A study of the phytotoxic effects of the aerial parts of *Senecio westermanii* Dusén (Asteraceae) on *Lactuca sativa* L. and *Allium cepa* L. seeds

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The objective of this study was to evaluate the effects of the ethanolic crude extracts and fractions of the species *Senecio westermanii* Dusén on *Lactuca sativa* L. (lettuce) and *Allium cepa* L. (onion) seeds. We assessed the germination, growth, root respiration and photosynthesis of the target species in Petri dishes (9.0 cm diameter) containing filter paper n°6. The study was conducted using 50 seeds per plate and held in 4 replicates per concentration of each sample. In the germination there was an inhibitory effect of fractions hexane (FH) and chloroform (FCl) at concentrations of 500 and 1000 µg/mL. There was a reduction in the radicle growth of lettuce by 14 to 24% and a reduction of hypocotilum by 14 to 28%. As for the radicle of the onion was up 74% reduction to the FCl and the coleoptile was 24 and 45% reduction for FH and FCl, respectively. Inhibitory effects in the root respiration of lettuce were detected in all the samples analyzed, with results ranging from 16 to 83%. For the seeds of *A. cepa,* there was an encouragement for the FCl and ethyl acetate fractions (FAE), with results ranging from 94 to 142% and 76 to 150%, respectively. With regard to the photosynthesis of *L. sativa,* there was no significant difference between the control, and as for the *A. cepa,* there was a strain in inhibition concentrations of 250 and 500 µg/mL, which ranged from 27 to 68%. The samples of *S. westermanii* caused changes in the target species and thus can be used as a natural herbicide.

Keywords: *Senecio westermanii*/extract/effects. *Lactuca sativa. Allium cepa.* Allelochemicals. Allelopathy.

INTRODUCTION

Allelopathy plays a key role in the detection of bioactive compounds of commercial importance. Several studies indicate that the allelochemicals of plant products are agrochemicals ideal for creating biological changes (El-Amier, Abdullah, 2014).

The presence of weeds among crops raises the cost of production for farmers, especially for intensive agriculture, as it induces changes in the population of these plants, changing the predominant species that best fits the middle. The interference of these plants in cultures of commercial interest occurs by the allelopathic effect and competition for water, light, CO_2 and nutrients, causing a qualitative and quantitative reduction in production (Ferreira, Souza, Faria, 2007).

The negative effects of allelopathy on germination lead to the non-uniformity of culture, as the allelochemicals can cause oxidative stress, forming reactive oxygen species and causing damage in the physiological processes and early development of seedlings (Silva, 2012).

The allelopathic effects and mechanism of action of allelochemicals are fundamental to understanding the interaction between plants. The production of allelopathic substances and the natural chemistry differ between plant species, as well as with regard to the sensitivity to allelochemicals of other plants. These differences may affect the species' specifics and quantitative composition of weed communities (Cruz-Silva *et al.*, 2015).

The main release of these components are leaching, volatilization and decomposition, which can change the membrane permeability, absorption of nutrients, growth,

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photosynthesis, enzymatic activity, and respiration. When these substances are released into the environment, they are capable of influencing the metabolism of the surrounding species (Silva, 2012).

The allelopathic compounds produced by higher plants are synthesized by the acetic acid and shikimic routes, and among the most active substances cyanogenic glycosides, phenolic, quinones, lactones, volatile organic acids and terpenes are present (Możdżeń, Repka, 2014).

The production of allelochemicals is influenced by factors such as temperature, humidity and soil type, and your summary is an evolutionary process as part of the defense system. These substances are present in the leaves, bark, roots, flowers, stems, fruits and seeds of various plant species (Borella, Pastorini, 2009).

Francescato *et al.* (2007) comment that one of the main reasons for the use of the genus *Senecio* L. in folk medicine is the different classes of secondary metabolites identified. In this genus, some species were able to change the development of other plant species through bioassays.

According to Ahmed, Wardle (1994), the species *S. jacobaea L.* was able to change the development of *Lolium perenne L.* (perennial ryegrass), *Trifolium repens L.* (white clover), *T. pratense L.* (red clover), *T. subterraneum L.* (subterranean clover) and *Medicago sativa L.* (lucerne). The species *S. brasiliensis* (Spreng) altered the germination and development of *Lactuca sativa L.* (lettuce), *Zea mays L.* (corn) and *Glycine max* (L.) Merr (soy) less (Cruz-Silva, Satorum, Bini, 2009). In addition to these species, *S. vulgaris* presented allelopathic effects against *Sorghum* bicolor (sorghum) (Schutt *et al.*, 1975).

Merino *et al.* (2015) detected the presence of alkaloids, flavonoids, iridoides, steroids, saponinic glycosides and amino group in *S. westermanii*, in a study aimed at evaluating the germination velocity index, growth, root respiration and photosynthesis of *Lactuca sativa L.* (lettuce) and *Allium cepa L.* (onions) in direct contact with crude *ethanolic* extract and fractions hexane, chloroform, ethyl acetate and hydroalcoholic remaining aerial parts of *S. westermanii* Dusén.

MATERIAL AND METHODS

Plant material

Senecio westermanii Dusén was collected at Quatro Barras in Paraná, Brazil, at coordinates 25°18'27" S and 48°56'37" W. The exsicata was deposited (MBM 379066) and identified in the Botanical Museum Municipal of Curitiba, Paraná. The project was regularized in accordance with resolution no. 35, of 27 April 2011, by the Ministry of environment, which provides for regulating access to genetic heritage activities.

Obtaining the crude extract and fractions

6300 g were dried plant at room temperature (25 °C) and crushed in knife and hammer mill. From this mass of crude extract obtained in ethanolic Soxhlet apparatus modified under the registration PI 0601703-7 and concentrated on the 150 rpm rotary evaporator at 65 °C. Then the crude extract (CE) was partitioned by liquid/liquid extraction with solvents of increasing polarity, obtaining fractions of hexane (HF), chloroform (CLF), ethyl acetate (EAF) and remaining (RHF) (Carvalho *et al.*, 2009).

Study of allelopathic activity

5 mL of crude extract and fractions (250 µg/mL, 500 µg/mL and 1,000 µg/mL) were added in Petri dishes (d: 9.0 cm) containing filter paper Whatman (nº. 1.0). Then, they were placed randomly on 50 diasporas of *Lactuca sativa* and *Allium cepa*. The plates containing the diasporas were placed in the germination chamber (BOD) with internal light conditions, constant 160 W, relative humidity (± 80%) and temperature of 25 °C. All testing was conducted in four replications, including distilled water control (Brasil, 2009).

Germination

The readings were taken at 12–12 hours for seven days, always at the same time, considering the germinated the seeds with visible radicle protrusion through the integument. It has been estimated the germination velocity index (GVI) noting daily the number of germinated seeds. In the end, the number of germinated seeds were added by day, by dividing by the number of days from seeding according to the formula: GVI = (G1/N1) + (G2/N2) + ... + (Gn/Nn), where "G" is the number of germinated seeds and "N" is the number of days from sowing to the 1st, 2nd, ..., umpteenth evaluation (Laboriau, 1983).

Growth

For the bioassay, the growth methodology described by Barnes *et al.* (1987) and Macias, Castellano, Molinillo (2000) was followed. After three days of root protrusion, the radicle elongation and hypocotilum (*Lactuca sativa*)/coleoptile (*Allium cepa*), with ten seedlings per plate, were measured with graph paper.

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Respiration of roots

The test was performed according to Steponkus, Lanphear (1967). 10 roots were cut and transferred to test tubes containing 5 mL of triphenyl tetrazolium hydrochloride (TTC) 0.6% (p/v) and 1 mL of buffered sodium phosphate (mono-and dibasic) 0.05 M (pH 7.0). Then, the tubes were left at room temperature for 2 hours. After this period, the tubes were transferred to an oven at 40 °C for 15 hours. The solution was drained and rinsed with distilled water and added with 7 mL of 95% ethanol (v/v) and packed in a water bath (±100 °C) for 15 mins. After cooling, 10 mL of ethanol 95% (v/v) was added, and the reading was performed in the 530 nm spectrophotometer.

Photosynthesis

10 primary leaves—whole and sectioned—were placed in test tubes containing 5.0 mL of DMSO, and the tubes were wrapped in aluminum foil and kept at room temperature (25 °C) for 24 hours. At the end of the period, the readings of chlorophyll a (645 nm) and b (663 nm) were performed with a spectrophotometer. The

total chlorophyll content was calculated according to the equation of Arnon (1949) and Lichtenthaler (1987):

Chlorophyll total: 20.2 x AbsA + 8.02 x AbsB

where: AbsA = absorbance of the chlorophyll a; AbsB = absorbance of the chlorophyll b.

Statistical analysis

From the results, the statistical analysis was performed by the Scott-Knott test (p <0.05), using the software Sisvar 5.4 (Build 80). The graphs were compiled using GraphPad Prism 6.01 software.

RESULTS AND DISCUSSION

According to the results presented with the extract and fractions of *S. westermanii*, the allelopathic pattern in *L. sativa* and *A. cepa* were different. *L. sativa* had an inhibition in the GVI treated with fractions HF and CLF (500 and 1000 µg/mL), while the GVI of *A. cepa* was unchanged by the samples (Figure 1).

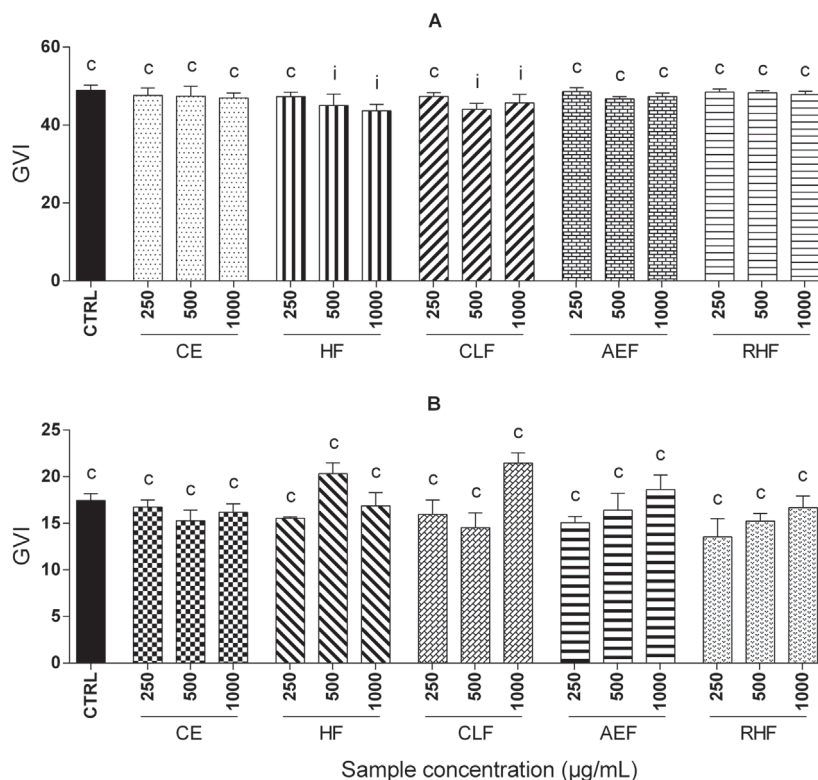The species *L. sativa* (lettuce) in the class of dicots

FIGURE 1 - Germination velocity index (GVI) of *L. sativa* (A) and *A. cepa* (B). Treatment means differ significantly (p< 0.05) when compared to control means [i]inhibiting or [e]stimulating germination velocity by Scott-Knott test. Treatment means do not differ significantly from [c]control means.

is widely used in allelopathic tests for being sensitive to various plant extracts and diminishing germination and changing the GVI (Wandscheer, Pastorini, 2008). While the target species *A. cepa* (onion) belongs to monocotyledons, it is also used in studies of allelopathic interference with extracts of plant species (Abdelgaleil, Hashinaga, 2007).

Several species of plants representing different genus feature allelopathic activity on germination of *L. sativa* as *Solanum megalochiton* Mart (Krause *et al.*, 2016), *Aristolochia esperanzae* O. Kuntze (Gatti, Perez, Lima, 2004), *Bidens pilosa and B. alba* (Lima *et al.*, 2011).

However, for the seeds of *A. cepa*, some studies have shown that this species of monocotyledon was not influenced by other species in germination. A study by Oliveira *et al.* (2012) with different extracts of *Solanum lycocarpum,* where different target species were studied, showed that only the seeds of *A. cepa* were indifferent in germination. The same was observed in Parvez *et al.* (2004) when studying the allelopathic effect with seeds and bark of *Tamarindus indica* L.

The change in germination index demonstrates that there occur changes in metabolic reactions that are part of the germination. But with the rise of that index, there is possibly a loss of sync in these reactions affecting germination (Maraschin-Silva, Aqüila, 2006).

According to Dodd, Donovan (2009), the germination and growth of seeds are critical stages and subject to high rates of failure, as the seeds are more susceptible and less tolerant to various environmental factors.

The allelochemicals inhibit and change the patterns of growth or development of plants, possessing a selective form of action. Plants act by modulating the action of these chemical compound forms, becoming the complex knowledge of attack of the allelochemicals (Gatti, Perez, Lima, 2004). The allelopathic action among species involves a complex chemical interaction between plants because of the low concentrations of allelochemicals in the environment; this demonstrates that the allelopathic action is due to the effect of various substances that act together, causing the allelopathic effect (Maraschin-Silva, Aqüila, 2006).

Belinelo *et al.* (2008) commented that the mechanism by which the allelochemicals act is related to the modification of physiological processes and biochemical processes. Souza, Velini, Maiomoni-Rodella (2003) pointed out that a series of compounds can affect the physiological activity of target plants, and there is a respiration that can be modified for aromatics, aldehydes, phenols, flavonoids and coumarins. In addition to these compounds, they may also have crystals, such as the raphids of calcium oxalate, and natural polymers, such as tannins, lignins and resins.

As the allelopathic effect is mediated by different classes of secondary metabolites, it becomes difficult to identify which of the groups comes from the occurred effect.

The allelopathic activity, as a result of the test, can be linked to the synergistic effect of various allelochemicals found in the species. In addition, the secondary compounds produced by plants are mostly applied in other species, modifying the development and effect of the organisms affected and reducing their populations (Moreira, Souza, Terrones, 2008).

In the studies with species of the genus *Senecio,* it was observed that secondary compounds were responsible for the change in the germination pattern of the target species. The β-caryophyllene compound isolated from *S. salignus* inhibited the germination of *Physalis ixocarpa* at the concentration of 150 µg/mL, but the caryophyllene oxide did not affect germination (Sánchez-Muñoz *et al.*, 2012). The hydroxylated eremophilanolide (1α, 6β, 10β-trihydroxy-8α-methoxyeremophil-7(11)-en-12,8β-olide) isolated from *S. candidans* and *S. magellanicus* reduced the germination of *L. sativa* by 25% (Reina *et al.*, 2012).

Burgueño-Tapia *et al.* (2007) evaluated six cacalolides and six eremophilanolides in *L. sativa* seeds, a cacalolide isolated from *S. madagascariensis* (14-isovaleryloxy-1,2-dehydrocacalol methyl ether **(1)**), two cacalolides from *S. barba-johannis* (13-hydroxy-14-oxocacalohastine **(2)** and 13-acetyloxy-14-oxocacalohastine **(3)**) and three eremophilanolides of *S. toluccanus* (6-hydroxyeuryopsin **(4)**, 1(10)-epoxy-6-hydroxyeuryopsin **(5)** and toluccanolide A **(6)**), in addition, with three cacalolides derivatives (methyl ether **(7)**, cacalol acetate **(8)** and 1-acetyloxy-2-methyloxy-1,2,3,4-tetradehydrocacalol acetate **(9)**) and three eremophilanolides derivatives (6-acetyloxyeuryopsin **(10)**, 6-acetyloxy-1(10)-epoxyeuryopsin **(11)** and toluccanolide A acetate **(12)**). The authors observed that compounds **2-6** and **12** inhibited germination by >50% within 24 hours.

In the root growth, both species have undergone changes in the pattern of growth with the inhibition of the radicle and hypocotilum (lettuce)/coleoptile (onion). For *L. sativa,* the radicle had a growth delay in all concentrations of CE, CLF and the largest concentrations of the other fractions. The results show that there has been a reduction in the development by 14 to 24% in the size of the radicle. The greatest influence occurs in hypocotilum because all the fractions and the CE delayed growth, and there has been a reduction from 14 to 28%. For the radicle of *A. cepa,* there was a similar inhibition pattern, but with a reduction in all concentrations of CE, HF and RHF, there was a reduction in the growth by up to 74% on CLF. As for coleoptile, there was

a reduction in the HF (250 μg/mL) and CLF (250 μg/mL), with a reduction of 24 to 45%, respectively (Figure 2). This shows that the species can change the growth of seedlings tested in early development.

The allelopathic effect of *Sorghum bicolor* on the growth of *L. sativa* and *Zea mays* L. plants that belong to different classes, different results. And for the seeds of lettuce, a reduction in cell elongation of the root was
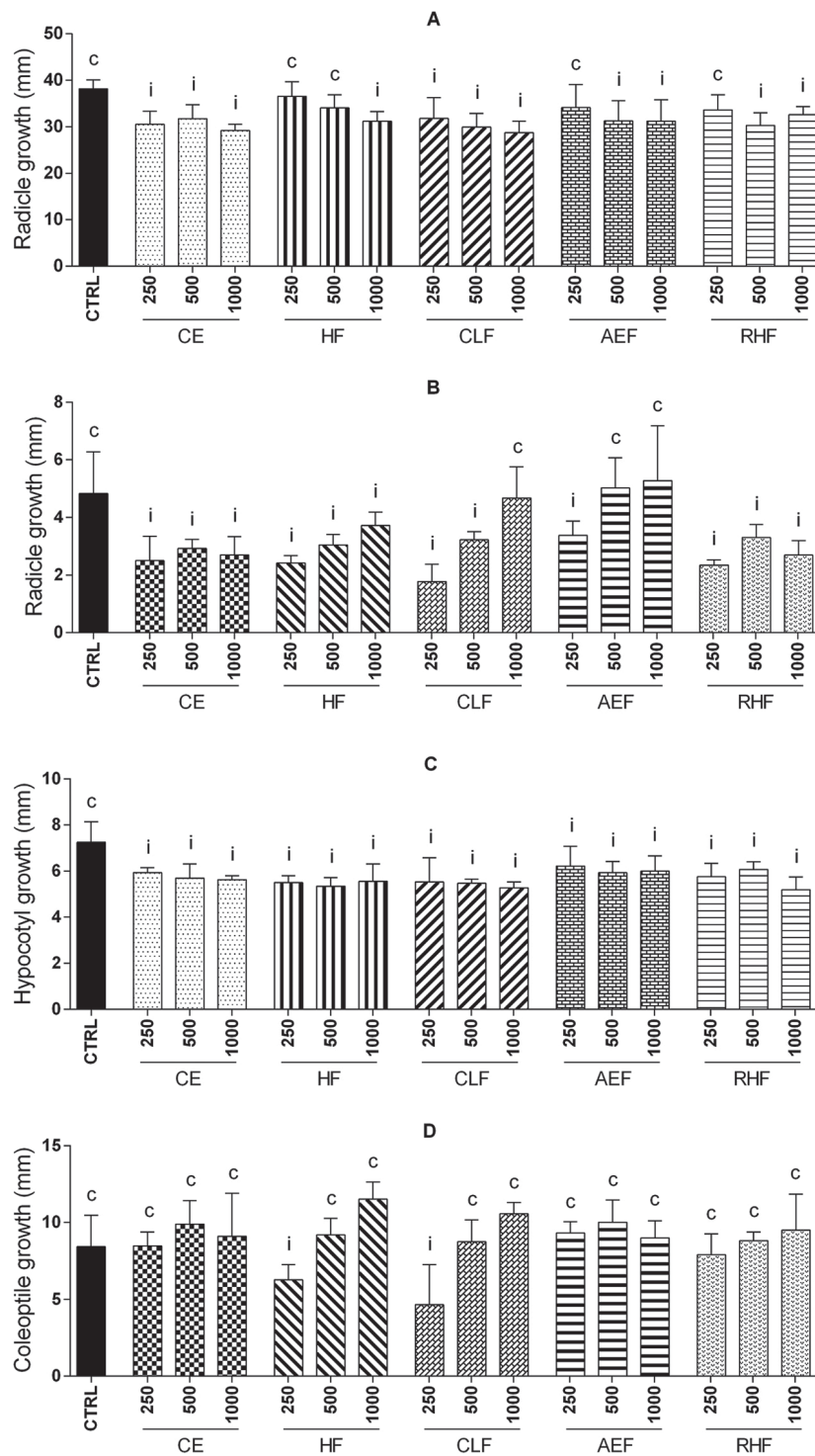

FIGURE 2 - Root growth of *L. sativa* (A and C) and *A. cepa* (B and D). Treatment means differ significantly (p< 0.05) when compared to control means ⁱinhibiting or ᵉstimulating root growth by Scott-Knott test. Treatment means do not differ significantly from ᶜcontrol means.

observed, while for corn there was no significant difference (Netzly *et al.*, 1988). Otherwise, the result demonstrated for the seeds of *A. cepa*, which is also monocots, with the samples of *S. westermanii* was that they were able to change the root development. This demonstrates that the action and the effect sense for species of the same class can present different answers.

The root growth and elongation of the aerial parts are due to the formation of the cambium and xylem, but they depend on the distribution of nutrients for the seedling. In addition, the development occurs through cell division (Hoffmann *et al.*, 2007).

Comparing the effect on the aerial parts with the roots of plants, phytotoxic effects are more pronounced in the root region due to the direct contact of the roots with the fractions, increasing the likelihood of influence of toxins in this region (Cândido *et al.*, 2010).

The bioassays of radicle elongation have been shown to be more sensitive to the allelopathic effects than the germination of seeds (Souza Filho, Guilhon, Santos, 2010). In addition, allelopathic studies have demonstrated inhibitory effects, mainly in primary root (Maraschin-Silva, Aqüila, 2006). The results also demonstrate that when comparing the results of germination and root growth for *L. sativa* and *A. cepa*, it is observed that the inhibitory effect was most prominent in roots compared to germination.

Several studies prove the interference of secondary compounds of the genus *Senecio* on the root growth of the target species. The β-caryophyllene compound isolated from *S. salignus* inhibited the root growth in *P. ixocarpa* by 42 to 53% at concentrations of 50 to 150 µg/mL, respectively, and inhibited the *Echinochloa crus-galli* root length by 30% in a concentration of 150 µg/mL. Moreover, caryophyllene oxide inhibited the elongation of *E. crus-galli* root by 23% and 30% with 100 and 150 µg/mL, respectively (Sánchez-Muñoz *et al.*, 2012).

In addition to the *S. candidans* and *S. magellanicus* species, several sesquiterpenes were isolated, and the derivatives of these compounds were obtained, using an eremophilanolide derivative with a carbonyl at carbon 1 and another with a double bond between carbons 1 and 10, which inhibited the elongation of the *L. sativa* radicle (Reina *et al.*, 2012). The secondary metabolite furanoeremophilane (6β-tigloyl-9-oxofuranoeremophilane) present in *S. macrotis*, *S. asperulus* (Portero *et al.*, 2012) and *S. magellanicus* (Reina *et al.*, 2012) were also able to delay the development of *L. sativa* radicle.

In Burgueño-Tapia *et al.* (2007), a study involving *S. madagascariensis*, *S. barba-johannis* and *S. toluccanus*

species, it was observed that the compounds **(1) (3) (5) (7) (8) (9)** and **(11)**, previously reported, obtained an inhibition on *L. sativa* radicle >50%. In addition to these compounds, the silphinene sesquiterpene isolated from *S. palmensis* (Reina *et al.*, 2002) inhibited the root length of *Amaranthus hypochondriacus* L. (Rivero-Cruz *et al.*, 2000).

Although most compounds delay root growth of the target species, the compounds 6β-hydroxy-8α-methoxyeremophila-1(10),7(11)-dien-12,8β-olide; Tolucanolide A and Tolucanolide C from *S. candidans* and *S. magellanicus* were able to stimulate the root development of *L. sativa* (Reina *et al.*, 2012).

With regard to the root respiration of *L. sativa,* there was inhibition in all the samples analyzed, ranging from 16 to 83%. As for the seeds of *A. cepa,* there was an encouragement for the fractional CLF and EAF with results that ranged from 94 to 142% and 150% to 76, respectively (Figure 3).

The allelochemicals present in plants are able to stimulate or inhibit cellular respiration, and this can occur according to the compounds present.

The allelochemicals alter the water balance and cellular respiration of plants exposed to plant extracts. The cellular respiration is affected because the secondary metabolites interfere in the steps of this process. A change in breathing may have occurred in the cell membranes, thus changing the functioning of mitochondria and contributing to the reduction of plant growth (Carmo, Borges, Takaki, 2007).

The allelopathic compounds present in the extracts can affect the processes of respiration as in the case of the aqueous extracts of *Ocotea odorifera* (Vell.) Rohwer was able to reduce the cellular respiration of root *Sorghum bicolor* L. (Carmo, Borges, Takaki, 2007). In soybean seeds submitted to germination in aqueous extract of tiririca bulbs stimulation of the enzyme glucose-6-phosphate dehydrogenase (6GPD) with an increase in the concentration of extracts. This enzyme is related with pentose phosphate oxidation and, according to the author, could accelerate the respiratory activity due to the presence of substances with such ability in the extract (Muniz *et al.*, 2007).

In the photosynthesis of *L. sativa,* there was no significant difference between the control, but there was a strain in the inhibition concentrations of 250 and 500 µg/mL, which ranged from 27 to 68% (Figure 4).

The reduction in the rate of photosynthesis can be explained by the closing of stomata, which decreases the absorption of CO_2 by these organelles. In addition, the reduction of Mg-porphyrin synthesis can be one of the reasons for the lower production of chlorophyll by *A.*
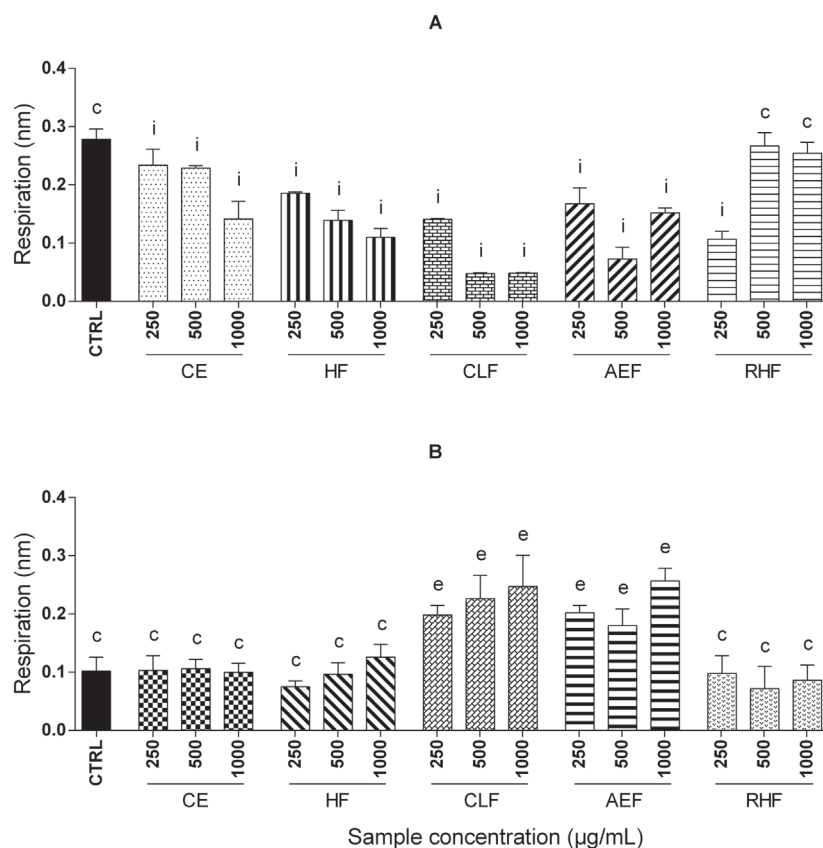
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FIGURE 3 - Root respiration of *L. sativa* (A) and *A. cepa* (B). Treatment means differ significantly (p< 0.05) when compared to control means [i]inhibiting or [e]stimulating root respiration by Scott-Knott test. Treatment means do not differ significantly from [c]control means.

cepa, or the reduction of chlorophyll by the oxidation of the secondary compounds present in the crude extract and fractions may also be the reason (Einhellig, 1986).

The sorgoleone compound extracted from *Sorghum bicolor* inhibited photosynthesis in chloroplasts of pea and soybean leaves (Einhellig *et al*., 1993). In addition to inhibiting photosynthesis, the same metabolite has the ability to reduce oxygenation and cause leaf bleaching (Nimbal *et al*., 1996).

The photosynthesis of the *P. ixocarpa* leaves was evaluated with the β-caryophyllene compound isolated from *S. salignus,* and the samples treated with 100 µg/mL of the isolate, which produced effects on the photosynthesis of the target species in 72 hours of analysis. It was observed that the electron transport per cross section and the maximum quantum yield of primary photochemistry decreased by 50%. In addition, the electron capture and transport decreased by about 50% (Sánchez-Muñoz *et al*., 2012).

Allelopathy is recognized as an important ecological process in natural and managed ecosystems, influencing the primary and secondary plant succession, structure, composition and dynamics of native or cultivated plant communities. In the latter case, the allelochemicals are seen as alternatives to synthetic agrochemicals, aiming at sustainable management and ecological agricultural production. Many allelopathic substances present great potential for use in the biological control of weeds. In contrast to power, the phytotoxic effects of germination and plant growth caused by allelochemicals are also of interest to agricultural management (Maraschin-Silva, Aqüila, 2006).

CONCLUSION

The species *S. westermanii* has phytotoxic activity on the species *L. sativa* and *A. cepa*, proven by the changes caused in the germination velocity index, growth, root respiration and photosynthesis. And for the seeds of *L. sativa,* the greatest influence was observed in radicle growth/root respiration and short embryonic inhibition in all the samples tested. As for *A. cepa,* the biggest influences were observed in the growth of the radicle/coleoptile and photosynthesis, with inhibition
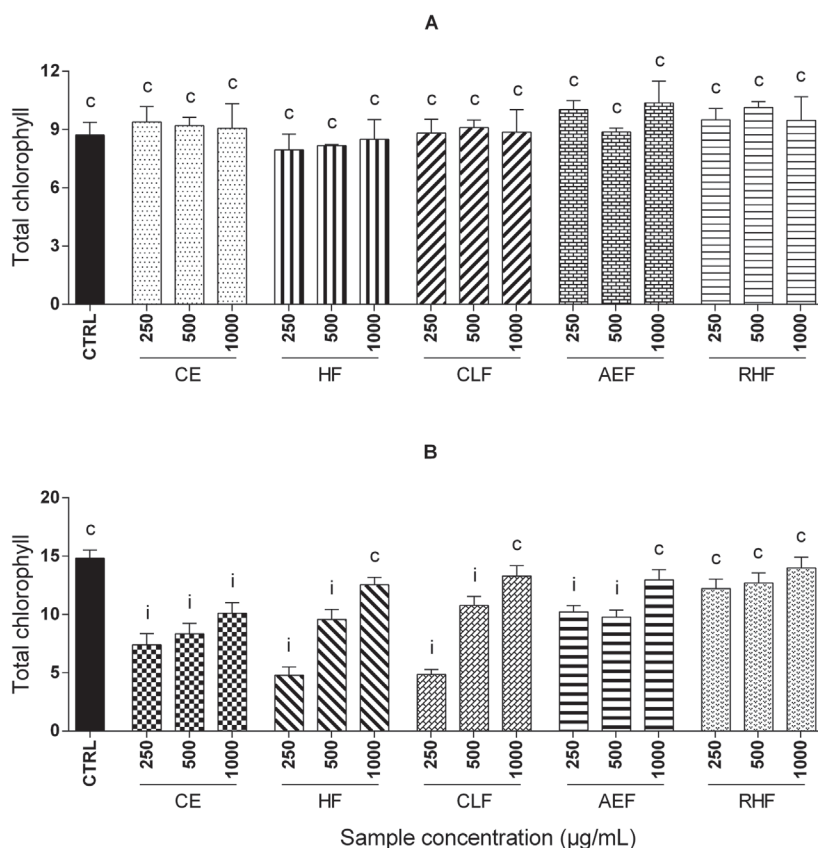
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FIGURE 4 - Photosynthesis of *L. sativa* (A) and *A. cepa* (B). Treatment means differ significantly (p< 0.05) when compared to control means [i]inhibiting or [e]stimulating photosynthesis by Scott-Knott test. Treatment means do not differ significantly from [c]control means.

in all the samples analyzed for root respiration, and there was an encouragement for the chloroform and ethyl acetate fractions. The identification of compounds present in the fractions is important to determine the components responsible for the side effects of inhibition and stimulation, thus making it essential for the isolation of these compounds in subsequent studies. In addition, the species *S. westermanii* is a potential species for use as a natural herbicide, decreasing the environmental impact caused by conventional herbicides.

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DECLARATION OF INTEREST STATEMENT

The author(s) have not declared any conflict of interests.

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