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 utilisation 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 high-performance liquid chromatography (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-deacetyl-cynaropicrin and 11,13-dihydroxi-8-deoxygrosheimin 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; Iliori 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 *Tursacum officinale* L. (Loughnan et al., 2014). *Cynara cardunculus* L. is an herbaceous perennial C3 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. *altiss* (DC.), along with their common progenitor wild cardoon [var. *sylvestris* (Lamk) Fiori] (Rotenberg 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 geno-
were randomly sampled from the 8-10th upper leaf of *Marsala* cultivar, a cardoon cultivar native to Navarra (Spain) and described as having high competitiveness in competitive environments. The cultivation of cardoon was carried out by spraying oxyfluorfen and imidacloprid, respectively. Weed and insect control, when required, was carried out using a mixture of oxyfluorfen and imidacloprid.

The local climate is typical Mediterranean, comprising hot-dry summers, mild-wet winters and average annual precipitation of approximately 600 mm. The soil is vertic and/or xerochrept soil (Soil Survey Staff, 1999) with clay (a.s.l.), 37° 25' N, 15° 30' E. The soil was characterised by a typic xerorthent and was used for further analyses.

**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 in Catania Plain, 10 m (a.s.l.), 37° 25' N, 15° 30' E. The soil was characterised by a xerorthent and/or xerochrept soil (Soil Survey Staff, 1999) with clay texture. The local climate is Mediterranean, comprising hot-dry summers, mild-wet winters and average annual precipitation of approximately 600 mm. The soil is vertic and/or xerochrept soil (Soil Survey Staff, 1999) with clay (a.s.l.), 37° 25' N, 15° 30' E. The soil was characterised by a typic xerorthent and was used for further analyses.

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**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. 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-C8 column (4.6×50 mm; 1.8 µm 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. Groshechin and ageriuin B were identified following Rial et al. (2014). The other sesquiterpene lactones and pinosinol were identified in accordance with Scavo et al. (2019b). Cynaratrile was detected using an isoameric mixture of chlororform/methanol (90:10 v/v, flow 3 mL min⁻¹, retention time 14 min). A mixture chlororform/methanol (95:5 v/v, flow 3 mL min⁻¹) was used for deaclycyanaropicrin (retention time 5.2 min) and 11,13-dihydro-deaclycyanaropicrin (retention time 4.7 min). Pinosinol...
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):

\[
RI = \begin{cases} 
(1 - \frac{T}{C}) \times 100 & \text{if } T \geq C \\
(\frac{C}{T} - 1) \times 100 & \text{if } T < C 
\end{cases}
\]  

(1)

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 litera-
ture (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 influ-

Figure 2. Effects of C. cardunculus leaf aqueous extracts on total dry weight of Amaranthus retroflexus, Portulaca oleracea and C. 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≤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.
enced (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, grosheimin, 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-glucoronic acid 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 deacylcynaropicrin 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-deacylcynaropicrin and 11,13-dihydroxy-8-deoxygrosheimin 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-glycoside, apigenin 7-O-glycoside and cynaratriol which were absent in globe artichoke extracts and which are indicated as allelochemicals for a wide range of target plant species (Macias et al., 1999; Basile et al., 2003; Beninger and Hall, 2005; Hosni et al., 2013). 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-deoxygrosheimin 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.

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ART</th>
<th>CC</th>
<th>WC</th>
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</thead>
<tbody>
<tr>
<td>Caffeoylquinic acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-O-cafeoylquinic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>5,5-O-dicaffeoylquinic acid</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Monosuccinylcaffeoylquinic acid</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Luteolin derivatives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteolin 7-O-glucoronicide</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Luteolin 7-O-malonylglucoside</td>
<td>Trace</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Luteolin</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Apigenin derivatives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apigenin 7-O-glucoside</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Apigenin 7-O-glucoronicide</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Apigenin malonylglucoside</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Apigenin</td>
<td>Trace</td>
<td>Trace</td>
<td>+</td>
</tr>
<tr>
<td>Sesquiterpene lactones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aguerin B</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cynaratriol</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cynaropicrin</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Deacylcynaropicrin</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Grosheimin</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11,13-dihydro-deacylcynaropicrin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>11,13-dihydroxy-8-deoxygrosheimin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lignans</td>
<td></td>
<td></td>
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<tr>
<td>Pinoresinol</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ and – indicate presence or absence, respectively. ART, globe artichoke extract; CC, cultivated cardoon extract; WC, wild cardoon 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-glycoside detected only in wild cardoon, apigenin 7-O-glucoside typic of globe artichoke, and 11,13-dihydro-deacylcynaropicrin and 11,13-dihydroxy-8-deoxygrosheimin 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.

**References**


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