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Original Paper

Germination inhibitory activity of aqueous extracts of native grasses from South America

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Abstract
Soluble allelochemicals have generated great interest since they can be used for the biological control of pests, especially of weeds. However, few studies have evaluated the effectiveness of soluble compounds of exudates on germination in relation to exposure time. Here we evaluate the inhibitory effect of aqueous root, stem and leaf extracts of five South American species of *Bothriochloa* on the percentage of seed germination of four target species (lettuce, lovegrass, maize and wintergreen paspalum) over three exposure periods (48, 120 and 168 h). Aqueous extracts of the five *Bothriochloa* species inhibited germination; germination inhibition was strongly correlated with exposure time, with the longest treatment period (168 h) being the one of greatest inhibitory activity. Inhibitory activity differed among types of aqueous extracts. The suitable management of allelopathy might improve crop productivity and environmental protection through biologically friendly control of weeds.
Key words: allelopathy, aqueous extracts, *Bothriochloa*, exposure time, germination.

Resumo
Aleloquímicos solúveis têm gerado grande interesse, pois podem ser utilizados para o controle biológico de pragas, principalmente de plantas daninhas. No entanto, poucos estudos avaliaram a eficácia de compostos solúveis de exsudatos na germinação com relação ao tempo de exposição. Aqui, avaliamos o efeito inibitório de extratos aquosos de raízes, caules e folhas de cinco espécies sul-americanas de *Bothriochloa* na porcentagem de germinação de quatro espécies-alvo (alface, choro de capim, milho e paspalum verde-inverno) ao longo de três períodos de exposição (48, 120 and 168 h). Extratos aquosos das cinco espécies de *Bothriochloa* inibiram a germinação; a inibição da germinação foi fortemente correlacionada com o tempo de exposição, sendo o período de tratamento mais longo (168 h) o de maior atividade inibitória. A atividade inibitória diferiu entre os tipos de extratos aquosos. O manejo adequado da alelopatia pode melhorar a produtividade das culturas e a proteção ambiental através do controle biológico de ervas daninhas.
Palavras-chave: alelopatia, extratos aquosos, *Bothriochloa*, tempo de exposição, germinação.

Introduction

Nowadays, there is a growing interest in the biological control of pests through the application of allelochemical compounds as herbicides, pesticides, insecticides, and antibacterial or antifungal products (Narwal 2010; Linde *et al.* 2010; Cheng & Cheng 2015; Jabran *et al.* 2015; Bey-Ould Si Said *et al.* 2016; Khan *et al.* 2016; Macías *et al.* 2019). In addition, allelochemicals are considered safe and beneficial for the environment and human population (El-Kenany & El-Darier 2013); however, its application in agriculture is limited (Cheema *et al.* 2013).

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Aqueous extracts and essential oils of some plants are sources of allelochemicals. Soluble allelochemicals in exudates can suppress the germination and growth of weeds; thus, they are an alternative to synthetic herbicides, which pollute and damage the ecosystem (Rodrigues Carmello & Cardoso 2018). The nature of allelochemicals is complex and includes simple phenolics, flavonoids, alkaloids, coumarins, quinones, triterpenes, steroids, diterpenes, sesquiterpenes, monoterpenes and benzoxazinoids (Macías *et al.* 2019).

Bothriochloa (Poaceae: Andropogoneae) species are tropical and subtropical perennial grasses characterized by essential oils rich in sesquiterpenes (Pinder & Kerr 1980; Zalkow *et al.* 1980; Melkani *et al.* 1984; Bhandari *et al.* 1993; Kaul & Vats 1998; Scrivanti *et al.* 2009); their aqueous extracts are recognized for their allelopathic property (Hussain *et al.* 1982; Hu & Jones 1999; Scrivanti 2010; Scrivanti *et al.* 2011). In addition, epi-α-cadinol, damascenone- (E) -β, E, E-farnesol, γ-gurjuneno and germacrene D sesquiterpenes, the main compounds in the essential oils of the South American species of *Bothriochloa*, inhibit germination and growth of other plants (Scrivanti & Anton 2018). However, the inhibitory effect of allelochemicals on germination and seedling growth in relation to exposure time has not been evaluated. Seed germination is the most critical stage for seedling establishment and survival (Rajjou *et al.* 2012). The aim of this work was to evaluate the inhibitory activity of the aqueous extracts of root, stem and leaf of five South American species of *Bothriochloa* (Poaceae) on the germination of four plants over three exposure periods. Our results may contribute to weed management through the application of allelopathy in modern agriculture.

Material and Methods

Plant materials

Five *Bothriochloa* species were collected from Córdoba province, Argentina, in January 2018: *B. barbinodis* (Lag.) Herter, *B. edwardsiana* (Gould) Parodi, *B. perforata* (Trin. *ex* Fourn.) Herter, *B. saccharoides* (Sw.) Rydb. subsp. *australis* Scrivanti and *B. springfieldii* (Gould) Parodi (Tab. 1). Voucher specimens are deposited in the Herbarium, Museo Botánico de Córdoba (CORD). Stem, leaf and root portions were separated and air dried. Aqueous extracts were obtained and examined to determine the allelopathic effect of different plant parts.

Aqueous extracts

Portions (5 g each) of dried culm, leaf-blade and root from each *Bothriochloa* plant were coarsely cut with a razor and gently crushed using a mortar and pestle to create openings in the surface tissues. Each sample was placed in a tube containing 50 mL deionized water and the mixture was kept in a refrigerator for 2 h; then it was stirred in a rotary shaker for 1 h and centrifuged (Eppendorf 5804R, Hamburg, Germany) at 1,500 rotations min^{-1} for 15 min (Scrivanti *et al*. 2011). The supernatant was recovered and stored in a refrigerator for further use as a crude water-soluble extract. The extracts were stored at 4 ºC.

Bioassay

The allelopathic effects of aqueous root, stem and leaf extracts were evaluated on 25 seeds of each of the following plants: lettuce (*Lactuca sativa* L.), maize (*Zea mays* L.), lovegrass [*Eragrostis curvula* (Schard.) Nees] and wintergreen paspalum (*Paspalum guenoarum* Arechav.). Seeds were

Table 1 – Locality and voucher number of the examined material.

Species	Collector and locality
Bothriochloa barbinodis (Lag.) Herter	L.R. Scrivanti 370. Argentina, Córdoba, Capital
Bothriochloa edwardsiana (Gould) Parodi	L.R. Scrivanti 358. Argentina, Córdoba, Punilla
Bothriochloa perforata (Trin. *ex* Fourn.) Herter	L.R. Scrivanti 368. Argentina, Córdoba, Punilla
B. saccharoides (Sw.) Rydb. subsp. *australis* Scrivanti	L.R. Scrivanti 375. Argentina, Córdoba, Punilla
B. springfieldii (Gould) Parodi	L.R. Scrivanti 371. Argentina, Córdoba, Punilla

placed in Petri dishes containing two layers of filter paper moistened with 4 mL of aqueous extracts of *Bothriochloa* species. The control Petri dishes were treated with 4 mL deionized water. These Petri dishes were kept at 23 °C under low light (300 μmol photons m^{-2} s^{-1}) photosynthetically active radiation (PAR) from fluorescent lamps Scrivanti *et al*. 2011). To determine the moment of highest inhibitory activity of the aqueous extracts, germination was measured 48, 120 and 168 hours after application. The treatments were replicated three times in a completely randomized design.

Data were standardized and subjected to an analysis of variance (ANOVA) followed by Tukey's test to determine significant differences among mean values at probability level of 0.05, using the InfoStat program version 2018 (Rienzo *et al*. 2018).

Results

The aqueous root, stem and leaf extracts of *B. barbinodis*, *B. edwardsiana*, *B. perforata*, *B. saccharoides* subp. *australis* and *B. springfieldii* inhibited the germination of maize and wintergreen paspalum after 48 h of exposure with respect to control ($p \leq 0.05$). In most treatments with the aqueous root and leaf extracts of the five species of *Bothriochloa*, the highest average percentages of inhibition occurred after 120 h, with no significant differences among treatments at 48 h and 120 h (Tab. 2). The inhibition percentage of aqueous root extracts treatments ranged from 36.38 to 78.33% for maize and from 38.46 to 97.30% for wintergreen paspalum (Tab. 2), whereas for aqueous leaf extracts the inhibition percentage ranged from 42.80 to 73.20% for maize (Tab. 2). In treatments with the aqueous leaf extract of the five *Bothriochloa* species the highest average inhibition percentages on wintergreen paspalum occurred at 168 h (Tab. 2). The treatments with the aqueous stem extracts of the five species of *Bothriochloa* showed the highest average percentages of inhibition at 168 h, with values ranging from 49.70 to 75.0% for maize and from 75.80 to 98.20% for wintergreen paspalum (Tab. 2).

Overall, the aqueous root, stem and leaf extracts inhibited germination of lettuce and lovegrass significantly at 168 h, with average values from 9.60 to 17.60% for lettuce and from 21.0 to 26.40% for lovegrass (Tab. 2).

The aqueous root, stem and leaf extracts of all *Bothriochloa* species on the four target plants differed in their inhibitory activity at the three exposure periods (Fig. 1a-c). The inhibitory

Table 2 – Effects of aqueous extracts of five South American species of *Bothrichloa* (Poaceae) on seed germination of lettuce, lovegrass, maize and wintergreen paspalum.

			Inhibition of germination (%) over control (%)*		
***Bothriochloa* (species)**	**Test species**	**Time (hours)**	**Root extract**	**Stem extract**	**Leaf extract**
Bothriochloa barbinodis	Lettuce	48	4.00 ± 2.35 a	3.20 ± 2.95 a	3.40 ± 2.61 a
		120	4.20 ± 2.17 a	4.20 ± 2.17 a	4.60 ± 1.82 a
		168	13.20 ± 2.77* b	9.60 ± 3.21* b	17.00 ± 3.81* b
	Lovegrass	48	7.00 ± 2.65 a	5.40 ± 3.44 a	5.80 ± 3.27 a
		120	9.00 ± 2.92 a	6.80 ± 2.17 a	7.60 ± 2.07 a
		168	22.60 ± 3.65* b	24.18 ± 1.94* b	24.84 ± 3.46* b
	Maize	48	45.93 ± 2.06* a	43.82 ± 2.35* a	43.94 ± 2.37* a
		120	50.92 ± 2.31* b	68.10 ± 5.94* b	72.80 ± 2.28* b
		168	64.26 ± 2.24* c	75.00 ± 1.87* c	73.20 ± 2.17* b
	W. Paspalum	48	73.18 ± 2.56* a	66.20 ± 2.07* a	67.00 ± 2.35* a
		120	81.24 ± 1.61* b	82.60 ± 2.88* b	83.40 ± 2.07* b
		168	82.32 ± 2.10* b	88.20 ± 2.05* c	88.00 ± 2.55* c

			Inhibition of germination (%) over control (%)*		
***Bothriochloa* (species)**	**Test species**	**Time (hours)**	**Root extract**	**Stem extract**	**Leaf extract**
Bothriochloa edwardsiana	Lettuce	48	3.60 ± 1.67 a	4.40 ± 2.41 a	3.90 ± 1.52 a
		120	4.56 ± 2.04 a	4.80 ± 2.59 a	4.80 ± 2.39 a
		168	13.28 ± 2.26* b	15.40 ± 2.70* b	15.20 ± 3.11* b
	Lovegrass	48	5.40 ± 2.30 a	5.80 ± 2.68 a	5.60 ± 2.07 a
		120	9.00 ± 3.39 a	8.50 ± 3.57 a	8.40 ± 3.05 a
		168	25.40 ± 4.22* b	24.40 ± 3.85* b	23.80 ± 2.59* b
	Maize	48	34.82 ± 2.33* a	49.36 ± 2.31* a	57.20 ± 3.11* a
		120	49.60 ± 2.07* b	57.84 ± 1.93* b	62.80 ± 2.28* b
		168	51.46 ± 2.78* b	71.38 ± 3.43* c	63.20 ± 2.17* b
	W. Paspalum	48	82.80 ± 2.17* a	66.60 ± 2.07* a	70.40 ± 2.30* a
		120	91.64 ± 1.77* b	95.20 ± 2.95* b	93.40 ± 2.07* b
		168	94.84 ± 1.96* b	98.20 ± 2.05* b	97.20 ± 1.64* c
Bothriochloa perforata	Lettuce	48	3.40 ± 2.51 a	3.40 ± 2.30 a	3.70 ± 2.11 a
		120	4.80 ± 2.39 a	3.80 ± 2.31 a	5.00 ± 2.55 a
		168	13.36 ± 2.13* b	15.00 ± 2.92* b	15.62 ± 2.67* b
	Lovegrass	48	6.20 ± 2.39 a	6.60 ± 2.19 a	4.00 ± 3.24 a
		120	10.20 ± 5.07 a	11.60 ± 2.88* b	9.24 ± 1.95* b
		168	26.40 ± 2.51* b	23.60 ± 2.92* c	21.00 ± 2.00* c
	Maize	48	71.34 ± 2.91* a	43.82 ± 2.35* a	43.94 ± 2.37* a
		120	73.51 ± 2.76* a	68.10 ± 5.94* b	72.80 ± 2.28* b
		168	78.33 ± 2.58* b	75.00 ± 1.87* c	73.20 ± 2.17* b
	W. Paspalum	48	59.32 ± 2.53* a	66.60 ± 2.07* a	67.00 ± 2.35* a
		120	73.04 ± 2.68* b	82.60 ± 2.88* b	83.40 ± 2.07* b
		168	74.72 ± 3.00* b	88.20 ± 2.05* c	88.06 ± 2.55* c
Bothriochloa saccharoides subsp. *australis*	Lettuce	48	3.80 ± 2.39 a	3.62 ± 2.60 a	3.60 ± 1.14 a
		120	4.20 ± 2.59 a	3.90 ± 0.74 a	4.00 ± 2.00 a
		168	16.54 ± 2.49* b	17.60 ± 2.70* b	13.00 ± 2.12* b
	Lovegrass	48	5.80 ± 2.39 a	5.50 ± 2.06 a	4.20 ± 2.95 a
		120	8.60 ± 2.61 a	8.24 ± 2.83 a	8.60 ± 3.36 a
		168	23.46 ± 2.12* b	24.96 ± 3.33* b	23.10 ± 2.25* b
	Maize	48	25.93 ± 2.55* a	38.38 ± 2.72* a	19.98 ± 2.33* a
		120	36.38 ± 2.79* b	44.10 ± 1.75* b	42.80 ± 2.28* b
		168	38.72 ± 3.02* b	49.70 ± 2.95* c	43.20 ± 2.17* b
	W. Paspalum	48	34.46 ± 5.80* a	60.38 ± 2.05* a	79.28 ± 2.31* a
		120	38.46 ± 2.45* ab	63.00 ± 2.35* a	80.61 ± 2.11* ab
		168	41.54 ± 2.95* b	76.40 ± 1.67* b	84.36 ± 2.71* b

			Inhibition of germination (%) over control (%)*		
***Bothriochloa* (species)**	**Test species**	**Time (hours)**	**Root extract**	**Stem extract**	**Leaf extract**
Bothriochloa springfieldii	Lettuce	48	2.60 ± 1.14 a	3.94 ± 2.17 a	4.40 ± 2.30 a
		120	4.20 ± 1.30 a	4.80 ± 2.04 a	3.70 ± 2.73 a
		168	16.72 ± 2.38* b	15.60 ± 3.91* b	13.00 ± 2.12* b
	Lovegrass	48	4.20 ± 1.64 a	6.05 ± 3.10 a	6.60 ± 3.78 a
		120	8.60 ± 2.61 a	8.24 ± 2.36 a	8.20 ± 2.39 a
		168	23.32 ± 2.70* b	22.96 ± 3.45* b	23.10 ± 2.25* b
	Maize	48	35.93 ± 2.55* a	37.80 ± 2.28* a	19.98 ± 2.33* a
		120	51.62 ± 2.96* b	42.90 ± 2.75* b	42.80 ± 2.28* b
		168	53.18 ± 2.65* b	50.34 ± 2.41* c	43.20 ± 2.17* b
	W. Paspalum	48	94.72 ± 2.34* a	59.80 ± 3.30* a	79.40 ± 1.95* a
		120	97.02 ± 1.61* a	62.48 ± 3.32* a	80.52 ± 1.88* ab
		168	97.30 ± 2.17* a	75.80 ± 2.77* b	84.20 ± 2.77* b

aValues (means ± SD) having different letter in the same column are significantly different from each other according to Tukey's multiple range test at P ≤ 0.05 (n = 25). * Indicate values significantly less than the respective control (P ≤ 0.05).

activity of treatments with aqueous root and stem extracts gradually increased from 48 h to 168 h, with inhibition percentages being highest at 168 h (Fig. 1a-b). In turn, the highest inhibitory activity percentage of the aqueous leaf extract treatment was recorded at 120 h, with no significant differences from values recorded at 168 h (Fig. 1c). The germination inhibitory activity increased with exposure time of the seeds to aqueous extracts (Tab. 2).

Discussion

Aqueous extracts of root, stem and leaf of the South American *Bothriochloa* species showed allelopathic activity that inhibited seed germination in lettuce, maize, lovegrass and wintergreen paspalum. The allelopathic effects of the aqueous vegetative extracts of the South American species of *Bothriochloa* have already been reported (Scrivanti 2010; Scrivanti *et al.* 2011; Scrivanti & Anton 2018). However, the inhibitory activity of allelochemicals on germination in relation to exposure time has not been evaluated so far. The aqueous extracts mainly inhibited germination after 120 h, with a peak at 168 h. This result suggests that the decrease in germination is favored by longer exposure to the allelochemicals present in the aqueous root, stem and leaf extracts of the South American species of *Bothriochloa*. The essential oils of *Bothriochloa barbinodis*, *B. edwardsiana*, *B. perforata*, *B. saccharoides* subsp. *australis* and *B. springfieldii* from South America were characterized by dominance of oxygenated sesquiterpenes such as E,E-farnesol, epi-α-cadinol and γ-gurjunene followed by E-β-farnesene, germacrene D and cis-nerolidol (Scrivanti *et al.* 2009). The E,E-farnesol, epi-α-cadinol, γ-gurjunene and germacrene D sesquiterpenes shown inhibitory activity on seed germination and seedling growth of other plants (Bede & Tobe 2000; Barbosa *et al.* 2007; Scrivanti & Anton 2018). Although sesquiterpenes are less water soluble cause strong inhibitory effects (Fischer *et al.* 1994; He *et al.* 2009). Therefore, the allelochemicals present in the aqueous extracts may be able to prevent seed germination by producing cell wall damage in radicle tips, which reduces cell proliferation and DNA synthesis in plant meristems; producing disorganization of organelles; reducing mitotic activity; disrupting mitotic microtubules; suppressing hormone activity; reducing the ion uptake rate; inhibiting protein formation ; reducing the permeability of cell membranes; and inhibiting enzyme activities among other mechanisms (Andrade *et al.* 2010; Cheng & Cheng 2015; Sitthinoi *et al.* 2017).

The allelochemicals of *B. alta*, *B. barbinodis*, *B. edwardsiana*, *B. perforata*, *B. saccharoides*

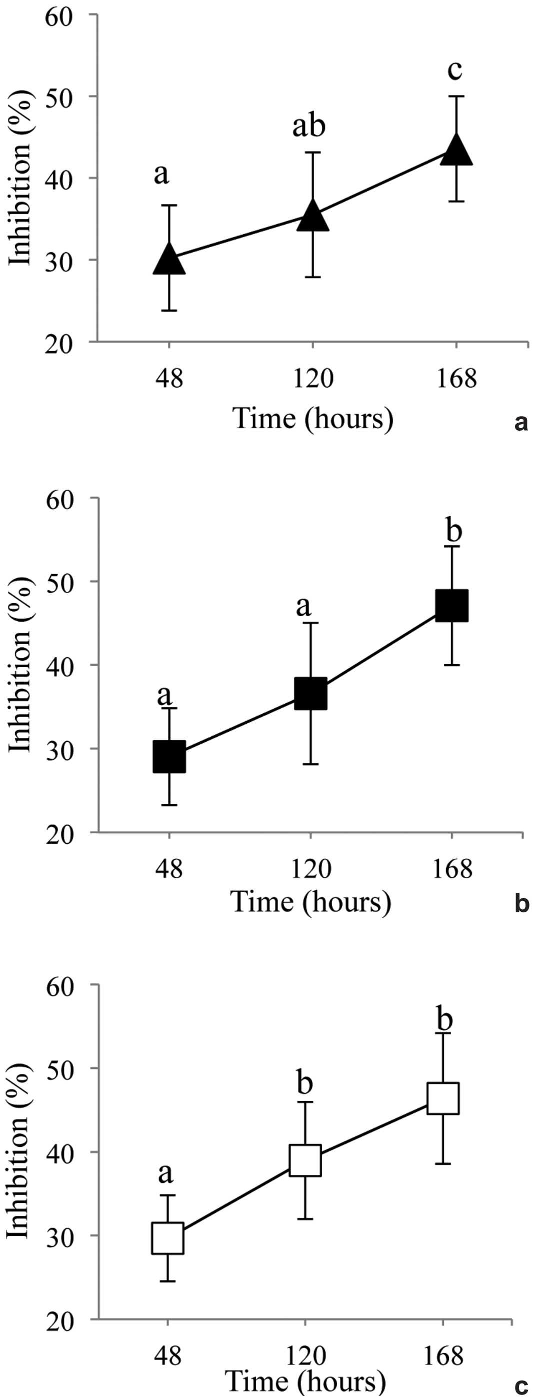

Figure 1 – a-c. Inhibitory activity of extracts of the five *Bothriochloa* species on seed germination of four target plants at 48, 120 and 168 h of exposure time – a. aqueous root; b. stem; c. leaf. Values with different letters are significantly different according to Tukey's multiple range test at $p \leq 0.05$ ($n = 25$).

subsp. *australis* and *B. springfieldii* can be applied as potential herbicides, since they inhibited seed germination and growth of the four tested plants from the first hours (48 h) of application, with germination inhibition peaking at 168 h.

Allelochemicals with negative allelopathic effects on seed germination and seedling growth do not have residual or toxic effects; therefore, they are a suitable substitute for synthetic herbicides (Cheng & Cheng 2015). The suitable management of allelochemicals might improve crop productivity and environmental protection through biologically friendly control of weeds. Nowadays, few natural herbicides derived from allelochemicals are marketed (Cheng & Cheng 2015), perhaps due to the lack of knowledge about allelopathic interactions. Therefore, it is necessary to support and stimulate scientific advances on the use of allelochemicals as herbicides, pesticides, insecticides, and antibacterial and antifungal agents to contribute to agricultural production, both in small farms and large-scale agronomic systems.

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