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CYTOTOXIC AND GENOTOXIC EFFECTS OF *Macroptilium lathyroides*

Efeito Citotóxico e Genotóxico da Espécie Macroptilium lathyroides

ABSTRACT - The monitoring of phytochemicals with potentially toxic properties can be assessed by observing the physiological and cellular alterations of the test organism exposed. This present study aimed to evaluate the cytotoxic and genotoxic potential of aqueous extracts of leaves and roots of *Macroptilium lathyroides* weed on the cell cycle of lettuce. Bioassays were conducted in a germinator (25 °C) with aqueous extract at concentrations of 0, 5, 10, 20 and 40% p v⁻¹, obtained from fresh leaves and roots. For biological, lettuce root meristems were used for the preparation of slides using the technique of squashing. All blades were observed with an optical microscope at a magnitude of 400x. A total of 5,000 cells were analyzed for each treatment, and the number of cells in each phase of mitosis was recorded. Possible presence of chromosomal abnormalities was verified, such as chromatid breaks, anafasic bridges, loss of whole chromosomes or micronuclei formation. These analyzes were conducted only on a qualitative level. Results showed that aqueous extracts of *M. lathyroides* mitotic caused reduced index with increased concentration. Genotoxic activity was also observed for both extracts tested, since composition resulted in cell cycle changes and chromosomal abnormalities.

Keywords: abnormalities, bioassays, cell division, phasey bean, mitotic index.

RESUMO - O monitoramento de fitoquímicos com propriedades potencialmente tóxicas pode ser avaliado por alterações fisiológicas e celulares do organismo-teste exposto. O presente estudo teve como objetivo avaliar o potencial citotóxico e genotóxico de extratos aquosos das folhas e raízes da planta invasora *Macroptilium lathyroides*, sobre o ciclo celular de alface. Os bioensaios foram conduzidos em câmara de germinação (25 °C), testando-se extratos aquosos da planta invasora, nas concentrações de 0, 5, 10, 20 e 40% p v⁻¹, extraídos das folhas e das raízes frescas. Para o ensaio biológico, meristemas de raízes de alface foram usados no preparo de lâminas através da técnica de esmagamento. Todas as lâminas foram observadas em microscópio óptico, a uma magnitude de 400x. Foram analisadas 5.000 células para cada um dos tratamentos, observando-se o número delas em cada fase da mitose. Foram observadas também as possíveis alterações cromossômicas, como quebras cromatídicas, pontes anafásicas, perda de cromossomos inteiros ou formação de micronúcleos. Essas análises foram feitas somente em nível qualitativo. Os resultados demonstraram que os extratos aquosos de *M. lathyroides* causaram redução no índice mitotótico em função do aumento da concentração. Observou-se também atividade genotóxica para ambos os extratos testados, uma vez que sua composição ocasionou alterações no ciclo celular e anormalidades cromossômicas.

Palavras-chave: anormalidades, biotestes, divisão celular, feijão-de-pombinha, índice mitótico.

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INTRODUCTION

The Phaseolinae Benth. (Leguminosae) subtribe is a polyphyletic group (Kajita et al., 2001) and comprises 21 genera and about 314 species, with pantropical distribution. Of these, about 118 are present in neotropics and subtropics, arranged mainly in eight genera (Lewis et al., 2005). In Brazil, six genera and about 32 species are present (Lima et al., 2010); Among them is the genus *Macroptilium* (Benth.) Urb.

Macroptilium, belongs to the family Fabaceae (alt. Leguminosae), comprises about 17 species, and is exclusive to the American continent, concentrating in the tropical region (Lewis et al., 2005). In Brazil, there are 12 species (Moura, 2010). Some species of this genus are used as fodder, as green manure and in folk medicine (Barbosa-Fevereiro, 1986).

Macroptilium lathyroides, popularly known as phasey bean, (Ramos, 2006) or wild bean, originates from the tropical part of South America and was introduced in tropical and subtropical India, Australia, Africa and Southeast North America (Ferreira, 2002). It is characterized as a legume with great nitrogen fixing capacity, and can be used as green fertilizer (Lorenzi, 2000); In several regions of Brazil it is used as forage for grazing (Vasconcelos et al., 2011). However, *M. lathyroides* has become a problematic invasive plant for soybean and maize crops and pasture areas (Concenço et al., 2012), due to its characteristics of adaptability to different environments and short reproductive cycle.

Biological assays on the bioactivity of extracts, fractions and plant-isolated compounds have often been applied to the identification and monitoring of potentially toxic substances (Noldin et al., 2003).

Plant-test systems have been used to study the effects of plant extracts for the detection of genotoxicity (Teixeira et al., 2003; Fachinetto et al., 2007). Biological tests of mutagenicity for the analysis of the cytotoxicity and genotoxicity of substances can be evaluated, respectively, through changes in the cell division process on the test organism, both by inhibiting the mitotic index and by verifying the occurrence of chromosomal mutations, such as chromatographic breaks, anaphase bridges, loss of whole chromosomes, or the formation of micronuclei (Bagantini, 2007).

The visible action of the allelochemicals on plants is only a secondary signaling of previous changes (Ferreira and Borghetti, 2004). In this present study, the effects of allelochemicals on the germination and/or development of plants are secondary manifestations of molecular and cellular processes (Ferreira and Aquila, 2000; Ferreira and Borghetti, 2004).

Silva et al. (2003) have reported that one way to monitor products from the secondary metabolism of some plants is to evaluate their genotoxic and cytotoxic potential through the mitotic index (MI) and the presence of chromosomal aberrations (CA), chromosomal breaks (CB), ring chromosomes (RC) and other errors in cell division.

The objective of this work was to evaluate the cytotoxic and genotoxic potential of the leaves and roots of *M. lathyroides* on lettuce cell cycle, in order to verify the ability of this species to inhibit and/or cause damage in others species of economic potential.

MATERIAL AND METHODS

This present work was carried out at the Laboratory of Seed and Weed Technology (Laboratório de Tecnologia de Sementes e Matologia, LaSeM) and in the Laboratory of Plant Genetics and Molecular Biology of Universidade do Estado de Mato Grosso – UNEMAT, Campus Universitário de Alta Floresta-MT.

For the root and leaf aqueous extracts, *Macroptilium lathyroides* plants were reproduced in a protected environment belonging to Universidade do Estado de Mato Grosso – UNEMAT, Campus Universitário de Alta Floresta-MT, using seeds from Batayporã-MS.

The extracts at the concentrations of 0, 5, 10, 20 and 40% p v⁻¹ were prepared from the crushing of fresh vegetable material, using a commercial blender in its maximum rotation for five minutes, at room temperature of 25 °C, Using 80 g of leaves or roots in 200 mL of distilled

water, for 40% concentration, and so on for lower concentrations, thus obtaining a weight-by-volume ratio.

Lettuce was the test organism used (*Lactuca sativa* L.), and Mimosa the cultivar choice, which seeds were purchased locally, without treatment with agricultural pesticides, and the germination test was previously carried out.

The experiments were organized in a 2 x 5 factorial scheme, with two plant materials (green leaves and roots) and five concentrations, with four replicates of 25 seeds. Gerbox acrylic boxes (11.0 x 11.0 x 3.5 cm) submitted to previous aseptic treatment were cleaned with sodium hypochlorite (10%), two hours before the assembling of the experiments. The seeds were placed to germinate in the acrylic boxes, on two sheets of germitest paper moistened with each extract (according to the treatments of each experiment), in the proportion of 2.5 times the dry substrate mass (Brazil, 2009), and (Table 1). The samples were then placed in a BOD-type germination chamber at a constant temperature of 25 °C, with a 12 hour light period system, using a set of four white bulbs, providing approximately 0.012 W m⁻² nm⁻¹ (Cardoso, 1995). Radicle collection was conducted daily, always at the same hour, until a total of seven days after sowing.

Table 1 - Frequency of the different phases of mitosis in the meristematic cells of *Lactuca sativa* root grown under different concentrations of aqueous extracts of leaves and roots of species *Macropitilium lathyroides*

EC (%)	NCM Ex. Sheet	NCM Root Ex.	% Of cells in phases			
			Prophase		Metaphase	
			Ex. Sheet	Root Ex.	Ex. Sheet	Root Ex.
0	399.3	391.7	19.62 aA	19.49 aA	2.89 aA	2.59 aA
5%	327.4	305.4	17.97 abA	17.73 abA	1.82 bA	1.97 aA
10%	277	317.1	16.57 bA	17.34 bA	1.48 bcA	1.74 bA
20%	194	292.5	13.82 cB	16.94 bar	1.34 bcA	1.59 bA
40%	0.00	237	0.00 dB	15.09 cA	0.00 cA	1.50 bA
DMS (F/R)			1.78		0.72	
DMS (EC)			1.28		0.51	
VC (%)			9.28		32.36	
EC (%)	NCM Ex. Sheet	NCM Root Ex.	% Of cells in phases			
			Anaphase		Telophase	
			Ex. Sheet	Root Ex.	Ex. Sheet	Root Ex.
0	399.3	391.7	2.17 aA	1.58aA	1.89 aA	2.09 aA
5%	327.4	305.4	1.12 bA	1.07 bA	1.22 bA	1.37 bA
10%	277	317.1	1.0 bA	1.0 bA	1.08 bA	1.19 bA
20%	194	292.5	1.04 bA	1.11 bA	1.19 bA	1.41 bA
40%	0.00	237	0.00 bA	1.12 bA	0.00 bB	1.40 bA
DMS (F/R)			0.34		0.57	
DMS (EC)			0.24		0.40	
VC (%)			22.65		32.97	

Means followed by the same letter, lowercase in the columns and upper case in the lines, do not differ from each other by the Tukey test at 5% probability. EC (concentration of extracts); NCM (number of cells in mitosis); Ex. Leaf (leaf extract); Ex. Root (root extract).

To determine the mitotic index, we used the crushing technique (Guerra and Souza, 2002). Test organism radicles were harvested and fixed in Carnoy (3: 1, ethanol: acetic acid) for 24 hours at room temperature and then stored in a freezer.

The preparation of the slides for later analysis of the mitotic index was carried out in the following order: distilled water for 5 minutes; HCl 5N for 15 minutes at room temperature; Distilled water for 5 minutes. After this procedure, the radicles were transferred to the slide, where the hood was extracted with a scalpel to obtain the apical meristem. Then, a 2% acetic orcein drop was added, and a cover slip was placed on the crushed material, which was subsequently heated.

The slides of the lettuce cells were observed under an optical microscope at a magnitude of 400x. A total of 5,000 cells were analyzed for each of the treatments, by observing the number of cells in each phase of mitosis. Mitotic index (MI) was obtained through the following equation (Prates et al., 2001):

$$IM = \left(\frac{m}{T} \right) \times 100$$

where: m = number of cells in mitosis; and T = total number of cells.

Possible chromosomal alterations were also observed, such as: chromatographic breaks, anaphase bridges, loss of whole chromosomes, or formation of micronuclei. These analyzes were performed only at a qualitative level.

The criteria for accepting the presence of the observed chromosomal changes were based on a study described by Picker and Fox (1986), and are reported as follows: chromatographic breaks equivalent to the loss of chromatid chromosomes or by fragmentation that occurs in chromatids during cell division; Not disjunction of the chromosomes at the end of the metaphase, forming structures denominated of anaphasic bridges – alterations observed at the beginning of the anaphase; Presence of micronuclei (MN) equivalent to a regular, round or oval contour structure, and contained within the cytoplasm of a cell; The MN being in the same plane of focus of observation and clearly separated from the nucleus.

Results were submitted to analysis of variance, and the means were compared by Tukey's test at 5% probability, using the statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

The aqueous extracts of *M. lathyroides* caused a cytotoxic effect on lettuce root meristem cells, with interaction between the factors ($p < 0.05$), what can be observed in the regression graph, which shows the reduction of the mitotic index with the concentration of extracts (Figure 1).

According to the results, it was observed that all concentrations of aqueous extracts of *M. lathyroides* caused reduction in the mitotic division index. However leaf extracts caused a

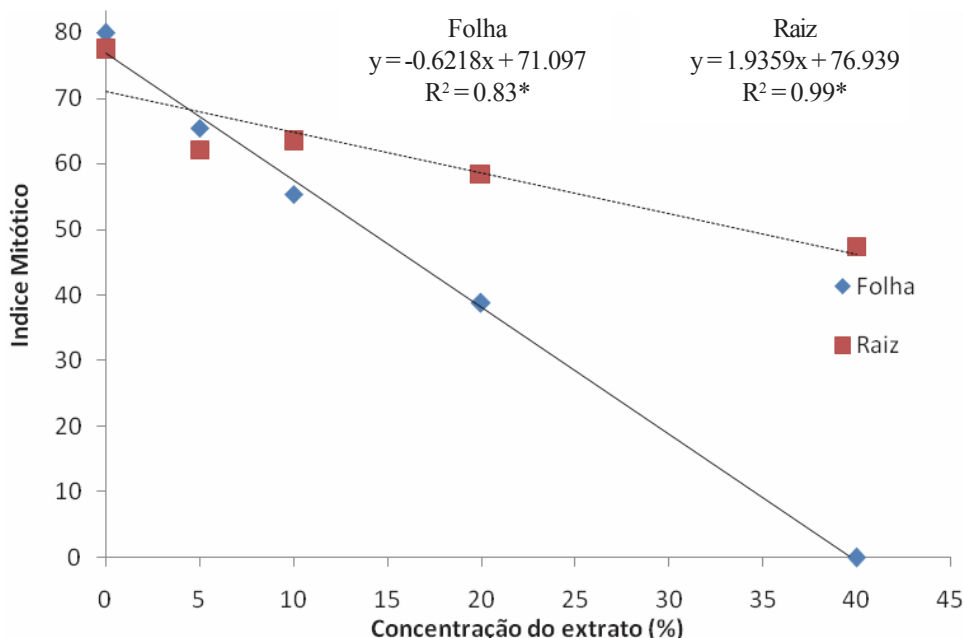


Figure 1 - Mitotic index of meristematic cells of lettuce roots exposed to different concentrations of aqueous extracts from fresh leaves and roots of the species *Macropodium lathyroides*.

reduction of approximately 50% in the concentration of 20%, with total inhibition at the highest concentration tested (40% p v-1). Moreover, in the root extracts the reduction was 38.74%, in this same concentration. Thus, it was found that leaf extracts provided greater cytotoxic effect.

Increased concentrations of the aqueous extracts cause a gradual reduction in the process of cell division of the test organism, but with a distinct physiological action of each of the extracts applied. According to Fiskesjö (1985), an agent can be considered toxic when it promotes reduction of more than 50% in the seed germination index of the test organism, as verified in the allelopathy experiment.

The analysis of the gradual reduction in the 80% MI found in the control to zero in the highest concentration of the aqueous extract of the leaves and 78% of the control to 37% in the higher concentration of the aqueous extract of the roots (1) suggests that the leaf extracts and roots of the *M. lathyroides* species, besides allelopathic action, have cytotoxic activity, causing inhibition in the cell cycle of other species. According to Carvalho et al. (2005), the cytotoxic action of a substance is determined by the increase or decrease of MI of the cells submitted to the exposure of the tested agent.

Similar results were found by Fachinetto and Tedesco (2009) on *Allium cepa* in the evaluation of the possible cytotoxic and genotoxic effect of aqueous extracts of *Baccharis trimera* and *Baccharis articulata*. Similarly, Luz et al. (2012) concluded that foliar extracts of *Plantago major* cause alteration in the cell cycle, by inhibiting the division of the *A. cepa* cells.

By analyzing each phase of the cell division of meristematic cells of lettuce roots under the influence of aqueous extracts of *M. lathyroides*, a significant difference of the different concentrations of both extracts tested was observed (Table 1).

In all treatments, it was observed a higher prophase frequency and a lower frequency of the other phases of cell division, with a marked drop in this process from the first concentration of leaves and roots 5%.

We have noted that in the highest concentration of the leaves oxidation of the root meristem of the seeds occurred, preventing the cellular divisions of the radicles; In extracts from the roots there was a gradual decrease of this variable.

Such data show that lettuce seeds are sensitive to the exposure of both aqueous extracts, showing that the decrease in mitotic index indicates the ability of the species to reduce or inhibit cell proliferation with allelopathic and cytotoxic effects. In general, chemical compounds with allelopathic action may also have genotoxic and mutagenic effects (Ferreira and Aquila, 2000).

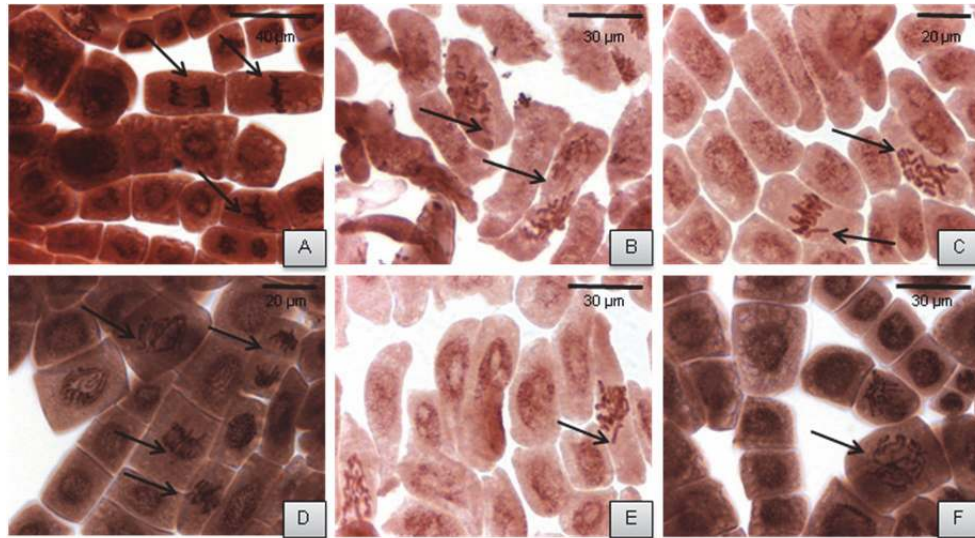
According to Pires et al. (2001), the interference in the phases of cell division caused by the action of the extractor probably represents a mechanism of action of this on the initial development of the test plant.

Our results corroborate with those found by Iganci et al. (2006), who observed considerable variation in the cell division of onion bulbs under the action of extracts of fresh leaves of *Plectranthus barbatus*, *Plectranthus amboinicus* and *Vernonia condensata*. Borges et al. (2011) also observed a growing cytotoxic effect on lettuce and onion seeds under influence of fresh and dried leaves extract of *Ricinus communis*.

When analyzing the results found for the genotoxic potential of *M. lathyroides*, by means of the index of chromosomal aberrations present in the meristematic cells of lettuce, a directly proportional relation was noted, so that the higher the concentration of the extracts, the greater the amount of disturbed cells.

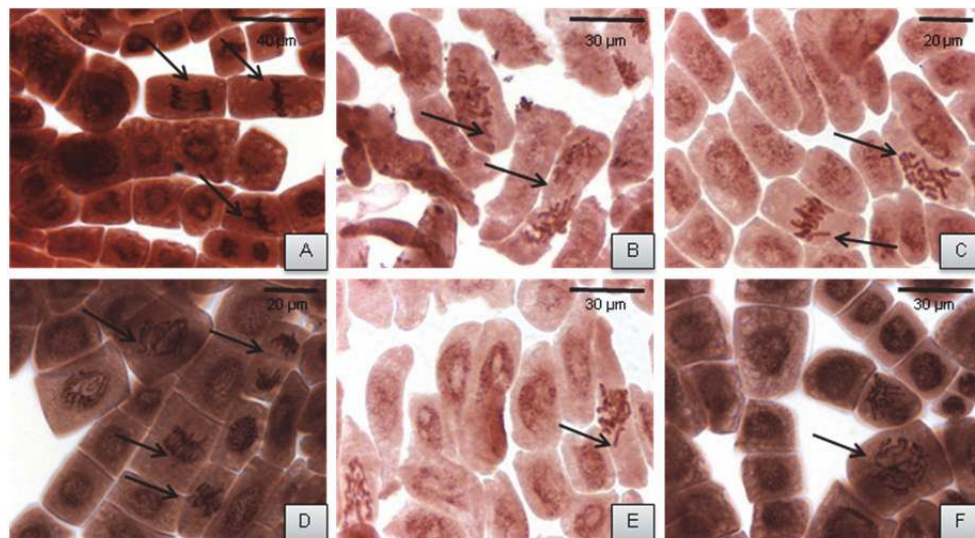
These changes may impair vital cell processes, leading to cell death or chromosomal changes, and are called genotoxic effects (Franke, 2003), as observed in leaf extract (Figure 2), such as: irregular metaphase and anaphase with chromosome adhesion (Figure 2A); Telophase with presence of micronucleus in one of the daughter cells (Figure 2B); Prophase with irregular chromosomes and anaphase with chromosome bridge (Figure 2C); Bridged anaphase and chromosome loss (Figure 2D); Prophase with irregular chromosomes (Figure 2E); And telophase with chromosome adhesion and anaphase with chromosome bridge (Figure 2F). In the root extract

(Figure 3), irregular metaphases and anaphase with chromosome bridge were observed (Figure 3A); C-metaphase and telophase with irregular chromosomes (Figure 3B); Prophase with irregular chromosomes and metaphase with chromosomal loss (Figure 3C); Irregular prophase, irregular metaphase, anaphase with chromosome adhesion and telophase with chromosome adhesion (Figure 3D); Prophase with chromosome loss (Figure 3E); And prophase with irregular chromosomes (Figure 3F). In the control, some anaphaseic bridges were observed, but at low frequency.



(A) irregular metaphase and anaphase with chromosome adhesion; (B) prophase with presence of micronucleus; (C) prophase with irregular chromosomes, prophase with presence of micronucleus and anaphase with chromosome bridge; (D) bridged telophase and chromosome loss; (E) prophase with irregular chromosomes and presence of micronucleus; (F) telophase with chromosome adhesion and anaphase with chromosome bridge.

Figure 2 - Meristematic cells lettuce in cell division, treated with different concentrations of aqueous extract of fresh leaves of *Macroptilium lathyroides*.



(A) irregular metaphases and telophase with chromosome bridge; (B) C-metaphase and telophase with irregular chromosomes; (C) prophase with irregular chromosomes and metaphase with chromosomal loss; (D) irregular prophase, irregular metaphase, anaphase with chromosome adhesion and telophase with chromosome adhesion; (E) prophase with chromosomal loss; (F) prophase with irregular chromosomes.

Figure 3 - Lettuce meristematic cells in cell division aqueous extract treated with different concentrations of fresh roots *Macroptilium lathyroides*.

The term genotoxic applies to the agent capable of promoting damage to the genetic material. These chromosomal changes are an indication that the substance has direct action on the genetic material of the test organism (Caritá and Marin-Morales, 2008), as observed in the present study, since the presence of micronuclei and the frequency of breaks Chromosomes are often used for mutagenicity evaluation (Fiskejő, 1985). These aberrations are a consequence of the genotoxic actions of chemical agents (Natarajan, 2002).

Agreeing with the results obtained in this present study, Dias et al. (2014), in a study with aqueous extracts of *Mikania cordifolia*, also observed presence of chromosomal aberrations, confirming mutagenic action in the cell cycle of *A. cepa*. Similarly, Knoll et al. (2006), working with different populations of *Pterocaulon polystachyum*, demonstrated that the increase in leaf infusion concentrations caused a genotoxic effect on the test organism.

The aqueous extracts of the leaves and roots of *Macroptilium lathyroides* have cytotoxic and genotoxic potential on lettuce cell cycle, reducing MI with increasing concentrations, able to cause inhibition in the development of other species of economic potential that share the same geographic space.

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REFERENCES

- Bagantini M.D., Silva A.C.F., Tedesco S.B. Uso do sistema teste de *Allium cepa* como bioindicador de genotoxicidade de infusões de plantas medicinais. **Rev Bras Farmacog.** 2007;17:444-7.
- Barbosa-Fevereiro V.P. *Macroptilium* (Bentham) Urban do Brasil (Leguminosae Faboideae – Phaseoleae – Phaseolinae). **Arq Jardim Bot Rio de Janeiro.** 1986;28:109-80
- Borges C.S. et al. Efeitos citotóxicos e alelopáticos de extratos aquosos de *Ricinus communis* utilizando diferentes bioindicadores. **Tecnol Ci Agropec.** 2011;5:15-20.
- Brasil. Ministério da Agricultura e Reforma Agrária. **Regras para análise de sementes.** Brasília: SAND/DNDV/CLAV, 2009. 365p.
- Cardoso V.J.M. Germinação e fotoblastismo de sementes de *Cucumis anguria*: influência da qualidade da luz durante a maturação e secagem. **Rev Bras Fisiol Veg.** 1995;7:75-80.
- Caritá R., Marin-Morales M. Induction of chromossome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. **Chemosphere.** 2008;72:722-5.
- Carvalho R.O. et al. Diesel emissions significantly influence composition and mutagenicity of the ambient particles: a case study in São Paulo, Brazil. **Environ Res.** 2005;98:1-7.
- Concenço G. et al. Controle de *Macroptilium lathyroides* com herbicidas aplicados em pré e pós-emergência. **Rev Bras Herbic.** 2012;11:11-23.
- Dias M.G. et al. Efeito genotóxico e antiproliferativo de *Mikania cordifolia* (L. F.) Willd. (Asteraceae) sobre o ciclo celular de *Allium cepa* L. **Rev Bras Plantas Med.** 2014;16:202-8.
- Fachinetto J.M. et al. Efeito anti-proliferativo das infusões de *Achyrocline satureioides* DC (Asteraceae) sobre o ciclo celular de *Allium cepa*. **Rev Bras Farmacog.** 2007;17:49-54.
- Fachinetto J.M., Tedesco S.B. Atividade antiproliferativa e mutagênica dos extratos aquosos de *Baccharis trimera* (Less.) A. P. de Candolle e *Baccharis articulata* (Lam.) Pers. **Rev Bras Plantas Med.** 2009;11:360-7.
- Ferreira A.G., Aquila M.E.A. Alelopatia: uma área emergente da ecofisiologia, **Rev Bras Fisiol Veg.** 2000;12:175-204.

- Ferreira A.G., Borghetti F. **Germinação**: do básico ao aplicado. Porto Alegre: Artmed, 2004. 323p.
- Ferreira D.F. Sisvar: a computer statistical analysis system. **Cienc Agrotec.** 2011;35:1039-42.
- Ferreira O.G.L. **Efeito do corte no estágio vegetativo e de épocas de colheita sobre o rendimento e qualidade das sementes de *Macroptilium lathyroides* (L.) Urb** [dissertação]. Pelotas: Universidade Federal de Pelotas, 2002.
- Fiskejç G. The Allium test as a standard in the environmental monitoring. **Hereditas.** 1985;102:99-112.
- Franke I.R. et al. Genotoxicidade dos agentes sintéticos e naturais. In: Silva J., Erdtmann B., Henriques J.A.P., organizadores. **Genética toxicológica.** Porto Alegre: Alcance, 2003. p.309-17.
- Guerra M., Souza M.J. **Como observar cromossomos**: um guia de técnicas em citogenética vegetal, animal e humana. São Paulo: Funpec, 2002.131p.
- Iganci J.R.V. et al. Efeito do extrato aquoso de diferentes espécies de boldo sobre a germinação e índice mitótico de *Allium cepa* L. **Arq Inst Biol.** 2006;73:79-82.
- Kajita T. et al. RbcL and legume phylogeny, with particular reference to Phaseoleae, Millettieae, and allies. **Syst Bot.** 2001;26:515-36.
- Knoll M.F. et al. Effects of *Pterocaulon polystachyum* DC. (Asteraceae) on onion (*Allium cepa*) root-tip cells. **Genet Mol Biol.** 2006;29:539-42.
- Lewis G.P. et al. **Legumes of the World.** Kew: The Royal Botanic Garden, 2005. [accessed on: 2016 dec. 20.]. Available at: <http://www.kew.org/science-conservation/research-data/resources/legumes-of-the-world>.
- Lima H.C. Phaseolus L. In: **Lista de Espécies da Flora do Brasil.** Rio de Janeiro. Jardim Botânico do Rio de Janeiro, 2010. [acesso em: 20 dez. 2016]. Disponível em: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB101162>.
- Lorenzi H. **Manual de identificação e controle de plantas daninhas**: plantio direto e convencional. 5ª.ed. Nova Odessa: Instituto Plantarum, 2000. 339p.
- Luz A.C. et al. Avaliação do potencial citotóxico e genotóxico de *Plantago major* L. em sistemas teste *in vivo*. **Rev Bras Plantas Med.** 2012;14:635-42.
- Moura T.M. *Macroptilium* L. In: **Lista de Espécies da Flora do Brasil.** Rio de Janeiro. Jardim Botânico do Rio de Janeiro, 2010. [acesso em: 20 dez. 2016]. Disponível em: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB18750>.
- Natarajan A.T. Chromosome aberration: past, present and future. **Mutat Res.** 2002;504:3-16.
- Noldin V.F., Monache F.D., Yunes R.A. Composição química e atividade biológica de *Cynara scolymus* L. cultivada no Brasil. **Quím Nova.** 2003;26:331-4.
- Picker J.D., Fox D.P. Do curried foods produce micronuclei in buccal epithelial cells? **Mutat Res.** 1986;171:185-8.
- Pires N.M. et al. Efeito do extrato aquoso de leucena sobre o desenvolvimento, índice mitótico e atividade da peroxidase em plântulas de milho. **Rev Bras Fisiol Veg.** 2001;13:55-65.
- Prates H.T. et al. Efeito do extrato aquoso de leucena na germinação e no desenvolvimento do milho. **Pesq Agropec Bras.** 2001;35:909-14.
- Ramos L.A., Cavalheiro C.C.S., Cavalheiro E.T.G. Determinação de nitrito em águas utilizando extrato de flores. **Quím Nova.** 2006;29:1114-20.
- Silva J., Erdtman B., Henriques J.A.P. **Genética toxicológica.** Porto Alegre: Alcance, 2003. 422p.
- Teixeira R.O. et al. Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millefolium* L. in *in vivo* assays. **Genet Mol Biol.** 2003;26:551-5.
- Vasconcelos W.A. et al. Germinação de sementes e desenvolvimento de plântulas de figo de pombo (*Macroptilium lathyroides*). **Rev Trópica.** 2011;5:3-11.