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CONYZA SUMATRENSIS ALLELOPATHY EFFECT ON BIDENS PILOSA (ASTERACEAE) SEED GERMINATION

EFECTO ALELOPÁTICO DE CONYZA SUMATRENSIS SOBRE LA GERMINACIÓN DE SEMILLAS DE BIDENS PILOSA (ASTERACEAE)

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Abstract
Background: Other tools for weed management should be considered in addition to the use of herbicides. In this context, the identification and use of allelopathic compounds deserves attention.
Question: To quantify phenolic compounds and evaluate the allelopathic potential of extracts of *Conyza sumatrensis* on the germination of *Bidens pilosa*.
Studied species: *Conyza sumatrensis* (Retz.) E.Walker, *Bidens pilosa* L. (Asteraceae)
Study site: Palotina, state of Paraná, Brazil.
Methods: The aqueous extracts were prepared with shoot and root portions of *C. sumatrensis* in concentrations: 0, 1, 5 and 10 %. Germination and germination speed index were evaluated. The total flavonoids and phenols of the tested extracts were also quantified.
Results: With increasing concentrations of the extracts there was an increased inhibition of germination of *B. pilosa* and delays in the germination process. The extracts from the shoot of *C. sumatrensis* had a more expressive effect on seed germination when compared to the extracts obtained from the root. At concentration of 10 %, the extract from the shoot reduced in 83 % the percentage of germination. This may be related to the higher concentration of total flavonoids and phenols found.
Conclusion: The allelopathic potential of *C. sumatrensis* plants can be considered since both shoot and root extracts inhibited and/or reduced the germination of *B. pilosa*.
Key words: Flavonoids, germination, interference, total phenol, weed.

Resumen
Antecedentes: Se deben considerar otras herramientas para el manejo de malezas además del uso de herbicidas. En este contexto, la identificación y el uso de compuestos alelopáticos merece atención.
Problema: Cuantificar compuestos fenólicos y evaluar el potencial alelopático de extractos de *Conyza sumatrensis* en la germinación de *Bidens pilosa*.
Especies de estudio: *Conyza sumatrensis* (Retz.) E.Walker, *Bidens pilosa* L. (Asteraceae)
Sitio de estudio: Palotina, estado de Paraná, Brasil.
Métodos: Los extractos acuosos se prepararon con porciones de brotes y raíces de *C. sumatrensis* en concentraciones: 0, 1, 5 y 10 %. Se evaluaron la germinación y el índice de velocidad de germinación. También se cuantificaron los flavonoides y fenoles totales de los extractos probados.
Resultados: Con el aumento de las concentraciones de los extractos hubo una mayor inhibición de la germinación de *B. pilosa* y retrasos en el proceso de germinación. Los extractos del brote de *C. sumatrensis* tuvieron un efecto más expresivo sobre la germinación de las semillas en comparación con los extractos obtenidos de la raíz. En la concentración de 10 %, el extracto del brote redujo en 83 % el porcentaje de germinación. Esto puede estar relacionado con la mayor concentración de flavonoides y fenoles totales encontrados.
Conclusión: El potencial alelopático de las plantas de *C. sumatrensis* puede considerarse ya que tanto los extractos de brotes como los de raíz inhibieron y/o redujeron la germinación de *B. pilosa*.
Palabras clave: Fenol total, flavonoides, germinación, interferencia, maleza.

In addition to the selection of herbicide resistant weed biotypes, it is necessary to use other tools to manage weeds, as well as to prevent the selection of resistant weeds (Heap & Duke 2018; Neve *et al.* 2018, Rosario-Lebron *et al.* 2019). In grain crops, weeds reduce yield and increase costs (Trezzi *et al.* 2015, Gazziero *et al.* 2019), a situation potentiated by the selection of resistant biotypes (Adegas *et al.* 2017). The decline in production is related to the competition for resources of the environment and may still be related to the release of allelopathic compounds (Souza-Filho 2014).

Allelopathy is the negative influence of a biomolecule, from a plant, on the morphophysiological development of other plants. When its interference is negative to the development of the same species, it can be classified as autotoxic, detrimental to the species itself, and/or heterotoxic when interfering with another plant species (Szczepański 1977, Mullik 2008). Allelopathic substances are released in the process of plant deterioration and/or root exudation as well as by volatilization. They may alter germination, because they cause disturbances to membrane permeability, DNA transcription and translation, affect the functioning of secondary messengers, respiration, the conformation of enzymes and receptors or their compilation (Ferreira & Áquila 2000). Phenolic compounds are the most abundant class in plants and one of the main allelopathic substances inhibiting/retarding the germination process (Taiz & Zeiger 2010).

Examples of allelopathy applied in agriculture have been observed in studies. Silva *et al.* (2016) observed the inhibition of lettuce germination using the extract of *Conyza bonariensis* (L.) Cronquist. In turn Reik (2018) observed that aqueous extracts of *Eragrostis plana* Nees reduced seed germination in *Euphorbia heterophylla* L. and *Conyza* sp.

Among the most diverse weed communities, those of the genus *Conyza* are composed of up to 80 species, which make up the family Asteraceae. They develop in the tropical and subtropical regions of America, as well as Europe and China (Sansom *et al.* 2013, Wang *et al.* 2017). The weeds hairy fleabane (*C. bonariensis*), horseweed (*Conyza canadensis* [L.] Croquist) and Sumatran fleabane (*Conyza sumatrensis* [Retz.] E.Walker) are among the most important in the world (Trainer *et al.* 2005). *Conyza* spp. annual, herbaceous plants (Lorenzi 2014) with high seed production, found in several agricultural environments, such as grain crops, one of the main weeds in these environments (Kissmann & Groth 2007, Moreira & Bragança 2011).

Studies on the allelopathic potential of *Conyza* spp. are important, since these plants are highly adaptable to current agricultural production systems. Besides that, studies indicate that allelopathy is one of the strategies for the success of weeds to reach the success in the process of interference with the cultivated plants, since compounds present in the shoot have an inhibiting/retarding effect on germination (Silva *et al.* 2016).

The eudicot weed hairy beggarticks (*Bidens pilosa* L. and *Bidens subalternans* DC.) is present in several crops in Brazil, with importance in grain and sugarcane crops (Kissmann & Groth 2007, Moreira & Bragança 2011, Lorenzi 2014). Studies highlight *B. pilosa* as a weed of importance in soybean crops, for example, Ferreira *et al.* (2015) observed reduced dry mass of shoots and photosynthetic parameters of soybean in competition with *B. pilosa*.

It is believed that extracts of *C. sumatrensis* may have allelopathic effects on the germination of *B. pilosa* seeds. Thus, the aim of this study was to evaluate the allelopathic effect of compounds present on the shoot and root of *C. sumatrensis* on the germination of *B. pilosa*, and to quantify phenolic compounds present in extracts from shoot and root of *C. sumatrensis*.

Materials and methods

Collection of C. sumatrensis *plants and site description.* The collection of *C. sumatrensis* was carried out in a commercial soybean crop during the off-season in the municipality of Palotina, state of Paraná (PR), Brazil (24° 20' 44.54" S 53° 51' 50.93" W), from July to August 2018. After collection, the plants were separated into shoots and roots. The samples were dried in a forced air circulation oven at 40 ºC for 48 hours, to constant mass.

The study was conducted in a greenhouse at the Laboratory of Plant Physiology and Nutrition, Department of Agronomic Sciences and in the Laboratory of Organic Chemistry, Department of Engineering and Exact Sciences, Federal University of Paraná - Sector Palotina, Brazil.

Preparation of aqueous extracts from the shoots and roots of C. sumatrensis*.* After drying the samples were ground in a Wiley knife mill (MA340, Marconi Equipamentos para Laboratórios Ltda, Piracicaba, SP, Brazil) with a 1 mm sieve and placed in externally black painted glass vials to avoid exposure to light. After grinding, distilled water was added to the samples and held for 24 h at room temperature. Then, they were filtered through cotton, thus obtaining cold aqueous extracts of the shoot and roots, separately.

Allelopathic effect of C. sumatrensis *extracts on the germination of* B. pilosa*.* The treatments consisted of aqueous extracts at the concentrations of 0, 1, 5 and 10 % (w/v) (for 0, we used distilled water only), with four replicates in a completely randomized design.

For the germination test, 50 seeds of *B. pilosa* were used per replicate, distributed in four gerbox plastic boxes, on two sheets of germination paper. The paper was moistened, at 2.5 x its weight, with the respective concentrations of the aqueous extracts of *C. sumatrensis*. The boxes were kept in a B.O.D. germination chamber (MA415, Marconi Equipamentos para Laboratórios Ltda, Piracicaba, SP, Brazil) under photoperiod variation of 12 h and diurnal temperature of 30 ºC and night temperature of 20 ºC.

The germination percentage and the germination speed index (GSI) were evaluated daily, up to 15 days after assembling the test. Seeds were considered as germinated when radicular protrusion exceeded 3 mm. To calculate the GSI, the equation quoted by Borghetti & Ferreira (2004) was used:

$$GSI = \sum D / d$$

In which:

$\sum D$ = total number of germinated seeds;

d = day of evaluation.

Data were analyzed according to Pimentel-Gomes & Garcia (2002). After checking the assumptions, no data transformation required, germination of *B. pilosa* seeds, under aqueous extract of root and shoot at 10 % of *C. sumatrensis*, was tested by analysis of variance (ANOVA) ($P < 0.05$), posteriorly the means were analysed by multiple comparisons by the Tukey's (1949) test ($P < 0.05$). For concentrations of roots and shoots extracts (separately) at germination and GSI, it was run regression analysis ($P < 0.05$). For this purpose, the Sisvar 5.6 software was used (Ferreira 2011).

In the model selection in the regression analysis, the following fit quality parameters were adopted: significant regression, regression deviations or lack of adjustment, significant t-test for all regression coefficients, residue analysis without trend, low coefficient of variation, high R, and biological explanation. It was adjusted a decreasing linear fit for effect of shoot extracts on seed germination, also for effect of shoot and root extracts on GSI. For effect of root extracts on seed germination, it was adjusted a quadratic curve.

Quantification of phenolic compounds in extracts from the shoots and roots (10 % concentration) of C. sumatrensis. For quantification of phenolic compounds, the following chemical reagents were used: Methyl Alcohol (lot: 2637-i, molecular weight 32.04g) (Alphatec Produtos Químicos Ltda., Santo André, SP, Brazil); Aluminum chloride (241.43g molecular weight) (Vetec Química Fina Ltda., Duque de Caxias, RJ, Brazil) and Folin-Ciocalteu reagent - LOT 94905 (Dinâmica Química Contemporânea Ltda., Indaiatuba, SP, Brazil).

Among the phenolic compounds total flavonoids and phenols were quantified in the *C. sumatrensis* shoots and roots extracts (10 % concentration). To determine the analytical curve for flavonoids, a stock solution of methanol-based quercetin (MeOH) at 500 ppm and a solution of aluminum chloride $AlCl_3$ at 5 % (w/v) were prepared.

The established curve points were at concentrations of 0.5; 5; 10; 50; 100; 300 and 500 ppm. For this, an Orion AquaMate spectrophotometer (Thermo Fisher Scientific Brasil, São Paulo, SP, Brazil) at a wavelength of 425 nm was used. The solvent used was distilled water. For determination of flavonoids, crude extracts were prepared 24 h in advance. The test solution was prepared adding the following to a 10 mL penicillin flask: 2.8 mL H_2O + 0.1 mL MeOH +1 mL 5 % $AlCl_3$ + 0.1 mL sample. Once the reaction became sensitive, test solution was stored for 1 h protected from light to stabilize the reaction and subsequent reading of the samples. Flavonoids content from the root and shoot extracts was obtained in ppm by triplicate. Data were tested by ANOVA ($P < 0.05$) using the Sisvar 5.6 software (Ferreira 2011).

For quantification of total phenols, the methodology of Folin-Denis (Shanmugam & Thangaraj 2013) was adopted. First, it became necessary to establish an analytical curve. To determine the curve, a stock solution of Gallic acid (500 ppm) in methanol (MeOH) was prepared. A solution of 5 % calcium carbonate (w/v) was also prepared. The established curve points were at 5; 10; 50; 100; 300 and 500 ppm. For this purpose, an Orion AquaMate spectrophotometer at a wavelength of 760 nm was used. The solvent used was distilled water.

For determination of total phenols, the crude extracts were prepared 24 h in advance. For the preparation of the test solution, 2.8 mL H_2O + 0.1 mL Folin-Ciocalteu + 1 mL 5 % Na_2CO_3 + 0.1 mL sample was added to a 10 mL penicillin flask. Once the reaction is sensitive, the samples were stored for 1 h protected from light, for stabilization of the reaction and subsequent reading of the samples. The triplicate data obtained in ppm of root and shoot extracts were tested by ANOVA ($P < 0.05$) using the Sisvar 5.6 software (Ferreira 2011).

Results

Allelopathic effect of C. sumatrensis *extracts on the germination of* B. pilosa. Aqueous extracts from the shoots of *C. sumatrensis* reduced the percentage of *B. pilosa* seed germination. It was possible a decreasing linear fit, with increasing concentration of the extracts, it was observed a reduction in germination (Figure 1). For the extracts from *C. sumatrensis* roots, there was a quadratic curve adjustment, with reduction for > 5 % of concentration (Figure 2). The shoot extract affected more the germination percentage of the seeds in comparison with the root extract (at 10 % concentration) (Table 1).

It was possible a decreasing linear fit for GSI, with increasing concentration of the extracts from the shoots of

C. sumatrensis, there was a reduction in the GSI of seeds of *B. pilosa* (Figure 3). The highest concentration (10 %) resulted in a higher delay in seed germination, with a value 5 times lower in comparison to control (0 %).

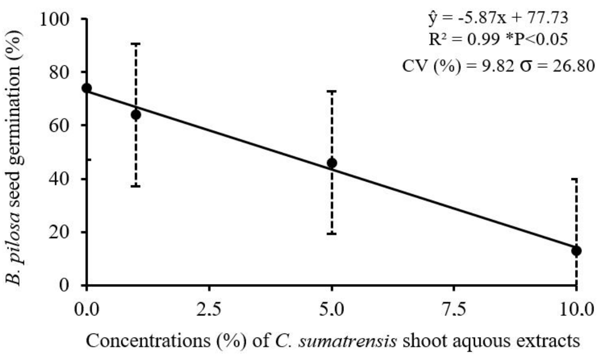

Figure 1. Percentage of *B. pilosa* seed germination under different concentrations of *C. sumatrensis*shoot aqueous extracts.

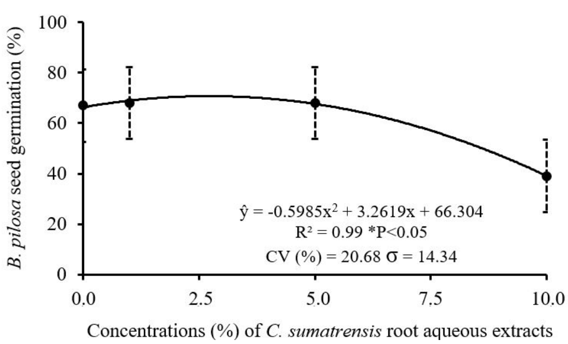

Figure 2. Percentage of *B. pilosa* seed germination under different concentrations of *C. sumatrensis*root aqueous extracts.

As for the shoot extract, results for the GSI of *B. pilosa* seeds using root extracts also fit a decreasing linear model. With increasing concentration of the extracts from the roots of *C. sumatrensis* plants, there was a reduction in the GSI of seeds of *B. pilosa* (Figure 4).

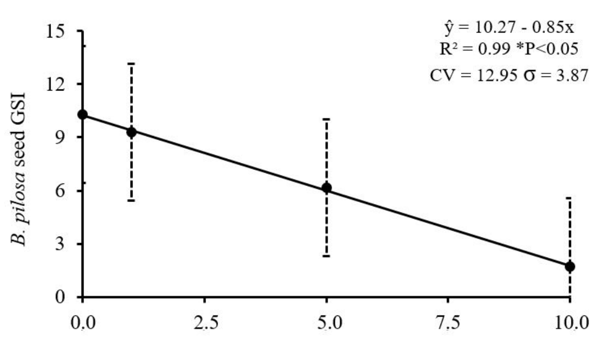

Figure 3. *B. pilosa* seed germination rate index under different concentrations of *C. sumatrensis*shoot aqueous extracts.

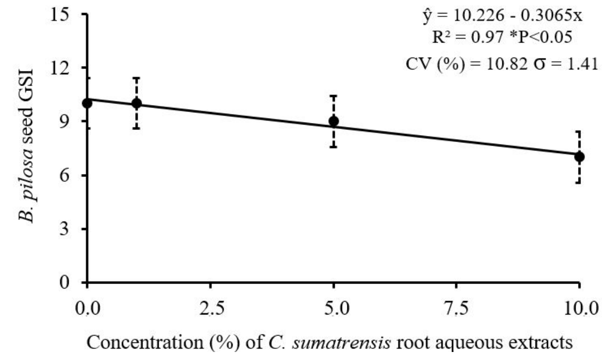

Figure 4. *B. pilosa* seed germination rate index under different concentrations of *C. sumatrensis*root aqueous extracts.

Quantification of phenolic compounds in extracts from the shoot and root (10 % concentration) of C. sumatrensis. The shoot extracts at 10 % concentration had a flavonoid content of 290 ppm. While for the roots extracts, the flavonoid content observed was 9.2 ppm, different values by the *F*-test ($P < 0.05$). The highest concentrations of total phenols were also identified in the shoot extracts, presenting 312.04 ppm, while the roots extract presented a concentration of only 108.04 ppm, different values by the *F*-test ($P < 0.05$) (Table 2).

Table 1. Mean values of germination percentage (% G) of *B. pilosa* seeds under aqueous extract of roots and shoot (10 % concentration) of *C. sumatrensis.*

Treatments	% G
Control	70 a
Extract 10 % of roots	39 b
Extract 10 % of shoot	13 c
Standard deviation	24.89
CV (%)	8.40
F	209.40 *

*Means followed by different letters in the column differ by Tukey's (1949) test ($P < 0.05$).

Table 2. Phenolic compounds (ppm) in extracts from the shoot and roots (10 % concentration) of *C. sumatrensis.*

Treatments	Flavonoids content	Total phenols
Extract 10 % of roots	9.20	108.04
Extract 10 % of shoot	290.00	312.04
Standard deviation	153.93	113.02
CV (%)	4.71	9.06
F	2,377.59*	172.46*

*Means, at same column, differ by *F*-test ($P < 0.05$).

Discussion

The reduction in the germination process can be related to the increase in the concentrations of toxic compounds. It is characterized the inhibitory allelopathic effect of *C. sumatrensis* plants on the germination of *B. pilosa*. Allelopathy is classified as interference, since there is no competition for essential factors for germination and seedling development, but rather the release of compounds into the environment that alter patterns and conformations at cellular levels of neighboring species, leading to suppression of these plants (Silva 2012).

Different studies demonstrate the sensitivity of plants of the genus *Bidens* to plant extracts. Results similar to those found in this study were reported by Rizzardi *et al*. (2008), in which aqueous extracts from the shoots of canola reduced the germination of *Bidens* sp. to 88 and 97 %, at 6 and 8 % (w/v) concentration, respectively. Reik (2018) also observed a reduction of germination percentages of *B. pilosa* under the influence of the crude aqueous extract of *Avena strigosa* Schreb. at concentrations above 30 % (corresponding to 7.5 % w/v).

Shaukat *et al*. (2003) evaluated the allelopathic potential of horseweed and found that the aqueous extract of *C. canadensis* seedlings at 7.5 (w/v) totally inhibited tomato germination. This result corroborates those obtained herein, showing that there is interference between different species.

The germination speed index (GSI) may be indicative of seed vigor according to its germination pattern. High GSI indicates a fast and uniform germination; in this situation the seeds are less exposed to soil pathogens, which ensures competitive advantages over the neighboring species (Piña-Rodrigues *et al*. 2004). The increased concentration of the extracts delayed the germination process. The finding of reduction in GSI indicates the interference of the allelochemicals contained in the extracts on germination.

The delay in germination according to the rates of the aqueous extracts is an advantage over the other neighboring species, with advantages in the competition. This may explain the fact that areas with dense populations of *Conyza* are practically free of other weeds (Djurdjević *et al*. 2011).

Flavonoid compounds, especially quercetin, may be responsible for the inhibitory effect on seed germination. Paszkowski & Kremer (1988), found that the aqueous extract of *Abutilon theophrasti* Medik. significantly inhibited/retarded the germination of cress, radish and soybean. The phenolic compounds identified in larger expressions in the extracts were: catechins, epicatechins and quercetin.

This proportion in the amount of total phenols and flavonoids present in the extracts from the shoots helps to understand the event of inhibition and retardation of the germinative process of the seeds tested. This indicates that there is an interaction of different secondary metabolites, which result in the inhibition of germination.

Although phenolic compounds are frequently released into the environment via the root system, stress bound to this organ will not necessarily increase the concentration of these compounds. The relationship in increasing phenolic compound production seems to be more associated with increases in solar radiation intensity, since phenolic compounds act to protect against UV radiation. Secondary metabolites, as the phenolic compounds studied, are expressed as a function of the chemical interface between the synthesizing organ and the environment (Gobbo-Neto & Lopes 2007), it may be due to this characteristic that the observed levels of the phenolic compounds in the extracts were very low compared to the extracts from the shoots.

The data obtained herein corroborate the results in the literature, where researchers have identified that phenolic compounds such as: caffeic acid, chlorogenic acid, gallic acid and flavonoid compounds, including quercetin, are responsible for the inhibition of germination (Cantanhede-Filho *et al*. 2017, Pereira *et al*. 2018).

Among the dysfunctions triggered by flavonoid compounds, stands out the modulation in the levels of reactive oxygen species, which leads to the peroxidation of membranes and fundamental enzymes in the germination process (Ferreira & Áquila 2000, Pereira *et al*. 2018). But also, phenols, which exert interference in the germination process as a whole, in addition to causing damage to membrane permeability by raising oxidative enzyme activity as well as early root lignification, interfering with the process of organ absorption and development (Bubna *et al*. 2011, Pereira *et al*. 2018).

In this way, this characterization helps us to understand and interpret the values obtained in germination tests. The most effective results regarding inhibition/delay were observed in the treatments testing extracts from the shoots, which are probably because such extracts come directly from the organ in which the compounds are synthesized.

The shoot and root extracts of *C. sumatrensis* presented an allelopathic potential in inhibiting and reducing the germination of *B. pilosa*. The extracts from the shoots reduced the germination more intensely, as well as had the highest concentrations of molecules of total flavonoids and phenols, which may be associated with the inhibitory effect. Our findings serve as support for research in the area of alternative weed control management.

As already mentioned, our results can explain the fact that areas with dense populations of *Conyza* are practically free of other weeds. This specie is an important weed, especially in grain crops, and its interference on crops, among other factors, may also be related to the release of allelopathic compounds.

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