Insight into organoselenium compounds as photosynthesis inhibitors

Investigação dos compostos de organosselênio como inibidores da fotossíntese

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ABSTRACT

The post-emergent herbicidal activities of diphenyl diselenide (**A1**) and 1,2-bis(4-chlorophenyl)diselenide (**A2**) were evaluated against *Senna obtusifolia* and *Ipomoea grandifolia* plants. On the *S. obtusifolia* experiment, compound **A1** showed the best activity at 100 µM, increasing basic fluorescence parameters $\mathbf{F_0}$, $\mathbf{F_0/F_M}$ and $\mathbf{F_J}$ by 36%, 30% and 58%, respectively, indicating the reduction of energy transfer from the antenna complex to the reaction centers. Additionally, compound **A1** reduced the plant vitality index parameters $\mathbf{PI_{abs}}$, $\mathbf{PI_{(CSm)}}$, $\mathbf{PSI_0}$ and $\mathbf{PHI(E_0)}$ by 49%, 45%, 24% and 18%, respectively, suggesting that the redox reactions of the electron transport chain through $\mathbf{Q_A^-}$ were interrupted, indicating that compound **A1** directly interfered on the PSII electron transport. On the *I. grandifolia* experiment, compound **A2** showed the best result at 200 µM, increasing the parameter $\mathbf{F_0/F_M}$ 21% and reducing the parameters $\mathbf{F_V}$, $\mathbf{F_J}$ and $\mathbf{F_I}$ by 14, 9% and 16%, respectively, compared to control. Compound **A2** also reduced the parameters $\mathbf{ABS/CS_M}$, $\mathbf{TR_0/CS_M}$ and $\mathbf{ET_0/CS_M}$ by 19%, 24% and 25%, respectively, indicating a decrease in the absorbed and trapped energy on the active reaction centers in the cross section. Furthermore, the best result on phytotoxicity experiment was achieved for compound **A2** in *I. grandifolia* weed at 100 µM and 200 µM, reducing the root length by 49% and 32%, respectively. Compound **A1** reduced the shoot length by 21% at 200 µM of *S. obtusifolia* weed. These phytotoxic results corroborate the PSII inhibitory activities demonstrated by the Chl *a* fluorescence assay, indicating that **A1** and **A2** have a selective action as a post-emergent herbicide.

Keywords: chlorophyll fluorescence, JIP-test, photosystem II, *S. obtusifolia*, *I. grandifolia*, herbicide.

RESUMO

A ação herbicida pós-emergente de disseleneto de difenila (**A1**) e disseleneto de 1,2-bis (4-clorofenil) (**A2**) foi avaliada em plantas de *Senna obtusifolia* e *Ipomoea grandifolia*. No experimento com a *S. obtusifolia*, o composto **A1** mostrou o melhor efeito a 100 µM, aumentando os parâmetros básicos de fluorescência $\mathbf{F_0}$, $\mathbf{F_0 / F_M}$ e $\mathbf{F_J}$ em 36%, 30% e 58%, respectivamente, indicando a redução da transferência de energia do complexo antena para os centros de reação. Além disso, o composto **A1** reduziu os parâmetros de índice de vitalidade da planta $\mathbf{PI_{abs}}$, $\mathbf{PI_{(CSm)}}$, $\mathbf{PSI_0}$ e $\mathbf{PHI\ (E_0)}$ em 49%, 45%, 24% e 18%,

respectivamente, sugerindo que as reações redox da cadeia de transporte de elétrons através do QA^- foram interrompidas, indicando que o composto **A1** interferiu diretamente no transporte de elétrons do PSII. No experimento com a *I. grandifolia*, o composto **A2** apresentou o melhor resultado a 200 µM, aumentando o parâmetro $\mathbf{F_0}$ / $\mathbf{F_M}$ em 21% e reduzindo os parâmetros $\mathbf{F_V}$, $\mathbf{F_J}$ e $\mathbf{F_I}$ em 14, 9% e 16%, respectivamente, comparados ao controle. O composto **A2** também reduziu os parâmetros **ABS / $\mathbf{CS_M}$**, $\mathbf{TR_0}$ / $\mathbf{CS_M}$ e $\mathbf{ET_0}$ / $\mathbf{CS_M}$ em 19%, 24% e 25%, respectivamente, indicando uma diminuição na energia absorvida e aprisionada nos centros de reação ativos por seção transversal. Além disso, o melhor resultado no experimento de fitotoxicidade foi obtido para o composto **A2** em plantas daninhas de *I. grandifolia* a 100 µM e 200 µM, reduzindo o comprimento da raiz em 49% e 32%, respectivamente. O composto **A1** reduziu o comprimento do caule em 21% a 200 µM da planta daninha *S. obtusifolia*. Esses resultados fitotóxicos corroboram as atividades inibitórias de PSII demonstradas pelo ensaio de fluorescência Chl *a*, indicando que **A1** e **A2** têm ação seletiva como herbicida pós-emergente.

Palavras chave: fluorecência da clorofila, teste JIP, fotossistema II, *S. obtusifolia*, *I. grandifolia*, herbicida.

1 INTRODUCTION

The development of society is directly related to innovations in agriculture. Methodologies that facilitate and increase agricultural production have always been present in human history. The competition for limited resources such as water, light and soil nutrients, makes weed control essential in commodity monocultures, since these species cause a decrease in agricultural production (Heap, 2014; Sheaffer and Seguin, 2003). Constant innovation in weed control methods is crucial, and chemical control is the most used strategy in monocultures. The accelerated metabolism and the high level of resistance to herbicides are some of the characteristics of weeds, making it necessary to constantly search for new bioactive molecules (Arato Ferreira et al., 2016).

Herbicides are a pesticide class that act on plant physiological processes, interfering with the metabolic energy flow and can inhibit the growth and development of weeds (Dayan et al., 2009). Photosynthesis is one of the fundamental physiological processes for the development of plants and has been widely studied to evaluate the performance of plants, as well as their behavior in stress environments such as high and low temperature, water deficit and flood, and herbicide (Paul and Pellny, 2003). In this context, chlorophyll *a* (Chl *a*) fluorescence is a sensitive and powerful technique for discovering bioactive compounds, plant and fungal extracts (Mendes et al., 2019; Moura et al., 2020; Sampaio et al., 2016; Veiga et al., 2013) and an indispensable analysis for assessing changes in plant ecophysiology (Dai et al., 2019; Digrado et al., 2018; Oukarroum et al., 2016), mainly

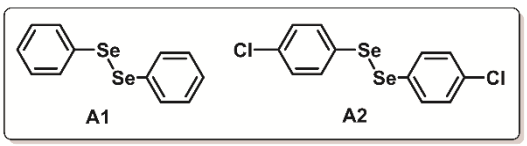
because it is a non-invasive measure of photosynthetic apparatus activity, providing valuable information through the OJIP test (Yang et al., 2018). Additionally, this analytical method allows to investigate the metabolic response of plants when exposed to stressors, since it quantifies fluorescence, vitality and electron transport indexes in the last few decades. (Franić et al., 2018).

Selenium is considered an essential micronutrient for the human diet, since it has antioxidant potential, in addition to being associated with delayed aging and helping in the treatment of various diseases (Brown and Arthur, 2001; Himoto et al., 2011). In last decades, it has been shown that adequate selenium concentrations can be associated with beneficial biological effects in plants (Chauhan et al., 2019; Hartikainen et al., 2000). For instance, selenium has been associated with plant growth stimulation and protective effect against oxidative stress induced by UV radiation (Pennanen et al., 2002). Organic selenium molecules have also shown promising effects as plant growth regulator in different bioassays (Tadino et al., 2003). However, plants exposed to excessive amounts of selenium showed stunted growth, chlorosis, leaf dehydration and a reduction on protein synthesis (Germ et al., 2005; Reich and Hondal, 2016).

Diorganoyl diselenides are organic selenium molecules that have attracted a lot of attention due to their diversified molecular structure and reactivity, producing anionic and radical species of organoselenium (Paulmier, 1986). In addition, the diselenide derivatives have showed important biological activities such as antioxidant, anti-inflammatory and antinociceptive (Nogueira et al., 2003). The antioxidant potential of diselenides is widely explored and has even been associated with protective effects against herbicide-induced toxicity in fish (De Menezes et al., 2012). Therefore, considering the molecular properties of diselenide derivatives, it is relevant to study the effects of these molecules as electron transport inhibitors on photosystem II (PSII).

Our aim in this work is the evaluation and determination of the diselenide derivative (**Figure 1**) effects on PSII and plant development through root and shoot growth assays.

Figure 1. Chemical structure of 1,2-diphenyldiselane (A1) and 1,2-bis(4-chlorophenyl)diselane (A2).



2 MATERIALS AND METHODS

2.1 GENERAL METHOD FOR THE SYNTHESIS OF DISELENIDES **A1** AND **A2**

According to a modified literature procedure (Reich et al., 2003), in a two-neck round-bottom flask equipped with magnetic bar, reflux condenser in nitrogen atmosphere, magnesium (22 mmol) and tetrahydrofuran (30 mL) were added. Then aryl bromide (20 mmol) was slowly added. After the magnesium consumption, elemental selenium (20 mmol) was added slowly. After 1 hour of reaction, a saturated ammonium chloride solution was added dropwise at 0ºC until complete neutralization. Finally, the system was exposed to atmospheric air to allow oxidation of selenol. The resulting residue was extracted with ethyl acetate and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure and the product recrystallizated using hexane.

1,2-diphenyldiselenide (**A1**)

Yellow solid. Yield: 74%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.61 – 7.57 (m, 4H), 7.25 – 7.21 (m, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 127.2, 129.3, 131.1, 132.9.

1,2- bis(4-chlorophenyl)diselenide (**A2**)

Yellow solid. Yield: 61%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.53 (d, *J* = 8.6 Hz, 4H), 7.25 (d, *J* = 8.6 Hz, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 134.22, 133.18, 129.55, 129.29.

2.2 CHLOROPHYLL A FLUORESCENCE MEASUREMENT IN VIVO ASSAY

According to a modified literature procedure (de Souza et al., 2020), the *in vivo* experiment was carried out employing *Senna obtusifolia* and *Ipomoea grandifolia* plants. Plastic pots (top diameter, 10.5 cm; bottom diameter, 7.5 cm; height, 7.0 cm) filled with approximately 100 g of a mixture of 50:50 w/w soil/vermiculite (plant growth medium), ten seeds were added and the pots were kept in a greenhouse under normal day/night illumination at 25-30°C. The plants of a similar size were selected and separated into three groups: control (DMSO), positive control (DCMU) and an experimental treatment using compounds **A1** and **A2** at 100 and 200 µM. A Chl *a* fluorescence measurement of intact leaves was performed 24 hours after application of the compounds. The experiment was carried out with mean 21 measurements. The Chl *a* fluorescence induction curves were measured at room temperature with a portable Hansatech Fluorescence Handy PEA (plant

efficiency analyzer) apparatus. The photosynthetic parameters associated with PSII were calculated using Biolyzer®-HP3 software (**Table 1**).

Table 1. Formulae and explanation of the technical data of the OJIP curves and the selected JIP-test parameters used in this study.

Specific energy fluxes (per cross section)	
DI_0/CS_m	Dissipated energy flux per excited cross section at t = 0
ABS/CS_m	Absorption flux per excited cross section at t = t_{Fm}
TR_0/CS_m	Trapping flux per excited cross section at t = t_{Fm}
ET_0/CS_m	Electron transport flux per excited cross section at t = t_{Fm}
DI_0/CS_m	Dissipated energy flux per excited cross section at t = t_{Fm}
Quantum yield	
$PHI(E_0)$	Quantum yield for electron transport at t = 0
PSI_0	Probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$PHI(D_0)$	Quantum yield for energy dissipation
Performance indexes	
$PI_{(abs)}$	Performance index on absorption basis
$PI_{(CSm)}$	Performance index defined on cross section basis at the moment of maximal fluorescence intensity

2.3 PLANT GROWTH ASSAY

To evaluate the effect of compounds **A1** and **A2** on *S. obtusifolia* and *I. grandifolia* growth, the plant length was measured after 15 days of treatment. The shoot and root measurements were performed using a ruler (± 0.1 cm) and the values obtained were statistically evaluated. Negative and positive controls were performed using DMSO and DCMU, respectively (Moura et al., 2020).

2.4 STATISTICAL ANALYSIS

Chl *a* fluorescence measurements and plant growth experiment were performed in a completely randomized design. Statistical analyses were performed using SPSS 17.0 (SPSS Inc.) Data were analyzed by means, including analysis of variance (ANOVA), Levene's test for homogeneity of variance and normality, and Tukey's-b ($p < 0.05$ and $p < 0.01$) for differences between averages in all tests (Mendes et al., 2019).

3 RESULTS AND DISCUSSION

3.1 DISELENIDE DERIVATIVES SYNTHESIS

The synthesis of diselenide derivatives **A1** and **A2** was based on the reaction of the corresponding Grignard reagent with elemental selenium, followed by mild oxidation in

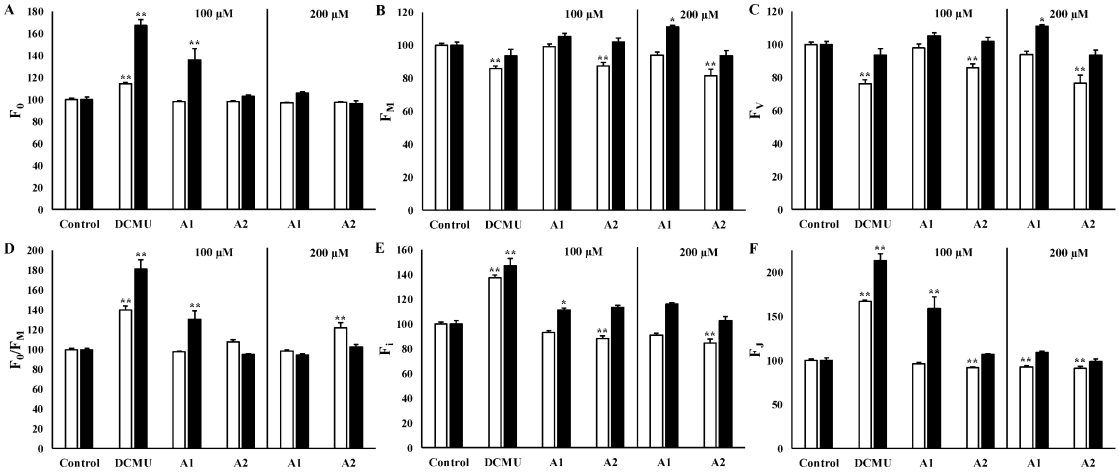
air. Compound **A1** and **A2** were obtained in good chemical yield, characterized by ^{1}H NMR and ^{13}C NMR spectroscopy, and the results corroborated literature data (Reich et al., 2003).

3.2 CHLOROPHYLL A FLUORESCENCE MEASUREMENT

Compounds **A1** and **A2** were evaluated in *in vivo* assay employing *S. obtusifolia* and *I. grandifolia* weeds at 100 µM and 200 µM. The effects of **A1** and **A2** on photosynthetic parameters were evaluated after 24 hours after treatment with the active compounds.

Among the diselenides derivatives, compound **A1** showed the best results against *S. obtusifolia* plants at 100 µM. Compound **A1** increased at 100 µM the basic fluorescence parameters $\mathbf{F_0}$, $\mathbf{F_0/F_M}$ and $\mathbf{F_J}$ by 36%, 30% and 58%, respectively (**Figure 2** – Charts **A**, **D** and **F**). The increase of $\mathbf{F_0}$ and $\mathbf{F_0/F_m}$ parameters demonstrated the stress effects on the plants (Slabbert and Krüger, 2011), indicating the reduction of energy transfer from the antenna complex to the reaction centers. These results are related to the antenna complex, photochemical efficiency and electron flow of the quinones, that suggests compound **A1** inhibits the redox process of the primary electrons in the PSII (Tongra et al., 2011).

Figure 2. Basic fluorescence parameters $\mathbf{F_0}$ (Chart **A**), $\mathbf{F_M}$ (Chart **B**), $\mathbf{F_V}$ (Chart **C**), $\mathbf{F_0/F_M}$ (Chart **D**), $\mathbf{F_I}$ (Chart **E**) and $\mathbf{F_J}$ (Chart **F**) of *S. obtusifolia* (black) and *I. grandifolia* (white), 24 hours after treatment with **A1** and **A2** at 100 µM and 200 µM. (*) and (**) indicate significant difference from control (DMSO) by $p < 0.05$ and $p < 0.01$, respectively.

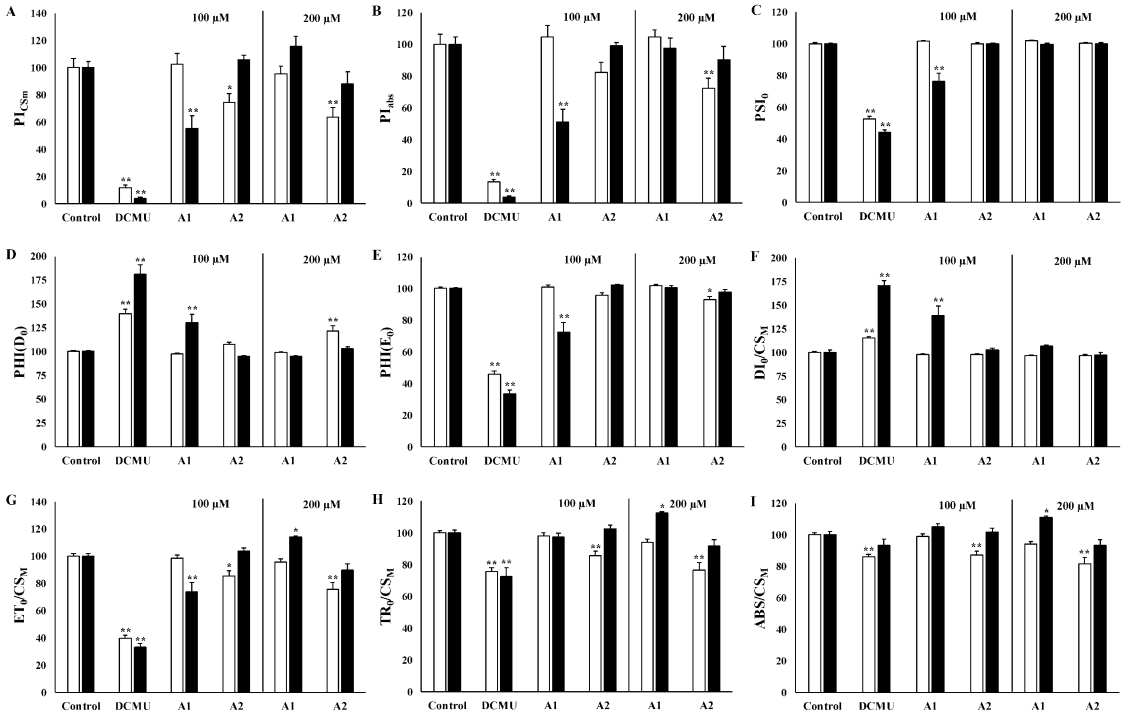


Compound **A1** at 100 µM reduced the plant vitality index parameters $\mathbf{PI_{abs}}$, $\mathbf{PI_{(CSm)}}$, $\mathbf{PSI_0}$ and $\mathbf{PHI(E_0)}$ by 49%, 45%, 24% and 18%, respectively, and increased the parameter $\mathbf{PHI(D_0)}$ by 30% compared to control (**Figure 3** – Charts **A-E**), which indicates the photosynthetic activity of *S. obtusifolia* plants was potentially inhibited (Oukarroum et al.,

2016). The **PI_{abs}** and **PI_{CSm}** parameters describe the general activity of PSII, and their reduction indicates that compound **A1** caused stress and irreversible damage to the photosynthetic apparatus (Slabbert and Krüger, 2011). The variation observed at photosynthetic quantum parameters **$PHI(D_0)$, PSI_0** and **$PHI(E_0)$** suggests that the redox reactions of the electron transport chain through **Q_A^-** were interrupted, indicating that compound **A1** directly interfered on the PSII electron transport (Chen et al., 2011).

The effects of compounds **A1** and **A2** on energy flow parameters in *S. obtusifolia* plants are displayed in **Figure 3** – Charts **F-I**. Compound **A1** increased the parameters related to cross section **DI_0/CS_M** by 39% at 100 µM, and the parameters **TR_0/CS_M** and **ABS/CS_M** by 12% and 11% at 200 µM, indicating the absorbed and trapped energy by the system was not used in the electron transport chain, as it was dissipated as heat (de Carvalho et al., 2016). Furthermore, compound **A1** reduced the electron transport by cross section parameter (**ET_0/CS_M**) by 26% compared to control, showing a blockage on electron transfer system, which is responsible for photosynthesis performing (Tongra et al., 2011).

Figure 3. Performance index parameters **PI_{CSm}** (Chart - **A**) and **PI_{abs}** (Chart - **B**), quantum yield parameters **PSI_0** (Chart - **C**), **$PHI(D_0)$** (Chart - **D**), **$PHI(E_0)$** (Chart - **E**), specific energy flux parameters **DI_0/CS_M** (Chart - **F**), **ET_0/CS_M** (Chart - **G**), **TR_0/CS_M** (Chart - **H**) and **ABS/CS_M** (Chart - **I**) of *S. obtusifolia* (black) and *I. grandifolia* (white), 24 hours after treatment with **A1** and **A2** at 100 µM and 200 µM. (*) and (**) indicate significant difference from control (DMSO) by $p < 0.05$ and $p < 0.01$, respectively.



Although compound **A2** showed no appreciable Chl *a* fluorescence results in *S. obtusifolia* plants experiment, compound **A1** demonstrated efficiency in the PSII inhibition at low concentration. Among the several factors that compound penetration into the living plant is impeded, the membrane permeability, compound solubility, subcellular compartmentalization, and a number of complicating physiological process potentially be reason for diselenides herbicide activity observed (Avram et al., 2014).

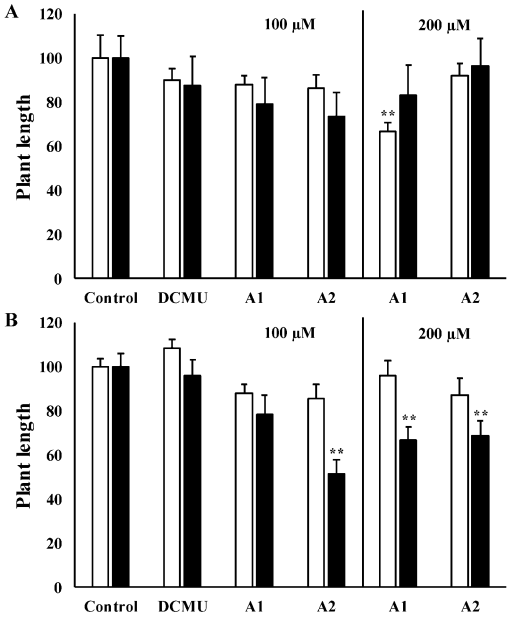
On the *I. grandifolia* experiment, compound **A2** showed the best result at 200 µM. Compound **A2** increased the parameter $\mathbf{F_0/F_M}$ 21% and reduced the parameters $\mathbf{F_V}$, $\mathbf{F_J}$ and $\mathbf{F_I}$ by 14, 9% and 16%, respectively, compared to control (**Figure 2** – Charts **C-F**). These results demonstrate the inhibitory effect of compound **A2** by blocking the electron transport on the PSII donor site (Aksmann and Tukaj, 2008). Additionally, compound **A2** reduced the vitality indexes parameters $\mathbf{PI_{abs}}$ and $\mathbf{PI_{CSm}}$ by 28% and 37%, respectively, showing any stress in the photosynthetic apparatus (**Figure 3 – Charts A-B**).

The reduction of PHI(E_0) by 9% and increase of PHI(D_0) by 21% and 10%, respectively (**Figure 3** – Charts **D-E**), confirm the electron transport chain was affected in the primary photochemical process leading to an accumulation of Q_A^- at PSII. The parameters related to maximum cross section $\mathbf{ABS/CS_M}$, $\mathbf{TR_0/CS_M}$ and $\mathbf{ET_0/CS_M}$ were reduced by 19%, 24% and 25%, respectively (**Figure 3** – Charts **G-I**), indicating a decrease in the absorbed and trapped energy on the active reaction centers in the cross section, which demonstrates the inhibition of electron transport chain on PSII (Tongra et al., 2011).

3.3 PLANT GROWTH

The phytotoxic activity of compounds **A1** and **A2** was evaluated through plant growth assays employing *S. obtusifolia* and *I. grandifolia* weeds. Plant length (shoot and root) was measured after 10 days of treatment with **A1** and **A2** at 100 µM and 200 µM. DMSO was used as control and DCMU was employed as positive control (**Figure 4**).

Figure 4. Shoot length (white) and root length (black) of *S. obtusifolia* (Chart - **A**) and *I. grandifolia* (Chart - **B**), 10 days after treatment with **A1** and **A2** at 100 µM and 200 µM. (**) indicates significant difference from control (DMSO) by $p < 0.05$.



On the *S. obtusifolia* weed (**Figure 4** – Chart **A**), compound **A1** showed the best result, reducing the shoot length by 21% at 200 µM compared to control. The shoot is responsible for nutrient and water transportation to the leaves, being a vital organ in plant development (Dotray and Young, 1993). The PSII inhibitory activity in addition to nutrient transportation reduction indicates the herbicidal activity of compound **A1** is potentially associated to the physiological processes.

The best result was achieved for compound **A2** in *I. grandifolia* weed at 100 µM and 200 µM, reducing the root length by 49% and 32%, respectively (**Figure 4** – Chart **B**). Compound **A1** decreased root length by 34% at 200 µM. The roots are responsible for the absorption of nutrients found in the soil, mass flow, plant transpiration, among other vital functions. The quantitative growth of weed roots is the main competitive factor for these plants (Dotray and Young, 1993).

These phytotoxic results corroborate the PSII inhibitory activities demonstrated by the Chl *a* fluorescence assay, indicating that **A1** and **A2** have a selective action as a post-emergent herbicide, blocking the electron transfer process at PSII in the weeds.

4 CONCLUSION

This work demonstrates the evaluation of diselenides derivatives as photosynthesis and plant growth inhibitors. The diselenide derivatives act as the post-emergent herbicide

prototype since the plant length was reduced through plant growth assays employing *S. obtusifolia* and *I. grandifolia* weeds. Compounds **A1** and **A2** decreased the phenomenological parameters $\mathbf{PI_{abs}}$ and $\mathbf{PI_{CSm}}$ indicating inhibition of electron transport chain on PSII. On the *S. obtusifolia* assay, compound **A1** reduced the shoot length by 21% at 200 µM compared to control. The best result was achieved for compound **A2** in *I. grandifolia* weed at 100 µM and 200 µM, reducing the root length by 49% and 32%, respectively. The plant growth assay corroborates the Chl *a* fluorescence results, since the electron chain blockage by these compounds may lead to diminished ATP synthesis and CO_2 fixation which interrupt the plant development.

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