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Phytotoxic potential of *Senna occidentalis* and *Senna obtusifolia*

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ABSTRACT. This work aimed to investigate the phytotoxic potential of the aerial and underground parts of *Senna occidentalis* and *S. obtusifolia* on the germination and initial growth of lettuce and onion. Four concentrations were used of each ethanol extract (0, 250, 500 and 1000 mg L^{-1}), with four replications of 50 seeds. From the investigated species, the aerial part of *S. occidentalis* interfered in onion germination and the aerial part of *S. obtusifolia* interfered in the germinations of lettuce and onion. The ethanol extract from the aerial and underground parts of the studied species inhibited the root growth of lettuce and onion. The hypocotyl/coleoptile growth in lettuce and onion was inhibited by the extract of *S. obtusifolia* aerial part and the underground part of *S. occidentalis* and S. *obtusifolia*. The results obtained make it possible to infer that the studied species contain substances that influence the germination and growth of the target seedlings.

Key words: ethanol extract, leguminosae, weeds.

RESUMO. Potencial fitotóxico de *Senna occidentalis* e *Senna obtusifolia*. O objetivo do presente trabalho foi investigar o potencial fitotóxico das partes aérea e subterrânea de *Senna occidentalis* e *S. obtusifolia* sobre a germinação e o crescimento inicial de alface e cebola. Utilizaram-se quatro concentrações de cada extrato etanólico (0, 250, 500 e 1000 mg L^{-1}), com quatro repetições de 50 sementes. Das espécies investigadas, a parte aérea de *S. occidentalis* interferiu na germinação de cebola e a parte aérea de S. *obtusifolia*, na germinação de alface e cebola. O extrato etanólico da parte aérea e subterrânea, das espécies em estudo, inibiu o crescimento da raiz de alface e de cebola. O crescimento do hipocótilo/coleóptilo de alface e cebola foi inibido pelo extrato da parte aérea de *S. obtusifolia* e da subterrânea de *S. occidentalis* e S. *obtusifolia*. Os resultados obtidos permitem inferir que as espécies em estudo contêm substâncias que influenciam a germinação e o crescimento das plântulas-alvo.

Palavras-chave: extrato etanólico, leguminosae, plantas daninhas.

Introduction

Plants can, favorably or unfavorably, affect other plants through chemicals released into the environment, named allelochemicals (BHOWMIK; INDERJIT, 2003). Allelochemicals are substances from secondary metabolism, such as terpenes, phenols and alkaloids that present an important ecological function. These substances may be produced in any vegetal organ but with a very low concentration and with intrinsic characteristics to the plant, for example, species, age and so forth (MACIAS et al., 2008). The allelopathy of the weeds is one of the most studied aspects in the last 30 years, mainly due to the losses in productivity that these plants may cause to crops.

Senna occidentalis (L.) Link (Leguminosae, Caesalpinioideae) is an annual, woody and very ramified plant, with 1.0 to 2.0 m height, native to tropical America, popularly known as "fedegoso", and which spreads exclusively through seeds. *Senna obtusifolia* (L.) Irwin and Barneby (Leguminosae, Caesalpinioideae) is an annual, subshrub, woody and erected plant, all out of root nodules that fix nitrogen, 70 - 160 cm height, probably native from the American continent, popularly known as "white fedegoso", and which only spreads through seeds (LORENZI, 2000).

Both species are weeds, quite frequent in pastures, perennial and annual cultures, orchards and vacant lots all over the Brazilian territory. They generally form large infestations and are rarely controlled, mainly in soya cultures, by selective herbicides used in that crop. In general, they are considered toxic for domestic animals, mainly for cattle, and are casually used in popular medicine (LORENZI, 2000).

Chemical studies performed with *S. occidentalis* leaves and seeds led to the isolation of anthraquinones, xanthones and flavonoids (GUPTA et al., 1995) and chemical studies from *S. obtusifolia* seeds, resulted in the isolation of anthraquinones and the emodine presented a larvicide activity against *Aedes aegypti*, *Aedes togoi* and *Culex pipiens* (HARRY-O`KURO et al., 2005).

The present work aimed to investigate the phytotoxic potential of the aerial and underground parts of *S. occidentalis* and *S. obtusifolia*, through the germination bioassays and the initial growth with lettuce and onion, in laboratory.

Material and methods

Plant material - *S. occidentalis* was collected from a degraded pasture at Boa Vista Farm, Rio Brilhante, Mato Grosso do Sul State (MS), Brazil, (21°45'S, 54°32'W), in November 2002. *S. obtusifolia* was also collected from a degraded pasture, at Núcleo de Ciências Agrárias of the Universidade Federal de Mato Grosso do Sul (UFMS), Dourados (Mato Grosso do Sul State), Brazil, (22°14'S, 54°49'W), in July 2002.

1413 g of *S. occidentalis* (aerial part and root) and 2309 g of *S. obtusifolia* (aerial part and root) were collected, and the plants from both species were in the flowering phase. A dry plant specimen of the species was incorporated to the collection of the Herbarium DDMS from UFMS, in Dourados (Mato Grosso do Sul State), under the following numbers: *S. occidentalis*, Brazil. Dourados: Mato Grosso do Sul, Boa Vista Farm, 10-XI-2002, *A. Sciamarelli* 212 (DDMS); *S. obtusifolia*, Brazil. Dourados: Mato Grosso do Sul, Universidade Federal de Mato Grosso do Sul, 20-VII-2002, *A. Sciamarelli* 213 (DDMS).

After the collection, the aerial (stem, leaves) and underground (roots) parts were separated, packed in plastic bags and frozen in a freezer (-7°C), until use.

Obtaining the crude ethanol extracts (CEEs) – The aerial and underground parts of *S. occidentalis* (1159 and 254 g) and of *S. obtusifolia* (2000 and 309 g), respectively, were broken up in small pieces and exhaustively extracted with commercial ethanol (m v^{-1}, 1:2) at room temperature. After seven days, the solution was filtered in filter-paper and the retained solid materials were discarded. After this, solvent evaporation (ethanol) was achieved under vacuum, in a rotary evaporator (± 40°C), obtaining the CEE from the aerial (ACEE) and underground (UCEE) parts of *S. occidentalis* (96.05 and 20.08 g) and *S. obtusifolia* (120.00 and 15.30 g), respectively. The water content of the CEE was pdetermined from their tax rates, submitted to drying (± 100°C) in a stove for 10h, until the mass was constant, during two consecutive days, to calculate the water mass present in the CEE.

Germination and growth bioassays – To prepare the solutions used in the bioassays, solubility tests of the CEEs were achieved with ethanol and acetone; verifying the best solubility with this one. According to Dayan et al. (2000), acetone is a transference solvent, widely used in bioassays with plants, with no physiological effect detected in solutions with up to 10% v v^{-1}.

The ACEE and UCEE from the two species were weighed, taking into consideration the water content, and dissolved in acetone solution at 5% v v^{-1} (RIMANDO et al., 2001), obtaining a 1000 mg L^{-1} stock solution. The 500 and 250 mg L^{-1} solutions were obtained by the dilution in acetone solution at 5% v v^{-1}. Acetone solution at 5% v v^{-1} was used as control.

The extracts from the vegetal species were tested with lettuce, *Lactuca sativa* cv. Grand rapids (Asteraceae, Magnoliopsida) and onion, *Allium cepa* cv. Baia periforme (Liliaceae, Liliopsida). The germination and growth bioassays were achieved at the Laboratories of Seeds and Chemistry from the Núcleo de Ciências Agrárias, at the Universidade Federal de Mato Grosso do Sul (UFMS), Dourados (Mato Grosso do Sul State, Brazil).

For the germination bioassays, the methodology described by Nishimura et al. (1984) was applied. The Petri dishes (6.0 cm diameter) received Whatman filter-paper disks, number 1.0 (5.5 cm diameter), which were moistened with 1.0 mL of the CEE solutions prepared at 0, 250, 500 and 1000 mg L^{-1} concentrations. Soon after, each filter-paper disk received 50 diaspores from the target-species (lettuce/onion), randomly arranged, with four replications for each treatment, according to Brasil (1992).

The Petri dishes that contained the diaspores were taken to a germination chamber (Mangelsdorf type to lettuce and B.O.D. type to onion) with light conditions (160 W), humidity (± 80%) and constant temperature (lettuce 25 ± 2°C and 12h photoperiod). The germination score was taken daily, having as standard, the root exertions with at least 2.0 mm length.

For growth bioassays, ten germinated seeds were selected and kept in the Petri dishes. After three days, measured the root and the hypocotyl/coleoptile elongations of the seedlings were measured using millimetric paper (BARNES et al., 1987).

The adopted experimental outline was totally randomized, involving four simple assays: CEE from the aerial and underground parts of *S. occidentalis* and

S. obtusifolia, with four treatments (0, 250, 500 and 1000 mg L^{-1}) in four replications. Each parcel consisted of 50 diaspores for germination and 10 for root and hypocotyl/coleoptile growth.

The germination percentage (G%) was calculated according to the methodology described by Labouriau (1983), where $G\% = (\Sigma n_i.N^{-1}).100$ (n_i is the number of germinated seeds in the interval of time $t_{i-1} \leftrightarrow t_i$ and N is the number of seeds used in each treatment). The germination speed index (GSI) was determined according to Maguire (1962), in Ferreira and Borghetti (2004), where $GSI = \Sigma(G_i/N_i)$, where G_i is the number of germinated seeds in the interval of time $t_{i-1} \leftrightarrow t_i$, and N_i is the number of days after sowing.

The data were submitted to the analysis of variance and, when the treatment effects presented significant results ($p < 0.05$) in relation to the control, the means were compared by Dunnet test. When one of assumptions demanded by the parametric model was not approved, non-parametric statistical tests, Kruskal-Wallis, were used as an alternative for the variance analysis and Mann-Whitney as an alternative for Dunnet test.

Results and discussion

On germination results, it is verified that the crude ethanol extract of the aerial part (ACEE) (Table 1) of *S. obtusifolia* reduced the germination speed index (GSI) of the lettuce, in all tested concentrations, observing a delay of 39% germination, in relation to the control, at 1000 mg L^{-1} concentration; but an effect was not observed on the final percentage of germination. The ACEE of *S. occidentalis* did not influence the GSI and the germination percentage of the lettuce (Table 1).

Table 1. Effect of crude ethanol extract from the aerial part (ACEE) of *Senna occidentalis* and *S. obtusifolia* on germination speed index (GSI), germination percentage (G%), root (R) and hypocotyl/coleoptile (H/C) growth from lettuce and onion.

	Lettuce				Onion			
Treatment	GSI[1]	G%[2]	R[1]	H[1]	GSI[1]	G%[2]	R[1]	C[1]
S. occidentalis								
Control	45.60	100.00	31.45	14.23	8.50	87.50	9.60	7.45
250 mg L^{-1}	46.00[ns]	99.00[ns]	33.38★	17.20★	16.40★	97.00★	6.73★	7.90[ns]
500 mg L^{-1}	44.90[ns]	99.00[ns]	31.55[ns]	16.40★	16.10★	96.00★	5.78★	8.00[ns]
1000 mg L^{-1}	43.50[ns]	99.00[ns]	23.05★	16.53★	15.70★	94.00★	5.28★	8.10[ns]
S. obtusifolia								
Control	45.60	100.00	31.45	14.23	8.50	87.50	9.60	7.45
250 mg L^{-1}	36.70★	99.00[ns]	32.45[ns]	14.55[ns]	15.90★	90.00[ns]	7.88★	7.55[ns]
500 mg L^{-1}	36.60★	98.50[ns]	28.00★	13.93[ns]	11.90★	91.50[ns]	7.23★	8.15★
1000 mg L^{-1}	28.00★	98.50[ns]	19.43★	10.23★	11.40★	92.00[ns]	8.18★	6.58★

★The mean of the treatment differ significantly ($p < 0.05$) in comparison to the mean of the control. [1]Dunnet test. [2]Mann-Whitney U test. [ns]The treatment mean does not differ significantly from the mean of the control ($p > 0.05$).

On onion, the ACEE of *S. occidentalis* increased the GSI (85%, 1000 mg L^{-1}) and the germination percentage (7%, 1000 mg L^{-1}) in relation to the control, in all tested concentrations. The ACEE of *S. obtusifolia* increased the GSI of the onion in all tested concentrations; being an increase of 34% at 1000 mg L^{-1} concentration in relation to the control, but it did not significantly influence the germination percentage (Table 1). No significant effect was verified on lettuce and onion germinations, when submitted to the CEE of the underground part (UCEE) on both evaluated plants (Table 2).

Vigor is accepted as a parameter to characterize physiological potential of the seeds, indicating the highest or the lowest probability of success after sowing. The simplest tests for the determination of seed vigor are the development speed tests, whose results may be obtained through the germination process, more specifically through GSI (MARCOS FILHO, 2005).

With these results, it is verified that only the aerial part of both plants contains chemicals that affect the germination of the target-species, observing that the effect varied, depending on the evaluated species. It may observe that ACEE of *S. occidentalis* influenced only on the onion germination. Yet, the ACEE of *S. obtusifolia* reduced the lettuce GSI and increased the onion one, but it did not influence germination development. In general, onion was more sensitive to the extracts of the evaluated species, as for the GSI parameter.

Table 2. Effect of crude ethanol extract from the underground part (UCEE) of *Senna occidentalis* and *S. obtusifolia* on germination speed index (GSI), germination percentage (G%), root (R) and hypocotyl/coleoptile (H/C) growth from lettuce and onion.

	Lettuce				Onion			
Treatment	GSI[1]	G%[2]	R	H	GSI[1]	G%[2]	R	C
S. occidentalis								
Control	45.60	100.00	33.62	18.40	8.50	87.50	7.07[ns]	6.47
250 mg L^{-1}	48.90[ns]	100.00[ns]	30.82★	17.50[ns]	9.50[ns]	89.50[ns]	7.40[ns]	6.60[ns]
500 mg L^{-1}	46.60[ns]	99.00[ns]	28.45★	16.25★	8.90[ns]	88.00[ns]	7.15[ns]	5.92[ns]
1000 mg L^{-1}	46.00[ns]	97.00[ns]	20.85★	15.62★	8.00[ns]	87.50[ns]	6.57★	5.52★
S. obtusifolia								
Control	45.60	100.00	33.62	18.40	8.50	87.50	7.07	6.47
250 mg L^{-1}	47.40[ns]	100.00[ns]	35.72[ns]	18.35[ns]	9.20[ns]	91.50[ns]	7.25[ns]	6.25[ns]
500 mg L^{-1}	46.80[ns]	100.00[ns]	27.15★	15.42★	9.00[ns]	89.50[ns]	7.15[ns]	6.05[ns]
1000 mg L^{-1}	46.20[ns]	99.00[ns]	21.20★	13.12★	7.90[ns]	88.50[ns]	6.27★	5.52★

★The mean of the treatment differ significantly ($p < 0.05$) in comparison to the mean of the control. [1]Dunnet test. [2]Mann-Whitney U test. [ns]The treatment mean does not differ significantly from the mean of the control ($p > 0.05$).

In relation to the initial growth of the seedlings, it is observed that the ACEE of *S. occidentalis* inhibited the root growth of the lettuce (27%) on the largest tested concentration and stimulated the hypocotyl growth (21%, 250 mg L^{-1}) in all tested concentrations (Table 1). On onion, the ACEE of *S. occidentalis* inhibited the root growth in all concentrations (45%, 1000 mg L^{-1}) but it did not affect coleoptile growth (Table 1). These phytotoxic effects may occur from the presence of phenolic substances on *S. occidentalis*, which have already been

reported in literature (VIEGAS JUNIOR et al., 2006).

The ACEE of *S. obtusifolia* inhibited root growth (38 and 25%, 1000 mg L^{-1}) and also inhibited hypocotyl/coleoptile growth (28 and 12%, 1000 mg L^{-1}) from lettuce and onion, respectively.

These results are important, since the marked reduction of the root may affect competitive capacity and plant productivity; and the reduction of the aerial part (hypocotyl/coleoptile) may diminish the capacity of the plant to compete for light (NINKOVIC, 2003).

The stimulatory effects may be caused by some substances on low concentrations, while on high ones, it is inhibitory. Most times, these substances may affect membrane permeability. In high concentrations, they may inhibit water and nutrient absorption; in low ones, they may facilitate the absorption of these substances (EINHELLIG, 2002).

In relation to the *S. occidentalis* and *S. obtusifolia* (Table 2), the inhibition of lettuce growth was observed, both on root (± 38%, 1000 mg L^{-1}) and hypocotyl (15 and 29%, 1000 mg L^{-1}), respectively. It is also observed that the increase on the UCEE concentration caused a higher inhibition on the growth of lettuce seddlings. This effect was followed by morphological changes on the roots, such as oxidation on the root apex, enlargement and absence by the absorbents. Lima and Morais (2008) also verified oxidized root caps on lettuce and tomato, when submitted to aqueous extracts of *Ipomoea fistulosa*.

On onion, the UCEEs from both plants (Table 2) inihibited significantly root and coleoptile growth on the largest tested concentration, with a mean inhibition of 9% on root and 15% on coleoptile.

Soares et al. (2002) observed that the aqueous extracts from leguminosae species present a strong inhibiting effect from the root development of the seedlings, followed by morphological changes on roots. In this work, this fact was observed for the ACEEs and UCEEs as well.

Regarding the results obtained on lettuce and onion bioassays, it may confirm that the growth bioassays were more sensitive than the germination ones, on which the root emergence is made at the expense of the seed reserves; being this fact mentioned on literature (MEDEIROS; LUCHESI, 1993). According to Miró et al. (1998), among the used tests, the root growth is more sensitive to the presence of allelopathic substances than the development of the aerial parts (hypocotyl/coleoptile) or than the germination, confirming the results presented in this work. The reduction of the root system is an important ecological aspect since, with the inhibition of the root development, there is a reduction on the competitive pressure of the plant, which favors the nearby species that may establish dominance aspects.

Most *Senna* species that occur in Brazil present piperidine alkaloids as majority constituents, besides glycosidic flavones, long chain aliphatic esters, glycosidic chromone and polyssaccharides (VIEGAS JUNIOR et al., 2006). Literature mentions the isolation of more than 350 secondary metabolites on species of this genus, distributed in tropical and subtropical regions, in several parts of the world. These studies proved the occurrence of substances from several classes, such as anthraquinones and flavonoids, which are the most frequent constituents on most of the species mentioned in literature (KIM et al., 2004; LUXIMON-RAMMA et al., 2002). It is important to emphasize that these classes of substances have already been mentioned in literature with an allelopathic potential (EINHELLIG, 2002).

It is confirmed that the two species of *Senna*, even though they are from the same family and subfamily, present different behavior in relation to the effects over the germination and growth of the target-species, evidencing that the products of the secondary metabolism are specific for each species.

The changes in germination and growth standards may result in various effects caused in primary level (GUSMAN et al., 2008). Among them, Ferreira and Áquila (2000) highlight changes in membrane permeability, transcription and translation of DNA, in the functioning of secondary messengers, breathing, by oxygen absorption, on the enzymes and receptor forms, or through the combination of these agents.

Conclusion

The results of this work indicate the presence of phytotoxicity promoted by the ethanol extract from aerial and underground parts of *S. occidentalis* and *S. obtusifolia*. This effect is broken up by the reduction in GSI and the inhibition of the initial growth of the seedlings from the evaluated species, as well as by the structural abnormalities found in the seedlings, which require later studies to isolate and identify the substance(s) responsible for the phytotoxic effect.

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