

Allelopathic potential of South American *Bothriochloa* species (Poaceae: Andropogoneae)

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ABSTRACT

In laboratory bioassays the aqueous extracts from different plant parts of six *Bothriochloa* species (*B. barbinodis*, *B. edwardsiana*, *B. perforata*, *B. saccharoides*, var. *saccharoides* and *B. springfieldii*) inhibited the seed germination and root and shoot growth of lettuce (*Lactuca sativa* L.), maize (*Zea mays* L.), wintergreen paspalum (*Paspalum guenoarum* Arechav.) and lovegrass [*Eragrostis curvula* (Schrud.) Nees] test species. Aqueous extracts were more inhibitory to shoot and root elongation than to seed germination. Significant inhibition in shoot length at all concentrations indicated the phytotoxic activity of extracts. The toxicity enhanced with increasing aqueous extract concentrations. Perhaps these extracts may contain allelochemicals, which interferes with seed germination and seedling growth of plants. Consequently, the study of these chemical interactions might be useful to develop new classes of environmentally safe herbicides.

Key Word: Allelopathy, aqueous extracts, *Bothriochloa*, inhibition, Poaceae, root growth, seed germination, shoot growth

INTRODUCTION

The phenomenon of allelopathy refers to chemical interactions between all types of plants, and sometimes includes microorganisms. In this process, the chemical exudates or leachates released from leaves, stems or roots of a donor plant species can inhibit the growth of a neighbouring one (28), and therefore they play an important role in regulating plant diversity (12). These organic allelopathic chemicals might subsequently inhibit and/or stimulate germination and the development of other plants, and therefore provide important mechanisms in the interactions between plants (1). Consequently, the study of these chemical interactions might be a useful approach to develop new environmentally safe herbicides (14).

The Poaceae family has shown allelopathic activity (34). In past few decades much research had been done to explore the herbicidal potential of allelopathic grasses (3,22,23). The root secretions of corn inhibited the growth of *Chenopodium album* L. weed (15) and soluble water extracts of wheat straw significantly inhibited the germination and growth of *Convolvulus arvensis* L. weed (9). Allelochemicals have been isolated and identified from rice owing to its potential application in weed management (42).

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The genus *Bothriochloa* (Poaceae: Andropogoneae) contains perennial aromatic grasses growing in tropical and subtropical regions. They differ in composition of their essential oils (7,25,29,31). Recently, essential oils compositions were studied in species from South America and the oxygenated sesquiterpenes *E,E*-farnesol, γ -gurjunene and epi- α -cadinol were the main chemotaxonomic markers (37). *E,E*-farnesol is a potent inhibitor of germination and shoot growth of lettuce, rice seedlings and *Cyperus* species (6). In addition, epi- α -cadinol has an allelopathic effect (5). *Bothriochloa pertusa* (L.) A. Camus suppresses and precludes the associated species (19,21). Nevertheless, nothing is known about the species from South America and their dominance on other plants.

This study aimed to investigate the allelopathic effects of South American *Bothriochloa* species on seed germination and early seedling growth of lettuce (*Lactuca sativa* L.) and maize (*Zea mays* L.), wintergreen paspalum (*Paspalum guenoarum* Arechav.) and lovegrass [*Eragrostis curvula* (Schard.) Nees] native grasses.

MATERIALS AND METHODS

Six *Bothriochloa* species were studied: *B. alta* (Hitchc.) Henrard, *B. barbinodis* (Lag.) Herter, *B. edwardsiana* (Gould) Parodi, *B. perforata* (Trin. Ex Fourn.) Herter, *B. saccharoides* (Sw.) Rydb. var. *saccharoides* and *B. springfieldii* (Gould) Parodi and were collected in April 2008 from the Province of Córdoba, Argentina. Vouchers specimens are deposited in the Herbarium, Museo Botánico de Córdoba (CORD). These plants were separated into stem, leaf and root portions and then air dried. Aqueous extracts were obtained and examined to determine the allelopathic potential of different plant parts.

Seeds of four test species, lettuce (*Lactuca sativa* L.) and maize (*Zea mays* L.), wintergreen paspalum (*Paspalum guenoarum* Arechav.) and lovegrass (*Eragrostis curvula* (Schard.) Nees) were used for germination assays. Lettuce and maize were standard test plants [these are sensitive and reliable species, hence, commonly used in bioassays (27, 36,38)]. The other two native grasses coexist with *Bothriochloa* species and these spp's allelopathic substances adversely affect the native grasses.

Aqueous Extracts: Five g of dried culms, leaf-blade and root materials 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 before stirring in a rotary shaker for 1 h and centrifuged at 1500 rotations min^{-1} for 15 min. The supernatant was recovered and stored in a refrigerator until being used as a crude water-soluble extract. Other test solutions (75, 50 and 25% v/v) were prepared by the dilution of stock solution with sterile distilled water. The extracts were stored at 4 °C.

Bioassay: To evaluate the allelopathic effects of aqueous root, stem and leaf extracts, 25 seeds each of lettuce, maize, lovegrass and wintergreen paspalum were placed in each Petri dish, which contained two layers of filter paper moistened with 4 mL of aqueous extracts of *Bothriochloa* species. The control Petri dishes received 4 mL deionized water. These Petri dishes were kept at 23°C under low light [300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

photosynthetically active radiation (PAR), from fluorescent lamps] for 3 days. The treatments were replicated thrice in complete randomised design.

Growth Measurements: Three days after sowing, germinated seeds were counted and root and shoot lengths of lettuce, maize, lovegrass and wintergreen paspalum plants were measured. The growth inhibition (%) of root and shoot lengths under the influence of aqueous *Bothriochloa* extract were calculated as under:

$$I (\%) = [1 - T/C] \times 100$$

Where, T: Length of treatment organs (mm) and C: Length of control organs (mm).

Data were subjected to an analysis of variance (ANOVA) followed by Duncan's test to determine significant differences among mean values at probability level of 0.05, using the InfoStat program version 1.1 (Group InfoStat 2002).

RESULTS AND DISCUSSION

Seed germination

Aqueous extracts of root, stem and leaf of *B. alta* were very toxic to the germinating wintergreen paspalum at all concentrations and maize at high concentrations (50, 75 and 100%) compared to control (Fig. 1).

Aqueous extracts of root and leaf of *B. barbinodis* reduced germination of winter paspalum and maize seeds at 75 and 100% respect to control (Fig. 1). Stem extract caused significantly effects on germination of winter paspalum seeds at all concentrations tested, while maize germination was reduced at 50, 75 and 100%.

Stem extract of *B. edwardsiana* decreased the germination of wintergreen paspalum at all concentration (Fig. 2), while high concentrations inhibited the germination of maize seeds. However, both root and leaf extracts reduce germination at high concentration in maize and wintergreen paspalum seeds.

All applied concentrations of stem and leaf extracts of *B. perforata* suppressed the germination of wintergreen paspalum than control, but they inhibited the germination of only maize seeds at 75 and 100% (Fig. 2). Aqueous root extract of *B. perforata* was comparatively less toxic, and reduced the germination of winter paspalum and maize seeds at the highest concentrations of 75 and 100%.

Both stem and leaf extracts of *B. saccharoides* at all concentrations reduced the germination of winter paspalum seeds (Fig. 3). The aqueous root and stem extracts at 75 and 100% were inhibitory to the germination of maize seeds. Root extract inhibited the wintergreen paspalum germination at 100%. Contrarily, the leaf extract did not affect the germination of maize seeds.

Root, stem and leaf extracts of *B. springfieldii* at higher concentrations (50, 75 and 100%) drastically inhibited the seed germination in wintergreen paspalum and maize (Fig. 3).

The inhibitory potential of aqueous extracts of *Bothriochloa* species was increased by increasing extract concentrations i.e. was concentration dependent. The germination of lettuce and lovegrass seeds was similar to control at test concentrations, stem and leaf extracts of *B. edwardsiana* and *B. perforata* at 100% conc. (Table 1).

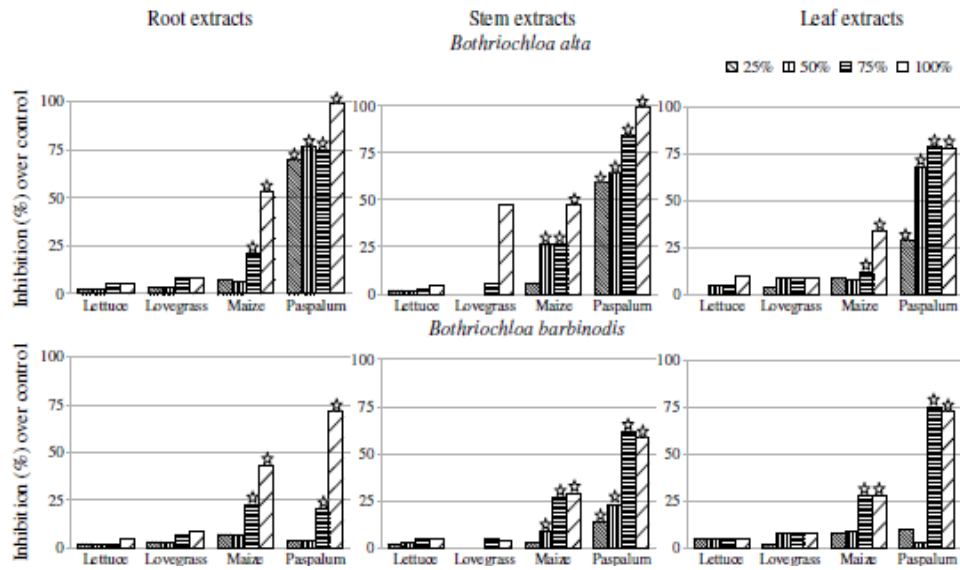


Figure 1. Inhibitory effects of *Bothriochloa alta* and *B. barbinodis* on germination of lettuce, lovegrass, maize and paspalum. Symbol indicate values significantly less than the respective control ($p \leq 0.05$).

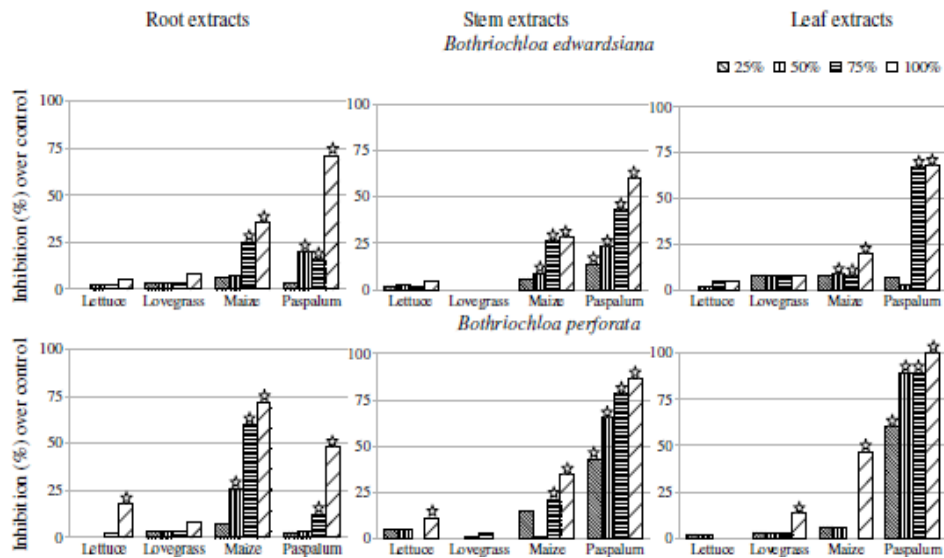


Figure 2. Inhibitory effects of *Bothriochloa edwardsiana* and *B. perforata* on germination of lettuce, lovegrass, maize and paspalum. Symbol indicate values significantly less than the respective control ($p \leq 0.05$).

Table 1. Mean Inhibitory effects of aqueous root, stem and leaf extracts of *Bothriochloa* species on germination and root and shoot growth of lettuce, lovegrass, maize and wintergreen paspalum

Test species	Root extract			Stem Extract			Leaf Extract		
	Inhibition (%) over control ^a								
	Germ	RL	SL	Germ	RL	SL	Germ	RL	SL
	<i>Bothriochloa alta</i>								
Lettuce	3.5	26.3	40.7	2.9	45.2	41.1	4.8	37.3	46.5
Lovegrass	5.6	57.1	45.5	13.2	47.4	44.0	6.9	48.4	39.5
Maize	22.0	38.3	97.9	26.5	44.4	86.2	15.5	40.4	90.0
Paspalum	80.1	87.9	98.9	77.1	75.7	90.0	63.2	69.4	90.0
Mean	27.8	52.4	70.8	29.9	53.2	65.3	22.6	48.9	66.5
	<i>Bothriochloa barbinodis</i>								
Lettuce	2.7	43.6	50.1	3.6	45.6	48.3	5.0	47.1	40.5
Lovegrass	5.2	48.3	47.0	2.3	46.9	37.3	6.5	43.7	40.0
Maize	19.5	45.9	89.0	16.7	43.4	71.2	17.8	50.1	81.5
Paspalum	24.7	72.6	85.0	39.3	74.0	73.9	40.0	77.9	84.6
Mean	13.0	52.6	67.7	15.5	52.4	57.6	17.3	54.7	61.6
	<i>Bothriochloa edwardsiana</i>								
Lettuce	2.2	43.1	42.3	2.9	42.1	40.1	3.0	38.6	39.0
Lovegrass	4.4	44.7	35.6	0.0	39.3	36.8	8.2	44.1	35.2
Maize	18.5	43.4	74.8	17.3	36.2	84.8	11.0	36.2	84.0
Paspalum	27.6	62.8	87.5	35.0	70.0	67.8	36.2	66.8	83.2
Mean	13.2	48.5	60.0	13.8	46.9	57.3	14.6	46.4	60.3
	<i>Bothriochloa perforata</i>								
Lettuce	5.0	18.1	49.4	5.3	34.7	47.6	1.0	30.6	37.3
Lovegrass	4.4	51.2	67.9	1.0	33.6	38.6	5.6	43.8	42.4
Maize	41.0	44.5	93.0	17.7	24.5	88.0	14.7	29.8	87.2
Paspalum	16.7	88.1	93.8	68.3	80.6	90.3	84.7	86.1	91.2
Mean	16.8	50.4	76.0	23.1	43.3	66.1	26.5	47.5	64.5
	<i>Bothriochloa saccharoides</i>								
Lettuce	1.7	18.1	49.4	4.0	34.7	47.5	2.7	30.6	37.3
Lovegrass	3.1	51.2	67.9	1.6	33.6	38.6	3.1	43.8	42.4
Maize	15.8	44.5	93.0	8.1	24.5	88.0	4.3	29.8	87.2
Paspalum	5.6	88.1	93.8	44.4	80.6	90.3	63.9	86.1	91.2
Mean	6.5	50.5	76.0	14.5	43.3	66.1	18.5	47.6	64.5
	<i>Bothriochloa springfieldii</i>								
Lettuce	2.2	60.0	51.4	1.5	39.7	45.8	2.0	35.8	37.7
Lovegrass	2.8	56.4	42.1	0.8	42.3	29.4	2.0	38.9	31.6
Maize	6.8	13.9	89.3	8.6	21.3	86.8	7.6	25.9	100.0
Paspalum	58.3	87.3	91.8	64.7	76.3	83.9	49.7	80.0	92.7
Mean	17.5	54.4	68.6	18.9	44.9	61.5	15.3	45.1	65.5

Germ: Germination, RL: Root length, SL : Shoot length length. ^a Data are expressed as percentage means from experiments with three replicates of 25 seeds each.

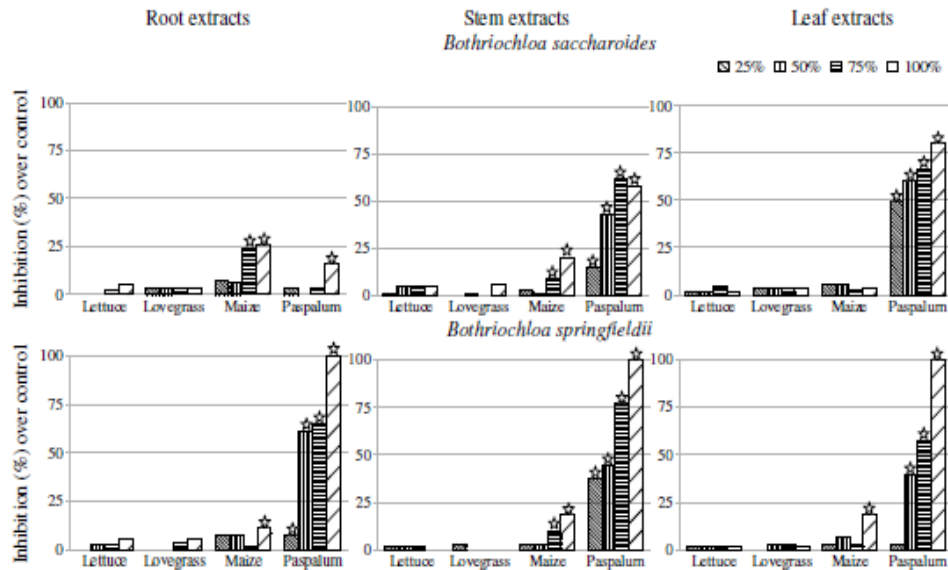


Figure 3. Inhibitory effects of *Bothriochloa saccharoides* and *B. springfieldii* on germination of lettuce, lovegrass, maize and paspalum. Symbol indicate values significantly less than the respective control ($p \leq 0.05$).

Root and shoot growth

Aqueous root, stem and leaf extracts of *B. alta*, *B. barbinodis* and *B. edwardsiana* inhibited the growth of lettuce, maize, wintergreen paspalum and lovegrass roots and shoots than control (Figs. 4, 5 and 6).

Aqueous root, stem and leaf extracts of *B. perforata* were phytotoxic to root and shoot growth (Fig. 7). Root extract at 75 and 100% conc. inhibited the root elongation of test plants compared to control but it reduced the root elongation of wintergreen paspalum and lovegrass at all concentrations. Stem and leaf extracts were more inhibitory to maize root growth. Aqueous root, stem and leaf extracts reduced the shoot growth of all test plants and the shoot growth was more sensitive than root growth.

All concentrations of aqueous root, stem and leaf extracts of *B. saccharoides* inhibited the root growth of all test plants but the root growth of lettuce and maize was reduced at highest concentrations respect to control (Fig. 8). The aqueous root, stem and leaf extracts of *B. saccharoides* reduced the shoot growth of test plants.

Aqueous extracts of *B. springfieldii* inhibited the root growth of lettuce, wintergreen paspalum and lovegrass (Fig. 9). However, aqueous root extract did not influence the maize root growth. Stem and leaf extracts were most inhibitory, except to maize root growth at 25 and 50% and lettuce at 25% conc.

In general, shoot length was more sensitive to treatments than root length and the degree of inhibition increased with increasing concentration of extract i.e. concentration dependent (3,10,22,24,26,39,40). Thus, it is important to determine the critical

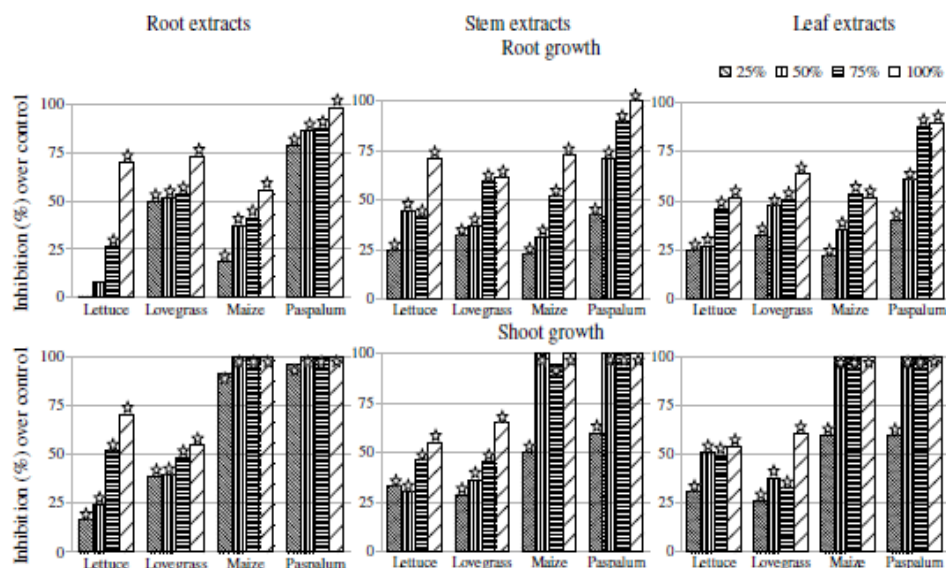


Figure 4. Inhibitory effects of *Bothriochloa alta* on root and shoot growth of lettuce, lovegrass, maize and paspalum. Symbol indicate values significantly less than the respective control ($p < 0.05$).

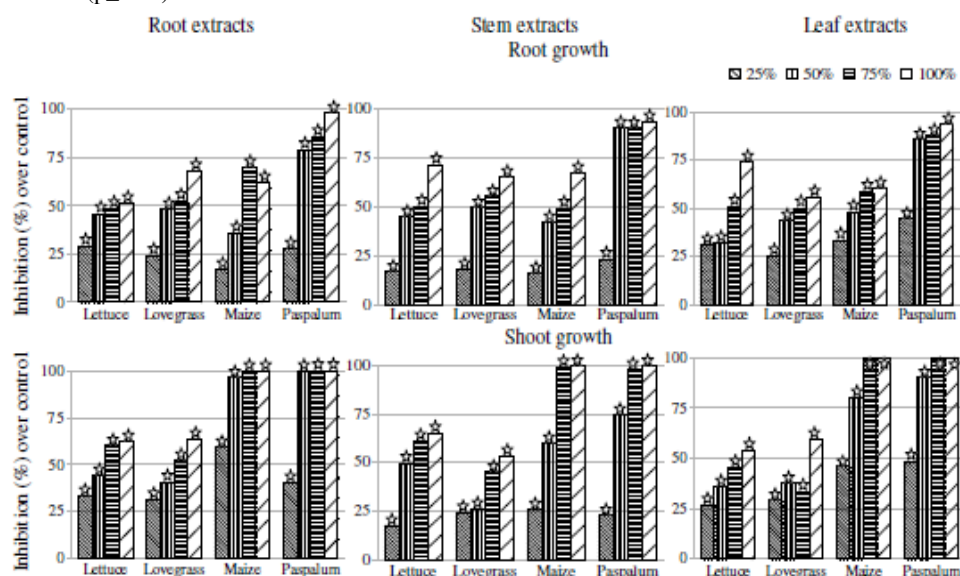


Figure 5. Inhibitory effects of *Bothriochloa barbinodis* on root and shoot growth of lettuce, lovegrass, maize and paspalum. Symbol indicate values significantly less than the respective control ($p < 0.05$).

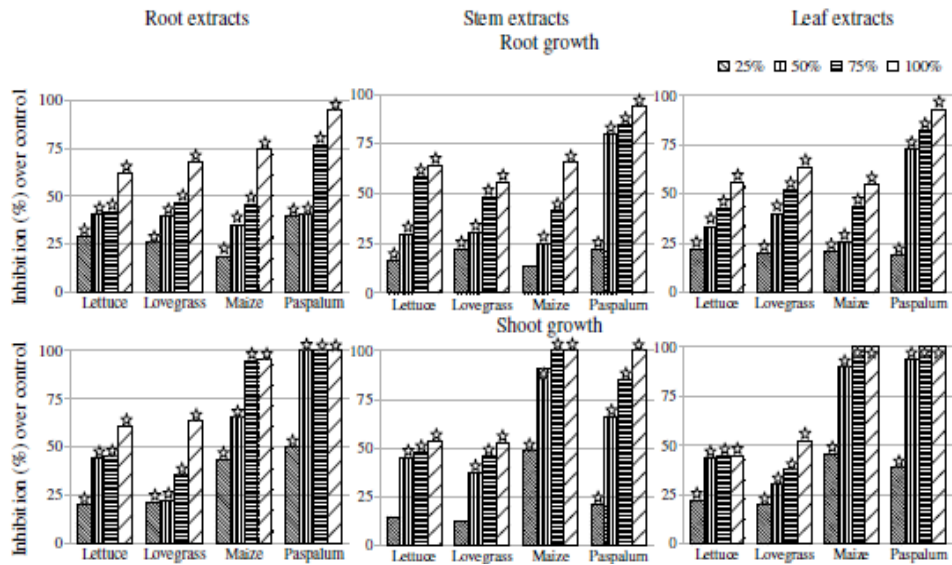


Figure 6. Inhibitory effects of *Bothriochloa edwardsiana* on root and shoot growth of lettuce, lovegrass, maize and paspalum. Symbol indicate values significantly less than the respective control ($p \leq 0.05$).

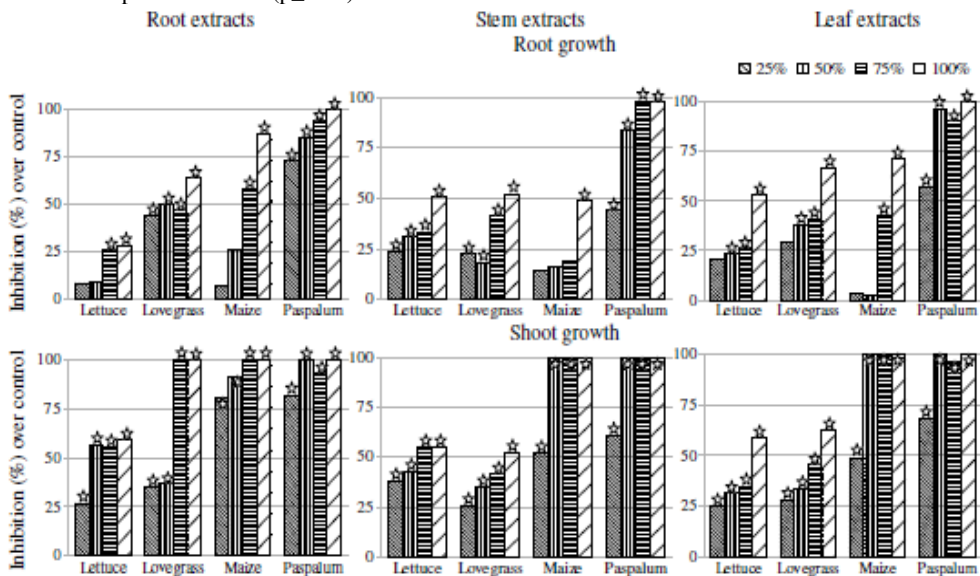


Figure 7. Inhibitory effects of *Bothriochloa perforata* on root and shoot growth of lettuce, lovegrass, maize and paspalum. Data Symbol indicate values significantly less than the respective control ($p \leq 0.05$).

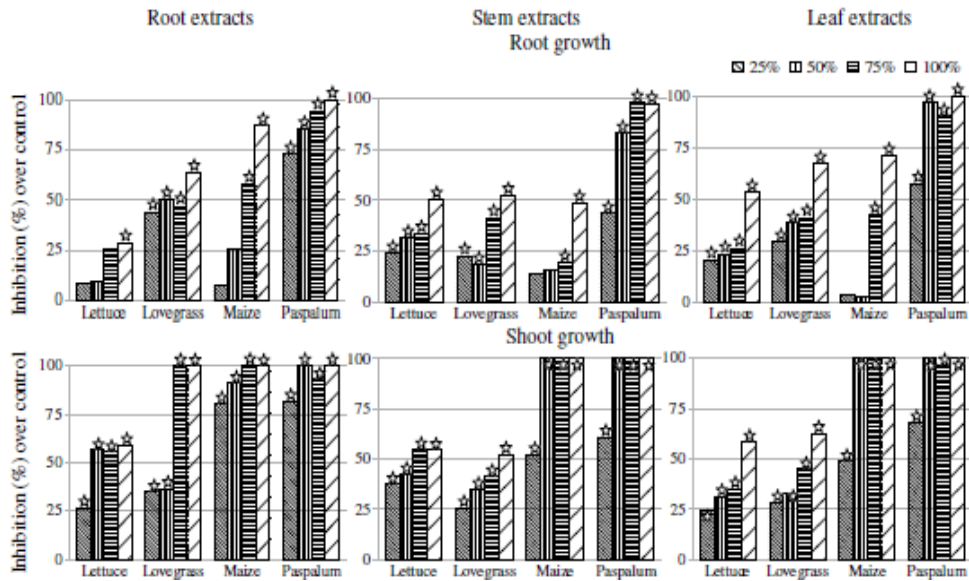


Figure 8. Inhibitory effects of *Bothriochloa saccharoides* on root and shoot growth of lettuce, lovegrass, maize and paspalum. Symbol indicate values significantly less than the respective control ($p \leq 0.05$).

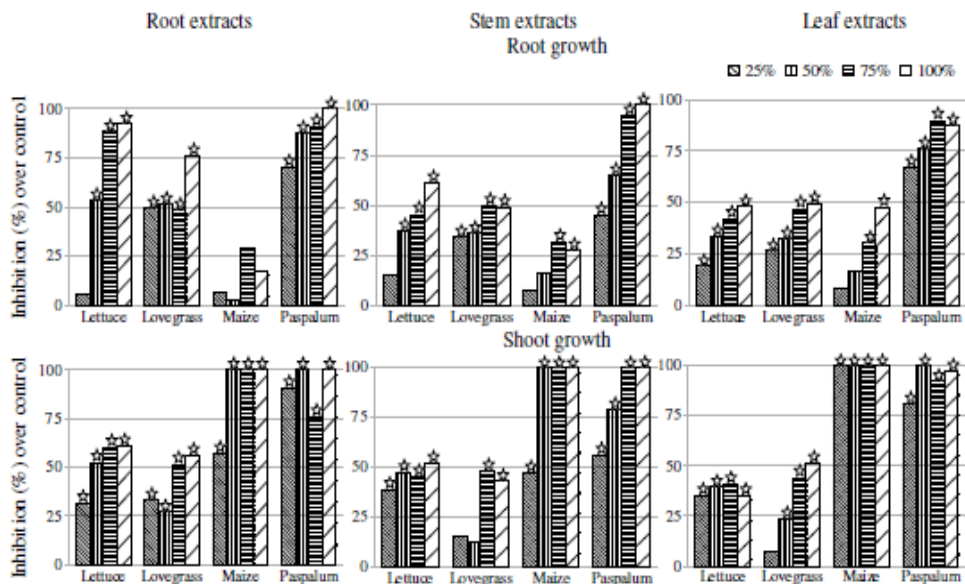


Figure 9. Inhibitory effects of *Bothriochloa springfieldii* on root and growth of lettuce, lovegrass, maize and paspalum. Symbol indicate values significantly less than the respective control ($p \leq 0.05$).

concentrations for weed management (4). The inhibitory potential of aqueous root, stem and leaf extracts of *Bothriochloa* on test species was similar. The root and shoot growth were more sensitive to treatments than germination to find the allelopathic effects of *Bothriochloa* species. Plant growth is more sensitive parameter to aqueous root, leaves and stems extracts of durum wheat than germination (30). The inhibitory effects observed on test plants without interference by soil microflora further indicate the allelopathic potential of *Bothriochloa* extracts (2).

Allelopathy has been widely reported in grasses (22) and our results are in agreement with previous reports on other *Bothriochloa* species (20,35). Aqueous extracts of *Bothriochloa* species contained seed germination and growth inhibitors that interfere with physiological and metabolic processes (photosynthesis, respiration, transpiration, cell division, cell elongation, protein and nucleic acid synthesis) in target plants, thus a reducing their growth and development (8,11,18,41). Several secondary metabolites have been isolated from *Bothriochloa* donor species, e.g. sesquiterpenes (E,E-farnesol, epi- α -cadinol, γ -gurjunene) α -cadinene, nerolidol cis, and E- β -farnesene (37), most of these compounds are allelopathic agents (32,34). Sesquiterpenes are less water soluble (18) and even then causes strong inhibitory effects (17). Biotic factors such as herbivory, disease and insect damage may influence accumulation of these allelochemicals and their release from the donor plant (16). It seems that allelopathic interference demonstrated in this study may also occur under field conditions and this may contribute to ecological behaviour of *Bothriochloa* of forming high density groups. The majority of the species inhabit disturbed ecosystems where the competition is great, the presence of these allelochemicals would be important for the establishment of the populations, inhibiting the growth of other species in the plant community. The allelochemicals can be washed from aerial parts of plants and so affect the germination and growth of other plants in vicinity. Allelopathy plays a significant role in natural and agricultural systems (10,11) and may be useful approach to develop of new classes of environmentally safe herbicides (13).

CONCLUSIONS

Bothriochloa species contain and release compounds responsible for reducing the germination and growth of other plants. Allelopathic potential did not vary with plant part used but varied with concentration of extract and was more inhibitory to shoots than roots in test plants. This study contributed to our understanding of allelopathic potential of *Bothriochloa* grasses in natural community and optimize the development of new environmentally safe herbicides.

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