PHYTOTOXICITY ACTIVITY OF *PIPTOCARPHA ROTUNDIFOLIA (LESS.)* – BAKER (CANDEIA) LEAF EXTRACTS ORIGINATING FROM ELUOTROPIC EXTRACTION.

ATIVIDADE FITOTÓXICA DE EXTRATOS FOLIARES DE *PIPTOCARPHA ROTUNDIFOLIA (LESS.)* – BAKER (CANDEIA), ORIUNDOS DE EXTRAÇÃO ELUOTRÓPICA.

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ABSTRACT

The Asteraceae phytotoxic activity is widely cited in literature but there are no information about the *P. rotundifolia* phytotoxicity. So, this study evaluated the phytotoxicity of extracts from *P. rotundifolia* on wheat (*Triticum aestivum L.)* coleoptile bioassays and root growth of sesame (*Sesamum indicum* L.) metaxylem cells. The extraction method employed five organic solvents in eluotropic order: hexane, dichloromethane, ethyl acetate, acetone and methanol. All extracts showed inhibitory activity in wheat coleoptile. Analyzes in metaxylem cells of sesame seedlings showed changes at cellular level in a dose-dependent manner in acetone extract. Based on the results, it is concluded that extracts have phytotoxic activity.

Keywords: Candeia. Extracts. Phytotoxicity. Coleoptile. Germination.

RESUMO

O potencial fitotóxico das Asteráceas são comumente citados na literatura, porém não há informações sobre a fitotoxicidade de *P. rotundifolia.* Assim, este estudo avaliou a fitotoxicidade de extratos de *P. rotundifolia* em bioensaios de crescimento de coleóptilos de trigo (*Triticum aestivum* L.) e em bioensaios de crescimento de células do metaxilema de raízes de gergelim (*Sesamum indicum* L.). O método de extração utilizou cinco solventes em ordem eluotrópica: hexano, diclorometano, acetato de etila, acetona e metanol. Todos extratos mostraram atividade inibitória em coleóptilo de trigo. Análises em células do metaxilema de plântulas de gergelim mostraram alterações a nível celular de um modo dose-dependente para extratos acetônicos. Com base nos resultados, conclui-se que os extratos e as frações possuem atividade fitotóxica.

Palavras-chaves: Candeia. Extratos. Fitotoxicidade. Coleóptilo. Germinação.

Piptocarpha rotundifolia (Less.) Baker, commonly known as candeia, is native from Brazil and belongs to the Asteraceae family, very common in the Brazilian savanna. According to the literature, this species is used on popular medicine but there are no information about the fitotoxicity of this species. So, this study evaluated the phytotoxic potential of extracts and fractions from *P. rotundifolia* leaves.

The plant material used was composed of mature leaves of *Piptocarpha rotundifolia* (Less.) Baker. The leaves were collected in savanna area in the campus of Federal University of São Carlos, São Carlos-SP, Brazil ($21^{o}\,58^{'}$ to $22^{o}\,00^{'}$ S and $47^{o}\,51^{'}$ to $47^{o}\,52^{'}$W). After the screening and washing, the plant material was dried at 40 °C for 72 hours and grinded using an industrial mill. The resulting powder was weighed and vacuum packed, and then used for obtaining the extracts and fractions of *Piptocarpha rotundifolia* mature leaves.

Five solvents were used in order of increasing polarity: hexane, dichloromethane, ethyl acetate, acetone and methanol. A total of 150 g of leaf powder and 1,8 L of each mentioned solventes were used. At each change of solvent, the same leaf powder was dried and reused in the next extraction, respecting the order of the solvents. The method used was made according Macias et al, 2010. The extracts were dried in exhaust hood until complete solvent evaporation and originated the extracts: A (hexane), B (dichloromethane), C (ethyl acetate), D (acetone) and E (methanol). After this process, all the extracts were weighed to have their yields calculated, and finally tested in the bioassays.

For the wheat coleoptile bioassays, wheat seeds were distributed in plastic boxes contained two sheets of filter paper, moistened with 10 ml of destilled water, capped and covered with aluminum paper. The boxes were kept in germination chamber (B.O.D oven) at 25 ° C in the dark for 72 h. Then, the boxes were taken into a room with green light for the selection of the wheat coleoptile to be selected and cut using a Van der Weij guillotine. The coleoptile apices were cut (2 mm) and discarded, while the rest was cut into 4 mm and used in the bioassay (Macias et al., 2010). The solutions were prepared from 10 mg of each extract pre-solubilized in dimethyl sulfoxide (DMSO, 5 uL ml-1) and diluted in buffer solution (pH = 5.6) containing monohydrate citric acid (1.05g / L), trihydrate potassium hydrogen phosphate (2.9 g / L) and 2% sucrose at concentrations of 0.2; 0.4 and 0.8 mg.mL-1. In the test tube were added 2 ml of the respective solutions containing five wheat coleoptiles (Macias et al., 2010). Two controls were performed: one negative with buffer solution and DMSO (5 uL / mL-1) and another positive with Goal® herbicide (240 uL / mL-1)

solubilized in control solution and DMSO (5 uL / mL) in same concentrations evaluated for fractions. These tubes were kept at 25 ° C in the dark and under constant rotation (1.2Hz), three replicates are used per treatment (Macias et al., 2010). After 24 h, the coleoptiles were measured with ImageJ program.

For the examination of metaxylem cells, extracts (A, B, C, D and E) were subjected to bioassay that avaluates the sesame (*Triticum aestivum*) metaxylem cells growth kept in contact with. After seven days, the primary root segments of the seedlings were removed and immersed in 70% alcohol (v/v) (Gatti et al., 2010). The modified Fuchs staining method was used (Kraus and Arduin, 1997), where the roots were immersed in alcohol (70%) for five days and placed in a solution of 25% NaOH at 50 °C for 48 h, until the material was clarified. Then, the root segments were immersed in lacmoid for 24 hours at a room temperature. After staining, the material was mounted on glass slides in Apathy's syrup (Kraus and Arduin, 1997), with the roots, for observation under an optical microscope (Olympus-BX41) coupled to a camera (Sony CCD-IRIS). Four primary roots of sesame seedlings grown in different concentrations of the extracts and control solutions were used. Half of the length of each root was photographed, starting from the central region toward the zone of cell diferentiation. From each photograph, 20 central cells of the metaxylem were measured at 20X magnification (Gatti, 2008). The measurements were made using the ImageJ program.

The coleoptile fragments and metaxylem cells lengths were calculated as a stimulus or inhibition percentage compared to the negative control, whereas positive values represent stimulus and negative values represent inhibition (Novaes, et al., 2013). The data were submitted to a test of normality (Shapiro-Wilk) and homogeneity (Levene). Normal and homogeneous data were subjected to analysis of variance (ANOVA) followed by Tukey test at 0.05 of significance. Abormal or non-homogeneous data was used Welch's test followed by non-parametric Kruskal-Wallis test.

All analyzes were performed using the statistical program v.2.17c PAST (Hammer. The Harper D. A. T., 2013).

In general, all extracts, in all concentrations, with the exception of the methanol extract (E) 0.4 $mg.mL^{-1}$ inhibited the growth of wheat coleoptile when compared to the negative control. The dichloromethane extracts (B) and ethylacetate (C) at concentrations of 0.4 and 0.8 $mg.mL^{-1}$ and the acetone extracts (D) at concentrations of 0.2 and 0.4 $mg.mL^{-1}$ and methanol (E) at the concentration 0.2 $mg.mL^{-1}$ showed inhibition values that are not statistically different values for the positive control at the same concentrations. The ethyl acetate extract (C) 0.8 $mg.ml^{-1}$ showed the greater percentage of inhibition (79.3%) (Figure 1).

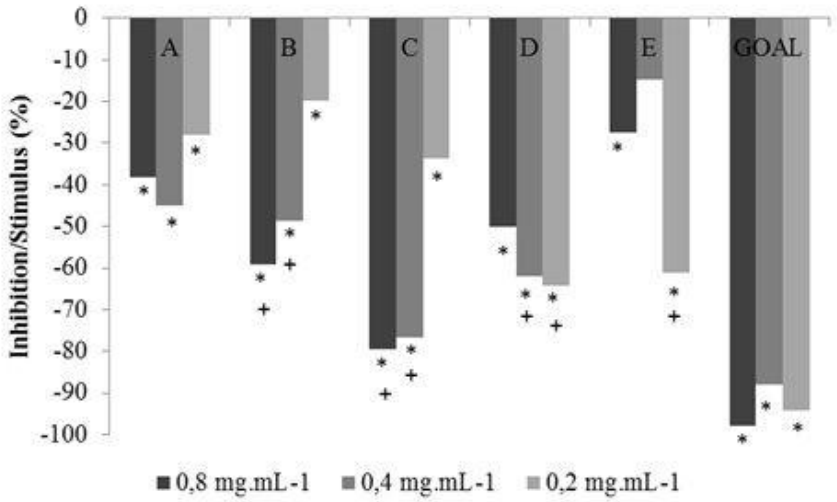

Figure 1. Effect of the extracts of *Piptocarpha rotundifolia* leafs on the lengh of wheat (*Triticum aestivum*) coleoptiles. (*) indicate treatments with significant difference when compared with negative control, and (+) indicate treatment with the same effect to positive control at the same concentration. The positive control corresponds to the solutions with the Goal®, and the negative, to the buffer solutions with DMSO.

Acording to Vattuone et al. (2009), compounds that has higher polarity, such as soluble simple sugars and polysaccharides are quite abundant in plants, reason why the methanol extract (E) and more polar fractions and subfractions showed higher yields. The wheat coleoptile bioassay has the advantage of being fast and sensitive to a wide variety of bioactive substances and very used to prove able to evaluate the stimulation or inhibition of coleoptiles growth when in contact with phytotoxic agents. Similar result obtained in the bioassay coleoptile with crude extracts were found by Macias et al. (2004), wherein fractions and compounds isolated from *Helianthus annuus* also caused inhibition in wheat coleoptile length. The same pattern was observed by Pereira et al. (2015) with wheat coleoptiles grown in solutions with *Serjania lethalis* extract.

The anatomical study of the roots of sesame seedlings allowed a better view of the phytotoxic effects of different extracts, at cellular level. The extracts were subjected to bioassay root metaxylem cell growth, to verify if the roots lengh reduction occurred from changes at the cellular level. There was a significant decrease in the length of metaxylem cells treated with extracts compared to negative controls and this reduction occurred in a dose-dependent way (Figure 2), with exeption of the extracts A (0, 2 mg.mL $^{-1}$) and B (0.2 and 0.4 mg.mL^{-1}) that not differed from positive control (Table 1). The extract more negatively influenced the growth of sesame metaxylem cells was ethyl acetate (C) at 0,4 mg.ml^{-1}. Thus, was not possible to measure the cell growth of the metaxylem roots treated with the positive control containing GOAL®, since they presented mostly dead or necrosed.

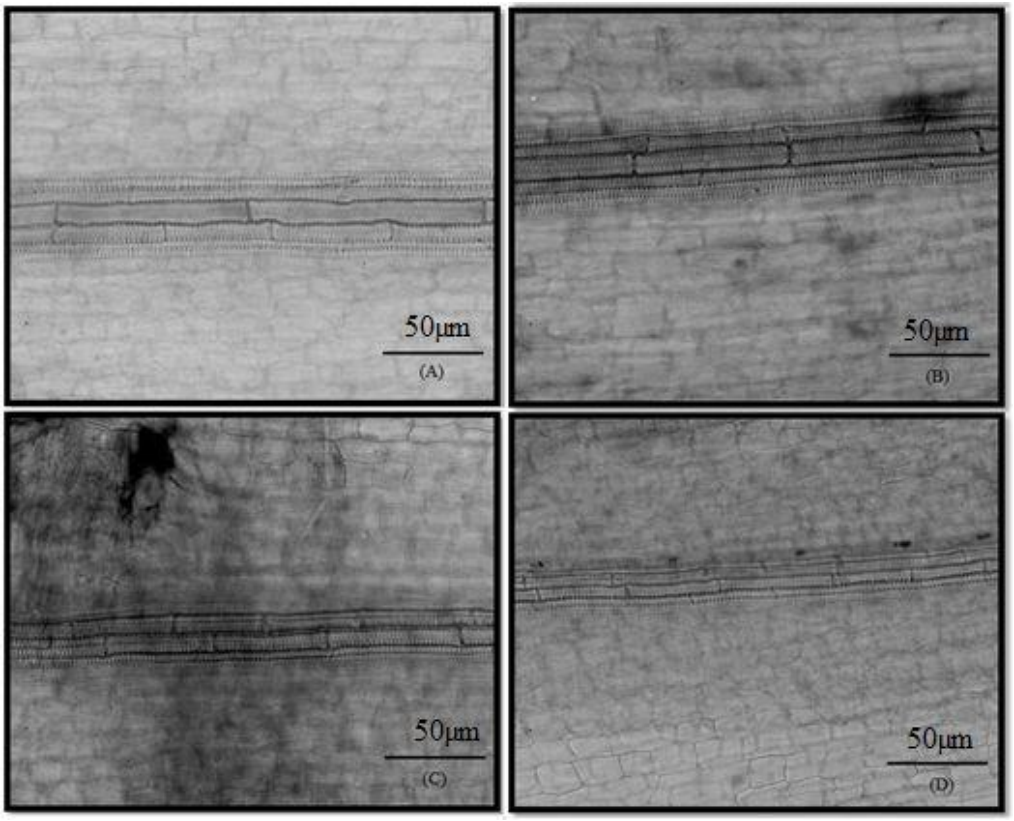

Figure 2. Photomicrographs of root metaxylem cells of sesame (*Sesamum indicum*) seedlings treated with the control solution (A), D extract at 0.2mg.mL^{-1} (B), D extract at 0.4mg.mL^{-1} (C) and D extract at 0.8mg.mL^{-1} (D). Scale = 50 µm.

Table 1. Size (µm) of root metaxylem cells of sesame (*Sesamum indicum*) seedlings grown in different concentrations of mature leaf extracts of *P. rotundifolia*.

	Concentrations		
Extract	**0.2mg.mL^{-1}**	**0.4mg.mL^{-1}**	**0.8mg.mL^{-1}**
Hexane (A)	170,41	136,87*	109,89*
Dichloromethane (B)	179,94	163,63	157,37*
Ethyl Acetate (C)	133,55*	105,85*	142,15*
Acetone (D)	133,55*	130,78*	130,82*
Methanol (E)	140,28*	132,50*	150,26*

*: Statistically different from negative control.

The reduction in root growth of sesame seedlings subjected to the action of *P. rotundifolia* extracts may be associated with the inhibition of the metaxylem cell elongation. According to Al-Wakeel et al. (2007) the cell elongation inhibition may be related to the direct action of allelochemicals, and these interfere in the process of cell division and balance between the different hormones.Auxin is responsible for controlling the process of cell elongation and growth and

differentiation of primary and vascular tissues, such as metaxylem and protoxylem (Aloni et al., 2006). Furthermore, this hormone is responsible for leading various processes of root growth, such as tropic responses to light and gravity, the general architecture of plants and cell growth (Strader et al., 2010; Tanimoto, 2005).

Thus, with the results obtained in this study, extracts shown to be potentially phytotoxic, as they were able to inhibit the wheat coleoptile and invasive species seedlings growth.

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