

Allelopathy of aqueous extracts of *Achyrocline satureioides* on the germination of *Bidens pilosa*

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ABSTRACT

The effects of aqueous extracts on seed germination can be assessed through allelopathic activity, which refers to the ability of certain secondary compounds produced by plants to interfere positively or negatively with the development of another. The aim of this study was to evaluate the effects of aqueous extracts of *Achyrocline satureioides* (Lam.) DC. on the germination of *Bidens pilosa* L. cypselas. The tests were carried out in a biochemical oxygen demand incubator at 26.5 °C, using aqueous extracts obtained by infusion of dry inflorescences at concentrations of 0.5, 5.0, and 10 g/100 ml and distilled water (control).

Five replicates of 100 cypselas each, totaling 500 cypselas per concentration, were used. After seven days of germination, the emerged seedlings were counted to calculate the germination percentage, the rootlet length were measured, and the data were analyzed. The analyzed extracts exerted allelopathic effects on the germination percentage of cypselas, but did not interfere with seedling growth. Aqueous extracts of *A. satureioides* inhibited the germination of *B. pilosa* cypselas at all concentrations, indicating the allelopathic ability of these extracts.

Keywords: bioactives, growth, marcela, picão-preto, weeds.

INTRODUCTION

Weed interference reduces the production potential of crops. Among weed species, *Bidens pilosa* L. stands out as a problematic agricultural weed throughout its range of distribution. It causes problems on lands cultivated with at least 30 different crops in over 40 countries and is known to significantly reduce crop yield (Guatimosin et al. 2015).

The most common method for controlling weed growth is the application of synthetic chemical pesticides (Lucio et al. 2018). However, due to the environmental and toxicological problems created by pesticides, it has become necessary to develop safe methods such as the use of allelochemicals (Palanivel et al. 2021), for the control of weeds (Abouziena and Haggag 2016).

Allelopathy is a natural phenomenon defined as the inhibition or promotion of growth in one plant species by chemicals produced by another species (Kamal 2020). Plants produce a variety of important bioactive molecules and secondary metabolites that can be used to control the germination and growth of weeds (Cabral and Maciel 2011).

These allelochemical substances may be present in all plant parts, including the leaves, flowers, fruits, roots, stems, and seeds (Nery et al. 2013).

Several plant species have been reported to affect the growth of others. Members from the family Asteraceae, including *Artemisia* L., *Cirsium* Mill., *Lactuca* L., and *Xanthium* L. species, have allelopathic effects (Chon and Nelson 2012). These species are natural inhibitors of weed growth and germination and can act as stimulants for the development of the root system of specific crops, including alfalfa, wheat, flax, soybean, and corn.

Achyrocline satureioides (Lam.) DC., belonging to the Asteraceae family, popularly known as marcela, is widely used for its sedative, anti-inflammatory, anti-spasmodic, and digestive properties and in the treatment of bronchitis and intestinal disorders (Gomes et al. 2018). It is native to the subtropical and temperate southeast regions of South America, including southern Brazil, Uruguay, Argentina, and Paraguay (Retta et al. 2012).

Phytochemical analyses of *A. satureioides*

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des have shown a high level of total phenolic compounds and flavonoids (Alves et al. 2021), tannins and saponins (Maciel et al. 2017), quercetin, luteolin, 3-O-methylquercetin, and achyrobichalcone (Bianchi et al. 2019). Experimental studies using this herb have demonstrated antimicrobial (Maciel et al. 2017), antiviral (Siqueira et al. 2021), anti-edematogenic, anti-inflammatory (Machado et al. 2020), antioxidant, anti-diabetic, and anti-obesity activities (Fernández-Fernández et al. 2021).

Studies have shown that *A. saturoioides* extracts have allelopathic effects on commercially cultivated species such as lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.), and chili (*Cap-sicum annum* L.) (Ferreira and Borghetti 2004; Souza et al. 2005). Another study found that aqueous extracts of *A. saturoioides* markedly inhibited cell division in the *Allium* L. test (Fachinetto et al. 2007). These authors observed an antiproliferative ability but a non-mutagenic ability. To the best of our knowledge, thus far, no studies have tested the effects of these extracts against *B. pilosa* or other weeds.

The present study, therefore, aimed to evaluate the allelopathic effect of aqueous extracts of the inflorescences of *A. saturoioides* on the germination and growth of *B. pilosa* cypselas, an important weed species of Brazilian cultures.

MATERIAL AND METHODS

Plant collection

Fresh inflorescences of *A. saturoioides* were collected from the rural area of Ijuí city, Rio Grande do Sul, Lat 28°28'45"S and Long 53°48'0"W. The specimen was collected on March 16, 2019, during the flowering period of the plant. The inflorescences were dried at room temperature in the Plant Biology Laboratory of the Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUI) and stored for six months until the beginning of the experiments. From this collection, a voucher was deposited at the Herbarium Rogério Bueno of UNIJUI (HUIRB 8040).

The cypselas of *B. pilosa* were collected on May 05, 2019, in a ryegrass field in Espumoso City, Rio Grande do Sul, Lat 28°49'07,7"S and Long 52°09'01,2"W. From this collection, a voucher was also deposited at Herbarium Rogério Bueno of UNIJUI (HUIRB 8041).

The genetic heritage access was registered on the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) (No. A465298).

Obtention of the aqueous extract

The inflorescences of *A. saturoioides*

were weighed and placed in boiling distilled water, autoclaved, and infused for 10 min. Infusions were prepared in three concentrations: 0.5 (C1), 5.0 (C2) and 10.0 g/ml (C3), maintained at room temperature.

Allelopathic activity

The cypselas of *B. pilosa* were placed in autoclaved Petri dishes (90 × 10 mm) on moistened cotton and filter paper to germinate and growth. No dormancy breaking or sterilization was done for the cypselas. Five replicates of 100 cypselas, totaling 500 cypselas, were used for each extract concentration and control group, totaling four treatments: control (distilled water), C1, C2, and C3.

The experiment started on October 7, 2019. The corresponding treatment liquid (4 ml) was poured into each dish, which was capped (with Petry dish lid) to prevent evaporation. The Petry dishes was placed in the biochemical oxygen demand incubator at a temperature of 26.5 °C and a 12 h light/dark cycle, until October 14, 2019. During the experiment, 1 ml of the corresponding extract from each group was poured daily to maintain the humidity necessary for seed germination (adapted from Cruz-Silva et al. 2015).

After 7 d of germination, all germinated cypselas were counted to calculate the germination percentage, and their rootlets (cm) were measured with a millimeter ruler.

Statistical analysis

Statistical analysis of the data was performed using the Chi-square test with a level of probability <0.05, for seed germination. For the size of the seedlings, a two-way ANOVA was performed, and the means were compared using Tukey's test at 5%. The statistical software BioEstat 5.0 (Ayres et al. 2007) was used for the statistical analyses.

RESULT AND DISCUSSION

The germination rates observed in this study were 1.6% (C3), 3.8% (C2), 4.6% (C1), and 10.2% (control). There was a significant difference between the treatments ($\chi^2 = 41,908$, $p < 0.0001$), and the inhibition of germination was verified with regard to the concentration of aqueous extracts, the data is show in Figure 1. The extract with the highest concentration exhibited the lowest germination rate. All concentrations differed significantly from those of the control group (C1: $\chi^2 = 11,441$, $p = 0.0033$; C2: $\chi^2 = 15,730$, $p = 0.0004$; C3: $\chi^2 = 33,304$, $p = 0.0001$). Among the extract concentrations, only C1 and C3 differed from each other ($\chi^2 = 7,490$, $p = 0.0236$).

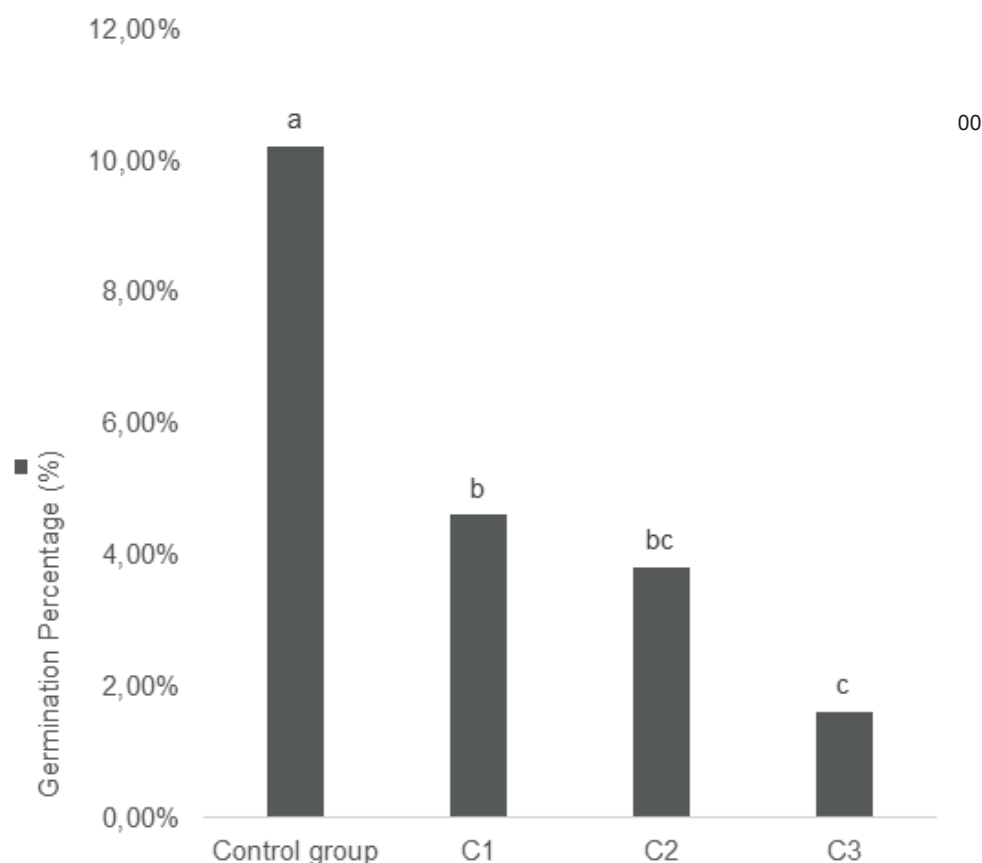


Figure 1. Germination percentage of *Bidens pilosa* in the presence of different concentrations of aqueous extracts of *Achyrocline satureioides*. Infusion concentrations: C1 = Concentration 1 (0.5 g/100 ml), C2 = Concentration 2 (5.0 g/100 ml) and C3 = Concentration 3 (10.0 g/100 ml). Values followed by the same letter do not differ significantly (as assessed by the χ^2 test; $p < 0.05$).

The mean length of rootlets was 5.37 cm in the control group, 4.20 cm in case of treatment C1, 4.69 cm in case of treatment C2 and 2.80 cm in case of treatment C3. Although the mean seedling length was greater in the control group, there was no significant difference between the treatments ($F = 3.0466$, $p = 0.9837$). Figure 2 shows the average length of *B. pilosa* seedlings that emerged at different concentrations of *A. satureioides* aqueous extracts.

As shown in Figure 1, it is possible to affirm that the aqueous extracts of *A. satureioides* suppressed the germination of *B. pilosa* cypselas at different concentrations, because the higher concentration presented, the greater the efficacy. However, the extracts did not interfere with seedling growth in the post-germination stage, as was observed in the length of the rootlets (Figure 2).

Brazil is the country with the highest plant genetic diversity in the world, representing 20% of the world flora, although plant diversity is far from being optimally exploited (Moreira-Araújo et al. 2019). Therefore, studies of chemical constituents are needed in order to test these compounds for

different purposes.

Allelopathy is a chemical interaction that occurs between plant specimens and plays an important role in several ecosystems (Kamal 2020). This type of interaction can also be defined as any direct or indirect harmful or beneficial effect that one plant exerts on another through the production and release of chemical substances.

In agriculture, allelopathic studies can be of great importance in uncovering the causes of failure of cultivars that do not achieve the expected performance; thus, these studies represent an important and advantageous tool for agronomy-associated research (Abouziena and Haggag 2016).

Several studies have reported the control of plants using plant extracts, for e.g., the verification of the allelopathic effects of aqueous extracts of the leaves of umburana-de-cheiro (*Amburana cearensis* A.C. Smith) and malva-santa (*Plectranthus barbatus* Andr.) on the germination of caruru (*Amaranthus deflexus* L.) (Lessa et al. 2017). In our study, aqueous extracts have also demonstrated allelopathic effects on *B. pilosa*. It

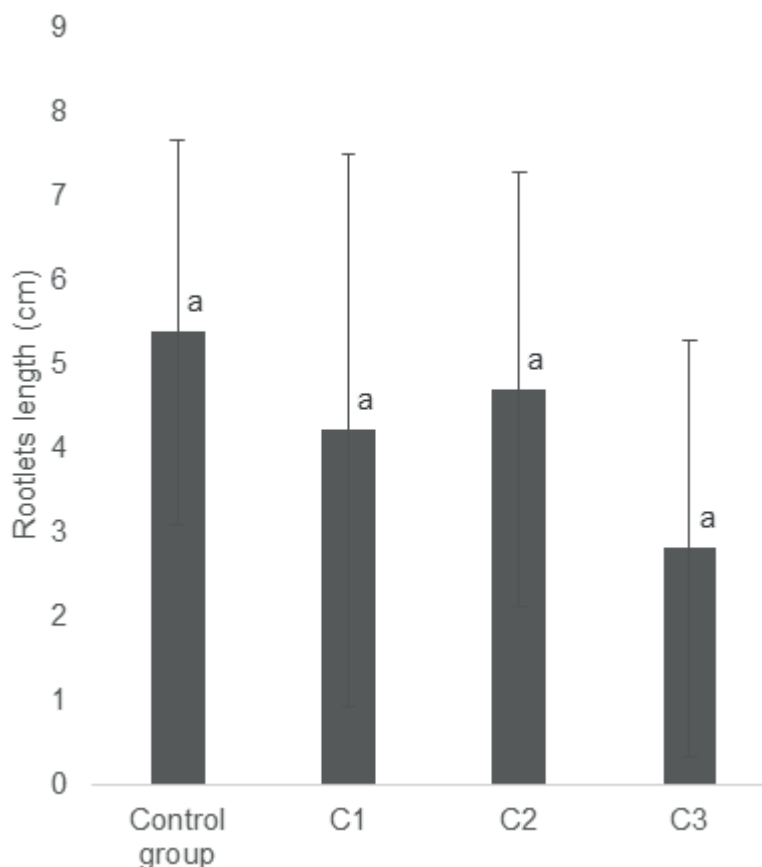


Figure 2. Average length (cm) of *Bidens pilosa* seedlings at different concentrations of aqueous extracts of *Achyrocline satureioides*. Infusion concentrations: C1 = Concentration 1 (0.5 g/100 ml), C2 = Concentration 2 (5.0 g/100 ml) and C3 = Concentration 3 (10.0 g/100 ml). Values followed by the same letter do not differ significantly (as assessed by ANOVA; Tukey test: $p < 0.05$). The bars in the columns represent the standard deviation.

is widely recognized that *B. pilosa* has become a problematic weed in many ecosystems worldwide and requires attention regarding emerging herbicide resistance (Chauhan et al. 2019).

It is widely known that losses caused by weeds exceed those caused by any category of agricultural pests (Abouziena and Haggag 2016). The most effective method for controlling these plants is the use of pesticides, which are popularly known as herbicides. In recent years, several studies regarding the different side effects of these products in humans, animals, cultures, and the environment have been published (Abouziena and Haggag 2016; Lucio et al. 2018). Consequently, the increased interest in the biological control of weeds is justified, as its expansion limits the excessive use of herbicides and solves the problem of resistance of biotypes to the products (Petrova et al. 2015).

Low germination of *B. pilosa* cypselas was observed in all treatments, including the control group, in which the samples were treated only with distilled water. According to a previous study, the low germination observed in *B. pilosa* cypselas

was due to dormancy, showing the rate of survival and viability of seeds from the seed bank under adverse conditions (Carmona and Murdoch 1995). Confirmation of the occurrence of dormancy cycles is a fundamental factor in weed management. In our study, although dormancy breaking was not performed, leading to low germination in the control group, the difference was sufficient to show the effect of the extracts.

In agreement with these results, we found that germination was inhibited in lettuce (*L. sativa*) and tomato (*S. lycopersicum*) at higher concentrations of aqueous extracts of marcela inflorescences (Souza et al. 2005). In another study, the effect of inflorescence storage was evaluated, and it was observed that the aqueous extracts of stored plants had a higher antiproliferative ability than that of fresh plants (Fachinetto et al. 2007).

The chemical composition of the *A. satureioides* extracts revealed the presence of numerous compounds that may have contributed to the effects observed in this study. The secondary compounds in plants are important for adaptation

and contribute to a good interaction between the plant and the environment (Cabral and Maciel 2011). According to some authors, phenolic compounds are considered one of the main biologically active secondary compounds in *A. satureioides* (Alves et al. 2021; Bianchi et al. 2019; Maciel et al. 2017). Some plant species have developed the ability to synthesize phenolic compounds that inhibit the growth of competing plants (allelopathic action) (Silva 2013). A previous study showed a high content of total phenolic compounds and flavonoids in *A. satureioides* (Alves et al. 2021), which reinforces the allelopathic potential of this species.

Other species from Asteraceae that also contain phenolic compounds have been reported to have allelopathic effects. A previous study using aqueous extracts of common ragweed (*Ambrosia artemisiifolia* L.), showed negative effect on the vigor of corn seeds (*Zea mays* L.) and the vigor and germination of soybean seeds (*Glycine max* (L.) Merr.) (Formigheiri et al. 2018).

Our findings show that the aqueous extracts of *A. satureioides* inhibited the germination of *B. pilosa* cypselas at all concentrations. This indicates the allelopathic ability of these extracts, which is in agreement with the results of previous studies regarding the germination of cultivated species. However, aqueous extracts do not affect the post-germination growth of weeds.

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AUTHORS' CONTRIBUTIONS

Conceptualization, C.B.P. and J.M.F.; Data curation, C.B.P., L.K., C.F.C. and J.M.F.; Formal analysis, C.B.P. and J.M.F.; Investigation, C.B.P.; Methodology, C.B.P. and J.M.F.; Project administration, C.F.C. and J.M.F.; Resources, J.M.F.; Supervision, C.F.C. and J.M.F.; Validation, C.B.P.; Visualization, C.B.P.; Writing – original draft, C.B.P. and L.K.; Writing – review & editing, L.K., C.F.C. and J.M.F.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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