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Allelopathic potential of leaf aqueous extracts from *Cynara cardunculus* L. on the seedling growth of two cosmopolitan weed species

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Abstract

The search for sustainable alternatives to synthetic herbicides for weed control, has led the scientific community to an increased interest for plant allelopathic mechanisms. The utilization of plant extracts as possible bioherbicides represents an important solution. In the present study, laboratory experiments were carried out to investigate, for the first time, the differences in the allelopathic activity of the three *Cynara cardunculus* L. botanical varieties (globe artichoke, cultivated and wild cardoon) leaf aqueous extracts on the seedling growth of *Amaranthus retroflexus* L. and *Portulaca oleracea* L. In addition, the autoallelopathic effect on wild cardoon growth and the qualitative profile of the aqueous extract through HPLC analysis were evaluated. Overall, the allelopathic effects were both genotype- and weed species-dependent. Wild cardoon showed the highest allelopathic potential (–23.4%), followed by cultivated cardoon and globe artichoke, and *P. oleracea* was the most sensitive target species (–32%). Besides, root system length was the most affected parameter (–32.6%). The autoallelopathic effect of wild cardoon extract was also demonstrated on root system length, hypocotyl and epicotyl length and total dry weight. *C. cardunculus* leaf aqueous extract was characterised by 5 sesquiterpene lactones, 2 caffeoylquinic acids, 6 flavones and 1 lignan. From the HPLC analysis we found that apigenin and luteolin 7-*O*-glucoronide were detected only in wild cardoon, apigenin 7-*O*-glucoside was typic of globe artichoke, and 11,13-dihydro-desacylcynaropicrin and 11,13-dihydroxi-8-desoxygrosheimin were characteristics of cultivated cardoon.

Introduction

Allelochemicals are secondary metabolites, produced by plants and microorganisms, belonging to many different chemical classes (*e.g.* phenolic compounds, terpenoids, cyanogenic glycosides, *etc.*) and playing a defensive role for the plant (Whittaker and Feeny, 1971). In order to find new eco-friendly strategies for weed control without the adoption of synthetic chemical products, the scientific community increased its interest for the manipulation of allelopathic mechanisms, mainly the selection of allelochemicals and their potential use as bioherbicides (Jabran *et al*., 2015). Their efficacy, of course, is clearly weak if done alone, becoming more effective when combined within an Integrated Weed Management System (IWMS) (Scavo *et al*., 2018a). Several studies have been published on the interaction between allelochemicals, especially as water extracts, and commercial herbicides in order to reduce the herbicide rate (Cheema *et al*., 2003; Jabran *et al*., 2010). Allelopathic species could be also integrated as cover crops, living or dead mulch, in intercropping or green manures, or even in rotational sequences with the cash crop under an IWMS (Farooq *et al*., 2011; Jabran *et al*., 2015).

The Asteraceae family has been widely studied for its allelopathic properties both in crop and weed species (Chon and Nelson, 2010; Ilori *et al*., 2010). In this regard, sunflower (*Helianthus annuus* L.) is referred as the most common Asteraceae member showing strong allelopathic activity (Alsaadawi *et al*., 2012). Among weeds, the most studied species for their allelopathic potential are *Artemisia annua* L. (Lydon *et al*., 1997), *Pluchea lanceolata* (DC.) Oliv. & Hiern (Inderjit and Dakshini, 1994) and *Taraxacum officinale* L. (Loughnan *et al*., 2014). *Cynara cardunculus* L. is an herbaceous perennial C_3 plant species, belonging to the Asteraceae family, naturally occurring in the semi-arid zones of the Mediterranean Basin. It is a complex species that comprises two domesticated forms, globe artichoke [var. *scolymus* (L.) Fiori] and cultivated cardoon (var. *altilis* DC.), along with their common progenitor wild cardoon [var. *sylvestris* (Lamk) Fiori] (Rottenberg and Zohary, 1996). In previous studies, *C. cardunculus* allelopathic activity has been reported both on weeds and bacterial species of food and agricultural interest (Mazzaglia *et al*., 2018; Scavo *et al*., 2019a). Scavo *et al*. (2018b) demonstrated the allelopathic effects of the three *C. cardunculus* botanical varieties on seed germination of six common weeds in Mediterranean agroecosystems. In particular, they indicated cultivated cardoon as the best genotype in terms of inhibitory activity (−64% on the average of the six weeds tested), followed by wild cardoon, with concentration-dependent phytotoxic effects. Its allelopathic potential is mainly determined by the sesquiterpene lactones cynaropicrin, aguerin B, grosheimin, *etc*. (Rial *et al*., 2014), as well as by caffeoylquinic acids and flavones (Pandino *et al*., 2012, 2015), particularly abundant in the leaves (Lombardo *et al*., 2009). Nevertheless, Scavo *et al*. (2019b) investigated the extraction method of cultivated cardoon allelochemicals and reported the sesquiterpene lactones cynaratriol, desacylcynaropicrin and 11,13-dihydro-desacylcynaropicrin in cultivated cardoon leaf extracts for the first time. However, the differences in the aqueous extract allelopathic profiles of the three *C. cardunculus* botanical varieties are unknown, as well as their phytotoxic activity on weeds growth. Moreover, wild cardoon is reported as a strong invasive weed in California and Australian grassland communities thanks to its high competitive behaviour and the massive production of wind-dispersed seeds (Marushia and Holt 2006). In particular, they found that, due to the large dimensions of seeds (20-60 mg), its dispersal distance is limited to less than 20 m in vegetated sites and more than 40 m in non-vegetated ones. In this regard, as suggested by Chon *et al*. (2006) for alfalfa, *C. cardunculus* may gain ecological advantages from its autotoxic potential through the improvement of its geographical distribution, the induction of dormancy and the prevention of seed and propagules decay (Scavo *et al*., 2018a). Autoallelopathy in *C. cardunculus* has been previously studied on seed germination, but the autotoxic effects on seedling growth are equally unknown.

Therefore, this study proposed: i) to investigate the differences in the allelopathic activity of globe artichoke, cultivated and wild cardoon leaf aqueous extract on the seedling growth of two cosmopolitan weed species (*Amaranthus retroflexus* L. and *Portulaca oleracea* L.); ii) to evaluate the autoallelopathic effect on wild cardoon growth; and iii) to determine the qualitative profile of the aqueous extracts through HPLC analysis.

Materials and Methods

Collection of plant material and preparation of extracts

Globe artichoke, cultivated and wild cardoon were grown in the Catania University experimental station [Catania Plain, 10 m (a.s.l.), 37° 25' N, 15° 30' E]. The soil was characterized by a typic vertic and/or xerochrept soil (Soil Survey Staff, 1999) with clay texture. The local climate is typical Mediterranean, comprising hot-dry summers, mild-wet winters and average annual precipitations of ~500 mm. Before planting, the experimental field was ploughed up to 30 cm, harrowed and fertilized with 50 kg N ha^{-1} (as urea), 80 kg P_2O_5 ha^{-1} (as double perphosphate) and 80 kg K_2O ha^{-1} (as potassium sulphate). A plant rate of 1 plant m^{-2} was applied, using an inter- and intra-row spacing of 1.25 and 0.80 m, respectively. Weed and insect control, when required, was carried out by spraying oxyfluorfen and imidacloprid, respectively.

Sixty expanded leaves of globe artichoke 'Violetto di Sicilia', cultivated cardoon 'Verde de Peralta' and wild cardoon ecotype 'Marsala' were randomly sampled from the 8-10th upper leaf of about 60 plants. 'Violetto di Sicilia' is the most popular and widespread Sicilian globe artichoke ecotype, 'Marsala' was obtained from a native stand in Marsala (Western Sicily) and 'Verde de Peralta' is a cardoon cultivar native to Navarra (Spain) and destined to both markets, fresh and food industry (Macua *et al*., 2004). The collection of plant material was done in November at the rosette developed and leaves cover 50% of ground growth stage, code 35 following the BBCH scale proposed by Archontoulis *et al*. (2010). Extract preparation was carried out following Scavo *et al*. (2018b). Aqueous extract solutions were diluted to 80%. Bidistilled water was used as control (C). Each extract was stored in a refrigerator (−10 °C) for further analyses.

Weed growth bioassay

Mature seeds of *A. retroflexus* and *P. oleracea* were collected from natural weed populations infesting the same Catania University experimental station. Seedling growth tests were carried out under a completely randomized block design with three replicates into dark plastic pots (Ø, 10 cm; height 8 cm). The substrate was a mixture fine sterilized sand/peat (50:50, v/v), with the addition of expanded clay (Combo) 8/15 mm, in order to achieve a better combination of aeration and extract percolation, and to avoid any extract stagnation. To evaluate the allelopathic effects of the different aqueous leaf extracts in post-emergence experiments, each pot was seeded with ten pre-germinated seeds (4 days old) of *A. reroflexus* and *P. oleracea* and moistened with 50 mL of extracts. After 20 days, 25 mL of extracts were added. For the autotoxicity evaluation, ten wild cardoon 'Marsala' pre-germinated seeds (5 days old) were used. Moreover, the pots were wrapped with parafilm with the aim to limit the evapotranspiration and allow the free-development of the plant. Each pot was incubated inside growth chambers in alternating light (dark/light cycle 12/12 h) at 25 ± 1°C for *A. retroflexus* and *P. oleracea*, and 20 ± 1°C for *C. cardunculus* var. *sylvestris*. After 40 days, the plants were removed from the pots and washed. Root system length (cm), hypocotyl length (cm), epicotyl length (cm) and total dry weight (mg) were measured. For the total dry weight determination, the plants were dried at 60°C until constant weight. All the determinations were repeated twice.

Allelochemicals identification

The identification of allelochemicals was performed using 20 μL of each extract for HPLC analysis and comparing their retention times and their UV spectra with those of standards. All analyses were repeated three times. The determination of caffeoylquinic acids, flavones and sesquiterpene lactones was carried out in a 1200 HPLC system (Agilent Technologies, Palo Alto, USA) equipped with ChemStation software (version: B.03.01).

For the determination of caffeoylquinic acids and flavones, a Zorbax Eclipse XDB-C_{18} column (4.6 × 50 mm; 1.8 lm particle size) operated at 30°C, with a 0.2 μm stainless steel in-line filter, was used. The mobile phase was 0.1% formic acid in a step gradient of water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.5 mL min^{-1}, in accordance with Lombardo *et al*. (2015).

Cynaropicrin was analysed according to Menin *et al.* (2012), using a step gradient of 0.1% formic acid with water and acetonitrile at a flow rate of 0.3 mL min^{-1}. Grosheimin and aguerin B were identified following Rial *et al*. (2014). The other sesquiterpene lactones and pinoresinol were identified in accordance with Scavo *et al*. (2019b). Cynaratriol was detected using an isocratic mixture of chloroform/methanol (90:10 v/v, flow 3 mL min^{-1}, retention time 14 min). A mixture chloroform/methanol (95:5 v/v, flow 3 mL min^{-1}) followed by *n*-hexane/acetone (75:25 v/v, flow 1 mL min^{-1}) and water/acetonitrile/methanol (70:20:10 v/v, flow 1 mL min^{-1}) was used for desacylcynaopicrin (retention time 5.2 min) and 11,13-dihydro-desacylcynaropicrin (retention time 4.7 min). Pinoresinol was identified using *n*-hexane/acetone (70:30 v/v, flow 1 mL min^{-1}, retention time 4.7 min). Chromatograms were recorded at 240, 254, 280, 310 and 350 nm from diode array data.

Statistical analysis

Once verified the homoscedasticity with the Bartlett's test and the normal distribution by log-transformation, all data were subjected to analysis of variance (ANOVA). Means were separated with the Duncan's test at the 0.05 probability level. The degree of inhibition and/or stimulation compared to control was calculated with the Allelopathic Effect Response Index

(RI), using the Equation suggested by Williamson and Richardson (1988):

$$RI = \begin{cases} \left(1 - \frac{C}{T}\right) \times 100 \; if \; T \geq C \\ \left(\frac{T}{C} - 1\right) \times 100 \; if \; T < C \end{cases}$$

where T is the treatment value and C is the corresponding control. Positive values indicate stimulating effects, while negative ones indicate inhibitory activity of the aqueous extracts.

Results and Discussion

The influence of *C. cardunculus* leaf aqueous extracts on the seedling growth of *A. retroflexus* and *P. oleracea* and the autoallelopathic effect on wild cardoon are shown in Figure 1. Our data revealed that on root system length all extracts had a better performance on *A. retroflexus* (−50% respect to the control), while all the extracts had a stimulatory effect on the epicotyl length. Stimulatory effects were also reported by Rejila and Vijayakumar (2011) for *Jatropha curcas* L. leaf extract on seed germination and shoot length of *Sesamum indicum* L. Here, only the globe artichoke extract had a significant inhibitory effect on *A. retroflexus* hypocotyl length. Inhibitory effects were observed in *P. oleracea* for all the parameters under study and, in particular, for hypocotyl and epicotyl length, with a mean reduction of 22 and 51%, respectively, if compared to control. Wild cardoon extract was the most phytotoxic for both root system and epicotyl length, while globe artichoke extract was the worst. Regarding the autoallelopathic effect on wild cardoon, on average of extracts, any statistical differences were recorded on the hypocotyl length, while the root system length was relatively more sensitive to autotoxic allelochemicals. Among extracts, globe artichoke ones stimulated the length of the epicotyl, but on the contrary it was the most phytotoxic on the root system. These results agree with Turk and Tawaha (2003), who reported that

Brassica nigra L. water extracts had more pronounced effects on radicle growth than on hypocotyl growth.

Regarding the total dry weight, *P. oleracea* was the most affected weed species with a mean reduction of 60%, while *A. retroflexus* was stimulated by all the extracts (Figure 2). The globe artichoke extracts were both the most phytotoxic on *P. oleracea* and the most stimulant on wild cardoon. Similar effects on total dry weight are present in literature. Tanveer *et al.* (2010) reported that *Euphorbia helioscopia* L. root, stem, leaf, and fruit water extracts reduced total dry weight of *Lens culinaris* Medic. seedlings, with a 100% reduction from the leaf extract. Chung *et al.* (2001) found that rice extracts decreased *Echinochloa crus-galli* P. Beauv. dry weight.

Overall, *P. oleracea* was the most sensitive weed species (RI = –32.3%) and the autoallelopathic effect on wild cardoon was greater than the inhibition activity on *A. retroflexus* (RI = –16.4% *vs*. –13.7%, respectively) (Figure 3). Therefore, in addition to the inhibition of seed germination, wild cardoon exerted also an autoallelopathic activity on its growth, mainly with reference to the epicotyl length. Wild cardoon's autoallelopathy is probably related to its wide diffusion in ecosystems. The effect of genotype on the expression of the allelopathic activity is widely reported in literature (Wu *et al*., 2000; Kabir *et al*., 2010; Alsaadawi *et al*., 2012; Scavo *et al*., 2018a). Alsaadawi *et al*. (2012), for example, reported that the phytotoxic potential of sunflower is strongly affected by the choice of variety; a similar trend was found by Kabir *et al*. (2010) and Wu *et al*. (2000) in the Poaceae family for rice and wheat, respectively. In the present study, on the average of the weed target species and the affected parameters under study, wild cardoon revealed the most phytotoxic genotype (RI = –23.4%), followed by cultivated cardoon (RI = –19.6%) and globe artichoke (RI = –18.8%), even if not statistical differences were observed (Figure 3). In a previous work, Scavo *et al.* (2018b) reported that cultivated cardoon leaf aqueous extracts showed the highest inhibitory activity among the three *C. cardunculus* botanical varieties on seed germination of six weeds. Overall, root system length was the most affected parameter (RI = –32.6%), followed by total dry weight (RI = –18%), while hypocotyl length was the least influenced (RI = –14.4%) (Figure 3). These results are in agreement with Turk and Tawaha (2003) and Han *et al.* (2008), who found that *Zingiber officinale* Rosc. aqueous extracts inhibited the seedling growth of soybean and chive, and radicle length was the most sensitive parameter with respect to hypocotyl length and dry weight.

The variability of *C. cardunculus* leaf aqueous extracts observed here is probably attributed to the different profiles of allelochemicals present in each extract. *C. cardunculus* allelochemicals belong to two major chemical classes: sesquiterpene lactones such as cynaropicrin, grosheminin, cynaratriol, aguerin B, *etc*. as well as polyphenols such as caffeoylquinic acids and flavones. In this study, we found some differences in the aqueous extract of the three *C. cardunculus* botanical varieties (Table 1). In particular, luteolin 7-*O*-glucoronide was detected only in wild cardoon extract, as well as apigenin, while apigenin 7-*O*-glucoside was typic of globe artichoke. Regarding the sesquiterpene lactones, aguerin B and grosheimin were absent in all botanical variety extracts, while cynaropicrin and desacylcynaropicrin were found in each extract. Cynaropicrin and its derivatives are reported as the main responsible of *C. cardunculus* phytotoxic activity (Rial *et al*., 2014; Scavo *et al*., 2019b). Moreover, cynaratriol was detected only in cardoon extracts and 11,13-dihydro-desacylcynaropicrin and 11,13-dihydroxi-8-desoxygrosheimin were absent in globe artichoke and wild cardoon. The major phytotoxicity of cardoon extracts could be related to the presence of pinoresinol, luteolin 7-*O*-glucoronide, apigenin 7-*O*-glucoronide and cynaratriol which were absent in globe artichoke extracts and which are indicated as allelochemicals for a wide range of target plant species (Basile *et al*., 2003; Beninger and Hall, 2005; Hosni *et al*., 2013; Macias *et al*., 1999). Generally, higher concentrations determine higher inhibitory effects (Ambika, 2013; Scavo *et al.*, 2018b). However, a compound may be not phytotoxic if alone, becoming allelopathic when combined with others due to synergistic effects (Einhellig, 1995). Studying the joint action of 17 binary mixtures from cynaropicrin, grosheimin, aguerin B and 11,13-dihydroxy-8-desoxygrosheim in *C. cardunculus*, Rial *et al.* (2016) obtained 25 additive interactions, 7 synergistic interactions and 2 antagonistic interactions. The fractionation-biodirected synergistic approach of major *C. cardunculus* sesquiterpene lactones indicated a prevalence of additive and synergistic effects and allow to explain the activity of a complex mixture of compounds like the aqueous extract.

Conclusions

This work represents, to our knowledge, the first study on the *in vitro* allelopathic activity of the three *C. cardunculus* botanical varieties on root system length, hypocotyl length, epicotyl length and total dry weight of *A. retroflexus* and P. *oleracea*. Moreover, wild cardoon showed an autoallelopathic effect on its growth. The allelopathic effects mostly varied in relation to the target weeds, with *P. oleracea* as the most sensitive. Wild cardoon was the most phytotoxic genotype, while globe artichoke showed the least activity. Regarding the parameters under study, root system length was the most sensitive for all weed species. Through HPLC analysis emerged that *C. cardunculus* leaf aqueous extract varied among the botanical varieties, with apigenin and luteolin 7-*O*-glucoronide detected only in wild cardoon, apigenin 7-*O*-glucoside typic of globe artichoke, and 11,13-dihydro-desacylcynaropicrin and 11,13-dihydroxi-8-desoxygrosheimin characteristics of cultivated cardoon. According to our data, *C. cardunculus* leaf extracts could represent a potential product to produce a future allelochemicals based bioherbicide also in post-emergence. Other efforts are required to understand the effect of biotic and abiotic factors on the allelopathic potential of *C. cardunculus*, to investigate its behaviour in field conditions both in intercropping and crop rotations, as well as to study other possible applications in sustainable agriculture.

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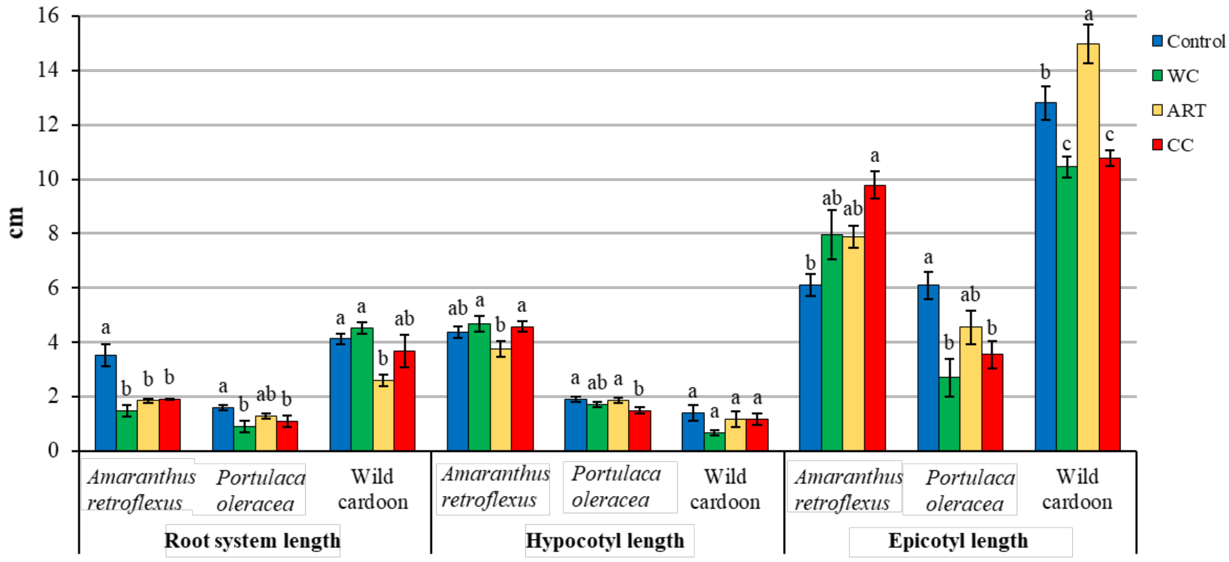

Figure 1. Effects of *C. cardunculus* leaf aqueous extracts on roost system length, hypocotyl and epicotyl length of *Amaranthus retroflexus*, *Portulaca oleracea* and *Cynara cardunculus* L. var. *sylvestris*. WC: wild cardoon extract; ART: globe artichoke extract; CC: cultivated cardoon extract. Values are given as means ± standard error. Different letters indicate statistical significance for $P \leq 0.05$.

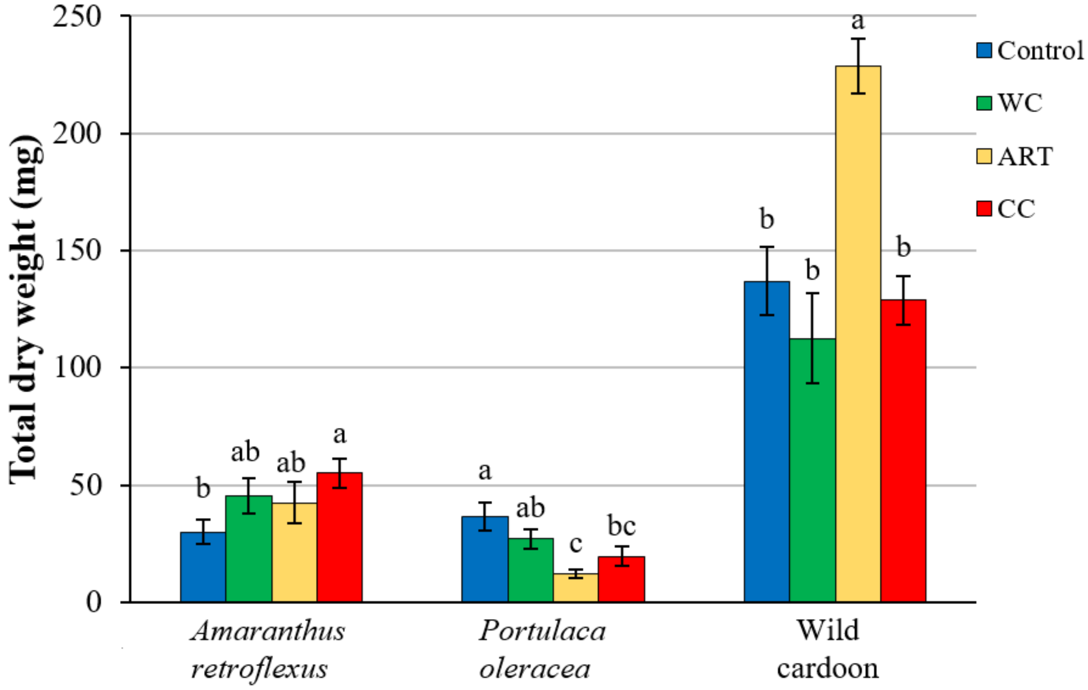

Figure 2. Effects of *C. cardunculus* leaf aqueous extracts on total dry weight of *Amaranthus retroflexus*, *Portulaca oleracea* and *Cynara cardunculus* L. var. *sylvestris*. WC: wild cardoon extract; ART: globe artichoke extract; CC: cultivated cardoon extract. Values are given as means ± standard error. Different letters indicate statistical significance for $P \leq 0.05$.

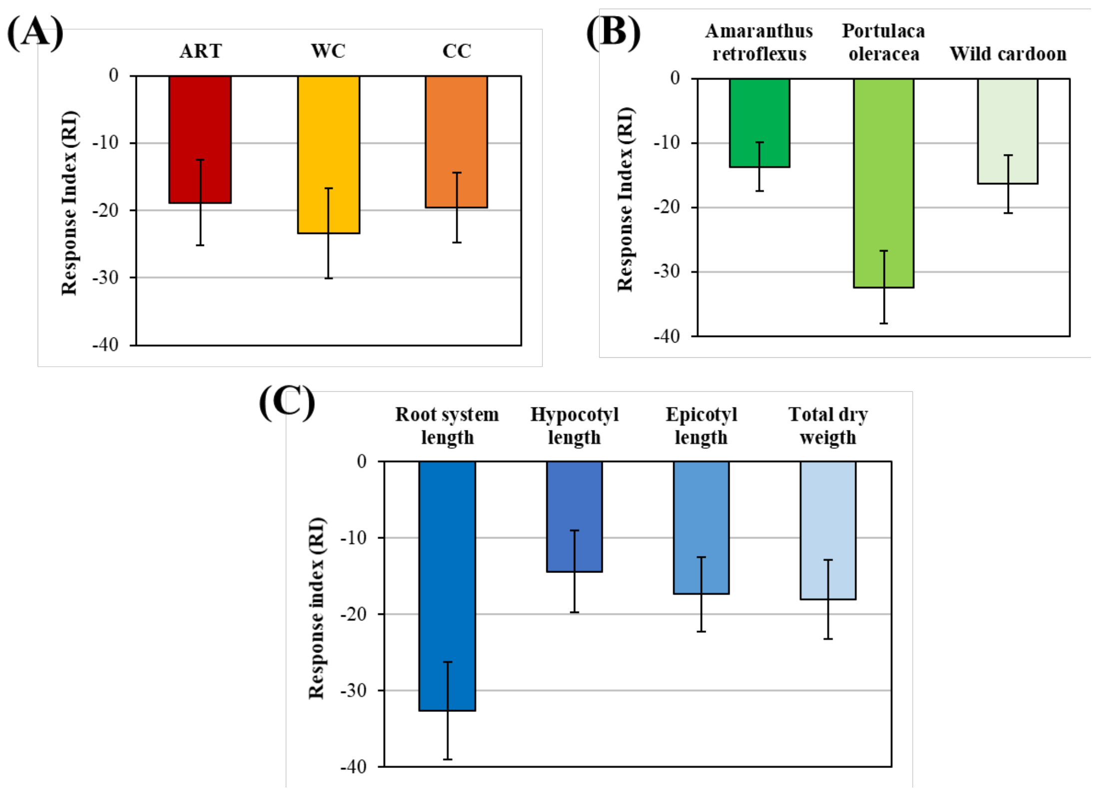

Figure 3. Allelopathic effect response index (RI) of *C. cardunculus* leaf aqueous extracts in relation to genotype (A), weed target species (B) and affected parameter under study (C). Values are expressed on the average of three weed species. WC: wild cardoon extract; ART: globe artichoke extract; CC: cultivated cardoon extract. Values are given as means ± standard error.

Table 1. Polyphenol and sesquiterpene lactone profile of leaf aqueous extract in *C. cardunculus*.

Compounds	ART	CC	WC
Caffeoylquinic acids			
5-*O*-caffeoylquinic acid	+	+	+
1,5-*O*-dicaffeoylquinic acid	+	+	–
Monosuccinildicaffeoylquinic acid	–	–	–
Luteolin derivatives			
Luteolin 7-*O*-glucoronide	–	–	+
Luteolin 7-*O*-malonylglucoside	trace	+	+
Luteolin	+	+	+
Apigenin derivatives			
Apigenin 7-*O*-glucoside	+	–	–
Apigenin 7-*O*-glucoronide	–	+	+
Apigenin malonylglucoside	–	–	–
Apigenin	trace	trace	+
Sesquiterpene lactones			
Aguerin B	–	–	–
Cynaratriol	–	+	+
Cynaropicrin	+	+	+
Desacylcynaropicrin	+	+	+
Grosheimin	–	–	–
11,13-dihydro-desacylcynaropicrin	–	+	–
11,13-dihydroxi-8-desoxygrosheimin	–	+	–
Lignans			
Pinoresinol	–	+	+

+ and – indicate presence or absence, respectively. ART, globe artichoke extract; CC, cultivated cardoon extract; WC, wild cardoon extract.