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Article *in* Allelopathy Journal · September 2017

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https://doi.org/10.26651/2017-42-1-1103 0971-4693/94 Euro 20.00
Allelopathy Journal 42 (1): 21-36 (September, 2017) *International Allelopathy Foundation* 2017
Table: 2, Figs: 4

Herbicidal activity of natural compounds from *Baccharis* spp. on the germination and seedlings growth of *Lactuca sativa* and *Bidens pilosa*

M.P. Dias, R.M. Nozari and E.R. Santarém
Laboratory of Plant Biotechnology, Faculdade de Biociências,
Pontifícia Universidade Católica do Rio Grande do Sul,
Porto Alegre, CEP 90619-900, RS, Brazil
E-mail: esantarem@pucrs.br

(Received in revised form: July 29, 2017)

ABSTRACT

We evaluated the effects of aqueous and ethanolic extracts from 3-*Baccharis* species (*B. dentata*, *B. uncinella* and *B. anomala*) on the germination and seedlings growth of *Lactuca sativa* (model species) and *Bidens pilosa* (weed), and by chemical analysis, the phenolic compounds were determined in extracts. Aqueous and ethanolic extracts of *B. dentata*, *B. uncinella* and *B. anomala* were tested at 2.5, 5, 7.5 and 10% concentrations. Allelopathic effects of three *Baccharis* species were variable. The *B. pilosa* was more sensitive to the extracts than *L. sativa* and germination was inhibited with ethanolic extracts of *B. anomala* and *B. uncinella* from 5 to 10%. Aqueous extract of *B. dentata* and *B. uncinella* at 10% concentration reduced the germination of *B. pilosa* by 80%. The ethanolic extracts of *B. uncinella* (2.5 to 10%) and *B. anomala* (5 to 10%) caused 100% mortality of seedlings. Total phenolic compounds were more abundant in aqueous extracts. Amongst the phenolics, catechin was most abundant (1.61 to 6.16 mg g^{-1} DM) in aqueous and ethanolic extracts of *Baccharis* species tested. This study showed that *Baccharis uncinella* may be used as an alternative bioherbicide to control the weeds in agroecosystems.

Key words: Allelochemicals, allelopathy, *B. anomala*, *B. dentata*, *Bidens pilosa*, *B. uncinella*, catechin, extracts, Herbicidal activity, *Lactuca sativa*, phenolic compounds, weeds

INTRODUCTION

Weeds negatively impact the crop plants for long time, causing yield reduction due to interference between weeds and crops. Herbicides are frequently used for weed control, but their use has caused several problems: development of herbicide-resistant weeds, harmful to human, animal and environmental health (7,24). This had led to the growing awareness of problems associated with the constant and intensive use of chemical herbicides and interest in developing effective biological weed management systems. Among different biological methods of weed control, allelopathy can be an important tool to cope with the challenges of environmental pollution and herbicide resistance development (30). Allelopathy is defined as beneficial or detrimental effect from a donor plant to the recipient by chemical pathways (44). Several plant species roots exudates contains secondary metabolism, which affects the growth and development of neighboring

*Corresponding author

plants (21,57). Among these natural compounds phenolic acids, benzoic acid, flavonoids, tannins, quinones, terpenes and alkaloids have shown allelopathic potential (57). Phenolic acids and terpenoids are common allelochemicals, involved in numerous metabolic and ecological processes (13). Nowadays, allelopathy offers potential for weed control through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots of living or decomposing plant materials. Under certain conditions, allelochemicals may be released in quantities sufficient to suppress developing weed seedlings (5). The use of allelopathy for controlling weeds could be either through the direct use of natural allelopathic interactions or by using allelochemicals as natural herbicides (3). Undoubtedly, it is important to find strong allelopathic plants that could be used for weed control.

Weeds have substantially adapted characteristics (e.g. prolific seed production, rapid seedling growth, quick maturation, dual modes of reproduction, environmental plasticity) that enable them to grow, flourish, invade and dominate natural and agricultural ecosystems. Weeds compete with crop plants for resources and thus reduce crop productivity (18).

Bidens pilosa, (hairy beggarticks, *Asteraceae* family) is native to the Americas and has invaded the Eurasia, Africa, Australia and Pacific Islands (47). In several countries it is recognized as very aggressive weed in annual and perennial crops (53). Studies have analyzed its phytotoxic effects on other plants and microorganisms (31,42).

Baccharis (*Asteraceae* family) is the largest genus with about 360 species, mainly distributed in Brazil, Argentina, Colombia and Mexico. In Brazil, about 120 species of *Baccharis* are located in the south and southeast regions. Its several species are extensively used as spasmolytic, diuretic, analgesic and anti-inflammatory and to treat wounds, ulcers, fever and gastrointestinal diseases (20,46). Phytochemicals of this genus are extensively studied and currently > 150 compounds have been isolated and identified (1,2). The most common compounds are flavonones, flavones and terpenoids (9,56,58). Unlike other species of the genus, few reports are available concerning chemical composition and biological properties of *Baccharis dentata* (Vell.) G. M. Barroso, *Baccharis uncinella* DC. and *Baccharis anomala* DC. (38,49,58). Despite the numerous medicinal studies on *Baccharis* genus species, few reports found their allelopathic effects (10,22,26), this biological property exists in several plants of *Asteraceae* family (3,41).

This study aimed to evaluate the allelopathic potential of *B. dentata, B. uncinella*, and *B. anomala* on the germination and early development of the crop weed *B. pilosa* and model plant *Lactuca sativa* and its relation with the phenolic compounds present in the extracts.

MATERIALS AND METHODS

Aerial parts (leaves and twigs) of *Baccharis dentata, B. uncinella* and *B. anomala* were collected at Pró-Mata Center for Research and Conservation of Nature (29°29'18.4"S, 50°12'23.5" W; Rio Grande do Sul, Brazil). Aerial parts were dried at 45 ºC in oven till fully dry, powdered in a mill and stored at -20 °C until use.

Ethanolic and aqueous extracts (10% m/v) were used in the experiments and prepared by mixing 10 g dry matter (DM) in 100 mL of ethanol (96 º) and sterile distilled

water, respectively. Aqueous extract was kept at 120 rpm for 5 h, followed by incubation for 24 h at 4 °C under dark. Crude extract was filtered under vacuum to remove fiber debris and final volume was adjusted to 100 mL with sterile distilled water. Ethanolic extract was kept on shaker for 5 days. After filtering, crude extract were dried on rotary evaporator and re-suspended in 100 mL of sterile distilled water. Afterwards, dilutions of 2.5%, 5% and 7.5% were prepared. All extracts pH was measured using a digital pH meter (Kasvi, Brazil), and the information was used to determine the effects of pH on germination and initial growth of test species (section 2.4).

Germination assay

Germination and initial growth assays were done in Petri dishes (9-cm diam) lined with 2-layers of filter paper (80 g m^{-2}). All Petri dishes were kept in growth chambers [26±2 °C and a 16 h photoperiod]. Germination was defined as the protrusion of the radicle > 2 mm (43). Diaspores of *L. sativa* and *B. pilosa* were surface disinfested with 2% sodium hypochlorite solution (0.5% active chlorine) for 15 min and rinsed thrice with sterile distilled water. Twenty diaspores of *L. sativa* or *B. pilosa* were sown in Petri dishes containing 7 mL of aqueous or ethanolic extracts as per treatments. Distilled water was used as control (0%). Five Petri dishes were used per treatment, totalizing 100 diaspores. The number of germinated diaspores was counted every 48 h until 8 days. Germination indices e.g., germination (%) and speed of germination index (SpGI) were calculated (32). SpGI was calculated as under:

$$\sum (n_i/t_i)$$

Where, n_i: Number of germinated diaspores at each time point ($_i$) and t_i: Time from the onset of experiment.

Initial growth assay

Diaspores of lettuce and *B. pilosa* were pre-germinated in sterile distilled water under the same conditions described above and 7-day old seedlings were used to determine the effects of *Baccharis* spp. extracts on initial development. Seedlings from both species were in average 3 cm long and were incubated in the presence of the extracts at 2.5%, 5%, 7.5% and 10%. Distilled water was used as control. Five Petri dishes were used per treatment with 10 ml of extract or water and 12 seedlings, totalizing 60 plantlets. The evaluated parameters for initial development of seedling were root and shoot length (cm) and mortality (%) at 8-days after the treatment. Mortality was identified by necrosis of root tip and browning of hypocotyl.

Effects of pH on germination and initial growth

The pH of extracts can mask the allelopathic effect of a species (8,49). Hence as per the extracts pH their effects were determined on the germination and initial growth of *L. sativa* and *B. pilosa*. The experiment was conducted with diaspores and seedlings grown in distilled water with pH adjusted to 4.7 and 6.2, using either HCl or KOH 0.1N. Germination test consisted of four plates per pH treatment. Each plate contained 20 diaspores and 10 mL of treatment solution. Diaspores from both species were treated 8-days and their germination was recorded daily. The pH effect on the initial development was assayed on five Petri dishes containing 12 plantlets each per treatment. Total germination (%), speed of germination index (SpGI) as well as the length of shoot and root (cm) were determined.

Quantification of secondary metabolites

Aqueous and ethanolic extracts (10%; v/v) were centrifuged (2,500 g; 20 min) and the supernatants were used for quantification of phenolic compounds. The colorimetric analysis was done as per Sartor *et al*. (49). Briefly, 100 µL extract was mixed with 2.5 mL Folin-Ciocalteu reagent and 0.7 M Na_2CO_3. Samples were incubated at 25 ºC in dark for 30 min and absorbance was measured at 765 nm. The contents of total phenolic compounds were calculated based on a gallic acid calibration curve and expressed as mg g^{-1} of DM.

Identification and quantification of the allelopathic related-phenolic compounds present in aqueous and ethanolic extracts from *Baccharis* spp. were done by High Liquid Performance Chromatography (HPLC), as per Sartor *et al*. (49) with modifications. Briefly, analyses were carried out in the system Sikam Chromatography TM S 600, chromatograph operated at 40 ºC, and separations were performed on a MetaSil ODS column (5 µm; 250 x 4.6 mm). Detection was achieved with a UV/Vis detector, Model 3345 DAD, set at 280 nm. Data was processed by Clarity Chromatography Software. A gradient was formed between two mobile phases: phase A consisted of 2.5% formic acid in MilliQ water, and phase B of 100% methanol. The analysis followed a linear gradient programmed as 40% of eluent B from 0 to10 min, 40 to 60% from 10 to 20 min, 60 to 100% from 20 to 21 min, and 100% from 21 to 30 min. The flow rate was kept constant at 1 ml min^{-1} and the injection volume was 20 µL. Extracts were analyzed at least in triplicates. HPLC analysis was performed using a five-point calibration curve generated with authentic phenolic standards (chlorogenic acid, gallic acid, 2-hydroxybenzoic acid, catechin, caffeic acid, benzoic acid, and coumarin). Co-chromatography was carried out to confirm the phenolic compounds found in the extracts.

Statistical analysis

The treatments (control, 2.5, 5, 7.5 and 10% concentrations) were replicated five times in a completely randomized block design, for the germination and initial development assays. Each experiment was repeated at least twice. Data were evaluated for variance homogeneity (Levene's Test). Percentage data were normalized using arcsine of square root of x. Data were subjected to One-way ANOVA and Tukey Test was used to compare means at α=0.05 as the level of significance. The results were presented as percentage of inhibition over the control. Statistical analyses were performed with the SPSS Statistical Software Program (SPSS v.17).

RESULTS AND DISCUSSION

Germination and initial growth assays

Both aqueous and ethanolic extracts from *Baccharis* spp. negatively affected the germination indices and initial growth of seedlings of both model (*L. sativa*) and invasive (*B. pilosa*) species tested. Variation in pH of extracts was recorded and regardless the species assayed ethanolic extracts had lower pH (4.6 to 5.2) than aqueous extracts (5.7 to 6.5). Germination and initial growth were evaluated at the minimum (4.6) and maximum (6.5) pH of extracts. The pH of extracts did not influence the analyzed parameters of germination and growth (Table 1).

Table 1. Effects of pH of extracts on germination and initial growth of *L. sativa* e *B. pilosa*.

Parameter	*L. sativa*		*B. pilosa*	
	pH 4.7	pH 6.2	pH 4.7	pH 6.2
Germination (%)	98.75 ± 1.44 a	98.75 ± 1.25 a	97.5 ± 1.44 a	100.0 ± 0.00 a
SpGI	7.56 a	7.65 a	9.61 a	10.87 a
Root length (cm)	3.34 ± 0.32 a	2.92 ± 0.42 a	1.2 ± 0.08 b	3.2 ± 0.23 a
Shoot length (cm)	0.48 ± 0.01 a	0.52 ± 0.02 a	0.8 ± 0.08 a	1.2 ± 0.72 a

Data are presented as the mean ± standard error. Means followed by the same letter in a line within a species do not differ significantly by Tukey test ($\alpha = 0.05$).

The ethanolic extract of *B. dentata* significantly reduced the germination of lettuce from the 5% concentration, reaching the drastic inhibition of 82.7% at 10%. However, aqueous extracts inhibited the germination only at 10% concentration (Fig. 1a). Compared to *L. sativa*, *B. pilosa* diaspores were more sensitive to both types of extracts, significantly reducing the germination to 38.5 and 23.1 % at the lowest concentration (2.5%) of aqueous and ethanolic extracts, respectively (Fig. 1b). Germination of *L. sativa* and *B. pilosa* was also affected by *B. uncinella* ethanolic extracts. Allelopathic activity was more significant with ethanolic extracts, whose 5 to 10% concentrations significantly inhibited the germination of both species (Fig. 2a, b). On the contrary, only the aqueous extracts at 10% inhibited the germination of lettuce diaspores, whereas, *B. pilosa* germination was reduced at 2.5% concentration (Fig. 2b). Extracts of *B. anomala* showed no allelopathic effects on germination of lettuce seeds except at the highest concentration of ethanolic extract, which reduced the germination (Fig. 3a). However, the effects of ethanolic extracts were stronger on germination of *B. pilosa* (Fig. 3b), the ethanolic extracts of 5 to 10% concentration, inhibited the 99% germination.

The most common physiological parameters used to determine the allelopathic effects are the germination (%) and the initial seedlings growth of target species. However, the mode~~(s)~~ of action of allelochemicals include membrane permeability, water and nutrients uptake, respiration, photosynthesis, protein and nucleic acid synthesis and growth regulation in susceptible plants (34). Concerning seed germination, Hanley and Whiting (25) reported that phytotoxic allelochemicals present in the extracts appear to mediate a disruption of normal cellular metabolism, affecting reserve mobilization, a process that usually takes place during early stages of seed germination (23). The aqueous extracts of *Cymbopogon citratus* were allelopathic to germination of *Bidens pilosa* and *B. subalternans* (33).

Germination capacity can be useful to obtain the ecological information in plant succession; however it is not sensitive enough to validate the allelochemical effects on the germination process (4,12). For this reason, other parameters need to be evaluated for the allelopathic effects. Amongst them, speed of germination is considered as the key indicator among germination indices in allelopathic studies (32). Similar to the results observed on germination, the effects of the extracts on the speed of germination index (SpGI) were more remarkable in *B. pilosa* than in *L. sativa*. In this species, with ethanolic extract of *B. dentata*, the SpGI was reduced from the 5% concentration, whereas in lettuce the SpGI was reduced at 10% and 5% of aqueous and ethanolic extracts, respectively (Fig. 1c, d). With *B. uncinella*, the SpGI was significantly reduced with highest concentration of

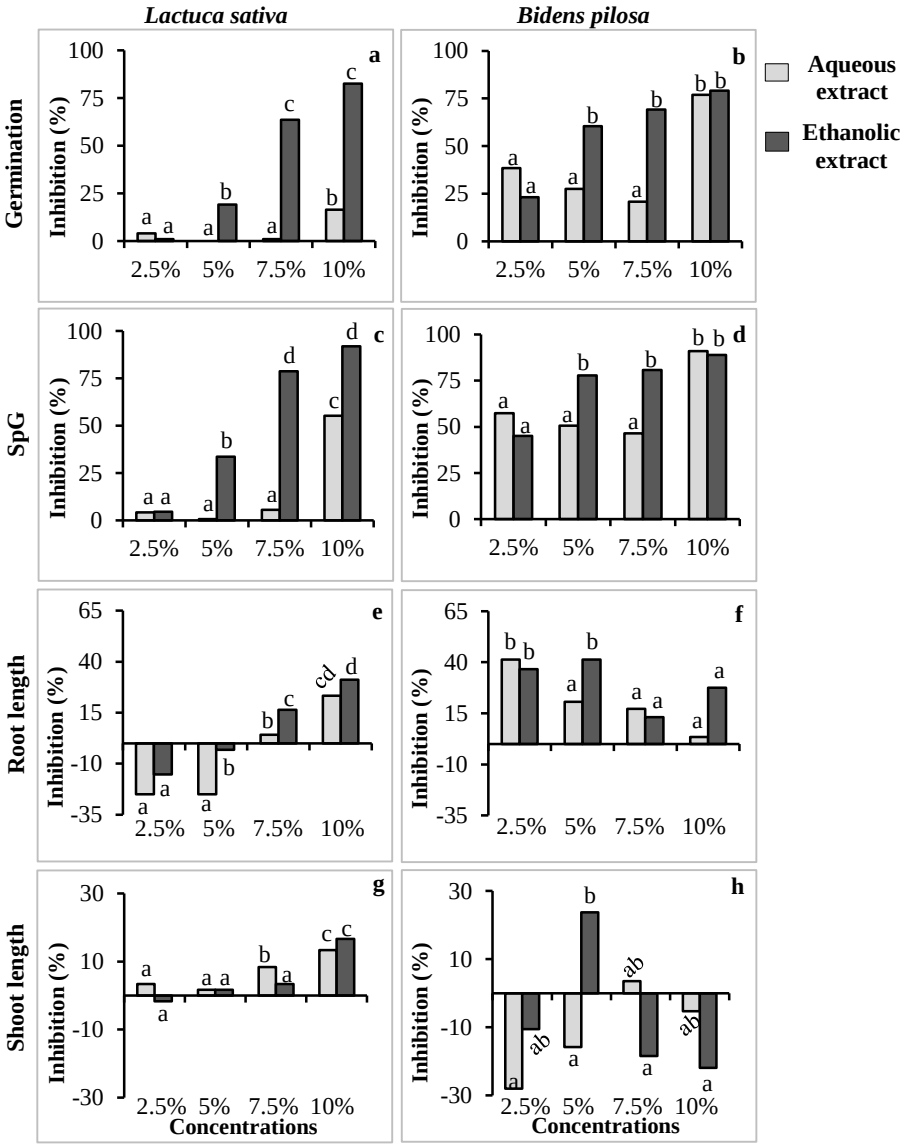

Figure 1. Effects of aqueous and ethanolic extracts of *B. dentata* on germination and seedling growth of *L. sativa* and *B. pilosa*, 8 days after sowing. Different letters indicate significant difference among treatments according to Tukey Test, $p< 0.05$

both types of extracts and target species (Fig 2c, d). In general, the allelopathic effects of *B. uncinella* extracts were more aggressive on *B. pilosa* than on *L. sativa*, leading to 98% inhibition of germination with ethanolic extract (10%) in comparison to 60% inhibition in

lettuce at the same concentration (Fig. 2c, d). Likewise, with *B. anomala* germination was delayed (Fig. 3c, d), being reduced to a rate of 0.1 seed per day (data not shown).

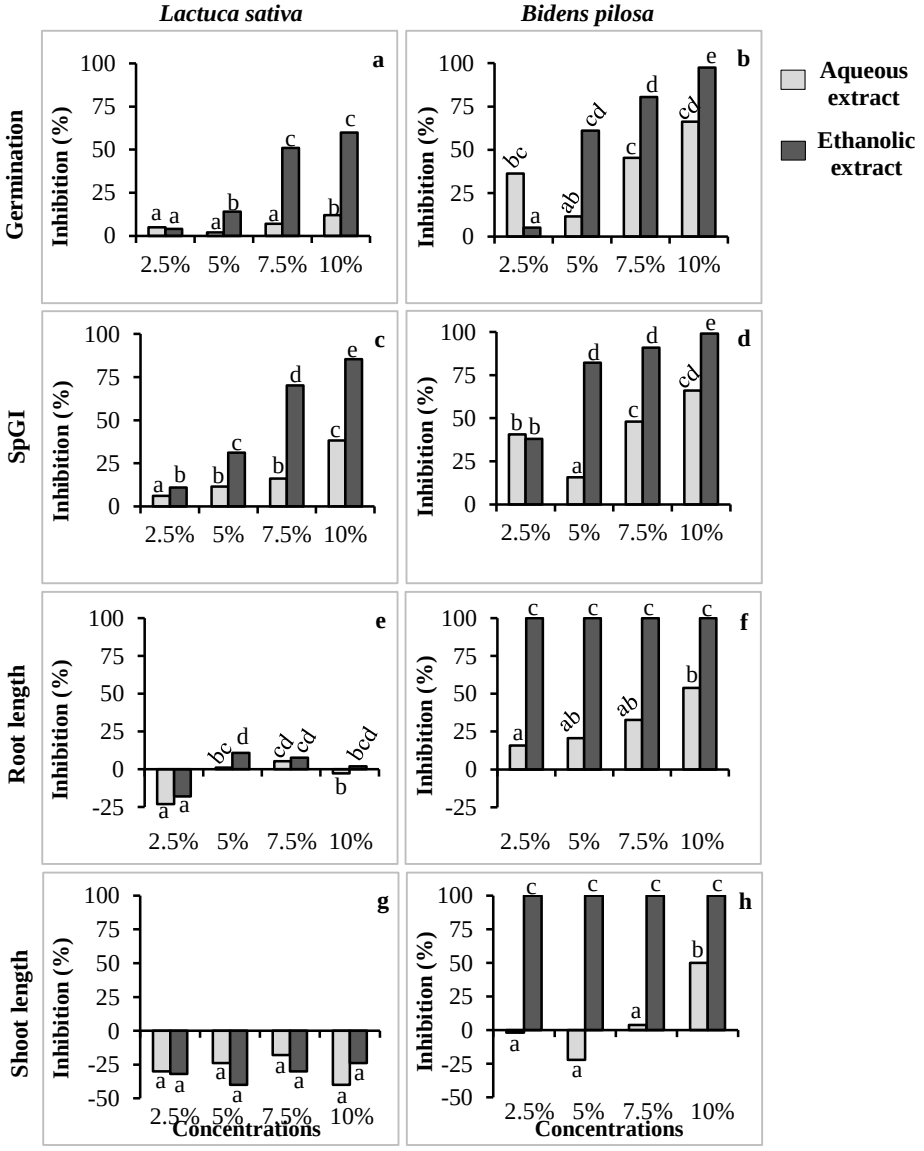

Figure 2. Effect of aqueous and ethanolic extracts of *B. uncinella* on germination and seedling growth of *L. sativa* and *B. pilosa*, 8 days after sowing. Different letters indicate significant difference among treatments according to Tukey Test, $p < 0.05$

The *Baccharis* spp. ethanolic extracts showed strong allelopathic effects on germination than aqueous extracts, regardless of the species tested. Therefore, the effects of *Baccharis* over *L. sativa* and *B. pilosa* indicated the presence of different types of allelochemicals, causing inhibition of germination of the target species. As verified in germination results (germination and SpGI), *B. pilosa* was more affected by *Baccharis* extracts than *L. sativa*. This result is intriguing because the *L. sativa* is model species for allelopathy studies, due to its fast germination and sensitivity (19). Thereby, the observed sensitivity of *B. pilosa* strengthens the possibility of using *Baccharis* as a natural herbicide.

The initial growth of target species is also an important parameter to evaluate the allelopathy potential. The allelopathic effects of *B. dentata* on initial development was observed in the *L. sativa* seedlings, causing significant reduction of root growth when aqueous and ethanolic (from 7.5% concentration) were tested (Fig. 1e). Lettuce shoots were less affected than the roots by *B. dentata* extracts and the inhibition of growth was only seen with 7.5% aqueous extracts and 10% ethanolic extracts (Fig 1g). Mortality of seedlings was not observed in this model species. On the other hand, in *B. pilosa* seedlings, the extracts were markedly toxic and mortality ranged from 58.33 to 96.67% (Fig. 4a). Regardless of the high mortality observed in *B. pilosa* seedlings, the remaining surviving plants were submitted to statistical analysis, and most of the treatments did not differ from the control regarding the length of roots and shoots (Fig. 1f, h), indicating some level of natural tolerance of *B. pilosa* to the allelochemicals from *Baccharis*. *B. uncinella* extracts led to little variation in root growth of lettuce seedlings, but there were no effects on shoot growth (Fig. 2e, g). Interestingly, aqueous extract at 2.5% concentration promoted the root growth (Fig. 2e). Nevertheless, when *B. pilosa* was assayed, no plants survived in ethanolic extracts (Fig. 2f). Aqueous extracts of *B. uncinella* showed dose-dependent effects on plant mortality (Fig. 4b). *B. anomala* ethanolic extracts from 5% concentration were also allelopathic to root growth of lettuce (Fig. 3f). Similar to *B. uncinella*, *B. anomala* aqueous extracts had no effect on shoot growth of lettuce plants (Fig. 2g and 3g). On the contrary, aqueous and ethanolic extracts significantly reduced the seedling growth of *B. pilosa*, causing mortality of plants in all concentrations tested (Fig. 4d).

Initial development of plantlets was severely affected by extracts of *Baccharis* spp. In *B. uncinella* and *B. dentata*, root length of lettuce seedlings was reduced with the increasing concentration of extracts, this effect was mostly observed with ethanolic extracts. However, the strongest allelopathic effect on initial development of roots and shoots was observed in seedlings of *B. pilosa* treated with ethanolic extracts of *B. uncinella* and *B. anomala*, causing 100% death. It is known that the growth of roots occurs by rapid cell division in the apical meristem and cell elongation in the elongation zone of the root tip and might be disrupted by allelopathic compounds, such as phenolics (36,37). The reduction in root length caused by *Baccharis* spp. may indicate that cell division was affected by allelochemicals present in the extracts. Tefera (56) reported allelopathic effects of the *Parthenium hysterophorus* weed on seed germination and seedling growth of *Eragrostis tef* with increasing concentrations of leaf extracts (1, 5 and 10%). Similar results were found with extracts of *Chrysanthemoides monilifera* on *L. sativa* and associated species (*Isotoma axillaris* and *Acacia mearnsii*) (4) as well as with extracts of *Helianthus annus*, *Chromolaena odorata* and *Tithonia diversifolia* on germination and

plant growth of *Vigna unguiculata* (27). Interestingly, aqueous extracts of *B. uncinella* at 2.5% concentration stimulated the root growth, but not the shoots growth. The likely presence of hydrophilic molecules, such as sugars and phytohormones may have caused the stimulatory effects observed on growth. Indeed, this effect has been previously reported, where allelochemicals at low concentrations stimulates the growth (4,14,54).

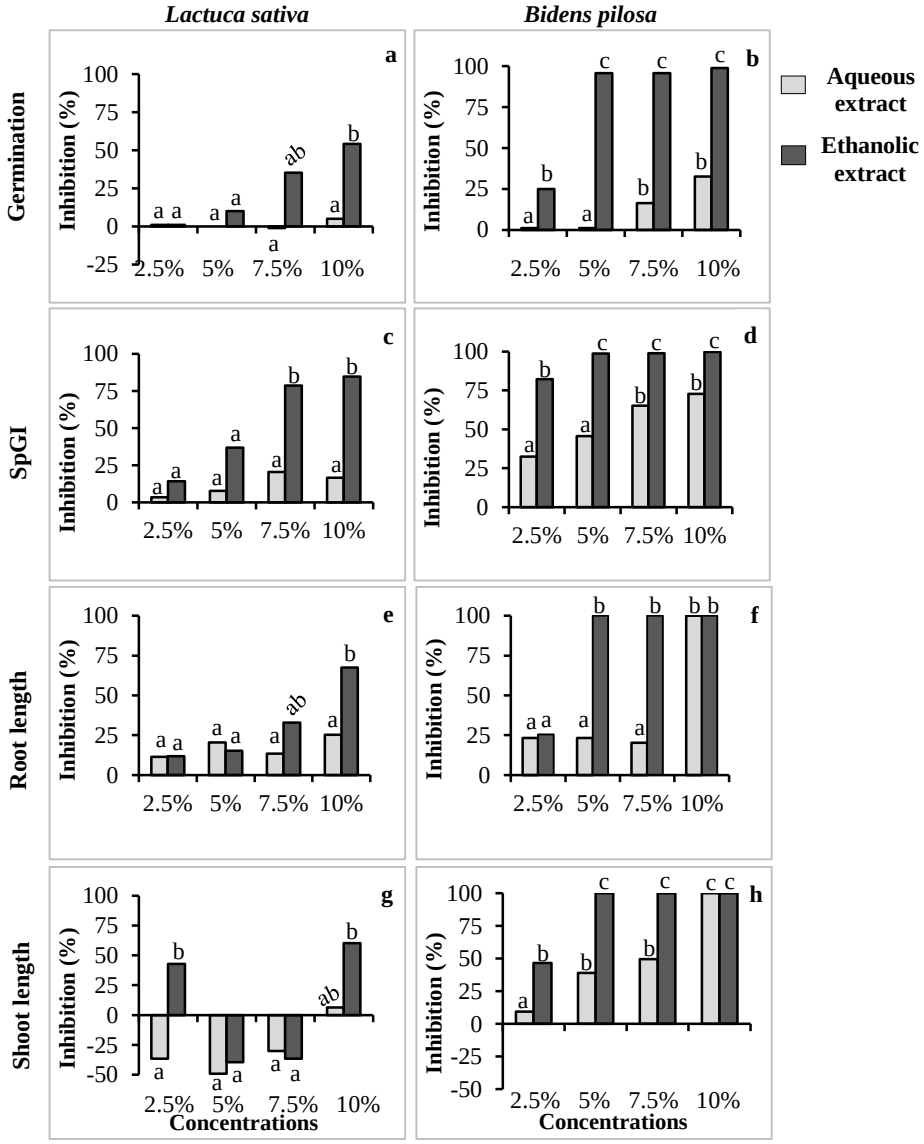

Figure 3. Effect of aqueous and ethanolic extracts of *B. anomala* on germination and seedling growth of *L. sativa* and *B. pilosa*, 8 days after sowing. Different letters indicate significant difference among treatments according to Tukey Test, $p < 0.05$.

Quantification of secondary metabolites

The genus *Baccharis* contains the large quantity of various secondary metabolites (1). The phytochemistry of *Baccharis* genus has shown the presence of diterpenoids, phenolic compounds and essential oils as the major classes of secondary metabolites (1,49,51,58). These compounds may play a role in the composition and dynamics of plant communities, facilitating competition between species, through allelopathy (6,34,55).

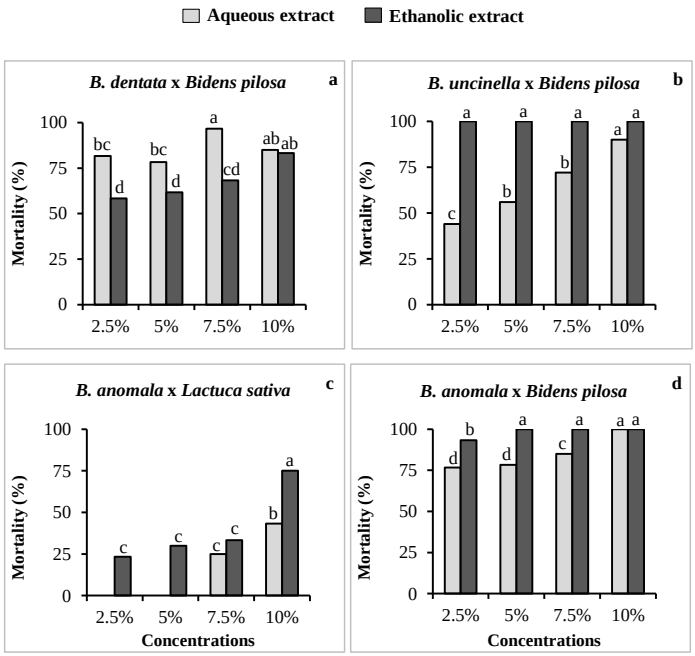

Figure 4. Seedling mortality (%) as result of treatment with aqueous and ethanolic extracts of *Baccharis* sp. on *L. sativa* and *B. pilosa*, 8 days after sowing. Different letters indicate significant difference among treatments according to Tukey Test, $p< 0.05$. *L. sativa* treated with *B. dentata* and *B. uncinella* (data not shown) showed no mortality.

Total phenolic compounds in the aqueous and ethanolic extracts of *Baccharis* spp. were quantified. Aqueous extract of *B. dentata* and *B. anomala* contained the highest concentrations of total phenolics followed by ethanolic extract of *B. uncinella* (Table 2). Some specific phenolic compounds in the extracts were identified and quantified by HPLC. Although variation had been detected in the profile of phenolic compounds, catechin was the most abundant phenolic compound in the aqueous and ethanolic extracts from the three species of *Baccharis* (Table 2). The highest concentrations of gallic acid were 1.38 and 1.17 mg. g^{-1} DM in ethanolic extracts of *B. uncinella* and *B. anomala*, respectively (Table 2). Caffeic acid was only found in *B. uncinella* aqueous extracts (0.50 mg. g^{-1} DM; Table 2). Chlorogenic acid and 2-hydroxybenzoic acid were detected in aqueous and ethanolic extracts of *Baccharis* spp. (Table 2). With the method used in this

study for extraction and identification of phenolic compounds coumarin and benzoic acid were not found in either of extracts. Phenolic compounds play role in plant interactions (9,13,34). They can inhibit the activity of α-amylase and gibberellic acid (40,52), consequently hampering the mobilization of seed reserves for germination. In the aerial parts of *Baccharis* species phenolic compounds were abundant and total phenolics ranged from 11.11 to 19.12 mg g^{-1} DM. The highest amounts were found in the aqueous extracts, regardless of the species. Several phenolics are found glycosylated, i.e., linked to sugars and thus, making the molecules more hydrophilic (11,36). Indeed, aqueous extracts of *Baccharis* spp. showed allelopathic properties in reducing the germination and seedling growth of *B. pilosa* and *L. sativa*.

Table 2. Contents of phenolic compounds in the aqueous and ethanolic (10%) extracts of *Baccharis* spp.

Type of extract/ Species	Total phenolic compounds (mg g^{-1} DM)	Phenolic compounds (mg g^{-1} DM)				
		CHL	GAL	HB	CAT	CAF
	Aqueous extract					
B. dentata	18.69 a	1.53±0.35	0.45±0.02	1.12±0.24	6.16±0.77	ND
B. uncinella	13.58 d	3.52±0.43	0.37±0.02	1.19±0.08	6.16±1.41	0.50±0.06
B. anomala	19.12 a	0.53±0.03	0.44±0.13	0.35±0.03	6.44±0.49	ND
	Ethanolic extract					
B. dentata	15.2 c	0.53±0.20	0.37±0.05	0.40±0.07	4.09±1.56	ND
B. uncinella	17.46 b	1.23±0.13	1.38±0.47	0.54±0.21	1.61±0.16	ND
B. anomala	11.14 e	0.72±0.27	1.17±0.17	0.71±0.32	1.70±0.15	ND

CHL: Chlorogenic acid; GAL: Gallic acid; HB: 2-hydroxibenzoic acid; CAT: Catechin; CAF: Caffeic acid; ND: Not detected

Phenolic compounds such as chlorogenic acid, gallic acid, 2-hydroxybenzoic acid and catechin, are recognized as allelochemicals (13,34,36,50), were found in the extracts of three species of *Baccharis*. Catechin was the most abundant phenolic compound in the aqueous and ethanolic extracts of *Baccharis* spp. Despite its known propriety as allelopathic molecule, its role as allelopathic has been intensively debated. (±)-catechin was first reported to play role as allelochemical from *Centaurea maculosa*, an Asteraceae species that invade North American grasslands (36). (+)-Catechin from *Fagopyrum tataricum* inhibits the root and shoot elongation of *Amaranthus palmeri*, *Trifolium repens* and *Lolium multifolium* at 50 to 100 µg mL^{-1} concentrations, but no inhibition at lower concentrations (28). Notwithstanding Duke *et al.* (16) reported that (±)-catechin is poor phytotoxin to many plant species in bioassays without soil, our *Baccharis* spp. extracts contained its high contents, which could be partly responsible, for the observed allelopathic effects of extracts.

Present in high concentrations in the ethanolic extracts of *B. uncinella* and *B. anomala*, gallic acid (3,4,5-trihydroxybenzoic acid) is recognized as allelochemical in several species. At concentration of 10^{-3} M gallic acid significantly reduced the dry matter production, leaf expansion, height, leaf production and net photosynthetic rate of soybean seedlings (39). Reigosa *et al.* (45) observed allelopathic effects of gallic acid on the germination and growth of six weed species and suggested that although this specific phenolic negatively affected the germination and growth, a mixture of six phenolics also showed allelopathic effects. According to the authors, in general, the phenolic acids affected the seedling growth more than germination. In *Baccharis* spp. extracts, the highest concentrations of gallic acid were found in *B. uncinella* and *B. anomala* species that showed the strongest allelopathic effects on the initial development of *Bidens pilosa.*

Besides catechin and gallic acid, chlorogenic and 2-hydroxybenzoic acids were found in *Baccharis* extracts in concentrations varying from 0.53 to 3.52 mg g^{-1} DM and 0.35 to 1.19 mg g^{-1} DM, respectively. Although concentration and production of phenolic acids may vary with species, phenology, plant organ and season (17), but they showed effects on initial growth of several species. Aqueous extracts of vegetative parts of *Conyza canadensis* reduced the seed germination and seedling growth of *Dactylis glomerata* and *Trifolium repens*, due to the presence of phenolic compounds *p*-coumaric, ferulic, *p*-hydroxybenzoic, vanillic and syringic acids (15).

CONCLUSIONS

Our results indicated that the high concentrations of catechin found in either aqueous or ethanolic extracts of *Bacharis* spp. may have contributed for the allelopathic activity of these species. In addition, we also suggest that the profile of phenolic compounds exerts more influence over the germination and initial development of a weed plant than the concentration of total phenolics. The allelopathic effect of the extracts could be result from the sum of individual effects as previously proposed (58). This would explain weaker allelopathic effect of *B. dentata*, which in spite of the low total phenolic compounds in ethanolic extract, showed strong phytotoxicity over *B. pilosa* diaspores. Our results may offer a potential use of these species in the weed control and *Baccharis uncinella* could be the candidate for a composition of a bioherbicide in organic agricultural field, through aqueous extracts. The possibility of using aerial parts as mulch could be tested for proposing a low cost alternative against *B. pilosa* invasion in crop fields. However, further studies with non-target species have to be carried out to confirm the safety of the use of *Baccharis* extracts.

ACKNOWLEDGMENTS

Authors thank Janaina Belquis da S. Pinto Langlois by her technical assistance. This work was supported by Pontifical Catholic University of Rio Grande do Sul, BPA Program (PUCRS, Brazil).

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