris* L. and *Solanum nigrum* L. Effects varied with the weed species and the concentrations of the extracts. On average, the aqueous leaf extracts significantly reduced the final percentage of seed germination compared to the control for *A. retroflexus* (–58.1%), *D. erucoides* (–43.9%) and *P. oleracea* (–42.5%). The rate of germination decreased with increasing extract concentration. In *C. cardunculus* L. var. *sylvestris* the autoallelopathic activity also was demonstrated. These results are very promising in order to produce a bioherbicide based on *C. cardunculus* allelochemicals.

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Introduction

The presence of weeds causes serious losses to the agricultural production, both in quantitative and qualitative terms, because they constantly compete spatially with crop plants, limiting the available amount of nutrients, light and moisture. Weeds are very good colonisers, reproduce faster, produce a large number of small seeds with very prolonged viability in soil and survive in the most adverse situations, becoming part of the persistent soil seedbank. On average, weeds cause a yield crop reduction estimated around 34% (Oerke, 2006). The increase in world population and the simultaneous decrease of the available resources, have led agriculture to an indiscriminate use of synthetic herbicides for weed management. This wide usage has lead to serious problems, such as the evolution of herbicide resistant weed populations and the negative impacts on environmental, human and animal health (Jabran *et al*., 2015). Therefore, there is an urgent need to explore eco-friendly strategies for weed control.

Putnam and Duke (1978) were the first ones to assess the possibility of using allelopathic crops for weed management in agriculture, minimising the serious problems of environmental impact. Allelochemicals includes terpenoids, N-containing compounds and phenolic compounds and can be found in different parts of plant: leaves, stems, roots, rhizomes, seeds, flowers and even pollen (Kruse *et al.*, 2000; Bertin *et al*., 2003). In contrast to a high proportion of synthetic agrochemicals, allelochemicals are biodegradable, mostly water-soluble and consist of non-halogenated molecules (Bhowmik and Inderjit, 2003). Besides, they present a wide chemical diversity, are selective (Dayan *et al*., 2012) and, thus, may offer new mode of actions (Macias *et al*., 2007).

According to Rial *et al*. (2014), cardoon allelochemicals (primarily aguerin B, grosheimin and cynaropicrin) possess a strong phytotoxicity on the germination and growth of standard target species (tomato, lettuce, onion and watercress) and weeds (barnyardgrass and brachiaria). Moreover, the joint action of binary mixtures of aguerin B, grosheimin and cynaropicrin and one non-active compound (11,13-dihydroxy-8-desoxygrosheimin) was also investigated on wheat coleoptide (Rial *et al*., 2016). *Cynara cardunculus* L. is a perennial diploid ($2n = 2x = 34$) member of Asteraceae family native to the Mediterranean Basin. According to Rottenberg and Zohary (1996), it includes the globe artichoke [var. *scolymus* (L.) Fiori], the cultivated cardoon (var. *altilis* DC.), and their progenitor wild cardoon [var. *sylvestris* (Lamk) Fiori]. The cultivated cardoon has been cultivated as a vegetable since ancient times, but nowadays the land area devoted to this crop is mainly localised in Spain, Italy, France and Greece (Portis *et al*., 2005; Mauromicale *et al*., 2014). Wild cardoon is a non-domesticated robust perennial plant, characterised by its rosette of large spiny leaves, branched flowering stems and blue-violet flowers.

The major product of the globe artichoke is the edible head, which is appreciated in both its fresh and processed forms (Baty-Julien and Hélias, 2012). The major globe artichoke producer is Italy (about 548 Kt per year), followed by Egypt and Spain (about 391 and 200 Kt per year, respectively) (FAO, 2013). The three types are a good source of caffeoylquinic acids and flavones as reported in previous works (Schütz *et al.*, 2004; Pandino *et al.*, 2012; Lombardo *et al.*, 2015). In particular, their leaves have been shown to represent a potentially productive source of polyphenols (Lombardo *et al.*, 2009, 2015), which have various industrial, pharmaceutical and cosmetic application (Pinelli *et al.*, 2007; Lattanzio *et al.*, 2009). Thanks to these compounds, the *C. cardunculus* species have been stimulated the scientific interest at the aim to evaluate these crops as promising sources of natural antioxidant for food and not food applications. Nevertheless, the potential use of *C. cardunculus* leaves extracts for weed management is at the beginning of investigation.

For this reason, the purpose of this study was to evaluate the possible effects of wild cardoon, cultivated cardoon and globe artichoke allelochemicals on seed germination and mean germination time of six common weeds in Mediterranean agroecosystems. Moreover, the autoallelopathic activity on wild cardoon was considered too.

Materials and methods

Sampling of *C. cardunculus* plant material and preparation of aqueous leaf extracts

Fresh material was sampled from cultivated cardoon, wild cardoon and globe artichoke plants at the 25th visible leaves growth stage (November 2014), randomly, from a field crop located in the Catania University experimental station farms situated in Catania Plain [10 m (a.s.l.), 37° 25' N, 15° 30' E]. The three botanical varieties were at the same phenological stage. The extraction was carried out according to Sarkar *et al.* (2012). In the laboratory, the plant material from each botanical variety (approximately 1 kg of leaves) was washed, cut and ground. Then, a portion of each gross material was mixed with distilled water (1:10 w/v). The mixture was kept under dark conditions for 48 h at room temperature and, then, filtered through filter paper (Whatman No. 2) to eliminate the solid fraction. From this solution, two different concentrations (40 and 80%), already investigated in our preliminary studies, were obtained for each botanical variety: wild cardoon ecotype *Marsala* (CW 40 - CW 80), cultivated cardoon cultivar *Verde de Peralta* (CC 40 - CC 80) and globe artichoke cultivar *Violetto di Sicilia* (ART 40 - ART 80). Each extract was compared using distilled water as control (C). The prepared aqueous extracts were transferred into a falcon flask and stored in a refrigerator (3°C) for further use. In a submitted work, we identified by HPLC analysis the phytotoxic compounds of *C. cardunculus*, such as caffeoylquinic acids and flavones (*e.g.* chlorogenic acid, luteolin 7-*O*-glucoronide, luteoilin, apigenin 7-*O*-glucoronide, apigenin, *etc.*).

Weed seed collection

Mature seeds from adult plants were collected from six common Mediterranean weed species in natural ecosystems as well as in field crops (Table 1). Collection sites were made from natural populations in the Catania Plane of Sicily (Italy, lat. 37° 28' N, long. 14° 57' E, at an average altitude of 50-150 m a.s.l.). The climate of this area is of a Mediterranean type, with a long, hot and dry summer, mild winter and rain falling mostly from late autumn to early spring. Daily mean temperature during the year ranges from 8.5°C to 26°C: minimum temperature is around 0°C, while maximum can peak at over 35°C. Annual precipitation is about 500 mm (Cristaudo *et al.*, 2007).

After collection, seeds were cleaned, kept in paper bags and dry-stored at room temperature until germination tests were performed. Seeds of each species were selected through the use of a stereomicroscope to achieve a homogeneity of the lots for size and colour.

Germination tests

Germination bioassays were arranged using 5 mL of leaf aqueous extracts at 40 and 80% and distilled water to humidify a double layer of sterilised filter paper (Whatman No. 2). Petri dishes, hermetically sealed with parafilm to prevent evaporation of the solution, were stored in incubators at the optimal conditions of temperature and photoperiod for single weed species tested. Germination tests were performed in continuous darkness and at a constant temperature of 35°C for *Amaranthus retroflexus* L. (Cristaudo *et al.*, 2007) and *Portulaca oleracea* L. (Singh, 1973), in continuous darkness and at a constant temperature of 20°C for *C. cardunculus* var. *sylvestris* (Lekić *et al.*, 2011), while *Diplotaxis erucoides* (L.) DC. (Gresta *et al.*, 2010) and *Lavatera arborea* L. were incubated in alternating light (dark/light cycle 12/12 h) at 20°C and 25°C respectively. Besides, *Brassica campestris* L. (Kondra *et al.*, 1983) and *Solanum nigrum* L. (Taab, 2009) were incubated in alternating light (dark/light cycle 8/16 h) at 20°C and 25°C respectively. Incubators maintained the designated temperature to within ±1°C and they were equipped with Osram cool white fluorescent lamps with an irradiance of 25 μmol m^{-2} s^{-1}, 400–750 nm.

For each treatment, four replications of 25 seeds were placed separately in 9 cm diameter plastic Petri dishes, transparent for dark/light alternating conditions and wrapped in sheets of aluminium foil for complete darkness. During the counting process, germinated seeds in continuous darkness treatments were manipulated under a green safelight (490-560 nm), while seeds in alternating photoperiod were counted during the 12-h light period (Cristaudo *et al.*, 2016).

Table 1. List of the plants used in the germination tests.

Common name	Scientific name	Family	Biological form	Corotype
Redroot pigwed	*Amaranthus retroflexus* L.	Amaranthaceae	T scap.	Cosmop.
Purslane	*Portulaca oleracea* L.	Portulacaceae	T scap.	Subcosmop.
White wall rocket	*Diplotaxis erucoides* (L.) DC.	Brassicaceae	T scap.	W-Medit. (Steno)
Tree mallow	*Lavatera arborea* L.	Malvaceae	H bien.	Steno-Medit.
Field mustard	*Brassica campestris* L.	Brassicaceae	T scap./H scap.	Medit.
Black nightshade	*Solanum nigrum* L.	Solanaceae	T scap.	Cosmop. (synanthrop.)
Wild cardoon	*C. cardunculus* L. var. *sylvestris*	Asteraceae	H scap.	Steno-Medit.

Germination was determined by counting and removing germinated seeds every 24 h. Germination was considered when the radicals were greater than or equal to 2 mm in length. All the determinations were performed twice and each value of a replicate is therefore a mean of the two readings.

Data analysis

The percentage of final germination (G %) was calculated as the ratio between the number of seed germinated and the total number of seeds used in each Petri dish. The corresponding proportions were analysed by way of a binomial generalised linear model with logit link (Sileshi, 2012). Plant species, allelopathic compounds and their interaction were included as factors in the model. Wherever necessary, contrasts between means were performed by using the procedures outlined in Bretz *et al*. (2011). The Mean Germination Times for each Petri dish were obtained by using the Kaplan-Meyer estimators (Onofri *et al*., 2010), together with mid-point imputation to comply with interval censoring (Law and Brookmeyer, 1992). Mean germination time (MGT) were analysed by using two-way ANOVA; a graphical inspection of residuals showed that no significant deviations with respect to the basic assumptions for ANOVA were found.

Allelopathic effect response index (RI) was calculated using the Equation (1) of Williamson and Richardson (1988):

$$RI = \begin{cases} 1 - \frac{C}{T} & if\ T \geq C \\ \frac{T}{C} - 1 & if\ T < C \end{cases} \quad (1)$$

where T is the seed germination (%) for the treated plants and C is the seed germination (%) for the corresponding control. RI ranges from –1 to +1, with positive values indicating the stimulation of germination by the aqueous extracts and negative values indicating the inhibition of germination, relative to the control.

Results and discussion

Many works report the inhibition of seed germination in presence of plant allelochemicals (Reigosa and Pazos-Malvido, 2007; Sbai *et al*., 2016). *C. cardunculus* secondary metabolites, such as chlorogenic acid and luteolin 7-*O*-glucoronide, have been reported to show allelopathic activity on different crops (Abdul-Rahman and Habib, 1989; Li *et al*., 1993; Hosni *et al*., 2013). In this experiment, RI was significantly affected by the interaction of species and compound ($P=9.7\times10^{-12}$). The RI of *C. cardunculus* leaf aqueous extracts was negative in all weed species under study, except in *S. nigrum* (and wild cardoon) (Figure 1). This variability among weed species could be attributed to the different combination of allelochemicals profile present in each extract, as well as by their level. Our hypothesis is corroborated by Ambika (2013), who found as a compound may be inhibitory at high concentration, stimulatory at low concentration, or have no effect at other concentrations.

Regardless of the weed species, all extracts reduced weed seed germination if compared with control (Figure 2). The best result was obtained with CC 80, which inhibited the weed seed germination by about 64%. On the contrary, CC 40 showed the worst allelopathic effect by reducing only 26% final seed germination, as well as the effects of ART 40, CC 40 and CW 40 appeared less marked (Figure 2). Overall, our results reported that the concentrated extract (80%) had major negative effect on weed species germination than the diluted one (40%) (45.5 *vs* 33.3% respectively). Similar trend was noted by Chung and Miller (1995) on selected weed species treated with alfalfa (*Medicago sativa* ssp. *sativa* L.) residues.

In *A. retroflexus*, all aqueous extracts significantly lowered seed germination with a decrease, on average, of 58.1% as com-

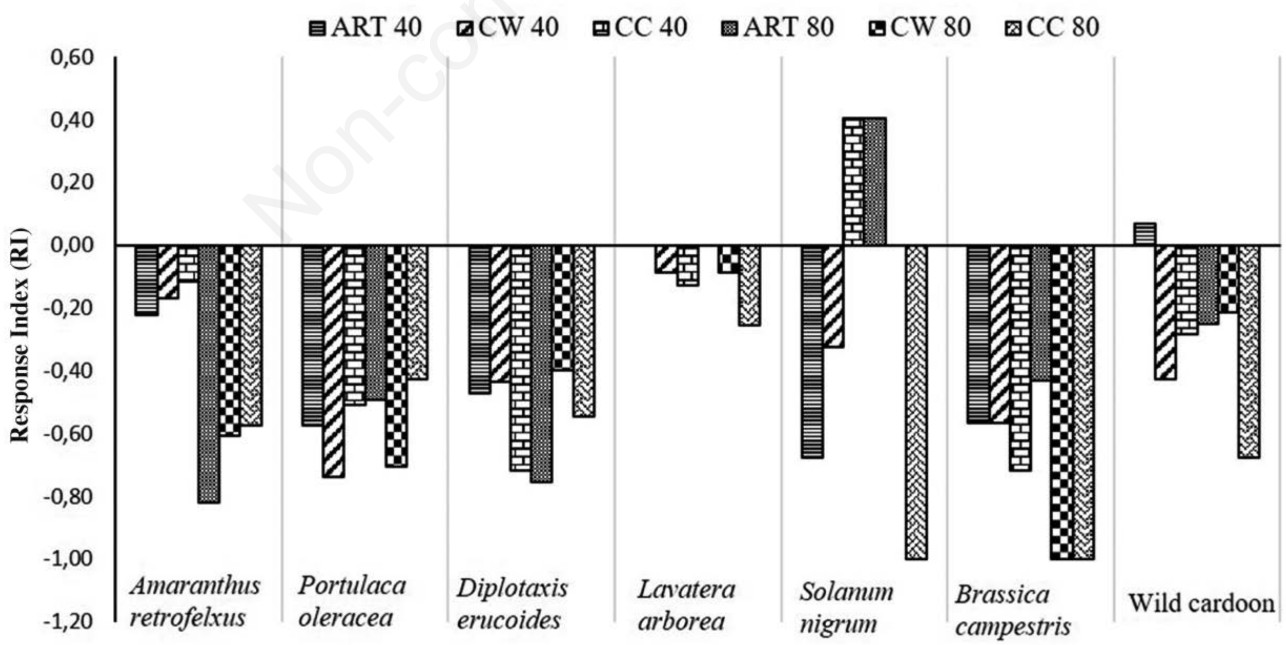

Figure 1. The influence of leaf aqueous extracts of *C. cardunculus* on the allelopathic effect response index (RI) in six weed species. The pooled standard error of the above means was 0.111. ART 40, globe artichoke extract 40%; CW 40, wild cardoon extract 40%; CC 40, cultivated cardoon 40%; ART 80, globe artichoke extract 80%; CW 80, wild cardoon extract 80%; CC 80, cultivated cardoon 80%.

pared to the control. ART 80 resulted the most efficient, since allowed only 17% of seed germination. Also in *P. oleracea* the allelopathic effect of the different solutions was significant, but not concentration-dependent. The percentage reduction of different types of extracts compared to the control was 42.5%. The lowest germination rates were obtained with CW 40 and 80 if compared with C (16 and 18% *vs* 61% respectively). These results are similar to many previous findings. According to Yarna *et al*. (2009), increasing of sorghum leaf extract concentration from 5 to 20% inhibited *A. retroflexus* germination from 70.76 to 92.77% and the germination time was extended too. Azizi and Fuji (2006) found that germination of *A. retroflexus* and *P. oleracea* was completely inhibited at a concentration of 0.7% (v/v) and higher of *Eucalyptus globulus* Labill. essential oils. Besides, they found that the undiluted hydro-alcoholic extract of *Hypericum perforatum* L. and *Salvia officinalis* L. had a significant inhibitory effect on seed germination percentage for *A. retroflexus,* but not for *P. oleracea*. Dadkhah and Asaadi (2010) reported that foliar aqueous extract of *Eucalyptus camaldulensis* Dehnh. not affected the germination percentage of *P. oleracea*, but severely reduced, especially at the

Table 2. Effects of leaf aqueous extract of *C. cardunculus* on seed germination (G%) of six weed species.

Leaf aqueous extract	*Amaranthus retroflexus*	*Portulaca oleracea*	*Diplotaxis erucoides*	*Lavatera arborea*	*Solanum nigrum*	*Brassica campestris*
Control	94.0±2.37[d]	61.0±4.88[d]	66.3±5.29[d]	47.0±4.99[a]	4.0±2.26[a]	8.8±3.16[a]
ART 40	73.0±4.44[c]	26.0±4.39[ab]	35.0±5.33[c]	47.0±4.99[a]	1.3±1.32[a]	3.8±2.12[a]
CW 40	78.0±4.14[c]	16.0±3.67[a]	37.5±5.41[c]	43.0±4.95[a]	2.7±1.86[a]	3.8±2.12[a]
CC 40	83.0±3.76[c]	30.0±4.58[bc]	18.8±4.36[ab]	41.0±4.92[a]	6.7±2.88[a]	2.5±1.75[a]
ART 80	17.0±3.76[a]	31.0±4.62[bc]	16.3±4.12[a]	47.0±4.99[a]	6.7±2.88[a]	5.0±2.44[a]
CW 80	37.0±4.83[b]	18.0±3.84[a]	40.0±5.48[c]	43.0±4.95[a]	4.0±2.26[a]	0.00
CC 80	40.0±4.90[b]	35.0±4.77[c]	30.0±5.12[bc]	35.0±4.77[a]	0.00	0.00

ART 40, globe artichoke extract 40%; CW 40, wild cardoon extract 40%; CC 40, cultivated cardoon 40%; ART 80, globe artichoke extract 80%; CW 80, wild cardoon extract 80%; CC 80, cultivated cardoon 80%. Values are given as means±standard error. [a-d]Different letters indicate statistical significance for P≤0.05.

Table 3. Effects of aqueous extract of *C. cardunculus* on mean germination time (MGT days) of four weed species.

Leaf aqueous extract	*Amaranthus retroflexus*	*Portulaca oleracea*	*Diplotaxis erucoides*	*Lavatera arborea*
Control	1.8[a]	3.7[a]	5.4[ab]	6.9[a]
ART 40	5.0[bc]	4.2[a]	2.3[a]	7.3[a]
CW 40	4.2[ab]	8.6[b]	4.5[ab]	7.8[a]
CC 40	7.5[c]	4.3[a]	5.4[ab]	6.5[a]
ART 80	7.0[bc]	4.4[a]	7.3d	6.3[a]
CW 80	5.0[abc]	6.3[ab]	7.2[b]	8.6[a]
CC 80	7.0[bc]	4.4[a]	6.1[b]	8.2[a]

ART 40, globe artichoke extract 40%; CW 40, wild cardoon extract 40%; CC 40, cultivated cardoon 40%; ART 80, globe artichoke extract 80%; CW 80, wild cardoon extract 80%; CC 80, cultivated cardoon 80%. Values are given as means with pooled standard error of a mean (SEM) =1.02. a-cDifferent letters indicate statistical significance for P≤0.05.

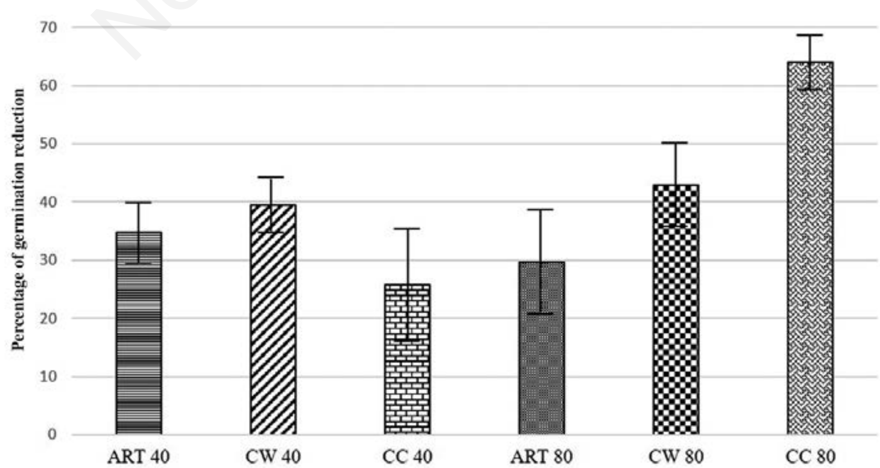

Figure 2. Percentage of reduction respect to control of weed seed germination in relation to extract of *C. cardunculus*. ART 40, globe artichoke extract 40%; CW 40, wild cardoon extract 40%; CC 40, cultivated cardoon 40%; ART 80, globe artichoke extract 80%; CW 80, wild cardoon extract 80%; CC 80, cultivated cardoon 80%.

higher aqueous extract concentration, the growth of young seedlings. Therefore, differently from other plant species, the *C. cardunculus* botanical varieties have a strong inhibitory effect on *P. oleracea*.

No studies about the allopathic effects on seed germination of *D. erucoides* have been published. Nevertheless, several works have been conducted upon the allelopathic activity of *D. erucoides* on field crops (Giordano *et al*., 2005; Qasem, 2007). In this experiment, germination of *D. erucoides* was significantly reduced by all aqueous extracts (66.3% control *vs* 29.1% average of aqueous extracts). The best results were obtained with ART 80 and CC 40 if compared with C (16.3% and 18.8% *vs* 66.3% respectively). However, the less marked effects were registered with CW 80. Results provide evidence of globe artichoke's foliar extracts strong allelopathic effect on *D. erucoides* seed germination. That is important under the applicative aspect because *D. erucoides* is one of the most harmful weed in Mediterranean environments.

Allelopathic effects of *C. cardunculus* extracts on *L. arborea* seed germination and mean germination time were not significant. Therefore, *L. arborea* cannot be considered a target plant for *C. cardunculus* foliar extracts. Also in *S. nigrum* and *B. campestris*, as well as in *L. arborea*, the germination percentage was not significantly affected by any extracts. The low seed germination percentages of *S. nigrum* and *B. campestris,* are probably due to the high seed dormancy of wild ecotypes. These results are in contrast with González *et al*. (1997) and Gao *et al*. (2009). The first found that the effects of six phenolics compounds obtained from the soil solution of nine pepper (*Capsicum annuum* L.) varieties on germination of *S. nigrum* were inhibitory only at a concentration of 10^{-2} M. The second reported that *B. campestris* seed germination and seed germination speed are strongly inhibited by *Hemistepta lyrata* Bunge water extract. *B. campestris* seed germination was inhibited (50%) also by aqueous extract of leaves of *Parthenium hysterophorus* L. at 2% concentration (Maharjan *et al*., 2007). Therefore, *S. nigrum* and *B. campestris*, are very sensitive to allelochemicals of some plant species, but not to *C. cardunculus* botanical varieties.

In addition to the reduction of germination, the delay in seed germination is crucial in weed control and can affect the ability of the seedlings to establish themselves in natural conditions (Escurdero *et al*., 2000; Chaves *et al*., 2001). Table 3 shows how *C. cardunculus* extracts increased MGT of *A. retroflexus*, *P. oleracea* e *D. erucoides*. Since *S. nigrum* and *B. campestris* seeds showed low germination percentage values, their MGTs were not considered. In *A. retroflexus*, all aqueous extracts increased MGT (from 1.8 d of control to 5.9 d on average of the aqueous extracts) and the best results were obtained with CC 40, CC 80 and ART 80 (7.5 d, 7.0 d and 7.0 d respectively). Also in *P. oleracea*, TMG was increased by all treatments (from 3.7 d of control to 5.4 d on average of treatments) and CW 40 showed the highest value (8.6 d). The most significant effect of *C. cardunculus* extracts on *D. erucoides* MGT was reached by using ART 80 and CW 80 (7.3 d and 7.2 d respectively).

In this experiment, the autoallelopathic effect on wild cardoon was also evaluated (Table 4). Our results showed that all the other leaf extracts, excluding ART 40, decreased the germination percentage if compared with C (19.6% *vs* 28% respectively). The most significant result (9%) was obtained with CC 80, followed by CW 40 (16%). Therefore, *C. cardunculus* L. shows an autoallelopathic capacity, although it is low. Autoallelopathy is probably a mechanism that helps *C. cardunculus* invasion in natural ecosystem through maintaining seed dormancy when conditions are not conducive to growth or by increasing plant resistance to pathogens (Friedman and Waller, 1985).

Table 4. Autoallelopathic effect of leaf aqueous extract of *C. cardunculus* on response index (RI), on seed germination (G%) and mean germination time (MGT days).

Leaf aqueous extract	Wild cardoon		
	RI	G %	MGT d
Control		28.0 ± 4.49^{c}	9.8 ± 1.02^{ab}
ART 40	0.07	30.0 ± 4.58^{c}	9.9 ± 1.02^{b}
CW 40	–0.43	16.0 ± 3.67^{ab}	9.5 ± 1.02^{ab}
CC 40	–0.29	20.0 ± 4.00^{bc}	7.9 ± 1.02^{ab}
ART 80	–0.25	21.0 ± 4.07^{bc}	6.9 ± 1.02^{a}
CW 80	–0.21	22.0 ± 4.14^{bc}	9.3 ± 1.02^{ab}
CC 80	–0.68	9.0 ± 2.86^{a}	9.5 ± 1.02^{ab}

ART 40, globe artichoke extract 40%; CW 40, wild cardoon extract 40%; CC 40, cultivated cardoon 40%; ART 80, globe artichoke extract 80%; CW 80, wild cardoon extract 80%; CC 80, cultivated cardoon 80%. Values are given as means±standard error. [a-c]Different letters indicate statistical significance for $P\leq0.05$.

Conclusions

The present study exploited the allelopathic effect of leaf aqueous extracts of *C. cardunculus* on six common Mediterranean weeds. Overall, the inhibitory effect was concentration-dependent, even if different behaviour was observed among the considered weed seeds. These results are very promising in order to produce a bioherbicide based on *C. cardunculus* extracts. It will make possible to reduce the use of synthetic herbicides and, as consequence, should have an impact positively on the environment and human health. Nevertheless, the trial was carried out *in vitro* conditions, thus, further investigations are needed to determine the effect of *C. cardunculus* allelochemicals in open field, in order to set up the best dose-response effect on common Mediterranean weeds. Future developments involve also an accurate study on the methodology for extraction of *C. cardunculus* allelochemicals, particularly on the utilisation of different solvents, on the stability of the extracts, as well as analysing the polyphenol profiles and investigating the phytotoxic effects of several genotype extracts from the three botanical varieties at different growth stages and stress conditions.

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