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Article

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PHYTOTOXIC POTENTIAL OF THE CRUDE EXTRACT AND LEAF FRACTIONS OF *Machaerium hirtum* ON THE INITIAL GROWTH OF *Euphorbia heterophylla* AND *Ipomoea grandifolia*

*Potencial Fitotóxico do Extrato Bruto e Frações Foliares de **Machaerium hirtum** sobre o Crescimento Inicial de Plântulas de **Euphorbia heterophylla** e **Ipomoea grandifolia***

ABSTRACT - Allelopathy is the term used to define any process involving secondary metabolites produced by plants and microorganisms that influence growth and development of agrobiological systems. Currently, it is sought to find allelochemicals of interest and know how to apply them in bio-herbicides to combat weeds. In this study, the effects of the crude leaf extract and fractions of *Machaerium hirtum* (Vell.) Stellfeld were analyzed on *Euphorbia heterophylla* L. (wild poinsettia) and *Ipomoea grandifolia* (Dammer) O'Donell (morning glory), as well as the occurrence of morphoanatomical changes. For this, 0.04 g of the crude extract and fractions were solubilized and diluted (50 mL) to concentrations of 0.1, 0.2, 0.4, and 0.8 g L^{-1} (m/v). Initial growth tests were performed on Petri dishes containing two paper sheets and seedlings of weed species with the respective treatments, being maintained in a germination chamber for 48 hours at 25 °C. Distilled water was used as a control. Shoot and root length was assessed in the initial growth. The percentage of inhibition was calculated based on the values obtained in the initial growth bioassays. Morphologically altered wild poinsettia seedlings were fixed and sectioned transversely for anatomical analysis. The results indicated significant changes in length, being wild poinsettia seedlings more sensitive when compared to those of morning glory. Morphologically altered seedlings presented root necrosis as the most frequent symptom. Anatomically, parenchymatic cells of the hypocotyl and roots of wild poinsettia seedlings presented smaller and irregularly shaped cells when compared to the control, causing significant reductions in the measured parameters.

Keywords: inhibition, allelopathy, morphoanatomy, weeds.

*RESUMO - Alelopatia é o termo utilizado para definir qualquer processo envolvendo metabólitos secundários produzidos por plantas e microrganimos que influenciem no crescimento e desenvolvimento de sistemas agrobiológicos. Na atualidade, busca-se encontrar aleloquímicos de interesse e saber como aplicá-los em bio-herbicidas para combater plantas daninhas. Neste trabalho foram analisados os efeitos do extrato foliar bruto e das frações de **Machaerium hirtum** (Vell.) Stellfeld em duas espécies daninhas: **Euphorbia heterophylla** L. (leiteira) e **Ipomoea grandifolia** (Dammer) O'Donell (corda-de-viola), assim como a ocorrência de alterações morfoanatômicas. Para isso, foram solubilizados e diluídos 0,04 g do extrato bruto e frações (50 mL) até as concentrações de 0,1, 0,2, 0,4 e 0,8 g L^{-1} (m/v). Os testes de crescimento inicial foram realizados em placas de Petri*

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contendo duas folhas de papel, e as plântulas das espécies daninhas com os tratamentos respectivos, mantidos em câmara de germinação por 48 horas a 25 ºC; utilizou-se como controle dos testes somente água destilada. Foi avaliado no crescimento inicial o comprimento radicular e aéreo. A porcentagem de inibição foi calculada com base nos valores obtidos nos bioensaisos de crescimento inicial. As plântulas de leiteira alteradas morfologicamente foram fixadas e seccionadas transversalmente, para análise anatômica. Os resultados indicaram alterações significativas no comprimento; as plântulas de leiteira foram mais sensíveis que as de corda-de-viola. Plântulas morfologicamente alteradas apresentaram necrose radicular como sintoma mais frequente. Anatomicamente, as células parenquimáticas do hipocótilo e das raízes das plântulas de leiteira apresentaram células menores e com formatos irregulares quando comparadas ao controle, o que ocasionou reduções significativas nos parâmetros mensurados.

Palavras-chave: inibição, alelopatia, morfoanatomia, invasoras.

INTRODUCTION

Weeds cause damage to the environment due to their invasion of cultivated and natural areas, representing one of the main problems of world agriculture. They are associated with threats to crop production and changes in ecological processes in the environment. One of the characteristics of weeds is a high seed production, showing a fast growth and reproduction after germination, being better competitors than other species, in addition to presenting a fast dispersion (Oliveira et al., 2016; Trognitz et al., 2016).

Invasive plants compete for resources with neighboring plants in their environment. In addition, plants produce, through their secondary metabolism, allelochemical compounds that cause to the environment a phenomenon known as allelopathy, affecting plant development. These allelochemicals can cause direct or indirect phytotoxic effects on agricultural and biological systems (Ferreira and Aquila, 2000; Souza et al., 2005; Cremonez et al., 2013; Cheng and Cheng, 2015).

The term allelopathy was coined by Hans Molisch (1937), who conceptualized the biochemical interactions between plants and microorganisms. Rice (1984) defined allelopathy as any detrimental or beneficial effect of one organism (plants or microorganisms) on another through the production of chemical compounds released into the environment (Rice, 1984; Einhellig, 1995). Allelochemicals may exhibit allelopathic effects when in neutral, acidic, and alkaline weak aqueous solutions or phytotoxic effects when in solutions with organic solvents (Reigosa et al., 2013).

At the same time that weeds release allelochemicals in the environment, they are also subject to the action of other allelochemicals of other plants. It is known that all plant organs can produce allelochemicals (Weston, 1996; Pelegrini and Cruz-Silva, 2012), which are released into the environment in various forms: volatiles, leachate, root exudation, and decomposition (Rice, 1984; Chou, 1992; Weir et al., 2004).

Currently, there is a growing interest related to allelochemicals, with researches that aim at identifying therapeutic potentials (Cechinel Filho and Yunes, 1998; Albuquerque and Hanazaki, 2006) or bio-herbicides (Vyvyan, 2002; Duke, 2010; Soltys et al., 2013). These researches with bio-herbicides aim at finding allelochemicals that have phytotoxic properties and can be used in weed management, as they are less harmful to the environment than synthetic chemicals due to their natural origin. They have in the composition elements that make them less toxic, but it is necessary to understand the mechanisms of action of these allelochemicals to properly understand the biochemical interactions that occur between plants in the natural environment (Duke et al., 2007; Duke, 2010).

The invasive plants *Euphorbia heterophylla* and *Ipomoea grandifolia* are harmful because they can affect crop productivity. It has been reported that they may resist to the use of glyphosate and herbicides that inhibit the enzymes acetolactate synthase (ALS) and protoporphyrinogen oxidase (PROTOX) (Trezzi et al., 2005, 2006; Vila-Aiub et al., 2008; Lorenzi, 2008; Pazuch et al., 2013; Vargas et al., 2013, 2016). Therefore, the research of new molecules with an herbicide potential, especially those of natural origin, can help in controlling weeds in a sustainable way.

Phytochemical studies and previous biological assessments performed by Ignoato et al. (2012) showed that chemical compounds produced by *Machaerium hirtum* have anti-inflammatory and cytoprotective properties (Ribeiro et al., 2015), but the presence of allelopathic and phytotoxic activity has not been studied to date. Thus, this study proposes to assess the phytotoxic potential of crude extract and leaf fractions of *M. hirtum* on the initial seedling growth of the invasive species wild poinsettia and morning glory, as well as the occurrence of morphoanatomical changes.

MATERIAL AND METHODS

Plant material and obtainment of crude extract and leaf fractions

Leaves of *M. hirtum* were collected in October 2008 in the Upper Paraná River Flood Plain, in Porto Rico, PR, Brazil, and taken to the laboratory, where they were dried in the open air, obtaining a leaf dry matter of 271.2 g. After the leaves were ground, an exhaustive methanol extraction (degree of distillate purity) was performed and a crude extract (CE 51.7 g) was obtained. An amount of 20 g of this extract was taken to be solubilized in an aqueous solution of 30% methanol and fractionated with solvents of increasing polarities, obtaining the hexanic, chloroformic, ethyl acetate, and hydromethanolic fractions. These fractions were evaporated in a rotary evaporator at 45 °C and the following masses were obtained for each fraction: hexanic (FH, 2.4 g), chloroformic (FCl, 1.5 g), ethyl acetate (FAc, 1.8 g), and hydromethanolic (FHm, 9.2 g). The fractions were stored at 4 °C until use (Ignoato et al., 2012).

Solubilization of the crude leaf extract and organic fractions of *Machaerium hirtum*

To perform the bioassays, 0.040 g of CE and of the fractions FH, FCl, FAc, and FHm were solubilized and diluted with distilled water to a volume of 50 mL (m/v). From this volume, an aliquot of 25 mL was taken and the remaining volume was diluted in order to obtain the concentrations 0.8, 0.4, 0.2, and 0.1 g L^{-1}.

Initial growth bioassays

For the initial growth bioassays, we used seedlings of the weed species wild poinsettia and morning glory purchased from Agro Cosmos (Cosmos Agrícola Produção e Serviços Rurais Ltda.). These seedlings were obtained after seed germination in distilled water. To break the dormancy, wild poinsettia seeds were immersed in water at ambient temperature for 30 min and then washed in running water for 5 min, according to the modified methodology by Vargas et al. (1999). Morning glory seeds were submitted to a chemical scarification with sulfuric acid (H_2SO_4 P.A.) for 40 min with stirring every 10 min, according to the modified methodology of Pazuch et al. (2015) and Azania et al. (2011). Subsequently, these seeds were washed in running water for 5 min and placed to germinate in a germination chamber with a 12 hour photoperiod (light and dark) at 25 °C for 48 hours for wild poinsettia (modified method by Kern et al., 2009) and 24 hours for morning glory (modified method by Azania et al., 2011).

After radicle protrusion (2 mm), 10 seedlings of wild poinsettia and morning glory were transferred to glass Petri dishes (9 cm) containing two filter paper discs and 5 mL of the irrigating solutions, with each treatment composed of five replications (totaling 50 seedlings). These plates were incubated in an Eletrolab climatic germination chamber model 102G under a 12 hour photoperiod (light and dark, illuminated with white fluorescent lamps of 20 W, daylight type), at a constant temperature of 25 °C for 48 hours. After incubation, the growth of the hypocotyl (aerial part) and primary root were measured in five seedlings (totaling 25 seedlings) using graph paper (Ferreira and Aquila, 2000; Ferreira and Borghetti, 2004). The remaining seedlings were used for the morphoanatomical assessments.

Percentage of initial growth inhibition

The percentage of inhibition was calculated based on the values measured in the initial growth (primary root and hypocotyl length) bioassays using the equation % inhibition =

(μT - μC/μC) x 100, where μT corresponds to the average values of treatments (primary root and hypocotyl length) and μC corresponds to the average values of the test control. The results are shown in bar graphs, where positive results imply a stimulation of the analyzed parameters and negative results express their inhibition (Oliveira et al., 2012).

Morphoanatomical assessment

For preparing the histological slides, five remaining wild poinsettia seedlings from the initial growth bioassays (CE 0.1 g L^{-1}, FAc 0.2 and 0.4 g L^{-1}, and FCl 0.1 g L^{-1}) were transferred to 1% glutaraldehyde fixing solution in 0.1 M phosphate buffer pH 7.2 (Karnovsky, 1965). After 48 hours, they were submitted to dehydration in an alcoholic series (Johansen, 1940). Dehydrated seedlings were included in a Leica historesin following the specifications of the manufacturer. After hardening, historesin blocks were cross-sectioned in a rotating microtome and subsequently stained with toluidine blue, as in O'Brien et al. (1964).

The photographs of hypocotyl and primary root sections were registered using a light microscope coupled to a Leica EZ4D digital camera. Images were processed by the software Leica Application Suite version 1.8. To verify changes in seedling morphoanatomy, root and hypocotyl diameter and their parenchyma tissue thickness were measured with the software Image-Pro Plus version 4.5.0.29.

Statistical analysis

The experimental design was a completely randomized design. The data obtained that met the assumptions of normality and homoscedasticity were analyzed by ANOVA and compared by the Tukey's test ($p>0.05$) using the software Statistica 7.0 and Assistat 7.7. When the assumptions of homoscedasticity and normality were not reached, the data were submitted to the Kruskal-Wallis non-parametric test and compared to the Simes-Hochberg post-test ($p>0.05$) using the statistical software Action Stat 3.1.

RESULTS AND DISCUSSION

There was no growth of wild poinsettia seedlings under CE and FCl at concentrations of 0.2, 0.4, and 0.8 g L^{-1} (Table 1), which resulted in high inhibitory rates of 63 and 46% (root/hypocotyl) and 83 and 56% (CE and FCl, respectively), indicating FCl as the most inhibitory (Figures 1 and 2).

Wild poinsettia seedlings maintained under FAc and FHm had dose-dependent root elongation, i.e. root length decreased as the concentrations significantly increased (Table 1). Hypocotyl growth of seedlings under FHm was lower when compared to control only at the highest concentrations (0.4 and 0.8 g L^{-1}), while under FAc all concentrations reduced hypocotyl growth.

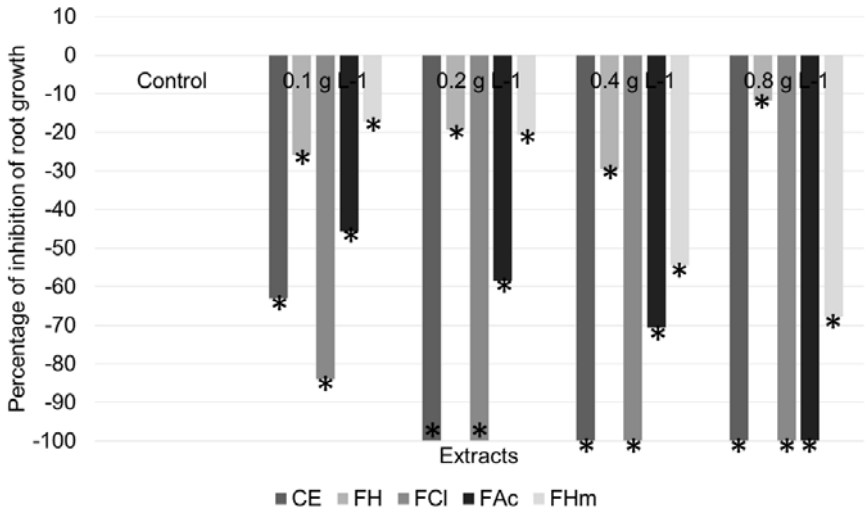
Morphological changes in wild poinsettia seedlings were not very evident, except under the action of CE and FCl, in which a darkening was registered in the root apex (Figure 3A). Anatomical sections of the root showed that CE (Figure 4D) and FAc (Figures 4F, G, H) changed the cell structure of the radicle parenchyma tissue and hypocotyl of wild poinsettia. Irregularly shaped parenchyma cells were observed (arrow), differing from those of the control, which presented a regular and uniform shape (Figure 4A, B).

In seedlings treated with FAc, a bulky and conspicuous presence of hairs was observed throughout the root extension at all tested concentrations (data not shown in the photographs). Root hair (trichoblasts) are specialized epidermal cell projections responsible for water absorption and fixation (Dolan and Davies, 2004; Yu et al., 2014). The hormone auxin is necessary for the formation and growth of root hair (Lombardo et al., 2006). Krasuska et al. (2016), Gniazdowska et al. (2015), and Araniti et al. (2016) argue that the inhibition of root growth by phytotoxins can induce an oxidative stress by the accumulation of reactive oxygen species (ROS) and cause changes in the enzymatic system, being root hair formation dependent on hormonal balance and metabolism. The allelochemicals present in FAc may have promoted a metabolic imbalance

Table 1 - Root (RL) and hypocotyl (HL) length (cm) of *Euphorbia heterophylla* (wild poinsettia) seedlings under the action of crude leaf extract and leaf fractions of *Machaerium hirtum* leaves

Target species	Fraction	Concentration	RL	HL
Euphorbia heterophylla	Crude extract (CE)	Control	2.94 a	0.96 a
		0.1 g L^{-1}	1.08 b	0.50 b
		0.2 g L^{-1}	–	–
		0.4 g L^{-1}	–	–
		0.8 g L^{-1}	–	–
	Hexanic (FH)	Control	2.06 a	0.84 a
		0.1 g L^{-1}	1.50 b	0.64 bc
		0.2 g L^{-1}	1.61 ab	0.65 bc
		0.4 g L^{-1}	1.37 b	0.63 c
		0.8 g L^{-1}	1.74 ab	0.84 ab
	Chloroformic (FCl)	Control	0. 92 a	0.92 a
		0.1 g L^{-1}	0.39 b	0.40 b
		0.2 g L^{-1}	–	–
		0.4 g L^{-1}	–	–
		0.8 g L^{-1}	–	–
	Ethyl acetate (FAc)	Control	2.94 a	0.96 a
		0.1 g L^{-1}	1.58 b	0.67 b
		0.2 g L^{-1}	1.14 c	0.52 b
		0.4 g L^{-1}	0.84 c	0.54 b
		0.8 g L^{-1}	–	–
	Hydromethanolic (FHm)	Control	2.94 a	0.95 a
		0.1 g L^{-1}	2.42 b	0.91 a
		0.2 g L^{-1}	2.32 b	0.94 a
		0.4 g L^{-1}	1.32 c	0.65 b
		0.8 g L^{-1}	0.94 c	0.64 b

* Means followed by the same lowercase letter in the column do not differ from each other by means of ANOVA compared to the Tukey's test ($p<0.05$) and the Kruskal-Wallis test compared to the Simes-Hochberg post-test ($p<0.05$).



* Significant differences in the test control using the Kruskal-Wallis test with the Simes-Hochberg post-test ($p<0.05$).

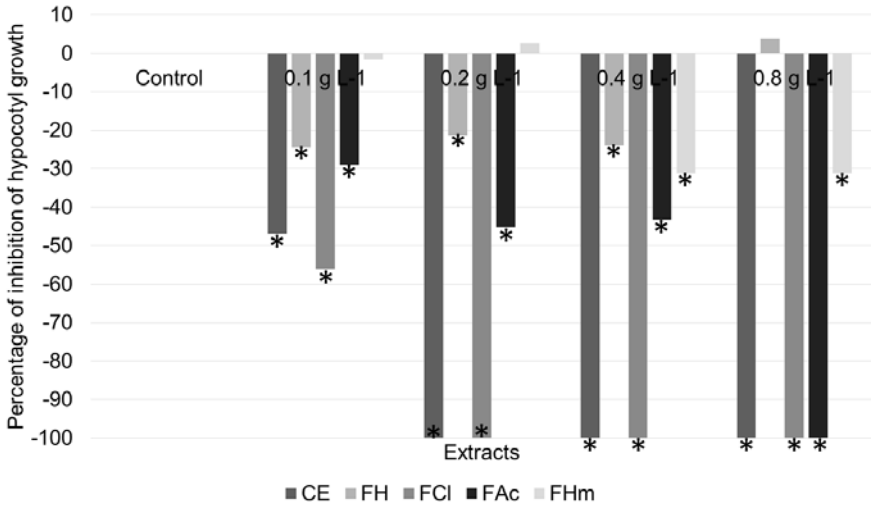
Figure 1 - Percentage of inhibition of the root growth of *Euphorbia heterophylla* (wild poinsettia) seedlings under the action of the crude leaf extract and leaf fractions of *Machaerium hirtum* leaves.

since the micrographs show alterations in the cellular structure of the parenchyma, so that this appearance of root hair may be related to the effects of allelochemicals on the metabolism (Figures 4F, H).

The bioassays demonstrated that morning glory seedlings are less sensitive than those of wild poinsettia since the growth of morning glory seedlings was registered in all treatments,

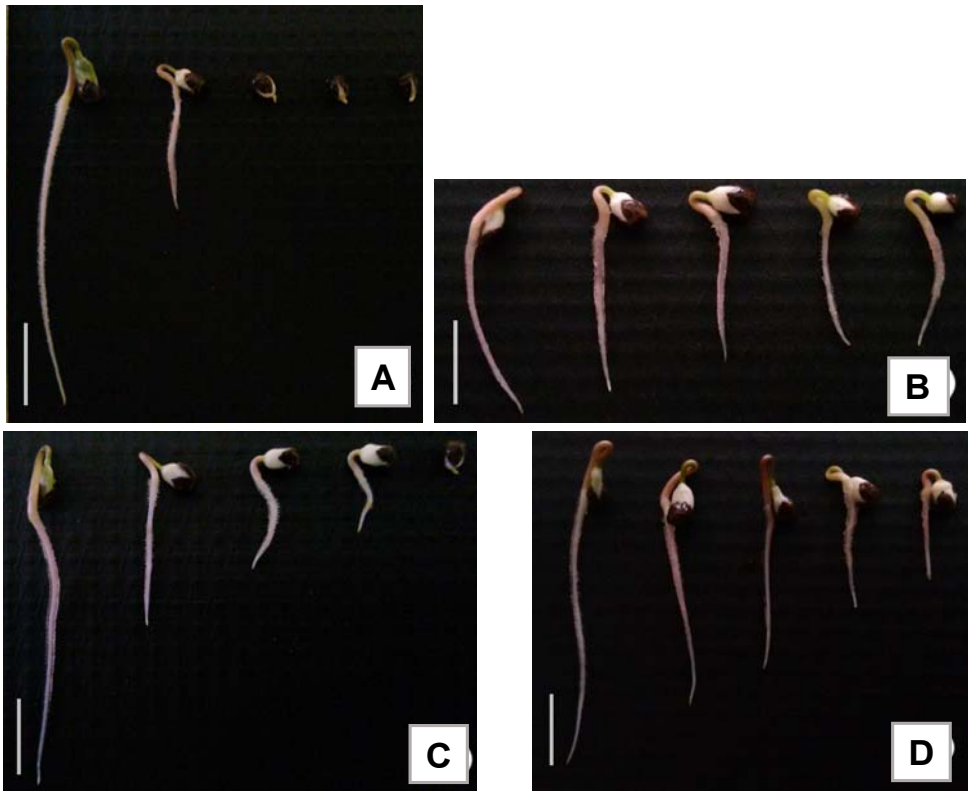
which is between 1.2<x<2.30 cm (Table 2). In contrast, wild poinsettia seedlings did not show growth when exposed to CE and FCl from a concentration of 0.2 g L^{-1}.

Root and hypocotyl growth of morning glory seedlings were reduced under CE at all assessed concentrations. In the fraction FH, reductions in root length were observed from the concentration of 0.2 g L^{-1}. Differences in hypocotyl growth were observed under FCl from the concentration of



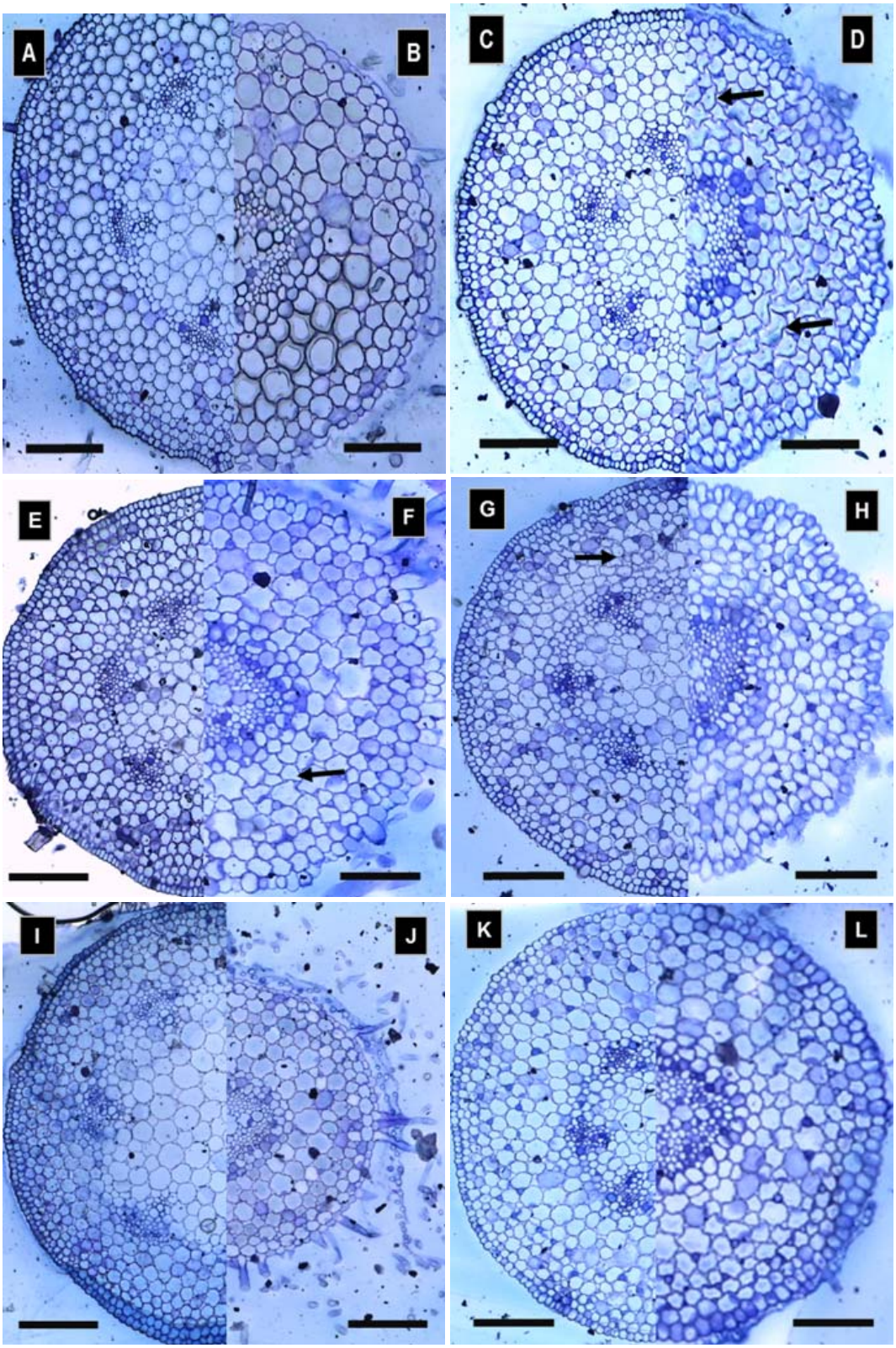
* Significant differences in the test control using the Kruskal-Wallis test with the Simes-Hochberg post-test ($p<0.05$).

Figure 2 - Percentage of inhibition of the hypocotyl growth of *Euphorbia heterophylla* (wild poinsettia) seedlings under the action of the crude leaf extract and leaf fractions of *Machaerium hirtum* leaves.



A – crude extract; B – hexanic fraction; C – ethyl acetate fraction; D – hydromethanolic fraction (Scale: 1 cm; respectively: control, 0.1, 0.2, 0.4, and 0.8 g L^{-1}).

Figure 3 - Initial growth of *Euphorbia heterophylla* under crude extract and fractions of *Machaerium hirtum*.



A – hypocotyl, control of crude extract and fraction ethyl acetate; B – root, control of crude extract and fraction ethyl acetate; C – hypocotyl, crude extract of 0.1 g L^{-1} , D – root, crude extract of 0.1 g L^{-1} ; E - hypocotyl, ethyl acetate fraction of 0.2 g L^{-1} , F – root, ethyl acetate fraction of 0.2 g L^{-1} ; G – hypocotyl, ethyl acetate fraction of 0.4 g L^{-1} , H – root, ethyl acetate fraction of 0.4 g L^{-1} ; I – hypocotyl, control chloroformic fraction, J – root, control chloroformic fraction; K – hypocotyl, chloroformic fraction of 0.1 g L^{-1} , L – root, chloroformic fraction of 0.1 g L^{-1} (Scale: 100 µm, indication of compromised cells).

Figure 4 - Cross sections of *Euphorbia heterophylla* seedlings under the action of the crude extract and fractions of *Machaerium hirtum*.

Table 2 - Root (RL) and hypocotyl (HL) length (cm) of *Ipomoea grandifolia* (morning glory) seedlings under the action of the crude leaf extract and leaf fractions of *Machaerium hirtum* leaves

Target species	Fraction	Concentration	RL	HL
Ipomoea grandifolia	Crude extract (CE)	Control	2.30 a	1.89 a
		0.1 g L^{-1}	1.75 b	1.06 b
		0.2 g L^{-1}	1.40 bc	1.09 b
		0.4 g L^{-1}	1.40 bc	0.94 b
		0.8 g L^{-1}	1.13 c	0.97 b
	Hexanic (FH)	Control	2.30 ab	1.89 a
		0.1 g L^{-1}	2.80 a	1.40 ab
		0.2 g L^{-1}	2.44 ab	1.11 c
		0.4 g L^{-1}	2.14 b	1.17 bc
		0.8 g L^{-1}	2.10 b	1.26 abc
	Chloroformic (FCl)	Control	2.06 a	0.98 a
		0.1 g L^{-1}	1.12 b	0.89 ab
		0.2 g L^{-1}	0.95 bc	0.75 b
		0.4 g L^{-1}	1.39 b	0.75 b
		0.8 g L^{-1}	0.9 c	0.73 b
	Ethyl acetate (FAc)	Control	2.30 a	1.89 ab
		0.1 g L^{-1}	2.17 a	1.47 b
		0.2 g L^{-1}	2.06 a	1.89 a
		0.4 g L^{-1}	1.97 a	1.65 ab
		0.8 g L^{-1}	1.84 a	1.15 c
	Hydromethanolic (FHm)	Control	2.30 b	1.89 c
		0.1 g L^{-1}	3.20 a	2.61 a
		0.2 g L^{-1}	2.76 a	2.16 ab
		0.4 g L^{-1}	1.55 d	1.83 bc
		0.8 g L^{-1}	1.74 c	1.88 bc

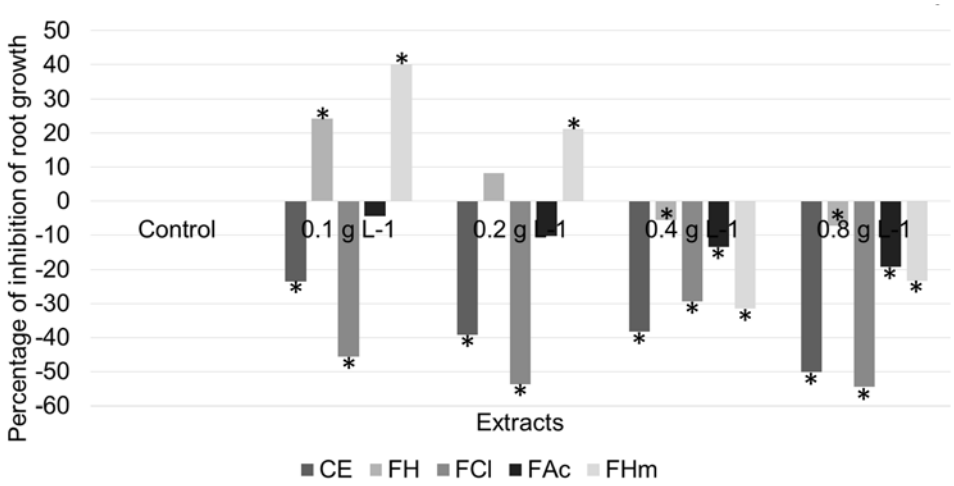
* Means followed by the same lowercase letter in the column do not differ from each other by means of ANOVA compared to the Tukey's test ($p<0.05$) and the Kruskal-Wallis test compared to the Simes-Hochberg post-test ($p<0.05$).

0.2 g L^{-1} whereas, under FAc, these differences were observed only at a concentration of 0.8 g L^{-1} (Table 2). The highest root and hypocotyl growth was observed under FHm when compared to that of the control (Figures 5 and 6), being this the fraction in which the highest morning glory seedlings were found, with an average growth of 4.38 cm; the lowest morning glory seedlings were found under FCl, with an average growth of 2.10 cm) (Table 2). The inhibition graphs show that concentrations of 0.4 and 0.8 g L^{-1} of all treatments promoted the highest and most significant inhibitory percentages of root growth in morning glory seedlings (Figures 5 and 6).

Morphologically, seedlings presented no evident changes, except for the presence of lateral roots in all treatments, including the control (Figure 7). Thus, the treatments did not promote, in this species, the appearance of lateral roots because in the control root growth was also observed. The shoot of seedlings was usually thick, sometimes long, as in FHm (Figure 7D), or short, as in CE (Figure 7A) and FH (Figure 7B).

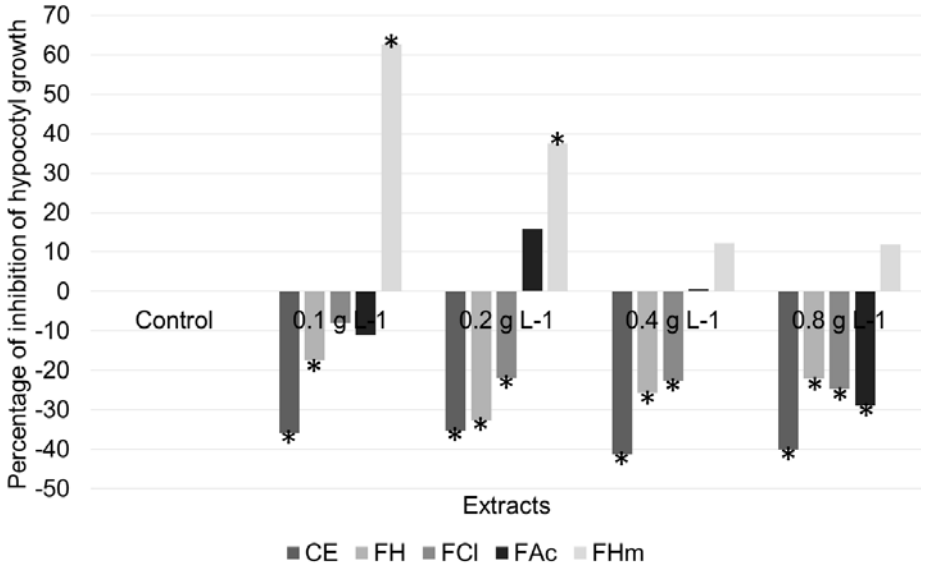
Other authors also observed a reduction of growth in morning glory seedlings when submitted to allelochemicals. In this sense, Grisi et al. (2013) reported that the aqueous extract of *Sapindus saponaria* caused morphological changes in seedlings of morning glory and barnyard grass (*Echinochloa crus-galli*), with the most severe changes in growth observed in the most concentrated extracts (7.5 and 10%), mainly on root length. This result was observed in our study, in which fractions of higher concentration inhibited root growth of morning glory seedlings. Significant reductions in root length of morning glory were also reported when submitted to the extract of *Leucaena leucocephala* at concentrations of 40% (Rosa et al., 2007; Mauli et al., 2009). Ribeiro et al. (2009) reported that root and shoot growth of morning glory, hairy beggarticks (*Bidens pilosa*) and barnyard grass (*Echinochloa crus-galli*) were significantly reduced under aqueous extracts of *Crinum americanum* at concentrations of 1 and 5%.

Among the parameters used to assess allelopathic effects, growth is the most sensitive to the effects of allelochemicals and seedling length is the parameter used to test these effects



* Significant differences in the test control using the Kruskal-Wallis test with the Simes-Hochberg post-test ($p<0.05$).

Figure 5 - Percentage of inhibition of the root growth of *Ipomoea grandifolia* (morning glory) seedlings under the action of the crude leaf extract and leaf fractions of *Machaerium hirtum* leaves.



* Significant differences in the test control using the Kruskal-Wallis test with the Simes-Hochberg post-test ($p<0.05$).

Figure 6 - Percent of inhibition of the hypocotyl growth of *Ipomoea grandifolia* (morning glory) seedlings under the action of the crude leaf extract and leaf fractions of *Machaerium hirtum* leaves.

(Ferreira and Borghetti, 2004). During growth, allelochemicals can induce the appearance of abnormal seedlings, with necrosis of radicle being one of the most common symptoms. Thus, to assess the effects of allelochemicals on growth is important to verify the occurrence of allelopathic effects (Ferreira and Aquila, 2000).

Many chemical constituents were identified in the genus *Machaerium*, with the presence of several classes of compounds such as steroids, alkaloids, benzoquinones, flavonoids, terpenoids, among others (El-Sohly et al., 1999; Muhammad et al., 2001; Ignoato et al., 2012; Ribeiro et al., 2015). The plant material used in our study was chemically studied and reported by Ignoato et al. (2012) and Ribeiro et al. (2015).

In the preliminary research conducted by Ignoato et al. (2012), five pure compounds produced by this legume were extracted and identified from the material used here. The steroids stigmasterol (24α-ethyl-cholest-5,22-dienol) and β-sitosterol (24α-ethyl-cholest-5-enol) were found in leaf FH. Reports of other studies using steroids as test substances can be found in the literature. According

to Ripardo Filho et al. (2012), the steroids spinasterol, spinasterone, and glucopyranosyl spinasterol presented inhibitory effects on *Mimosa pudica* and *Senna obtusifolia*. Kpoviessi et al. (2006) tested the steroids found in *M. hirtum* (stigmasterol and β-sitosterol) and reported allelopathic effects on the growth cowpea (*Vigna unguiculata*). Macías et al. (2006) reported the presence of phytotoxic effects of steroids extracted from *Oryza sativa* (rice) on seedlings of barnyard grass (*Echinochloa crus-galli*). According to data obtained with bioassays performed with FH, the steroids stigmasterol and β-sitosterol may have presented allelopathic effects in the studied plants, but less sensitive to the action of the allelochemicals present in FH.

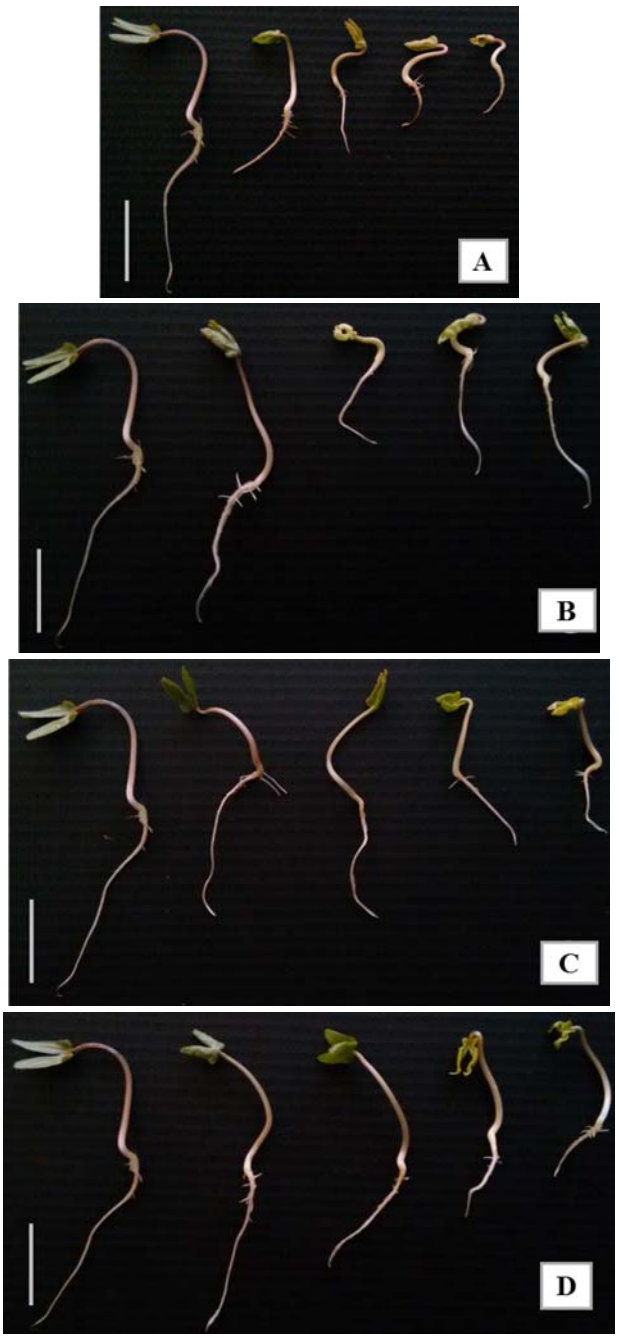
Two compounds of the flavonoid class were extracted from FAc: flavone swertisin (7-O-methyl-6-C-β-D-glucopyranosyl-apigenin) and isovitexin (6-C-β-D-glucopyranosyl-apigenin) (Ignoate et al., 2012). Flavonoids constitute the largest class of secondary plant metabolites, having several functions. They can inhibit ATP synthesis and phosphorylation in mitochondria, affect root growth, reduce cell division in meristems, suppress root hair formation, alter germination patterns, influence soil microbes, and act as hormonal regulators, especially the auxin polar transport (Rice, 1984; Einhellig, 1995; Deng et al., 2004; Taylor and Grotewold, 2005).

The loss of the normal gravitropic orientation may be the result of some flavonoids inhibiting auxin polar transport, resulting in growth disturbances, which may affect the ability to acquire resources (Weston and Mathesius, 2013). Thus, according to the results of the bioassays, flavonoids present in FAc may have affected the growth of wild poinsettia seedlings. In fact, root growth decreased as concentrations increased so that the effects were dose-dependent. Morning glory seedlings were less sensitive to FAc exposure.

Other authors, such as Yan et al. (2014), tested flavonoids of *Stellera chamaejasme*, which had effects on the development of *Arabidopsis thaliana*. Silva et al. (2013) found flavonoids that significantly affected the germination of *Mimosa pudica*. Aslani et al. (2016) tested the inhibitory effects of the flavone isovitexin on *Echinochloa crus-galli* seedlings and found a median degree of inhibition (flavone is constituent of FAc).

Micrographs of sections of wild poinsettia seedlings showed that FAc fraction affected cell structure. Tarahovsky et al. (2014) stated that flavonoids influence the stability of cell membranes, affecting permeability and fluidity. Ferrarese-Filho et al. (2009) report that altered membranes can cause cell death due to the leakage of cell electrolytes.

Because the structuring of parenchyma cells was affected by allelochemicals present



A – crude extract; B – hexanic fraction; C – ethyl acetate fraction; D – hydromethanolic fraction (Scale: 1 cm; respectively: control, 0.1, 0.2, 0.4, and 0.8 g L^{-1}).

Figure 7 - Initial growth of *Ipomoea grandifolia* under the action of the crude extract and fractions of *Machaerium hirtum*.

in CE and FAc (Figure 5D, F, G), not only the plasma membrane but also the cell wall was affected, a structure that must remain intact to ensure that the internal pressure exerted by the plasma allows the cell to grow and divide. According to Hoffman et al. (2007), the elongation of root and shoot is dependent on the formation of cambium and xylem vessels, which are dependent on the partition of nutrients by seedling, being the root system more sensitive to the action of allelochemicals since they are the first to emerge after germination, being directly exposed to the action of allelochemicals (Tanveer et al., 2012). Thus, a deficient root formation could affect the physiological state and prevent the establishment of weeds (Grisi et al., 2015).

Allelochemicals also alter the stability of proteins, which consequently affects the cytoskeleton, which is composed of protein microtubules that control cell expansion and, when affected by the action of some drug that interferes with this process, can lead to the appearance of cells with abnormal and irregular shapes, as registered in the micrographs obtained in our study (Dolan and Davies, 2004; Gniazdowska and Bogatek, 2005). It is believed that the cell elongation of wild poinsettia roots was inhibited, confirming the statistical differences observed in the hypocotyl diameter of wild poinsettia seedlings (Table 3).

Table 3 - Quantitative anatomy of root and hypocotyl cross sections of *Euphorbia heterophylla* (wild poinsettia) seedlings under the action of the chloroformic fraction of *Machaerium hirtum* leaves. ØR – root diameter; TR – root parenchyma thickness; THy – hypocotyledonary parenchyma thickness. Unit of measurement: µm (micrometers)

Target species	Fraction	Concentration	ØR	ØHy	TR	THy
Euphorbia heterophylla	–	Control	499.25	1056.41	156.87	225.02
	Crude extract (EB)	0.1 g L^{-1}	533.43	711.53*	225.21	209.06
	Ethyl acetate (FAc)	0.2 g L^{-1}	488.22	688.2*	178.36	175.74
		0.4 g L^{-1}	503.15	819.61*	153.83	211.03
	Chloroformic (FCl)	Control	582.2	929.82	158.65	201.35
		0.1 g L^{-1}	477.01	567.81*	174.5	141.4

* Significant differences in the test control using the Kruskal-Wallis test with the Simes-Hochberg post-test ($p<0.05$).

Ignoato et al. (2012) extracted and identified from FHm the non-protein amino acid alkaloid compound 4-hydroxy-N-methyl-proline. There are reports of some non-protein amino acids such as L-canavanine in *Canavalia ensiformis*, which has a chemical structure analogous to that of the arginine and may be mistakenly incorporated into a protein, which becomes nonfunctional (Rosenthal, 1986; Klack et al., 2012).

Leucaena leucocephala, a species widely used in urban afforestation, also produces a non-protein amino acid called mimosine, which showed biological activity in bioassays (Souza Filho et al., 1997; Pires et al., 2001; Rosa et al., 2007; Mauli et al., 2009). L-DOPA (L-3,4-dihydroxyphenylalanine) is a known non-protein amino acid found in root exudates and seeds of velvet bean (*Mucuna pruriens*), being reports of its activity in tests with corn (*Zea mays*) causing reductive effects on root growth due to changes in the enzymatic activity (Siqueira-Soares et al., 2013; Soares et al., 2014).

In plants, non-protein amino acids have protective functions, especially against herbivory and pathogens (Vranova et al., 2011). In the tests with FHm, wild poinsettia and morning glory seedlings had root elongation inhibited so that root growth was more sensitive when compared to shoot elongation. The leaf hydroalcoholic extract of *M. hirtum* is rich in glycosylated flavonoids (Ribeiro et al., 2015), which are polar compounds produced by plants and found in alcoholic fractions. In contrast, lipophilic flavonoids are found in nonpolar fractions, such as the chloroformic fraction (Harborne, 1989), and FH, as reported by Ignoato et al. (2012).

The phytotoxic effects found in the bioassays with the studied species are attributed to allelochemicals present in the crude extract and fractions since the previously reported compounds are members of bioactive classes with varied biological activities, such as the allelopathic activity. In general, the bioassays revealed that wild poinsettia seedlings showed to be more sensitive when compared to morning glory since, in addition to showing morphological changes, seedlings

did not survive the deleterious effects of concentrations of 0.2, 0.4 and 0.8 g L^{-1} of CE and FCl. Among the tested fractions, FCl showed the highest inhibitory effects, with the lowest growth values found in both weeds.

Changes in growth observed in weeds suggest that *M. hirtum* appears to be promising in the fight against biological invasion, and may compromise the maintenance of populations of unwanted plants. However, future researches are necessary, mainly to assess the individual effects of isolated compounds produced by this legume.

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