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THAMMYRES DE ASSIS ALVES

ECO-FRIENDLY HERBICIDE POTENCIAL OF DIFFERENT *Psidium* SPECIES: MECHANISMS OF ACTION, PHYTOTOXICITY AND CYTO-GENO-TOXIC APPROACHES

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Thesis presented to the Graduate Program in Genetics and Improvement at the Center of Agricultural Sciences and Engineering at the Federal University of Espírito Santo, as a requirement for obtaining the title of *Doctor Scientiae* in Genetics and Breeding, in the area of Evolutionary Biology and Cytogenetic.

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THAMMYRES DE ASSIS ALVES

Eco-friendly herbicide potencial of different *Psidium* species: mechanisms of action, phytotoxicity and cyto-geno-toxic approaches

Tese apresentada ao Programa de Pós-Graduação em Genética e Melhoramento do Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo, como requisito para obtenção do Título de Doutora em Genética e Melhoramento.

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Specially, to my father, Adeilton Alves,

To my mother Marcelina Victor de Assis Alves,

To my sister, Thaynnara de Assis Alves,

To my brother, Thayllon de Assis Alves,

To my nephew, João Alves Monteiro,

To Maikon Keoma da Cunha Henrique,

"Blessed is the man who trusts in the Lord, And whose hope is the Lord." Jeremiah 17.7

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BIOGRAPHY

THAMMYRES DE ASSIS ALVES, daughter of Marcelina Victor de Assis Alves and Adeilton Alves, was born on January 29, 1993, in the city of Alegre, State of Espírito Santo. She completed high school at E.E.E.F.M. “Professor Pedro Simão” in Alegre. In August 2010, she joined the Biological Sciences Degree course at the Center for Agricultural Sciences at the Federal University of Espírito Santo, graduated in February 2015. In March 2015, she joined the master's degree by the Postgraduate Program in Genetics and Breeding at the Center for Agricultural Sciences and Engineering at the Federal University of Espírito Santo, in the area of Biotechnology, submitting to the defense of a dissertation in February 2017. In March from 2017, she joined the Doctorate course in the Postgraduate Program in Genetics and Breeding at the Center for Agricultural Sciences and Engineering at the Federal University of Espírito Santo, in the line of evolutionary biology and cytogenetic.

SUMMARY

RESUMO

Desde o início das atividades agronômicas, doenças, pragas e ervas daninhas começaram a surgir. O desenvolvimento de agroquímicos foi de suma importância para o aumento da produtividade agrícola, acarretando na diminuição dos custos e no aumento do rendimento das produções. Entretanto, pesquisas tem demonstrado que os agroquímicos têm promovido prejuízos à saúde humana e ao ambiente. Por essa razão, os agroquímicos ambientalmente amigáveis têm ganhado a atenção dos pesquisadores. Para a avaliação de tal potencial são utilizados os bioensaios com sistemas modelo, tendo destaque os bioensaios vegetais, os quais são altamente sensíveis a agentes tóxicos, possibilitam diferentes análises simultaneamente (fito-cito-genotoxicidade), além de possuírem alta correlação com outros organismos incluindo humanos. Assim, a presente pesquisa avaliou e comparou a toxicidade dos extratos foliares aquosos e etanólicos de *Psidium cattleyanum*, bem como dos óleos essenciais de *Psidium acidum* e de *Psidium cauliflorum* investigando seus potenciais bioherbicida e seus mecanismos de ação celulares por meio de bioensaio vegetal de toxicidade, utilizando as plantas modelo *Lactuca sativa* e *Sorghum bicolor*. Para isso, os óleos essenciais foram extraídos, os extratos foram preparados e os produtos químicos foram caracterizados. Para os ensaios biológicos, sementes das plantas modelo foram tratadas com os óleos essenciais das duas espécies nas concentrações de 3000, 1500, 750, 375 e 187.5 mg mL^{-1} e com os extratos nas concentrações de 100, 50, 25 e 12.5 mg mL^{-1}, água destilada, diclorometano e glifosato foram usados como soluções controle. As variáveis: porcentagem de germinação (GP), índice de velocidade de germinação (GSI), crescimento radicular (RG), crescimento aéreo (AG), índice mitótico (MI), alterações cromossômicas (CA) e alterações nucleares (NA) foram avaliadas. Flavonois, flavononois, flavonas, flavonoides, alcaloides, resinas, xantonas e glicosídeo de antraquinona foram identificados no extrato etanólico. Ambos os extratos avaliados, na maior concentração, inibiram o desenvolvimento inicial das espécies utilizadas como modelo. Além disso, todos os extratos provocaram alterações nas fases mitóticas e inibiram o MI. Ademais, os tratamentos promoveram aumento das CA e NA. O mecanismo de ação apresentado foi aneugênico, clastogênico e determinou em alterações epigenéticas. O extrato etanólico foi mais citotóxico, uma vez que apresentou efeito mais expressivo em menor concentração. Apesar da citotoxicidade dos extratos em estudo, eles promoveram alterações em níveis menores do que o controle positivo glifosato. Assim, ambos os extratos apresentam menor citotoxicidade que o

herbicida comercial glifosato. Em relação aos óleos essenciais avaliados, ambos se mostraram fito-citotóxicos. O óleo essencial de *P. acidum* inibiu o RG de sorgo e o AG da alface, enquanto o de *P. cauliflorum* reduziu o GP, GSI, RG e AG de ambos os modelos. Sendo assim, *P. cauliflorum* se mostrou mais fitótoxico comparado a *P. acidum*. A investigação citotóxica indicou que o óleo de ambas as espécies inibiu o MI da planta modelo. Observou-se, por meio das CA, que o óleo de *P. cauliflorum* apresenta mecanismos de ação aneugênico e clastogênico, e que ambos os óleos determinaram em alterações epigénetica observadas pelo aumento da CA aderência cromossômica. Os resultados indicam o potencial bioherbicida do óleo essencial de *P. cauliflorum*, o qual, além de ser não seletivo e demonstrar taxa de inibição semelhante ao glifosato, apresentou menor taxa de NA que o herbicida comercial.

PALAVRAS-CHAVE: bioensaios, mutagênese, óleos essenciais, Myrtaceae.

ABSTRACT

Since the beginning of agronomic activities, diseases, pests, and weeds started to appear. Thus, development of agrochemicals was especially important for increasing agricultural productivity, resulting in lower costs and increased efficiency of production. However, research has shown that agrochemicals have been damaging human health and the environment. In this way, environmentally friendly agrochemicals have gained the attention of researchers. For the evaluation of such potential, bioassays with model systems are used, with emphasis on plant bioassays, which are overly sensitive to toxic agents, allowing different analyzes simultaneously (phyto-cyto-genotoxicity), in addition to having a high correlation with other organisms including mammals. Thus, the present research evaluated and compared the toxicity of the aqueous and ethanolic leaf extracts of the species *Psidium cattleyanum*, as well as of the essential oils of *Psidium acidum* and *Psidium cauliflorum*, investigating their potential bioherbicides and their cellular mechanisms of action through plant toxicity bioassay, using the plants model *Lactuca sativa* and *Sorghum bicolor*. For this, the essential oils were extracted, the extracts prepared, and the chemicals compounds were characterized. For the biological tests, seeds of the model plants were treated with the essential oils of the two species in the concentrations of 3000, 1500, 750, 375 and 187.5 mg mL^{-1} and with the extracts in the concentrations of 100, 50, 25 and 12.5 mg mL^{-1}, distilled water, dichloromethane and glyphosate were applied as controls. The variables: germination percentage (GP), germination speed index (GSI), root growth (RG), aerial growth (AG), mitotic index (MI), chromosomal changes (CA) and nuclear changes (NA) were evaluated. Flavonoids, flavonoids, flavones, flavonoids, alkaloids, resins, xanthones and anthraquinone glycoside were identified in the ethanolic extract. Both extracts evaluated, in the highest concentration, inhibited the initial development of the both plants used as models. In addition, all extracts caused changes in the mitotic phases and inhibited the MI. In addition, the treatments promoted an increase in CA and NA. The mechanism of action presented was aneugenic, clastogenic and determined in epigenetic changes. The ethanolic extract was more cytotoxic since it had a more expressive effect at a lower concentration. Despite the cytotoxicity of the extracts under study, they promoted changes at lower levels than the glyphosate positive control. Thus, both extracts have less cytotoxicity than the commercial herbicide glyphosate. Regarding the essential oils evaluated, both were shown to be phyto-cytotoxic. The essential oil of *P. acidum* inhibited

the sorghum RG and the lettuce AG. While *P. cauliflorum* reduced the GP, GSI, RG and AG of both models, being, therefore, more phytotoxic than *P. acidum* and non-selective. The cytotoxic investigation indicated that oil from both species inhibited the MI of the model plant. It was observed, through the CA, that the essential oil of *P. cauliflorum* has mechanisms of aneugenic and clastogenic action and that both oils determined in epigenetic changes observed by the increase of the chromosomal adherence. The results indicate the bioherbicidal potential of the essential oil of *P. cauliflorum*, which, in addition to being non-selective and demonstrating an inhibition rate like glyphosate, presented a lower NA rate than the commercial herbicide.

KEYWORDS: bioassays, essential oil, mutagenesis, Myrtaceae.

1. General introduction

The pests, weeds and diseases of agriculture have caused many damages to the economy and the food security since the primordium of the agriculture production. In the Bible, have many reports related to insects' invasion, causing problems to healthy and to economy. In 1845, thousands of people died by hungry in Ireland, in consequence of the "potato blight", a disease promoted by *Phytophthora infestans*, a fungus that infect potatoes and tomatoes. Diseases caused by fungus also promoted hungry and/or economic/social problems in India, Sri Lanka and Brazil (TURK, 1989; MAFFIA and MIZUBUTI, 1999; ZAMBOLIM, 1999).

To control these pests, weeds and diseases, many types of agrochemicals were introduced in the system of agricultural production, which the number and efficacy increase (SENENT, 1979). Thus, the application of agrochemicals is necessary due to which enables most of the rural productive systems, since their use compensates for losses in agricultural productivity (VEIGA, 2007).

Although agrochemicals reduce the index of disease to the human and animals and increased the agricultural production, these chemical compounds can remain actives in the environment for a long time, causing damages in the ecosystems. These effects represent a big risk to the healthy, being necessary the monitoring of these products in air, soil and food, as well as, if possible, avoid their use (JAVARONI et al., 1991).

One alternative to decrease the application of these synthetic agrochemicals caused of environment injuries is by mean of the discovery and development of natural compounds with potential agrochemical (DAYAN and DUKE, 2014). These compounds are produced and found naturally in plants and microorganisms (ALVES et al., 2003, 2004). Thus, molecules produced to the defense of the plants (allelochemicals) can have the potential to be applied as eco-friendly agrochemicals needing be evaluate (SIMÕES et al., 2017).

The *Psidium* genus have being attention of the researchers, because its potential to be used as a eco-friendly agrochemical and the other biological activities as: medicinal (FENNER et al., 2006; GUTIÉRREZ et al., 2008; CECÍLIO et al., 2012), antifungal (SUWANMANEE et al., 2014), antimicrobial (CHANDA and KANERIA, 2011), antiviral (BIRDI et al. 2011), allelopathic (HISTER et al., 2016), phytotoxic (LUBER et al., 2015), cytotoxic (ALMEIDA et al., 2006; LUBER et al., 2015), genotoxic (LUBER et al., 2015). These proprieties can have relation with their phytochemical characteristics.

Once, compounds as mono and sesquiterpenes, flavonoids, sesquiterpenoid alcohols, triterpenoid, tannin acids, terpinen-4-ol, γ-terpinene, α-terpinene, limonene, α-pinene, 1,8-cineole (eucaliptol), α-terpineol, β-terpinene, β-pinene, p-cimene e α-terpinolene, α -copaene, δ-cadinene, α-selinene, were found in species of this genus, which can contribute to their biological activity (YÁÑEZ et al., 2002; IHA et al., 2008; SCUR et al., 2016).

To evaluate the level of toxicity of synthetic compounds in the living beings, as well as, to confirm the potential agrochemical of natural substances, the use of bioassays with models' organisms is indicated (BADERNA et al., 2011; ANDRADE-VIEIRA et al., 2014; ARAGÃO et al., 2015; BERNARDES et al., 2015; PINHEIRO et al., 2015; ALVES et al., 2018). These model organisms used in bioassays act as bioindicator systems of the toxicity and environment pollution. In this way, the plant bioassays are appointed as complementary tool of data physic-chemistries in these studies (BADERNA et al., 2011).

The use of plant bioassays has been consolidated in the scientific community as an efficient way to evaluate the toxicity and the potential of new eco-friendly agrochemicals (POHREN et al., 2013; PINHEIRO et al., 2015; ARAGÃO et al., 2015, 2017; ALVES et al., 2018; SANTOS et al., 2018). This has occurred because the results found in plant bioassays are enough to alert the risks caused by toxic substances. Besides that, the high plants have correlation with other organisms, include the mammals and humans (BAGATINI et al., 2007; CARITÁ and MARIN-MORALES, 2008; LEME and MARIN-MORALES, 2009; ARRAES and LONGHIN, 2012; FIRBAS and AMON, 2013; TEDESCO et al., 2015). In addition, these organisms show high sensibility to toxics compounds, allow the simultaneous analysis of the phyto-cyto-genotoxicity and the several action mechanisms, besides the evaluation in different tissues (GRANT, 1994; LEME and MARIN MORALES, 2009).

Thus, the plant bioassays have being applied with the finality of the evaluated the potential of natural substances to be used in the agronomic activities as a substitute of the synthetic compounds, searching an eco-friendly alternative to the maintenance of the agricultural production (PINHEIRO et al., 2015; ARAGÃO et al., 2015, 2017; ALVES et al., 2018; SANTOS et al., 2018).

2. Objectives

2.1 General objective

Evaluated the eco-friendly herbicide potencial: mechanisms of action, phytotoxicity and cyto-geno-toxic of the essential oil of *Psidium acidum* (DC.) Landrum and *Psidium cauliflorum* Landrum & Sobral, and the extracs of *Psidium cattleyanum* Sabine.

2.2 Specifics objectives

(i) To evaluate the chemical composition of the ethanolic and aqueous extracts of *P. cattleyanum;*

(ii) To evaluate the chemical composition of the essential oil of *P. acidum* and *P. cauliflorum*;

(iii) To evaluate the phytotoxicity of the essential oil of *P. acidum* and *P. cauliflorum*, and extracts of *P. cattleyanum* in *Lactuca sativa* and *Sorghum bicolor*;

(iv) To evaluate the cyto-geno-toxicity and the action mechanisms of the essential oil of *P. acidum* and *P. cauliflorum*, and extracts of *P. cattleyanum* in *Lactuca sativa*.

3. References

ALVES, C. C. F.; ALVES, J. M.; SILVA, T. D.; CARVALHO, M. D.; NETO, J. J. Atividade alelopática de alcalóides glicosilados de *Solanum crinitum* Lam. **Floresta e ambiente**, Seropédica, v. 10, p. 93 -97, 2003.

ALVES, M. D. C. S.; MEDEIROS FILHO, S.; INNECCO, R.; TORRES, S. B. Alelopatia de extratos voláteis na germinação de sementes e no comprimento da raiz de alface. **Pesquisa agropecuária brasileira**, v.39, p.1083-1086, 2004.

ALVES, T. A.; PINHEIRO, P. F.; PRAÇA-FONTES, M. M.; ANDRADE-VIEIRA, L. F.; CORRÊA, K. B.; ALVES, T. A.; DA CRUZ, F. A.; LACERDA JÚNIOR, V.; FERREIRA, A.; SOARES, T. C. B. Toxicity of thymol, carvacrol and their respective phenoxy acetic acids in *Lactuca sativa* and *Sorghum bicolor*. **Industrial Crops and**

Products, v. 114, p. 59-67, 2018.

ANDRADE-VIEIRA, L. F.; BOTELHO, C.M.; PALMIERI, M.J.; LAVIOLA, B.G; PRAÇA-FONTES, M.M. Effects of *Jatropha curcas* oil in *Lactuca sativa* root tip bioassays. **Anais da Academia Brasileira de Ciências (Impresso)**, v. 86, p. 373-382, 2014.

ARAGÃO, F. B.; ANDRADE-VIEIRA, L. F.; FERREIRA, A.; COSTA, A. V.; QUEIROZ, V. T.; PINHEIRO, P. F. Phytotoxic and cytotoxic effects of *Eucalyptus* essential oil on lettuce (*Lactuca sativa* L.). **Allelopathy Journal**, v. 35, p. 259-272, 2015.

ARAGÃO, F. B.; QUEIROZ, V. T.; FERREIRA, A.; COSTA, A. V.; PINHEIRO, P. F.; CARRIJO, T. T.; ANDRADE-VIEIRA, L. F. Phytotoxicity and cytotoxicity of *Lepidaploa rufogrisea* (Asteraceae) extracts in the plant model *Lactuca sativa* (Asteraceae). **Revista de Biologia Tropical**, v. 65, p. 1-10, 2017.

ARRAES, A. I. O. M.; LONGHIN, S. R. Otimização de ensaio de toxicidade utilizando o bioindicador *Allium cepa* como organismo teste. **Enciclopédia Biosfera**, v.8, p. 1958-1972, 2012.

BAGATINI, 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. **Revista Brasileira de Farmacognosia**, v. 17, p. 444-447, 2007.

BADERNA, D.; MAGGIONI, S.; BORIANI, E.; GEMMA, S.; MOLTENI, M.; LOMBARDO, A.; COLOMBO, A.; BORDONALI, S.; ROTELLA, G.; LODI, M.; BENFENATI, E. A combined approach to investigate the toxicity of an industrial landfill's leachate: Chemical analyses, risk assessment and in vitro assays**.** **Environmental Research**, v. 111, p. 603–613, 2011.

BERNARDES, P. M.; ANDRADE-VIEIRA, L. F.; ARAGÃO, F. B.; FERREIRA, A.; Ferreira, M. F. S. Toxicity of Difenoconazole and Tebuconazole in *Allium cepa*. **Water, Air and Soil Pollution**, v. 226, p. 1-11, 2015.

CARITÁ, R; MARIN-MORALES, M.A. Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. **Chemosphere**, v. 72, p. 722–725, 2008.

CRUZ, C. D. Genes: a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum. Agronomy**, v. 35, n. 3, p. 271-276, 2013.

DAYAN, F. E.; DUKE, S. O. Natural compounds as next generation herbicides. **Plant**

physiology, p. pp. 114.239061, 2014.

DOYLE, J. J. Isolation of plant DNA from fresh tissue. **Focus**, v. 12, p. 13-15, 1990.

FIRBAS, P.; AMON, T. *Allium* Chromosome Aberration Test for Evaluation Effect of Cleaning Municipal Water with Constructed Wetland (CW) in Sveti Tomaž, Slovenia. **Journal of Bioremediation & Biodegradation**, v. 4, p. 189, 2013.

GRANT, W. F. The present status of higher plant bioassay for detection of environmental mutagens. **Mutation Research**, v. 310, p. 175-185, 1994.

IKPEFAN, E. O.; AYINDE, B. A. Comparative Growth Inhibitory Assay Of The Methanol Extract Of The Leaf And Seed Of Persea americana Mill (Lauraceae). **Journal of Pharmacognosy and Phytochemistry**, v. 1, n. 6, 2013.

JAVARONI, R.C.A; TALAMON, J; LANDGRAF, M.D.; REZENDE, M.O.O., Estudo da degradação de lindano em solução aquosa através da radiação gama. **Química Nova**, v.14, p.237-239, 1991.

LEME, D.M.; MARIN-MORALES, M.A. *Allium cepa* test in environmental monitoring: A review on its application. **Mutation Research**, v. 682, p. 71-81, 2009.

MAFFIA, L. A.; MIZUBUTI, E. S. G., Fitopatologia x sociedade. **Ação Ambiental**, v. 2, p. 9-12, 1999.

MCHUGH, Mary L. Multiple comparison analysis testing in ANOVA. **Biochemia medica: Biochemia medica**, v. 21, n. 3, p. 203-209, 2011.

PINHEIRO, P. F.; COSTA, A. V.; ALVES, T. A.; GALTER, I. N.; PINHEIRO, C. A.; PEREIRA, A. F.; OLIVEIRA, C. M. R.; PRAÇA-FONTES, M. M. Phytotoxicity and cytotoxicity of essential oil from leaves of *Plectranthus amboinicus*, carvacrol and thymol in plant bioassays. **Journal of Agricultural and Food Chemistry**, v. 63, p. 8981-8990, 2015.

POHREN, R. S; COSTA, T. C; VARGAS, V. M. F. Investigation of sensitivity of the *Allium cepa* test as an alert system to evaluate the genotoxic potential of soil contaminated by heavy metals. **Water, Air, & Soil Pollution**, v. 224, 2013.

SANTOS, F. E.; CARVALHO, M. S. S.; SILVEIRA, G. L.; CORREA1, F. F.; CARDOSO, M. G.; ANDRADE-VIEIRA, L. F.; VILELA, L. R. Phytotoxicity and cytogenotoxicity of hydroalcoholic extracts from *Solanum muricatum* Ait. and *Solanum betaceum* Cav. (Solanaceae) in the plant model *Lactuca sativa*. **Environmental Science and Pollution Research**, v. 25, p. 1-11, 2018.

SENENT, J. **A poluição**. Rio de Janeiro: Salvat, 1979.

SILVEIRA, G. L.; LIMA, M. G. F.; DOS REIS, G. B.; PALMIERI, M. J.; ANDRADE-

VIERIA, L. F. Toxic effects of environmental pollutants: Comparative investigation using Allium cepa L. and Lactuca sativa L. **Chemosphere**, v. 178, p. 359-367, 2017.

SIMÕES, C. M. O.; SCHENKEL, E. P.; DE MELLO, J. C. P.; MENTZ, L. A.; PETROVICK, P. R. **Farmacognosia: do produto natural ao medicamento**. 1. ed. Porto Alegre: Artmed, 2017.

TEDESCO, S. B.; KUHN, A. W.; BOLIGON, A. A.; LAUGHINGHOUSE, H. D.; ATHAYDE, M. L.; DA SILVA, A. C. F.; TEDESCO, S. B. Chromatographic analysis, antiproliferative effect and genotoxicity of aqueous extracts of *Citrus sinensis* (L.) Osbeck on the *Allium cepa* L. test system. **Bioscience Journal**, v. 31, p. 1213-1221, 2015.

TURK, J. **Introduction to environmental studies**. 3.ed. New York: Saunders College, 1989.

VEIGA, M. M. Agrotóxicos: eficiência econômica e injustiça socioambiental. **Agricultura**, v. 379, p. 145-152, 2007.

ZAMBOLIM, L. Fungicidas: benefícios x riscos. **Ação Ambiental**, v. 2, p. 24-27, 1999.

CHAPTER 1: Phytotoxicity and Cytogenetic Action Mechanism of Leaf Extracts of *Psidium cattleyanum* Sabine in Plant Bioassays

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Title: Phytotoxicity and Cytogenetic Action Mechanism of Leaf Extracts of *Psidium cattleyanum* Sabine in Plant Bioassays

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ABSTRACT:

The search for more environmental friendly herbicides, aiming at the control of agricultural pests, combinated with less harmfulness to human health and the environment has grown. An alternative used by researchers is the application of products of secondary plant metabolism, which are investigated due to their potential bioactivities. Thus, species belonging to the Myrtaceae family are potential in these studies, since this family is recognized for having high biological activity. A species belonging to this genus is *Psidium cattleyanum*, which has a medicinal effect and its fruits are used in human food. Thus, the objective of this research was to evaluate and compare the phyto-cyto-genotoxicity of aqueous and ethanolic leaf extracts of the specie *P. cattleyanum*, from plant bioassays, as well as to identify the main classes of compounds present in the extracts. For this, the extracts were prepared, characterized and biological tests were carried out by evaluating, in seeds and seedlings of lettuce and sorghum, the variables: percentage of germination (GP), germination speed index (GSI), root growth (RG) and aerial growth (AG). And in meristematic lettuce cells the variables: mitotic phases, mitotic index (MI), nuclear alterations (NA) and chromosomal alterations (CA) in lettuce. Flavones, flavonones, flavonols, flavononols, flavonoids, alkaloids, resins, xanthones and anthraquinone glycoside were identified in the ethanolic extract. Both evaluated extracts, in the highest concentration, inhibited the initial plant development. All treatments caused alterations in the mitotic phases and inhibited MI. In addition, the treatments promoted an increase in CA and NA. The mechanism of action presented was aneugenic, clastogenic and determined in epigenetic alterations. The ethanolic extract was more cytotoxic, since it had a more expressive effect at a lower concentration. Despite the cytotoxicity of the extracts under study, they promoted alterations at lower levels than the glyphosate positive control. Thus, both extracts have less cytotoxicity than the commercial herbicide glyphosate.

KEYWORDS: aqueous extract, ethanolic extract, *Lactuca sativa*, Myrtaceae, *Sorghum bicolor*.

1. Introduction

Most of the known organic compounds can be found in nature, and plants are the collaborators in the formation and supply of these molecules. The production is through the natural synthesis of phytochemicals in the plant, which are commonly known as primary and secondary metabolites. This classification is carried out considering the role played by each compound in the plant [1].

The molecules and substances necessary for plant growth, such as chlorophyll, sugars, proteins, amino acids, lipids, among others, are considered primary metabolites. The compounds intended to defend the individual's biotic and abiotic stresses, such as essential oils, alkaloids, tannins, flavonoids, saponins, terpenoids, phenolic compounds, among others, are the secondary metabolites. These phytochemicals products of secondary metabolism have biological activities, being studied due to this potential [1-2].

Studies have been developed seeking to prove the effects of the different uses and applications of plant materials. In this way, plant chemical compounds have been applied in various activities of human interest, such as in the synthesis of agrochemicals, cosmetics, medicines and condiments [2-4].

Different ways of extracting and obtaining plant products are described [5-8], in order to optimize your applications. One of the ways uses water as a solvent, obtaining aqueous extracts as a product. This type of extract is the most friendly to the environment, in addition, water is the most accessible and inexpensive solvent, making it very applicable to the extraction of bioactive vegetable compounds [9]. This type of extraction determines the greater obtaining and release of hydrophilic compounds [10]. Another way is ethanol extraction, which allows greater access to lipophilic substances, such as phenolic acids, aromatic acids, flavonoids and terpenes [10]. Thus, research that evaluates the bioactivity of the products of the different methods of obtaining plant metabolites is relevant and can provide illuminating results related to the different applications of these compounds.

Moreover, the understanding of bioactivity, as well as the differential effect of aqueous and ethanolic extract is important for the establishment of cultures, the study of possible bio-chemicals, in addition to allowing knowledge about the allelopathic activities of organisms.

To investigate the bioactivity of natural products against plant development, bioassays with model plants are used [3], for elucidating the different levels of toxicity of the test

agent and indicating potential agrochemicals [3, 11-12]. In addition, they feature fast response and are low cost [3]. The species *Lactuca sativa* L. (lettuce) and *Sorghum bicolor* (L.) Moench are applied as a model in bioassays, as they are easily found agricultural supply stores and have a large amount of small-sized seeds [13], in addition to germinating within 24 hours [3].

The Myrtaceae family agroup taxon with high biological activity. Among the species of this family with potential phytochemical content is the araça *Psidium cattleyanum* Sabine [14], which presents fruits used in human food and has bioactive compounds previously proven in studies that investigated its medicinal activity [14-16].

Given the above, the objective of the present study was to evaluate and compare the phyto-cyto-genotoxicity of aqueous and ethanolic leaf extracts of the species *P. cattleyanum*, from bioassays with model plants, as well as to identify the main classes of compounds present in the extracts.

2. Material e métodos

2.1 Material Vegetal

Young leaves were collected from adult individuals of *Psidium cattleyanum* at a height of 1.30 m, in the month of February (summer), in the morning period, at the experimental field of the Center of Agricultural Sciences and Engineering (CCAE) (altitude 254 m, coordinates 20°45' 41°31') at the Federal University of Espírito Santo (UFES), and used as test agents.

Seeds of two species were adopted as plant models [3]:

a) *Lactuca sativa* L. 'Crespa Grand Rapids' (Isla Pak) (eudicot), with germination rate of 97%, purity of 100% and within the validity period indicated by the supplier;

b) *Sorghum bicolor* L. Moench 'AL Precioso' (BR Seeds) (monocot), with germination rate of 87%, purity of 99.7% and within the validity period indicated by the supplier.

2.2 Extract preparation

To obtain the test extracts, the collected leaves were dried in forced air circulation oven at 60°C for 72 h and subsequently ground in a blender.

a) Aqueous extract

30 g of the dried leaf powder were weighed and 300 mL of distilled water at 100°C were added. After 10 minutes, the infusion was filtered, yielding the extract at the concentration of 100 mg^{-1} [17-18], from which dilutions were made to obtain the concentrations of 50, 25 and 12.5 mg mL^{-1}.

b) Ethanolic extract

10 g of the dried leaf powder were weighed and 100 mL of 70% ethanol were added, being kept on a shaker for three days. Subsequently, the solution was filtered and placed in a rotary evaporator, yielding a concentrate of 500 mg mL-1 (concentration not tested). From this solution, dilutions were made to obtain the tested concentrations of 100, 50, 25 and 12.5 mg mL-1 (the same as tested with the aqueous extract).

2.3 Chemical characterization of aqueous and ethanolic extracts

The phytochemical screening to determine the main classes of secondary metabolites present in aqueous and ethanolic leaf extracts were performed as described in the literature for phenols, hydrolyzable tannins, condensed tannins, anthocyanins, anthocyanidins, leucoanthocyanidins, flavones, flavonols, flavononols, flavanones, xanthones, chalcones, aurones, catechins, steroids, triterpenoids, saponins, strong fixed acids, resins and alkaloids [19]; for anthraquinone glycosides [20]; and for cardiac glycosides [21].

2.4 Phytotoxicity assay

The experiment was established following the method of direct treatment application in completely randomized design using five repetitions per treatment, with 25 seeds per repetition [22]. Distilled water was used as negative control (C-) and the commercially

available herbicide glyphosate (0.1%) as positive control (C+) [12]. The following variables were analyzed [3, 23]:

a) Germination percentage (GP) – number of germinated seeds after 48 h of exposure to the treatments, calculated by the ratio between the number of germinated seeds times 100 divided by the total number of exposed seeds per repetition.

b) Germination speed index (GSI) – number of germinated seeds counted every 8 h during the first 48 h of exposure to the treatments, calculated by the following formula:

$$(N\ 1\ *1) + (N\ 2\ -N\ 1)\ *1/2 + (N\ 3\ -N\ 2)\ *1/3 + \ldots (N\ y\ -\ (N\ y\text{-}1))\ *1/y,$$

Where: Ny refers to the number of seeds germinated within a given period; y: represents the total number of time intervals [24].

c) Root growth (RG) – measured (in mm) with the aid of a digital caliper after 48 h of exposure to the treatments.

d) Aerial growth (AG) – measured (in mm) after 120 h of exposure to the treatments with the aid of a digital caliper.

2.5 Cyto-genotoxicity Assay

To assess cyto-genotoxicity, tip of roots of lettuce were fixed in an methanol: acetic acid fixative (3:1/vv^{-1}) after 48h of exposure to treatments [3, 25], and then they were stored at -20ºC. Two fastener changes were made; the first after ten minutes of fixation and the second after 24 hours. The roots remained fixed for at least 24 hours, until the end of the last fixation step.

The roots were washed three times, for ten minutes each, in distilled water and hydrolyzed in 5N HCl at 25ºC for 18 minutes. For each slide, semi-permanent and prepared by the crushing technique, two root meristems were used, which were cut, stained with 2% acetic orcein for 15 minutes and sealed with colorless enamel. For each treatment, five slides were prepared and 1000 cells were evaluated per slide, totaling 5000 cells per treatment. The following variables were evaluated:

a) Mitotic index (MI) - refers to the number of cells that are dividing, calculated by the ratio between the number of cells in division and the total number of cells observed [26].

b) alteretions (CA) - refers to the changes observed at the chromosomal level, calculated by the ratio between the number of cells with CA and the total number of cells observed [26].

c) Nuclear alterations (NA) - refers to the changes observed at the nuclear level, calculated by the ratio between the number of cells with NA and the total number of cells observed [26].

The CA and NA were assessed separately according to their categories and their frequencies were measured individually[12]:

a) The CA are – c-metaphase, adherence, bridge, lost chromosome, chromosome not oriented, fragmentation, polyploidization, multipolarity, the frequency of each alteration being calculated individually by the ratio of the number of cells with each CA to the total number of cells in division.

b) The NA are – micronucleus and condensed nucleus the frequency of each alteration being calculated individually by the ratio of the number of cells with each NA to the total number of cells observed.

2.6 Statistical Analysis

The values obtained in each observation were tabulated. The means were obtained by analysis of variance and submitted to the Tukey test ($p<0.05$) using the statistical program Genes and the graphics were plotted in the program R, version 3.3.2 [27-28]. Regression analysis was used to assess the mitotic index (MI). The polynomial regression models were adjusted according to the significance of ANOVA F and the quality of the models was assessed by the coefficient of determination (R^2). The analysis were performed using the R computational environment [28].

3. Results and discussion

1.1 Chemical characterization of the aqueous and ethanolic extracts

Leucoanthocyanidins, catechins, anthocyanins, anthocyanidins, aurones, chalcones, condensed tannins and triterpenoids were not observed in any of the samples (Table 1). This observation corroborates another investigation that in which neither the aqueous or the ethanolic extract of *P. cattleyanum* presented anthocyanins, anthocyanidins, aurones and chalcones, confirming this finding for the specie [29].

Table 1. Classes of secondary metabolites found in the aqueous and ethanolic extracts of *Psidium cattleyanum*. The signals (+) and (-) respectively indicate the presence or absence of the chemical classes in the analyzed plant material.

Chemical class	Aqueous extract	Ethanolic extract
Strong fixed acids	+	+
Alkaloids	-	+
Catechins	-	-
Steroids	+	+
Simple phenols	+	+
Flavonoids	-	+
Anthocyanins	-	-
Anthocyanidins	-	-
Aurones	-	-
Chalcones	-	-
Flavones	-	+
Flavanones		+
Flavonols	-	+
Flavononols	-	+

Leucoanthocyanidins	-	-
Resins	-	+
Saponins	+	+
Condensed tannins	-	-
Hydrolyzable tannins	+	+
Xanthones	-	+
Anthraquinone glycosides	-	+
Cardiac glycosides	+	+
Triterpenoids	-	-
Terpenoids	+	+

Strong fixed acids, steroids, simple phenols, saponins, hydrolyzable tannins, cardiac glycosides and terpenoids were identified in both evaluated extracts (Table 1). Saponins – previously described in the composition of aqueous and ethanolic extracts of *P. cattleyanum* [29] – show insecticide activity, causing destruction of hemolymph components, leading to alterations in the coagulation, leakage, and ultimately death of the insect [30].

Alkaloids, resins, xanthones and anthraquinone glycosides were identified only in the ethanolic extract (Table 1). Alkaloids, previously described in the composition of the ethanolic extract of *P. cattleyanum*, but not in the aqueous one [29], have been described as insecticides owing to their detrimental effect on the nervous system of most insects, acting in the ganglion-cerebral disorientation, culminating in alteration of the insects' perception [30].

Also flavones, flavonones, flavonols, flavonnnols and flavonoids were only identified in the ethanolic extract of *P. cattleyanum* (Table 1). Different types of quercetin in the chromatographic profile of *P. cattleyanum* leaves further demonstrated [31]. Thus, there are possibly different types of quercetin among the occurring flavonoids, which are related to different biological activities [32].

Considering the above, several compound classes with biological activity already described in the literature were observed in the studied extracts, demonstrating their potential in investigations of novel biological activities.

3.2. Phytotoxicity assay

The lettuce seeds treated with ethanolic extract at the concentrations of 100, 50 and 25 mg mL^{-1} did not germinate, being completely inhibited (Figure 1a).

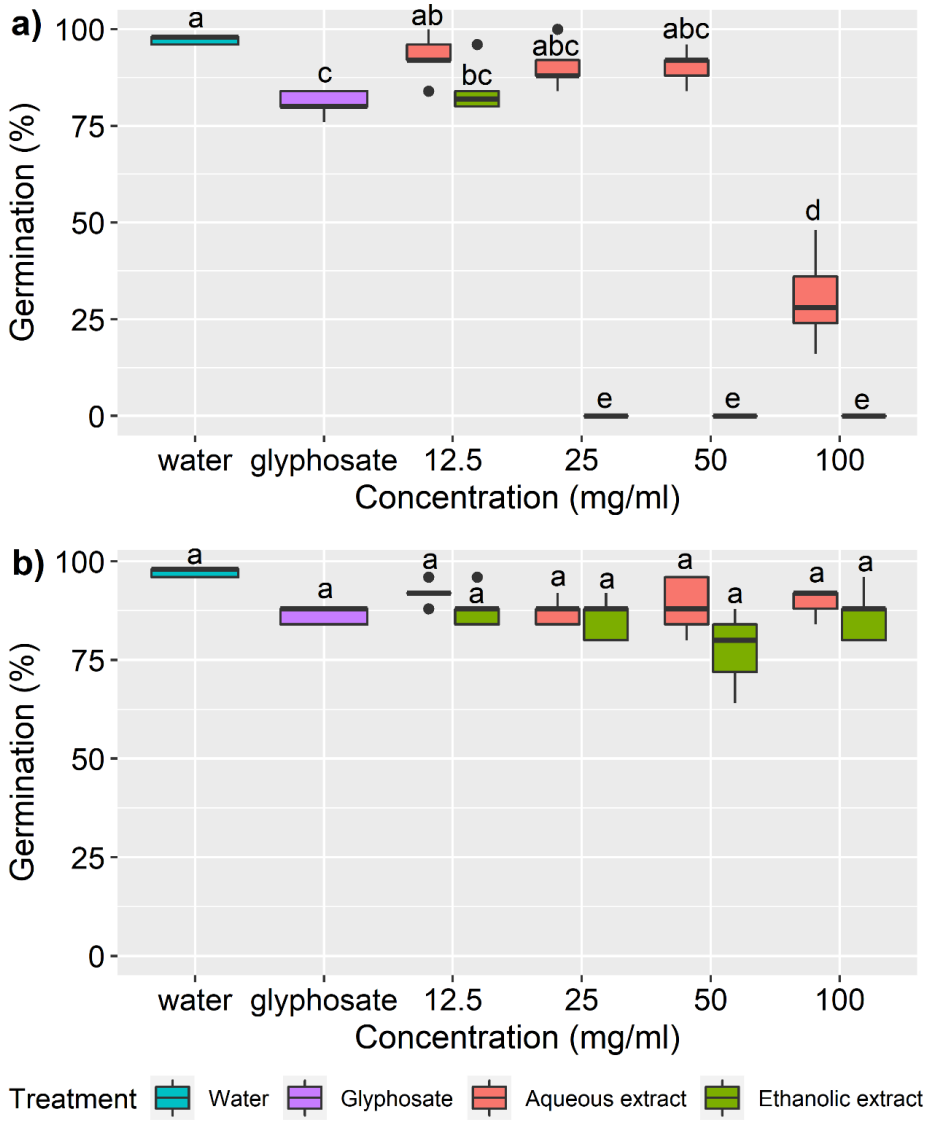

Figure 1. Effect of the aqueous and ethanolic extracts of *Psidium cattleyanum* on the germination percentage of (a) *Lactuca sativa* and (b) *Sorghum bicolor*. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

Allelochemical compounds exert direct and indirect action on the plant metabolism [33], The production of metabolites is dependent on the environmental conditions, such as nutrient availability, soil biota, chemical characteristics of the soil, interaction between the different populations, among others. Changes in these conditions that are caused by the presence of the allelochemical are considered as indirect action. In turn, the direct action comprises alterations at the level of cells and plant metabolism, including changes in physiological processes (e.g. respiration, photosynthesis) and in cell functioning (e.g. membrane permeability), among others [34-36].

In this way, when such effects of phytotoxic activity are compared to the phytochemical screening, it is noted that the treatment with aqueous extract at the highest concentration was the only that allowed germination of lettuce seeds (Figure 1a). This extract did not present flavonoid compounds, such as flavones, flavonols and flavononols (Table 1). Since flavonoids are allelochemicals known to promote inhibition of plant development, by direct and indirect action [37], it can be concluded that the observed result is related to the absence/presence of these chemical constituents in the extracts.

The variable GP remained similar among all treatments in the sorghum model (Figure 1b), whereas in the lettuce model it was significantly inhibited by all treatments compared to C- (Figure 1a). However, greater effectiveness of the ethanolic extract is observed in lettuce compared to the aqueous extract, as total inhibition of GP occurred at its three highest concentrations. This greater effectiveness of the ethanolic vs. aqueous extract may be associated to the presence of alkaloids only in the first (Table 1). This metabolite class has been described to promote rupture of the cell membrane [38], allowing leakage of electrolytes, thus inhibiting the development of seeds/plantlets in a direct way.

It is important to highlight that C+ reduced the GP in lettuce by approximately 20% when compared to C-, with the reductions caused by the extracts being superior to that promoted by C+ (Figure 1a). Studying the allelopathic effect of the aqueous extract of *P. cattleyanum*, other study also reported inhibition of the germination of lettuce seeds treated at the concentration of 75 g L^{-1}, associating this inhibition to the presence of allelopathic compounds, which act by interfering with membrane permeability, cell division and enzyme activation [39].

The variable GSI in the sorghum model did not present significant difference between the treatments, including C+ (Figure 2b). However, the lettuce model displayed several significant alterations (inductions and inhibitions), also in relation to C+ (Figure 2a), demonstrating that the extracts are more effective in lettuce than in sorghum. Alterations in the GSI evince the occurrence of changes in the metabolic processes related to germination [33]. In addition, highlighted that allelochemicals selectively inhibit and alter the growth or development patterns of the plants [40]. Thus, such effect can be related to (1) the different responses of the seeds in the germination process of monocots and eudicots, and (2) the greater sensitivity of lettuce to the metabolites produced by *Psidium*. Studying the phytotoxicity of essential oils from different species of this genus, also reported greater sensitivity of lettuce in comparison to sorghum [41].

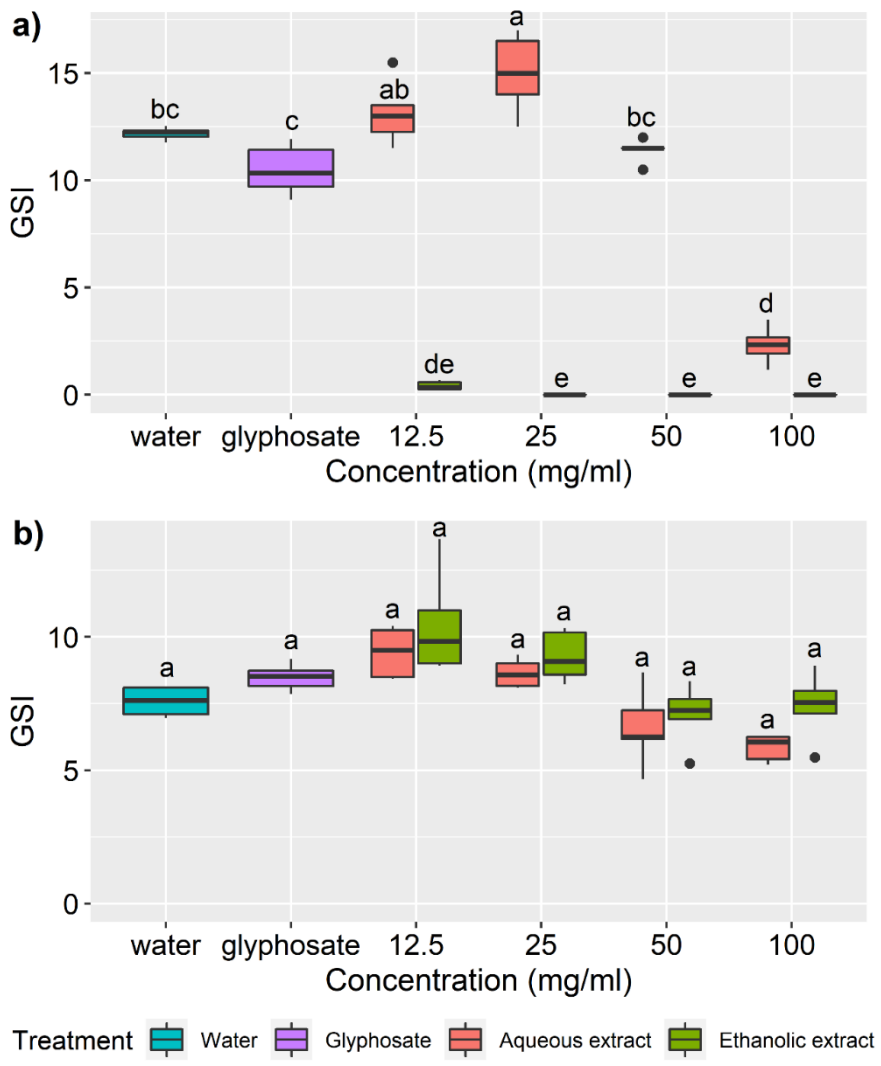

Figure 2. Effect of the aqueous and ethanolic extracts of *Psidium cattleyanum* on the germination speed index (GSI) of (a) *Lactuca sativa* and (b) *Sorghum bicolor*. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

For the variable RG, significant inhibition was observed in both plant models (Figure 3). Comparing both test agents, the ethanolic extract was more efficient in lettuce (comparing the highest concentration), whereas the aqueous extract was more efficient in sorghum (Figure 3). Some authors have already reported differential response for monocots and eudicots, relating similar results with the physiology of the plants [3, 12, 41].

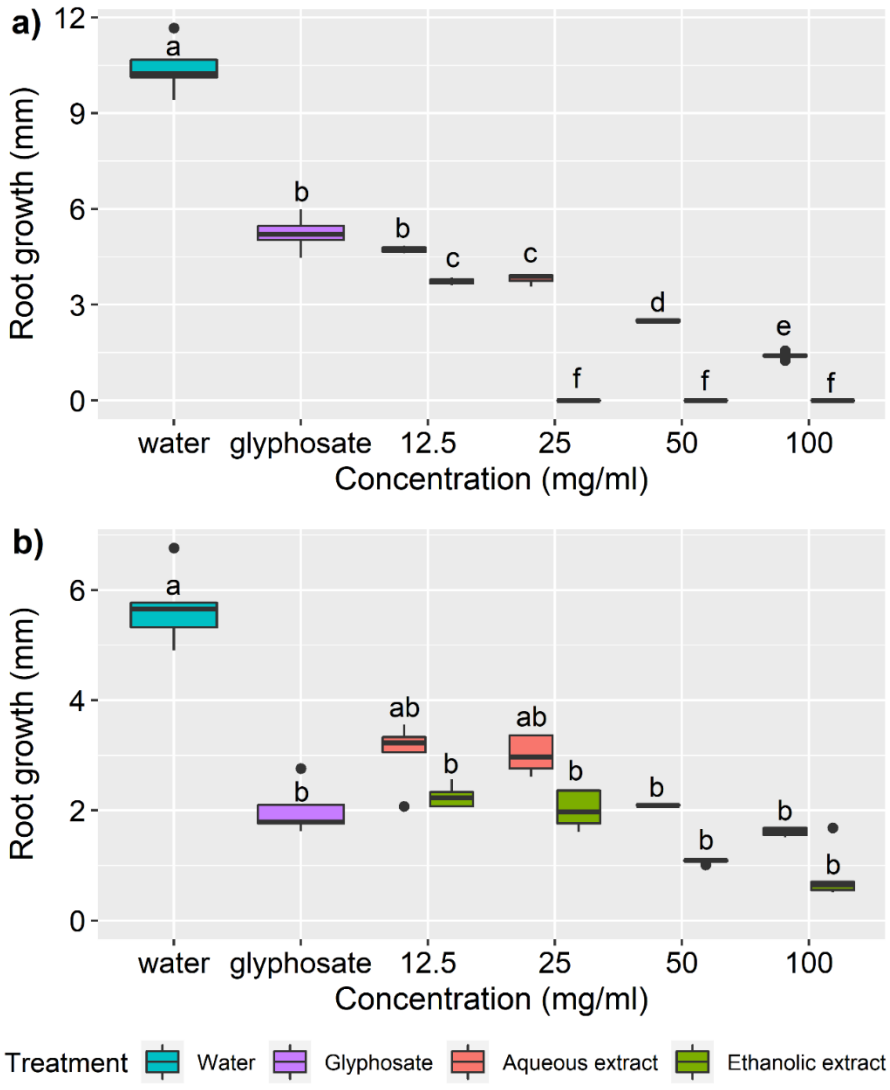

Figure 3. Effect of the aqueous and ethanolic extracts of *Psidium cattleyanum* on the root growth of (a) *Lactuca sativa* and (b) *Sorghum bicolor*. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

For the lettuce model, inhibition of AG was observed in the treatment with ethanolic extract only at the concentration of 12.5 mg mL^{-1}, in 35.4% of the plantlets, compared to C- (Figure 4a). The concentrations of aqueous extract that presented inhibitory effect were 25, 50 and 100 mg mL^{-1}, reaching 31.6%, 43.4% and 63.4% of the plantlets, respectively, in comparison to C- (Figure 4a). Several factors are determining for RG and AG, including nutritional and cellular conditions. In order for the plantlets to grow, it is necessary that cell multiplication and/or elongation occur. Moreover, the initial development is dependent on the formation of the cambium and xylem, which occurs according to the availability and distribution of nutrients in the plantlets [40].

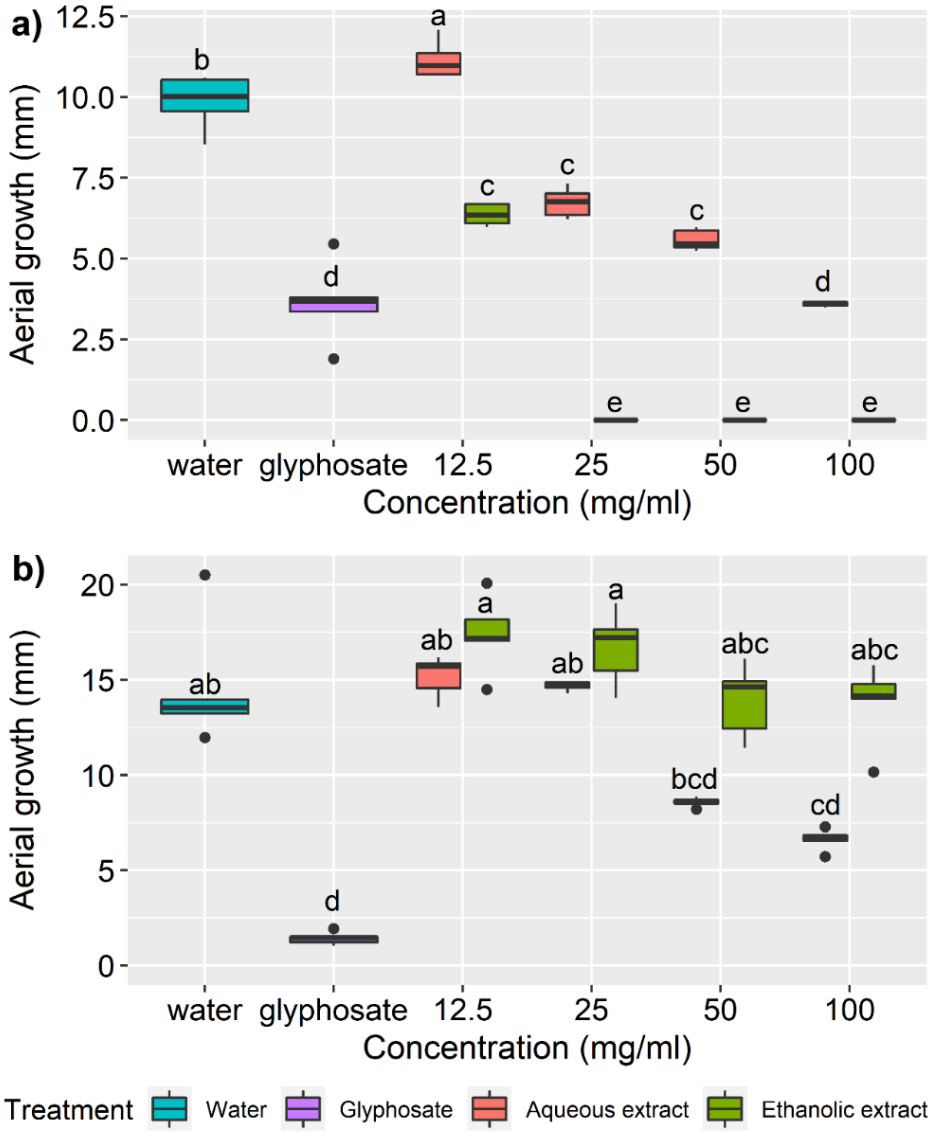

Figure 4. Effect of the aqueous and ethanolic extracts of *Psidium cattleyanum* on the growth of the aerial part of (a) *Lactuca sativa* and (b) *Sorghum bicolor*. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

The AG of sorghum was inhibited by the aqueous extract at the concentration of 100 mg mL^{-1} in 54.8% of the plantlets, compared to C- (Figure 4b). The evaluation of germination parameters and of the initial growth of the plantlets is considerably elucidative regarding the toxicity of compounds, since these are critical stages of the plant development and subject to high error rates, owing to the seeds presenting lower tolerance to different conditions imposed by the environment [40].

Overall, higher toxicity of the evaluated extracts was observed for the variable RG than for AG (Figures 3 and 4). The greater sensitivity of RG was also described by Cândido et al. [42]. These authors highlighted that, when comparing the action of phytotoxic agents in roots and aerial parts of plantlets, the effects are more prominent in the roots, as they remain in direct contact with the allelochemical, which increases the possibility of toxic agents influencing the development of this region.

Considering all variables of the assay, it was demonstrated that the ethanolic extract was the most toxic agent for lettuce, whereas the aqueous extract was the most efficient in the sorghum model.

3.3. Cyto-genotoxicity Assay

The three largest concentrations (100, 50 and 25 mg mL^{-1}) of ethanolic extract completely inhibited the emission of roots in the model plant (lettuce). Thus, they could not be evaluated for cytotoxic parameters.

The treatments evaluated with ethanolic extract and those of 25, 50 and 100 mg mL^{-1} with aqueous extract reduced the cells in prophase (Figure 5). Prophase is the first phase of mitotic division, which is the phase subsequent to the checkpoint of the G2 phase of the interphase[43]. Thus, the reduction in the number of cells in prophase indicates possible mistakes in the cell cycle, in order to activate interphase checkpoints. This check may be taking place in phase G1, before DNA duplication - or in phase G2, after DNA duplication [43]. However, regardless of the point at which the cell cycle is prevented from proceeding, this reduction demonstrates that the cell is identifying changes, whether metabolic, molecular or physiological, which put the tissue at risk, and therefore, its division, and consequently the perpetuation “mistakes” is inhibited.

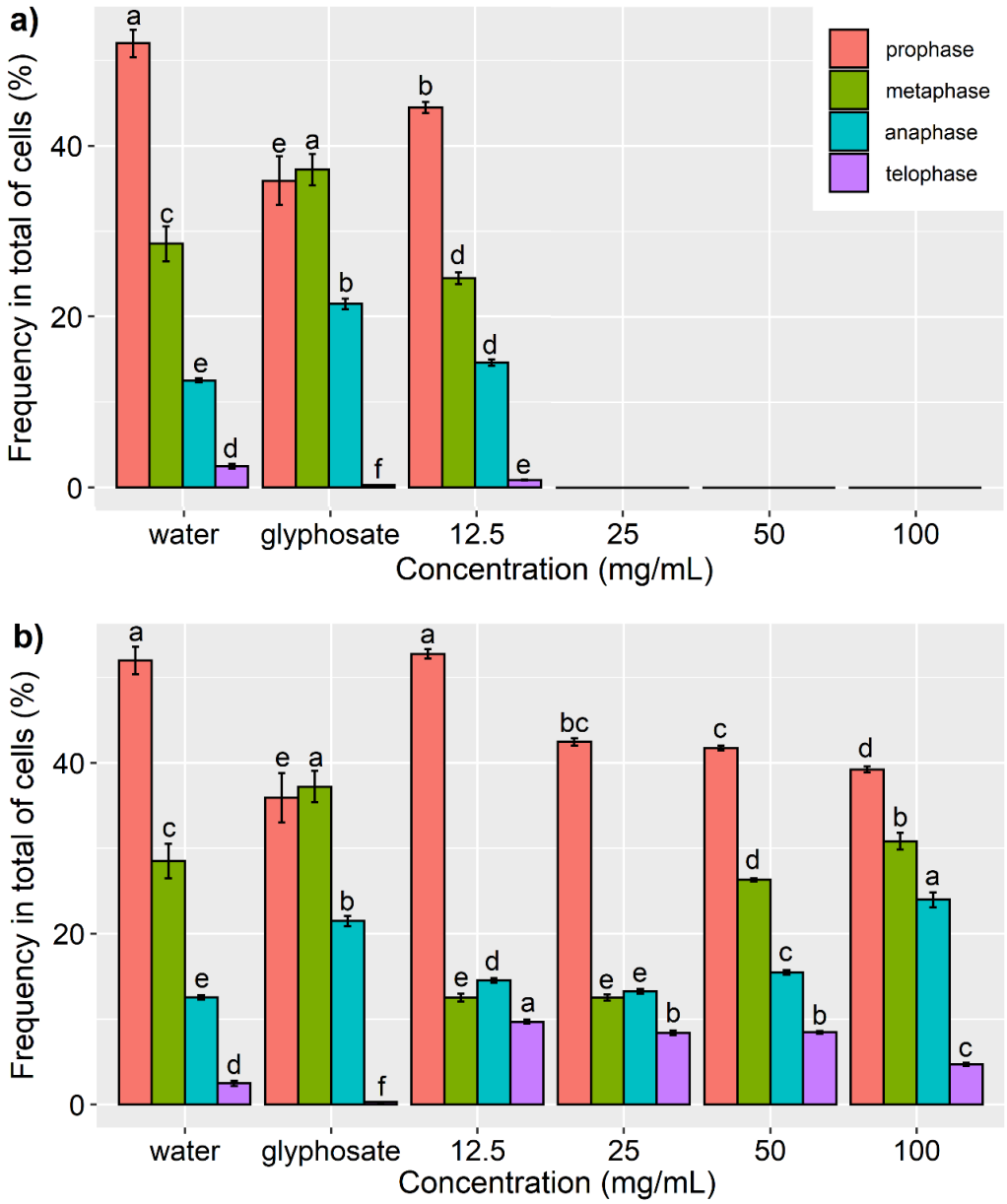

Figure 5. Effect of (a) ethanolic extract and (b) aqueous extract of *Psidium cattleyanum* on the mitotic phases of meristematic cells at the tip of the root of *Lactuca sativa*. The small letters above the bars indicate significant differences between treatments by the Tukey test (p<0.05).

Meristem systems treated with ethanolic extract in the concentration of 12,5 mg mL^{-1}, as well as with aqueous extracts in concentrations of 12,5, 25 e 50 mg mL^{-1} showed a reduction in metaphase cells (Figure 1). This reduction may be a reflection of the reduction in the number of cells that divide and pass to the following phases. However, the aqueous extract in the concentration of 100 mg mL^{-1} promoted an increase in

metaphase cells (Figure 5). According Aragão et al.[44], the increase in cells in metaphase is associated with changes in the mitotic spindle, which results in cell stagnation at this stage.

Treatments with aqueous extract in the concentrations of 12,5, 50 e 100 mg mL^{-1}, and treatment with ethanolic extract determined an increase in anaphase cells (Figure 5). The mitosis checkpoint occurs in anaphase [43]. In view of the increase in cells at this stage of the division, it is concluded that this checkpoint is also being inhibited, preventing the passage of cells to the next phase, resulting in the accumulation of anaphases.

The number of cells in telophase was inhibited in the treatment with ethanolic extract (Figure 5), which may be due to the inhibition of the other phases and the mitosis checkpoint. However, the aqueous extract in all tested concentrations determined the increase in cells in telophase (Figure 1). This increase may be related to a delay in the division time, which may be due to the inhibition of the mitosis checkpoint. Thus, cells that should have ended the cycle are experiencing delayed phase change, resulting in an increase in telophase cells in these treatments.

The mitotic index (MI) of all evaluated treatments, suffered a significant reduction, less than 50%, when compared to the C- (Figure 2). According Fiskejö [45], an effectively cytotoxic agent has MI inhibition greater than 50%, as is the case with the C+ used in the study, which showed a 73.7% reduction when compared to C- (Figure 6).

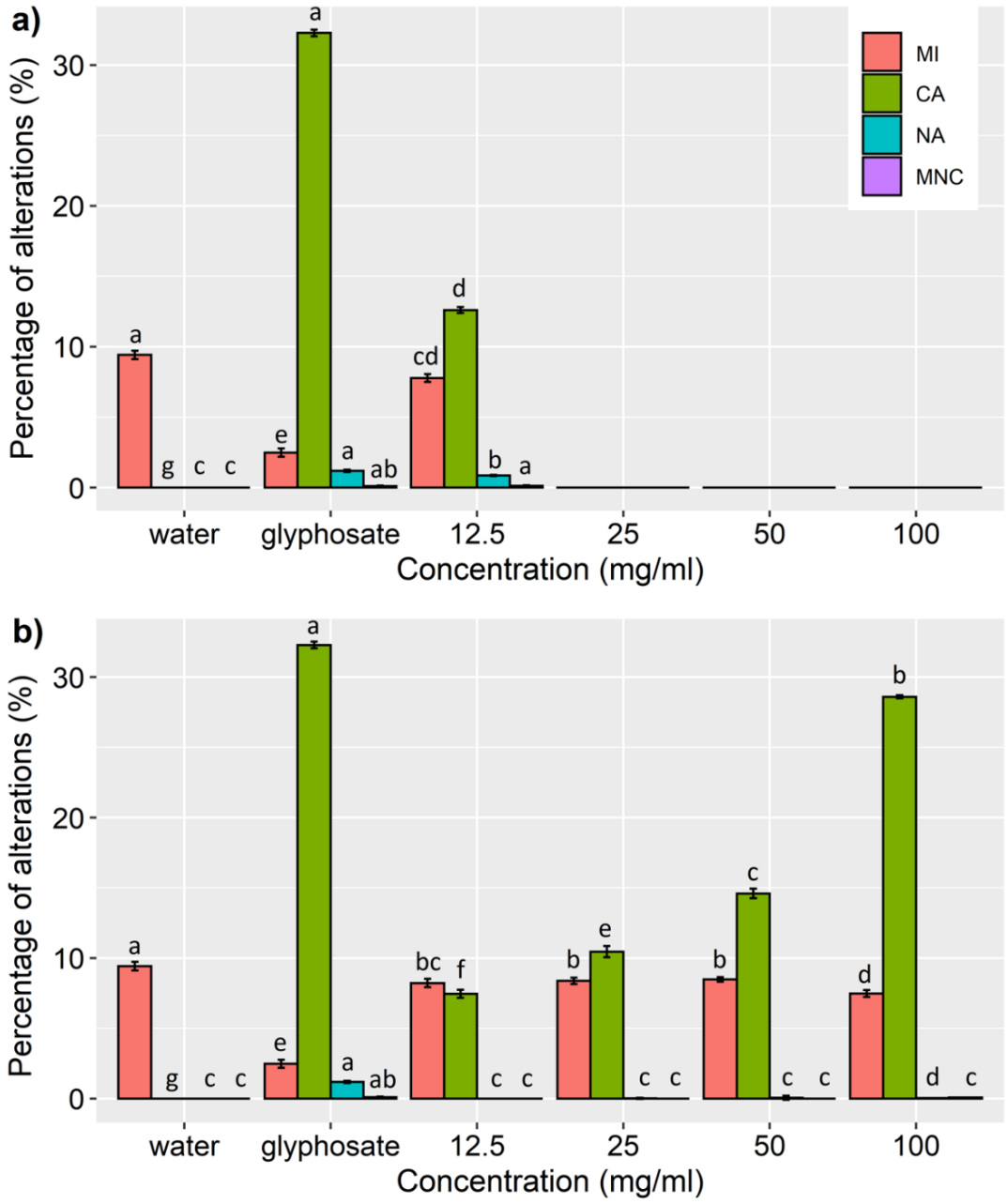

Figure 6. Percentage of observed alterations in the cell cycle of meristematic cells from the tip of the *Lactuca sativa* root exposed to (a) ethanolic extract and (b) aqueous extract from *Psidium cattleyanum* leaves. The small letters above the bars indicate significant differences between the treatments by the Tukey test (p<0.05). CA = chromosomal alterations, NA = nuclear alterations, MNC = micronuclei.

The most inducing treatment for chromosomal alterations (CA) was C+, although all other treatments promoted more CA than C- (Figure 6), showing that the treatments are

less toxic to chromosomes/DNA than glyphosate. This result is important, since, it is sought, compounds with high biological activity and that promote lesser impacts to the environment [3, 12-13, 44].

The treatments with aqueous extract did not promote an increase in nuclear alterations (NA) (Figure 6), with the opposite being observed with the ethanolic extract (Figure 6). NA are those that alter the cell nucleus of the cell metabolically or morphologically or are also related to the appearance of DNA in a compartmentalized way, as if new nuclei were being formed, as is the case of micronuclei [3, 13, 38, 46]. These changes reflect "mistakes" that are occurring during the split. In some cases, such as micronuclei (MNC), the goal is to reestablish the DNA content inside the nucleus; in others, as in the formation of condensed nuclei (Figure 7b), the damage is large enough to activate the cell death process, the latter being considered cytological evidence of the occurrence of cell death [46-47].

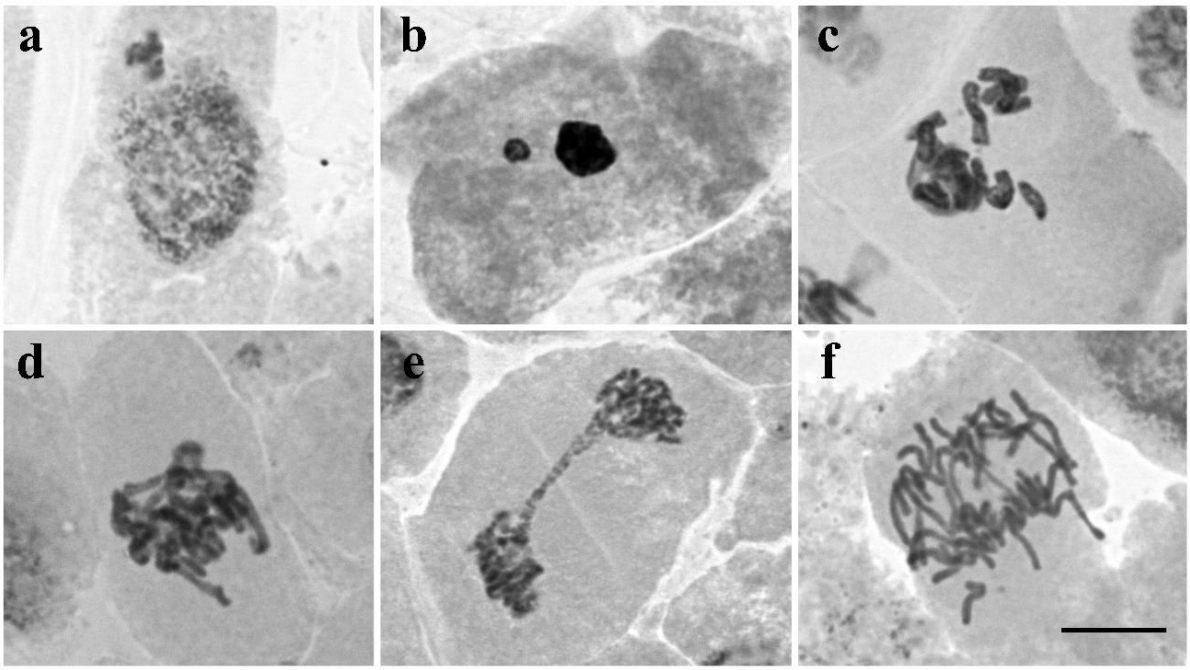

Figure 7. Alterations observed in meristematic cells of *Lactuca sativa* treated with aqueous and ethanolic extracts of *Psidium cattleyanum*. Where it is illustrated: (a) micronucleus, (b) condensed nucleus; (c) c-metaphase, (d) adherent, (e) bridge in telophase, (f) anaphase bridge with lost chromosome. Bar = 10µm.

The types of chromosomal alterations observed in plant bioassays allow classify the cellular action mechanism of the evaluated test agent. Thus, test agents that promote chromosomal changes resulting from changes in the formation of the mitotic spindle are considered aneugenic [3, 12, 48], since they will change the chromosomal number of the daughter cells, but they will not affect their DNA sequences. Whereas, test agents that determine chromosomal changes that alter the DNA sequence of daughter cells, such as bridges and chromosomal fragments, are considered clastogenic [41, 49]. Test agents that cause chromosomal alterations that modify chromosomal signaling, such as adherence, are considered as epigenetic action mechanisms because it is triggered by changes in the phosphorylation pattern of serine 10 in histone 3 [3, 50]. It is important to note that test agents can have more than one action mechanism simultaneously.

Both NA MNC (Figure 7a) and CA bridge (Figure 7e) showed an increase in treatments: ethanolic extract and aqueous extract (100 mg mL^{-1}) (Figures 6 and 8). The increase in these alterations are related, since the chromosome bridge is associated with the break-fusion-break cycle. In this case, telomeres are lost by fragmentation leaving cohesive ends of the chromosomes exposed. Thus, ends of different chromosomes connect, and at the time of chromosomal segregation, the formation / visualization of chromosomal bridges occurs. These linked chromosomes, which are being pulled to opposite poles, undergo “traction” by depolymerizing the microtubules, so that a new break occurs giving continuity to the break-fusion-break cycle [23, 38, 51]. These fragments formed in the cycle are organized in MNC, after the end of the division, to be exported from inside the cells [23, 46, 52], resulting in the formation of MNC.

The frequency of c-metaphases (Figure 7c) increased in all treatments evaluated when compared to C- (Figure 8). This alteration derives from the total dysfunction of the mitotic spindle, showing that microtubule polymerization is not occurring, consequently, the chromosomes are not organized in the cell's equatorial plane [23, 38, 51].

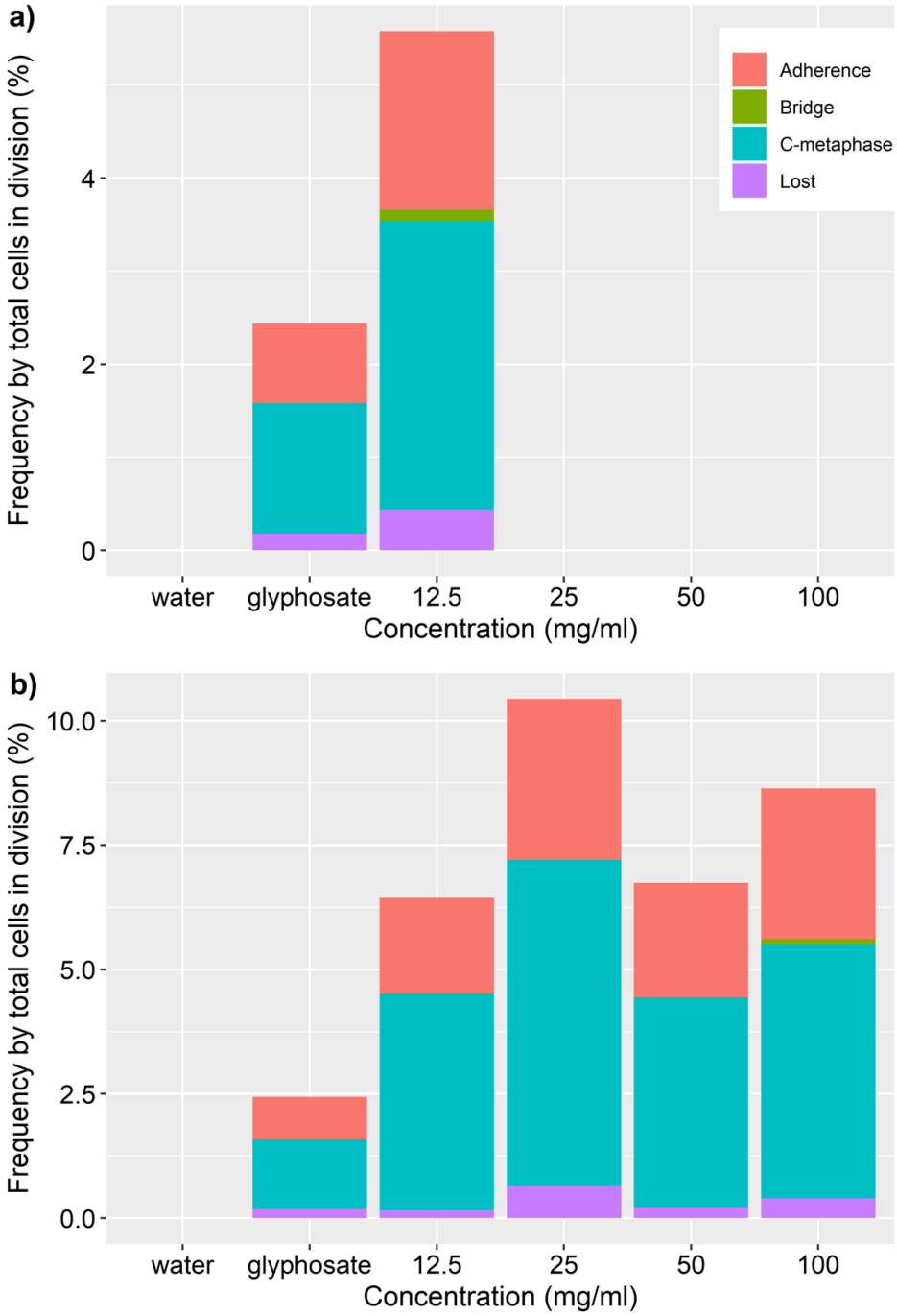

Figure 8. Distribution of observed chromosomal alterations in the cell cycle of meristematic cells from the tip of the *Lactuca sativa* root exposed to (a) ethanolic extract and (b) aqueous extract of *Psidium cattleyanum*.

The lost chromosomes (Figure 7f) were observed, significantly, in the cells treated with the evaluated concentration of the ethanolic extract and in the concentrations of 25 and 100 mg mL^{-1} of the aqueous extract (Figure 8). The lost chromosomes, as well as the

c-metaphases, refer to the bad organization of the spindle. However, the lost chromosomes are due to a partial change, while the c-metaphases of a total change in the formation of spindle fibers [38, 48].

Chromosomal adherence (Figure 7d) was observed, significantly, in the cells treated with all evaluated extracts (Figure 8). This alteration refers to a series of changes that occur. This alteration indicates changes in the functioning of the mitotic machinery, alteration in the chromosomal constitution, as well as alteration in the phosphorylation of amino acids that constitute the chromosomes, thus modifying its signaling [3, 23, 38, 50].

The observed CAs elucidated the action mechanism of the treatments. Thus, all treatments were aneugenic, as well as having an epigenetic effect, acting on mitotic machinery and chromosomal signaling. In addition, ethanolic extract in the investigated concentration and aqueous extract in the concentration of 100 mg mL^{-1} were clastogenic, altering the DNA sequence of the cells.

The knowledge of biological activity in the cell cycle, as well as the cellular action mechanism of plant extracts is important for directing studies and understanding relational dynamics between organisms. In addition, it helps to elucidate possible adverse health effects, assisting in the indication and restriction of the daily use of plants, whether for nutrition, body care and / or as a herbal medicine.

4.Conclusion

Elucidating the differential effects of extracts according to the applied extraction method and the target organism is important for the varied applications of natural products. This work contributed in the context of showing that the choice of extraction method should be in accordance with the aimed purpose; accordingly, the ethanolic extract favors the extraction of flavones, flavonones, flavonols, flavonnnols and flavonoids, alkaloids, resins, xanthones and anthraquinone glycosides.

With regard to phytotoxic potential, the ethanolic extract was more effective in lettuce, whereas the aqueous extract was more effective in inhibiting the sorghum model. Thus, differential toxicity was observed between the two used extraction methods and for the both model species.

The evaluated extracts were shown to be cytotoxic, with mechanisms of aneugenic action and promoters of epigenetic alterations, against the lettuce cell cycle. The ethanolic extract and the higher concentration of the aqueous extract promoted a significant increase in nuclear alterations, when compared to water. In addition, these treatments proved to be clastogenic. These results demonstrate the bioactivity of these extracts, as well as their toxic potential for biological applications.

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References

1. Mohan, B.; Saxena, H.; Kakkar, A.; Mishra, M., Determination of antioxidant activity, total phenolic and flavonoid contents in leaves, stem and roots of Uraria picta Desv. *Environment Conservation Journal* **2019,** *20* (3), 1-8.

2. Oszmiański, J.; Wojdyło, A.; Juszczyk, P.; Nowicka, P., Roots and Leaf Extracts of Dipsacus fullonum L. and Their Biological Activities. *Plants* **2020,** *9* (1), 78.

3. de Assis Alves, T.; Fontes Pinheiro, P.; Miranda Praça-Fontes, M.; Fonseca Andrade-Vieira, L.; Barelo Corrêa, K.; de Assis Alves, T.; da Cruz, F. A.; Lacerda Júnior, V.; Ferreira, A.; Bastos Soares, T. C., Toxicity of thymol, carvacrol and their respective phenoxyacetic acids in Lactuca sativa and Sorghum bicolor. *Industrial Crops and Products* **2018,** *114*, 59-67.

4. Aparecida Braga, M.; Silva de Abreu, T.; Cardoso Trento, M. V.; Henrique Andrade Machado, G.; Lopes Silva Pereira, L.; Assaid Simão, A.; Marcussi, S., Prospection of Enzyme Modulators in Aqueous and Ethanolic Extracts of Lippia sidoides Leaves: Genotoxicity, Digestion, Inflammation, and Hemostasis. *Chemistry & biodiversity* **2019,** *16* (3), e1800558.

5. Ferreira-Dias, S.; Valente, D. G.; Abreu, J. M., Comparison between ethanol and hexane for oil extraction from Quercus suber L. fruits. *Grasas y Aceites* **2003,** *54* (4), 378-383.

6. Alothman, M.; Bhat, R.; Karim, A., Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry* **2009,** *115* (3), 785-788.

7. Ozer, M. S.; Sarikurkcu, C.; Tepe, B., Phenolic composition, antioxidant and enzyme inhibitory activities of ethanol and water extracts of Chenopodium botrys. *RSC advances* **2016,** *6* (69), 64986-64992.

8. Saklani, S.; Mishra, A. P.; Chandra, H.; Atanassova, M. S.; Stankovic, M.; Sati, B.; Shariati, M. A.; Nigam, M.; Khan, M. U.; Plygun, S., Comparative evaluation of polyphenol contents and antioxidant activities between ethanol extracts of Vitex negundo and Vitex trifolia L. leaves by different methods. *Plants* **2017,** *6* (4), 45.

9. Vuong, Q. V.; Hirun, S.; Roach, P. D.; Bowyer, M. C.; Phillips, P. A.; Scarlett, C. J., Effect of extraction conditions on total phenolic compounds and antioxidant activities of Carica papaya leaf aqueous extracts. *Journal of herbal medicine* **2013,** *3* (3), 104-111.

10. Kubiliene, L.; Jekabsone, A.; Zilius, M.; Trumbeckaite, S.; Simanaviciute, D.; Gerbutaviciene, R.; Majiene, D., Comparison of aqueous, polyethylene glycol-aqueous and ethanolic propolis extracts: antioxidant and mitochondria modulating properties. *BMC complementary and alternative medicine* **2018,** *18* (1), 165.

11. Luber, J.; Palmieri, M. J.; Botelho, C. M.; Rinaldo, D.; Andrade-Vieira, L. F., Investigation on the effects of guava (Psidium guajava L.) infusions on germination, root tips and meristematic cells of Latuca sativa. *Anais da Academia Brasileira de Ciências* **2015,** *87* (2), 903-913.

12. Pinheiro, P. F.; Costa, A. V.; Alves, T. d. A.; Galter, I. N.; Pinheiro, C. A.; Pereira, A. F.; Oliveira, C. M. R.; Fontes, M. M. P., Phytotoxicity and Cytotoxicity of Essential Oil from Leaves of Plectranthus amboinicus, Carvacrol, and Thymol in Plant Bioassays. *Journal of Agricultural and Food Chemistry* **2015,** *63* (41), 8981-8990.

13. Aragão, F. B., Palmieri, M.J., Ferreira, A., Costa, A.V., Queiroz, V.T., Pinheiro, P.F., Andrade-Vieira, L.F., Phytotoxic and cytotoxic effects of Eucalyptus essential oil on lettuce (Lactuca sativa L.). *Allelopathy Journal* **2015,** *35* (1), 259-272.

14. Vinholes, J.; Reis, S. F.; Lemos, G.; Barbieri, R. L.; de Freitas, V.; Franzon, R. C.; Vizzotto, M., Effect of in vitro digestion on the functional properties of Psidium

cattleyanum Sabine (araçá), Butia odorata (Barb. Rodr.) Noblick (butiá) and Eugenia uniflora L.(pitanga) fruit extracts. *Food & function* **2018,** *9* (12), 6380-6390.

15. dos Santos Pereira, E.; Vinholes, J.; Franzon, R. C.; Dalmazo, G.; Vizzotto, M.; Nora, L., Psidium cattleyanum fruits: A review on its composition and bioactivity. *Food chemistry* **2018,** *258*, 95-103.

16. McCook-Russell, K. P.; Nair, M. G.; Facey, P. C.; Bowen-Forbes, C. S., Nutritional and nutraceutical comparison of Jamaican Psidium cattleyanum (strawberry guava) and Psidium guajava (common guava) fruits. *Food chemistry* **2012,** *134* (2), 1069-1073.

17. de Almeida, K. C.; Barbosa, T. R.; da Silva, R. N. R.; da Silva Jacques, D.; Freire, R. B., Efeito citotóxico do infuso aquoso de Psidium guajava L.(Myrtaceae). *Rev. Bras. Farm* **2006,** *87* (2), 60-62.

18. Prichoa, F. C.; Leyser, G.; Oliveira, J. V. d.; Cansian, R. L., Comparative allelopathic effects of Cryptocarya moschata and Ocotea odorifera aqueous extracts on Lactuca sativa. *Acta Scientiarum. Agronomy* **2013,** *35* (2), 197-202.

19. Matos, F. J. d. A., *Introdução à fitoquímica experimental*. 2009; p 150.

20. Joshi, A.; Bhobe, M.; Sattarkar, A., Phytochemical investigation of the roots ofGrewia microcosLinn. *Journal of Chemical and Pharmaceutical Research* **2013,** *5* (7), 80-87.

21. Ayoola, G.; Coker, H.; Adesegun, S.; Adepoju-Bello, A.; Obaweya, K.; Ezennia, E.; Atangbayila, T., Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research* **2008,** *7* (3), 1019-1024.

22. Alves, T. A.; Roberto, C. E. O.; Pinheiro, P. F.; Alves, T. A.; Henrique, M. K.; Ferreira, A.; Clarindo, W. R.; Praça-Fontes, M. M., Searching an auxinic herbicide to use as positive control in toxicity assays. *Anais da Academia Brasileira de Ciências* **2021,** *93* (1).

23. Silveira, G. L.; Lima, M. G. F.; Reis, G. B. d.; Palmieri, M. J.; Andrade-Vieria, L. F., Toxic effects of environmental pollutants: Comparative investigation using Allium cepa L. and Lactuca sativa L. *Chemosphere* **2017,** *178*, 359-367.

24. Maguire, J. D., Speed of germination—Aid in selection and evaluation for seedling emergence and vigor 1. *Crop science* **1962,** *2* (2), 176-177.

25. Quezia Pains Dutra, J. A. C., Tatiana T avares C arrijo,; Thayllon de Assis Alves, T. d. A. A., Luiza Alves Mendes,; Praça-Fontes, M. M., Phytocytotoxicity of volatile

constituents of essential oils from *Sparattanthelium* Mart. species (Hernandiaceae). *Scientific Reports* **2020**.

26. FISKESJÖ, G., The Allium test as a standard in environmental monitoring. *Hereditas* **1985,** *102* (1), 99-112.

27. Cruz, C. D., GENES: a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy* **2013,** *35*, 271-276.

28. Core Team, R., R: A language and environment for statistical computing (Computer software). Vienna, Austria. Retrieved from https://www. R-project. org: 2020.

29. Gavilla, A. A.; Muro, L. V., Tamizaje fitoquímico de los extractos alcohólico, etéreo y acuoso de las hojas del guayabo fresa (Psidium cattleyanum Sabine). *Agricultura Tropical* **2018,** *4* (1).

30. Cárdenas Tello, C., Plantas alelopáticas. Universidad de las Fuerzas Armadas ESPE. Ciencias de la Vida.: 2014.

31. Wang, L.; Wu, Y.; Bei, Q.; Shi, K.; Wu, Z., Fingerprint profiles of flavonoid compounds from different Psidium guajava leaves and their antioxidant activities. *Journal of separation science* **2017,** *40* (19), 3817-3829.

32. Díaz-de-Cerio, E.; Verardo, V.; Gómez-Caravaca, A. M.; Fernández-Gutiérrez, A.; Segura-Carretero, A., Health effects of Psidium guajava L. Leaves: An overview of the last decade. *International journal of molecular sciences* **2017,** *18* (4), 897.

33. Maraschin-Silva, F.; Aquila, M., Potencial alelopático de espécies nativas na germinação e crescimento inicial de Lactuca sativa L.(Asteraceae). *Acta Botanica Brasilica* **2006,** *20* (1), 61-69.

34. Reigosa, M. J.; Sánchez-Moreiras, A.; González, L., Ecophysiological approach in allelopathy. *Critical reviews in plant sciences* **1999,** *18* (5), 577-608.

35. Rice, E., Allelopathy. 2nd (ed.) Acad. Press. *Inc. Orlando. Florida, USA* **1984**.

36. Rizvi, S.; Haque, H.; Singh, V.; Rizvi, V., A discipline called allelopathy. In *Allelopathy*, Springer: 1992; pp 1-10.

37. Carvalho, M. S. S.; Andrade-Vieira, L. F.; dos Santos, F. E.; Correa, F. F.; das Graças Cardoso, M.; Vilela, L. R., Allelopathic potential and phytochemical screening of ethanolic extracts from five species of Amaranthus spp. in the plant model Lactuca sativa. *Scientia horticulturae* **2019,** *245*, 90-98.

38. dos Santos, F. E.; Carvalho, M. S. S.; Silveira, G. L.; Correa, F. F.; das Graças Cardoso, M.; Andrade-Vieira, L. F.; Vilela, L. R., Phytotoxicity and cytogenotoxicity of hydroalcoholic extracts from Solanum muricatum Ait. and Solanum betaceum

Cav.(Solanaceae) in the plant model Lactuca sativa. *Environmental Science and Pollution Research* **2018**, 1-11.

39. Hister, C. A. L.; Trapp, K. C.; Tedesco, S. B., Potencial alelopático e antiproliferativo de extratos aquosos das folhas de Psidium cattleyanum Sabine sobre Lactuca sativa L. *Revista Brasileira de Biociências* **2016,** *14* (2).

40. Merino, F. J. Z.; Ribas, D. F.; Silva, C. B. d.; Duarte, A. F. S.; Paula, C. d. S.; Oliveira, M. d.; Dias, J. d. F. G.; Miguel, M. D.; Miguel, O. G., A study of the phytotoxic effects of the aerial parts of Senecio westermanii Dusén (Asteraceae) on Lactuca sativa L. and Allium cepa L. seeds. *Brazilian Journal of Pharmaceutical Sciences* **2018,** *54* (3).

41. Vasconcelos, L. C.; de Souza Santos, E.; de Oliveira Bernardes, C.; da Silva Ferreira, M. F.; Ferreira, A.; Tuler, A. C.; Carvalho, J. A. M.; Pinheiro, P. F.; Praça-Fontes, M. M., Phytochemical analysis and effect of the essential oil of Psidium L. species on the initial development and mitotic activity of plants. *Environmental Science and Pollution Research* **2019,** *26* (25), 26216-26228.

42. Cândido, A. C. d. S.; Schmidt, V.; Laura, V. A.; Faccenda, O.; Hess, S. C.; Simionatto, E.; Peres, M. T. L. P., Potencial alelopático da parte aérea de Senna occidentalis (L.) Link (Fabaceae, Caesalpinioideae): bioensaios em laboratório. **2010**.

43. Bruce Alberts, D. B., Karen Hopkin, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter, Ardala Elisa Breda Andrade Gaby Renard, *Fundamentos da Biologia Celular*. 4ª ed.; 2017.

44. Barroso Aragão, F.; Tebaldi Queiroz, V.; Ferreira, A.; Vidal Costa, A.; Fontes Pinheiro, P.; Tavares Carrijo, T.; Fonseca Andrade-Vieira, L., Phytotoxicity and cytotoxicity of Lepidaploa rufogrisea (Asteraceae) extracts in the plant model Lactuca sativa (Asteraceae). *Revista de Biología Tropical* **2017,** *65*, 435-443.

45. Fiskesjö, G., The Allium test—a potential standard for the assessment of environmental toxicity. In *Environmental Toxicology and Risk Assessment: Second Volume*, ASTM International: 1993.

46. Andrade-Vieira, L.; Gedraite, L.; Campos, J.; Davide, L., Spent Pot Liner (SPL) induced DNA damage and nuclear alterations in root tip cells of Allium cepa as a consequence of programmed cell death. *Ecotoxicology and environmental safety* **2011,** *74* (4), 882-888.

47. Costa, A. V.; Oliveira, M. V. L. d.; Pinto, R. T.; Moreira, L. C.; Gomes, E. M. C.; Alves, T. d. A.; Pinheiro, P. F.; Queiroz, V. T. d.; Vieira, L. F. A.; Teixeira, R. R.,

Synthesis of Novel Glycerol-Derived 1, 2, 3-Triazoles and Evaluation of Their Fungicide, Phytotoxic and Cytotoxic Activities. *Molecules* **2017,** *22* (10), 1666.

48. Fernandes, T. C. C.; Mazzeo, D. E. C.; Marin-Morales, M. A., Origin of nuclear and chromosomal alterations derived from the action of an aneugenic agent—Trifluralin herbicide. *Ecotoxicology and environmental safety* **2009,** *72* (6), 1680-1686.

49. Bernardes, P. M.; Andrade-Vieira, L. F.; Aragão, F. B.; Ferreira, A.; da Silva Ferreira, M. F., Toxicity of Difenoconazole and Tebuconazole in Allium cepa. *Water, Air, & Soil Pollution* **2015,** *226* (7), 207.

50. Freitas, A. S.; Cunha, I. M. F.; Andrade-Vieira, L. F.; Techio, V. H., Effect of SPL (Spent Pot Liner) and its main components on root growth, mitotic activity and phosphorylation of Histone H3 in Lactuca sativa L. *Ecotoxicology and environmental safety* **2016,** *124*, 426-434.

51. Costa, A. V.; Moreira, L. C.; Pinto, R. T.; Alves, T. A.; Schwan, V. V.; de Queiroz, V. T.; Praça-Fontes, M. M.; Teixeira, R. R.; Moraisa, P. A.; de Jesus Júniord, W. C., Synthesis of Glycerol-Derived 4-Alkyl-Substituted 1, 2, 3-Triazoles and Evaluation of Their Fungicidal, Phytotoxic, and Antiproliferative Activities. *Journal of the Brazilian Chemical Society* **2019**, 1-12.

52. Leme, D. M.; Marin-Morales, M. A., Allium cepa test in environmental monitoring: a review on its application. *Mutation Research/Reviews in Mutation Research* **2009,** *682* (1), 71-81.

CHAPTER 2: Chemical Composition and Bioherbicidal Activity of Essential Oils of *Psidium acidum* (DC.) Landrum and *Psidium cauliflorum* Landrum & Sobral in Plant Bioassays

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Title: Chemical Composition and Bioherbicidal Activity of Essential Oils of *Psidium acidum* (DC.) Landrum and *Psidium cauliflorum* Landrum & Sobral in Plant Bioassays

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Abstract

The application of synthetic products in crops, aiming at controlling weeds, has been essential for maintaining productivity. However, the emergence of resistant weeds, the compromise in food quality and environmental damage and human health have strengthened discussions related to the need to develop alternative control methods. Thus, the search for bioherbicides has been encouraged and plants used in food or that have bioactivity have been studied. Plants of the genus *Psidium* are potential, both for having biological activity and for being used in human food. In this way, the present research investigated the bioherbicidal potential of the essential oils of *Psidium acidum* and *Psidium cauliflorum* by means of a plant toxicity bioassay, using the plants model *Lactuca sativa* and *Sorghum bicolor*. For this, seeds from the model plants were treated with the essential oils of the two species in the concentrations of 3000, 1500, 750, 375 and 187.5 mg mL^{-1} and with the controls, distilled water, dichloromethane, and glyphosate. The variables: germination percentage (GP), germination speed index (GSI), root growth (RG), shoot length (SL), mitotic index (MI), chromosomal alterations (CA) and nuclear alterations (NA) were evaluated. The essential oils of the two species proved to be phyto-cytotoxic. *P. acidum* oil inhibited sorghum RG and lettuce SL. While that of *P. cauliflorum* reduced the GP, GSI, RG and SL of both models, being, therefore, more phytotoxic than *P. acidum* and non-selective. The cytotoxic investigation indicated that oil from both species inhibited the MI of the model plant. It was observed, through CA, that *P. cauliflorum* oil has action mechanisms of aneugenic and clastogenic and that both oils determined in epigenetic alterations observed by the increase in CA chromosomal adherence. The results indicate the bioherbicidal potential of the essential oil of *P. cauliflorum*, which, in addition to being non-selective and demonstrating an inhibition rate similar to glyphosate, presented a lower NA rate than the commercial herbicide.

Keywords: Cytogenotoxicity, Mutagenesis, Phytotoxicity, *Psidium acidum*, *Psidium cauliflorum*.

1. Introduction

The growing increase in the demand for food has been decisive for the modernization of agricultural techniques, machines and products[1]. In this sense, herbicides have received prominence, as the most applied in the field, when compared to other classes of agrochemicals. Its application aims to control weeds, which are considered to be invasive in agricultural crops and, if not properly controlled, jeopardize production profits[2-3].

On the other hand, there are records of plants resistant to synthetic commercial herbicides. The use of these products has compromised the quality of food, contaminated the environment and generated discussions related to the risks caused to human health. Thus, alternative methods to perform weed control have been studied[4-5].

One of the methods currently studied involves the application of secondary metabolites such as bioherbicides, which are less harmful to man and the environment. This use can be given by obtaining new products containing the natural compound as a base or by applying the secondary metabolite directly to crops[1]. Secondary metabolites are naturally produced by plants and found, among other ways, in essential oils, and their use is recognized as an environmentally favorable and recommended alternative[6-8].

Myrtaceae is a family of the Angiosperms that highlights between the others of this plant group due to its representants present essential oil. Besides that, fruits of different species belonging to the genus *Psidium* are used in human food, both in natura and after processing and manufacturing products such as jams and juices. These food applications and the high production of essential oils in its leaves make this an important commercial gender. For fruit production, individuals need regular pruning, resulting in a large volume of leaf residues. Thus, aiming at the use of these leaf residues from pruning, studies of the applications of essential oils of *Psidium* species have been carried out and their potentials have been demonstrated[2].

Among the species belonging to the genus *Psidium* include *Psidium cauliflorum* Landrum & Sobral *and Psidium acidum* (DC.) Landrum. *Psidium cauliflorum* is found in the Atlantic Forest, it was the first species described for the genus with cauliflorous inflorescence, and because it was described in 2006 it presents few studies[9]. *Psidium acidum* is reported in Brazil as an indigenous specie cultivated in various parts of the country[10-11]. This is due to, its fruits that are used in human food (Landrum, 2016).

Landrum[11], reevaluating the *P. acutangulum* taxon and performing a new

combination in the classification of species of the genus *Psidium*, proposed the separation of *P. acutangulum* and *P. acidum*, based on their morphological specificities such as: characters of leaves, anthers, fruits and seeds. In the same paper, the author highlighted the probable mistake of authors and researchers in classifying Brazilian specimens as *P. acutangulum*, and also emphasized the need for revision and correction of the taxonomic classification[11]. Based on this information, some works listed in the literature of the present research are classifield as *P. acutangulum*, however have a similar morphological description of the *P. acidum*.

One way of evaluating the bioherbicidal potential of products from secondary plant metabolism is through plant bioassays, using model plants. These tests can be performed in laboratories following macroscopic variables related to seedling germination and initial growth, as well as microscopic parameters such as the mitotic index and nuclear and chromosomal alterations. In these tests, seeds of pre-established plants, such as *Lactuca sativa* and *Sorghum bicolor* are used as models because they have small seeds, fast growth and are easily accessed in agricultural stores[1, 12].

In view of the above, the present investigation aimed to elucidate the effects of the essential oil of *P. acutanculum* and *P. cauliflorum* leaves on the initial development of *S. bicolor* and *L. sativa*, as well as on the mitotic cycle of *L. sativa* meristematic cells, investigating bioherbicidal potential.

2. Material and Methods

2.1. Plant Material

The essential oils (test agents) studied were obtained from leaves of adult individuals of *Psidium cauliflorum* Landrum & Sobral collected in Cachoeiro de Itapemirim, Espírito Santo, Brazil, location: 20 ° 75’19.09’’S / 41º23’16.39 ”W; and *Psidium acidum* (DC.) Landrum collected in Alegre, Espírito Santo, Brazil, location: 20 ° 45'37.8 "S / 41 ° 27'24.8" W.

Seeds of eudicotyledonous *Lactuca sativa* L. (lettuce) 'Crespa Grand Rapids' (Isla Pak) and monocotyledonous *Sorghum bicolor* L. Moench (sorghum) 'AL Precioso' (BR Seeds) were used as model plants[12].

2.2. Essential Oils Extraction

The leaves were frozen at -20ºC, weighed and crushed with water. The material obtained was placed in a Clevenger apparatus. Hydrodistillation extraction took 5 hours

and the oil obtained was weighed. The oil yield was calculated by the ratio of 100 times the weight of the oil to the weight of the leaves[12].

2.3. Essential Oils Characterization

Oil samples were evaluated by gas chromatography using a mass and flame spectrometer. The identification of the compounds was carried out by comparing the results obtained with the program library[12].

2.4. Phytotoxicity Assays

The experiment was carried out in a completely randomized design, with five replications, consisting of 25 seeds in each. The treatments were composed of the essential oils of *P. acidum* and *P. cauliflorum* in the concentrations of 3000, 1500, 750, 375 and 187.5 mg mL^{-1} and containing as a positive control the commercial herbicide glyphosate in a concentration of 0.1% and as negative controls were distilled water and the solvent dichloromethane (DCM) used to dilute essential oils[12].

The seeds were distributed in Petri dishes previously cleaned and covered with film paper. The plates were treated, sealed with film paper and packed in a germination chamber (BOD), with a 12h photoperiod and at 25ºC±2ºC[1].

Seed germination was evaluated every 8 hours during the first 48 hours, obtaining the seed germination speed index (GSI). After 48h of exposure to the test agent, the percentage of seed germination (GP) and root growth (RG) were evaluated. The plates were put back in the BOD until 120h, then measuring the aerial growth (AG)[1].

2.5. Cytotoxicity Assay

Seeds of *L. sativa* were selected as a model for this assay because they are considered suitable for microscopic analysis, aiming to evaluate toxicity in meristematic cells[13]. The treated seeds were exposed to the test agents for 48 hours. Then, the emitted roots were collected and fixed in ethyl alcohol: acetic acid (3:1). Two changes were performed. The first 10 minutes after fixation and the second 24h after fixation. The slides were prepared after a minimum of 24 hours of fixation[14].

To prepare each slide, two root meristems were used. The roots were submitted to 3 baths of 10 minutes in distilled water. Subsequently, hydrolysis was carried out in 5N HCl for 18 minutes in 25°C. The slides were prepared by squashing and stained the root tips with acetic orcein 2%[1].

Evaluated 1000 cells per slide, adding 5000 cells per treatment. Mitotic phases were observed, as well as possible chromosomal (CA) and nuclear (NA) alterations. The mitotic index (MI) was obtained from the number of cells in interphase and cells in mitosis. The CA and NA were obtained by the ratio of the number of cells with alterations to the total number of cells evaluated; as well as the frequency of micronucleus (MNC), condensed nucleus (NC). The frequency of each CA: c-metaphase (c-met), lost, adherence, bridge were determined by the ratio of the number of cells with alterations to the total number of cells in division[15].

2.6. Statistics

The results obtained were submitted to analysis of variance and the means were submitted to the Tukey test ($p<0.05$) using the Genes program as a statistical tool[16]. Regression analysis was used to assess the mitotic index (MI). The polynomial regression models were adjusted according to the significance of ANOVA F and the quality of the models was assessed by the coefficient of determination (R^2). The regression analyzes and plotting of the graphs were performed using the computational environment R[17].

3. Results

3.1. Chemical Characterization of Essential Oils

The major compound of the essential oil of *P. cauliflorum* was α-pinene with 49.16% (Table 1). The essential oil of *P. acidum* presented four major compounds, trans-caryophylene (18.43%), β-elemene (18.36%), germacrene A (16.83%) and α-copaene (11.67%) (Table 2). The compounds α-copaene and Δ-cadinene were found in the same proportion in both oils (Tables 1 and 2), while α-pinene showed very different concentrations, approximately 49% in *P. cauliflorum* and 4% in *P. acidum* (Tables 1 and 2).

Table 1 - Identification of *Psidium cauliflorum* essential oil compounds by the LTPRI Index and Mass Spectrometry (GC/MS)[a].

Compound[b]	Retention time (min)	Relative area (%)[c]	Calculated Retention Index[d]	Literature Retention Index
α-Pinene	8.369	49.16	932	932
D-Limonene	12.421	4.82	1027	1024
β-Ocimene	13.434	3.19	1049	1044
Borneol	18.765	4.24	1165	1165
α-Terpineol	19.966	7.45	1191	1186
α-Copaene	28.107	11.39	1374	1374
α-Bisabolene	33.644	3.05	1508	1506
Δ-Cadinene	34.205	3.65	1523	1522
(E)-Nerolidol	35.841	13.05	1565	1561

[a] The compounds were identified by the LTPRI Index (GC / FID) and by Mass Spectrometry (GC / MS) using a column Rtx®-5MS.

[b] Tabulated Retention Index (Adams, 2007; El-Sayed, 2016; NIST, 2011).

[c] Only compounds with relative areas > 2% were identified.

[d] Retention index calculated from data obtained by sampling saturated n-alkanes (C7-C40).

Table 2 - Identification of essential oil of *Psidium acidum* compounds by the LTPRI Index and Mass Spectrometry (GC/MS)[a].

Compound[b]	Retention time (min)	Relative area (%)[c]	Calculated Retention Index[d]	Literature Retention Index
α-Pinene	8.344	4.19	931	932
β-Pinene	10.085	2.17	973	974
β-cis-Ocimene	13.438	2.06	1049	1044
α-Copaene	28.124	11.67	1374	1374
β-Elemene	28.871	18.36	1391	1389
trans-Caryophyllene	29.943	18.43	1417	1417
α-Humulene	31.305	2.56	1451	1452
γ-Muurolene	32.456	2.83	1479	1478
β-Selinene	32.649	2.82	1483	1489
Δ-selinene	33.036	4.22	1493	1492
germacrene A	33.467	16.83	1504	1508
α-Farnesene	33.679	2.00	1509	1505
Δ-Cadinene	34.215	3.66	1523	1522
Caryophyllene oxide	36.420	2.52	1580	1582
NI[e]	39.175	5.68	1654	-

[a] The compounds were identified by the LTPRI Index (GC / FID) and by Mass Spectrometry (GC / MS) using a column Rtx®-5MS.

[b] Tabulated Retention Index (Adams, 2007; El-Sayed, 2016; NIST, 2011).

[c] Only compounds with relative areas > 2% were identified.

[d] Retention index calculated from data obtained by sampling saturated n-alkanes (C7-C40).

[e] Unidentified compound.

3.2. Phytotoxicity Assay

The essential oil of *P. acidum* did not promote significant inhibition in the germination percentage in either of the two models studied (Figure 1a). The essential oil of *P. cauliflorum* at concentrations of 3000 and 1500 mg mL^{-1} determined the inhibition of lettuce GP by 19.33% and 15.97%, respectively (Figure 1b) and the concentrations of 3000, 1500 and 750 mg mL^{-1} inhibited the sorghum GP, respectively, at 35.34%, 25.86% and 18.10% at (Figure 1b).

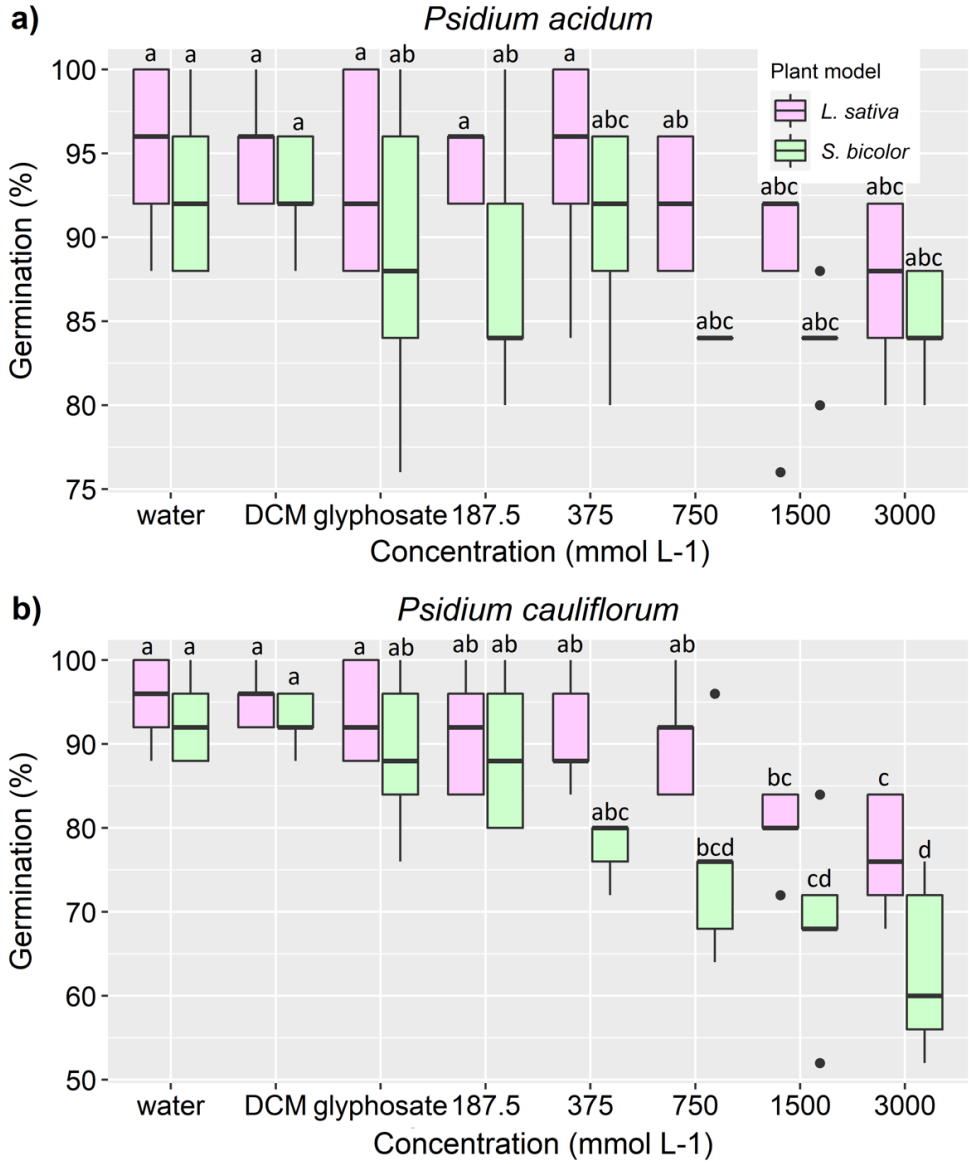

Figure 1. Effect of the essential oil of (a) *Psidium acidum* and (b) *Psidium cauliflorum* on the germination percentage of *Lactuca sativa* and *Sorghum bicolor*. The small letters

above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

The essential oil of *P. acidum* did not alter the sorghum GSI and the highest concentration tested inhibited 25.87% of the lettuce GSI (Figure 2a). The essential oil of *P. cauliflorum* in the concentrations of 3000 and 1500 mg mL^{-1} inhibited, respectively, 65.95% and 50.36% of the lettuce GSI and the concentrations of 3000, 1500, 750 and 375 mg mL^{-1} inhibited the sorghum GSI in 36.05%, 24.95%, 19.31% and 21.41%, respectively (Figure 2b).

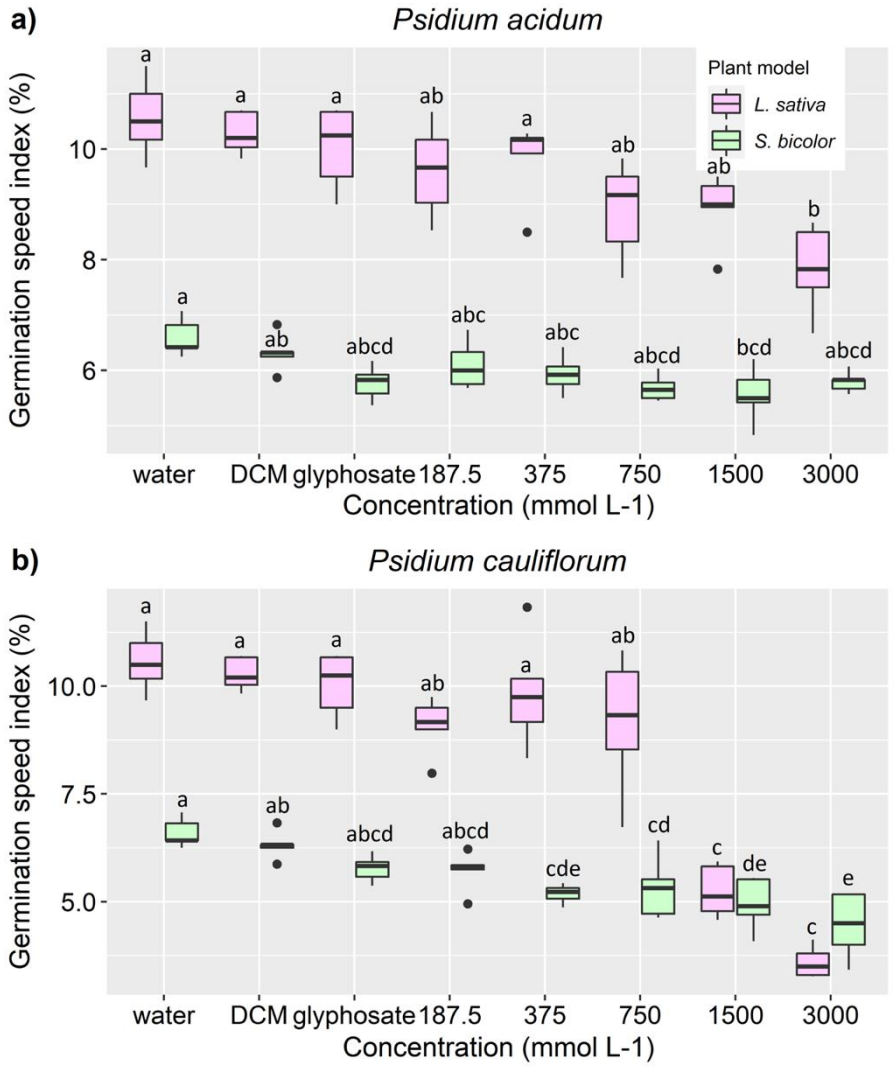

Figure 2. Effect of the essential oil of (a) *Psidium acidum* and (b) *Psidium cauliflorum* on the germination speed index of *Lactuca sativa* and *Sorghum bicolor*. The small letters

above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

The essential oil of *P. cauliflorum* proved to be more toxic compared to the two parameters of germination evaluated, in both models used (Figure 1 and 2).

The sorghum RG was 42.32% inhibited by the higher concentration of *P. acidum* oil and the same variable was not changed in the lettuce model (Figure 3a). The highest and the second greater concentrations of *P. cauliflorum* inhibited, respectively, more than 70% and 50% of the RG of both models (Figure 3b). The sorghum model was inhibited by the four highest concentrations tested and the lettuce by the two highest (Figure 3b).

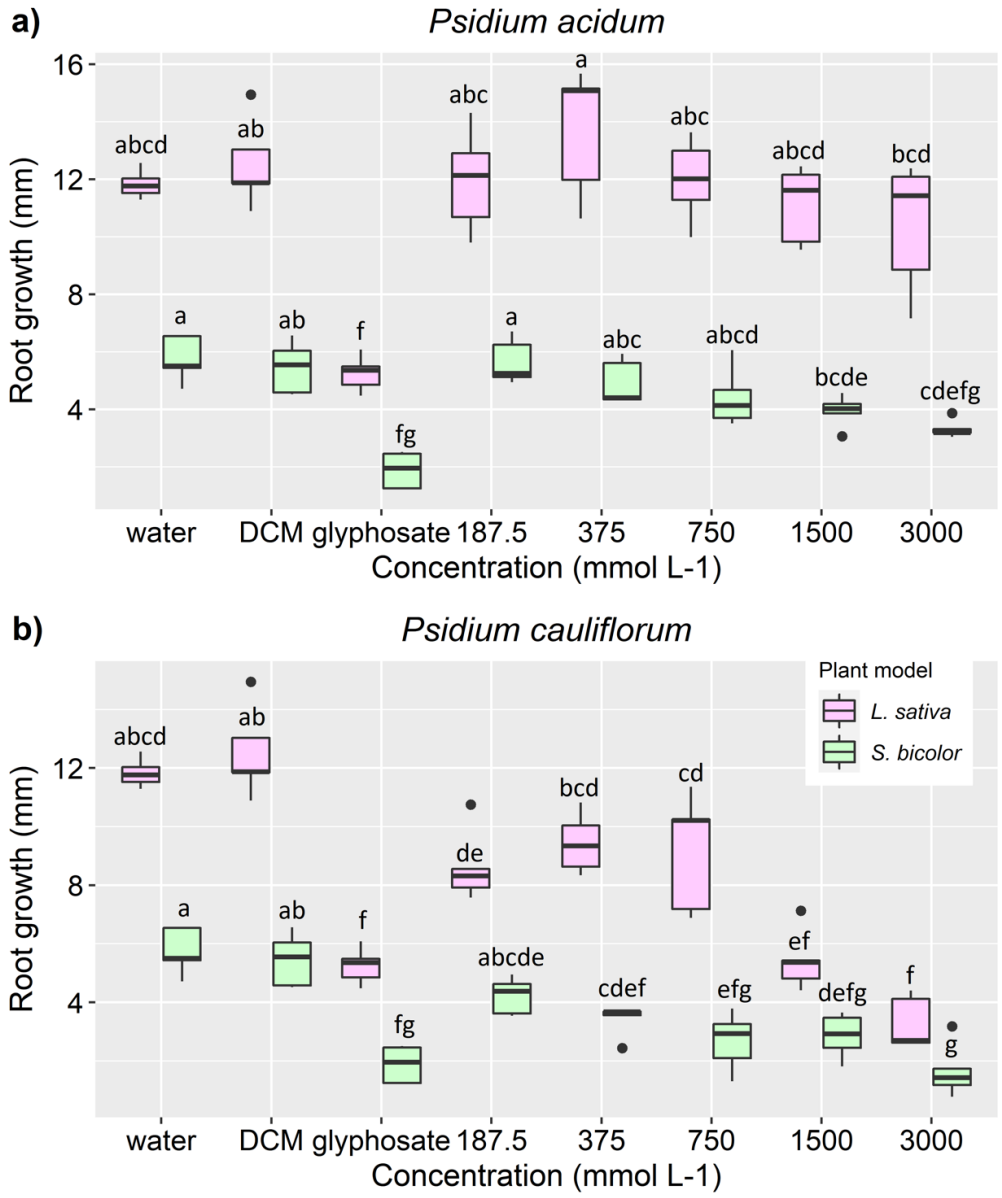

Figure 3. Effect of the essential oil of (a) *Psidium acidum* and (b) *Psidium cauliflorum* on the root growth of *Lactuca sativa* and *Sorghum bicolor*. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

The AG of the sorghum model had 41.14% inhibition in the highest concentration of *P. cauliflorum* oil (Figure 4b). The same variable and oil in the lettuce model was inhibited by 61.17% and 54.20% in the highest concentrations. The oil of *P. acidum* (3000 and 750 mg mL^{-1}) inhibited the lettuce SL by 71.44% and 57.72%, respectively (Figure 4a).

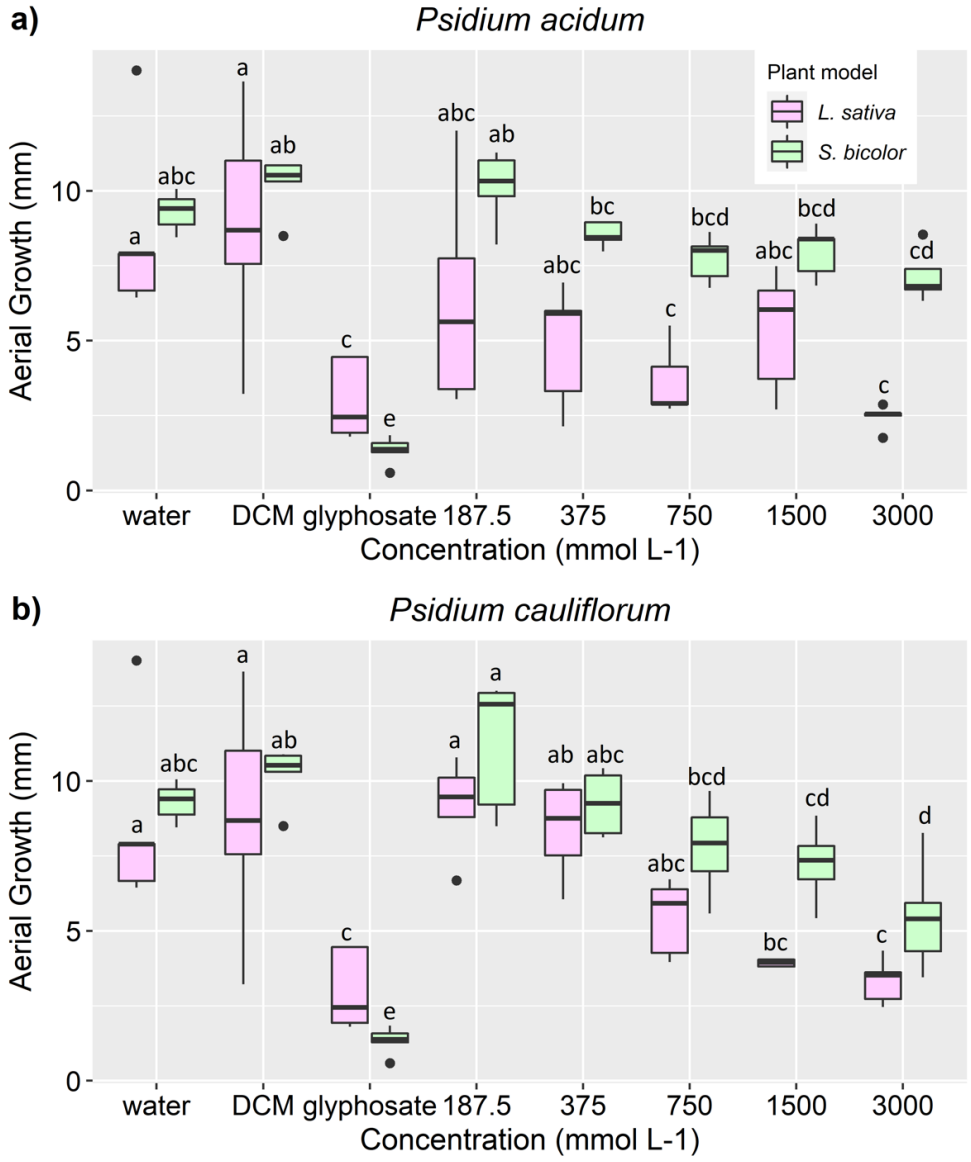

Figure 4. Effect of the essential oil of (a) *Psidium acidum* and (b) *Psidium cauliflorum* on the aerial growth of *Lactuca sativa* and *Sorghum bicolor*. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

3.3. Cytotoxicity Assay

The MI of the root meristems treated with the three highest concentrations of the essential oil of *P. cauliflorum* and with the concentrations 1500, 750 and 375 mg mL^{-1} of *P. acidum* was significantly decreased, compared to negative controls, but no treatment reduced more than 50% the MI (Figure 5).

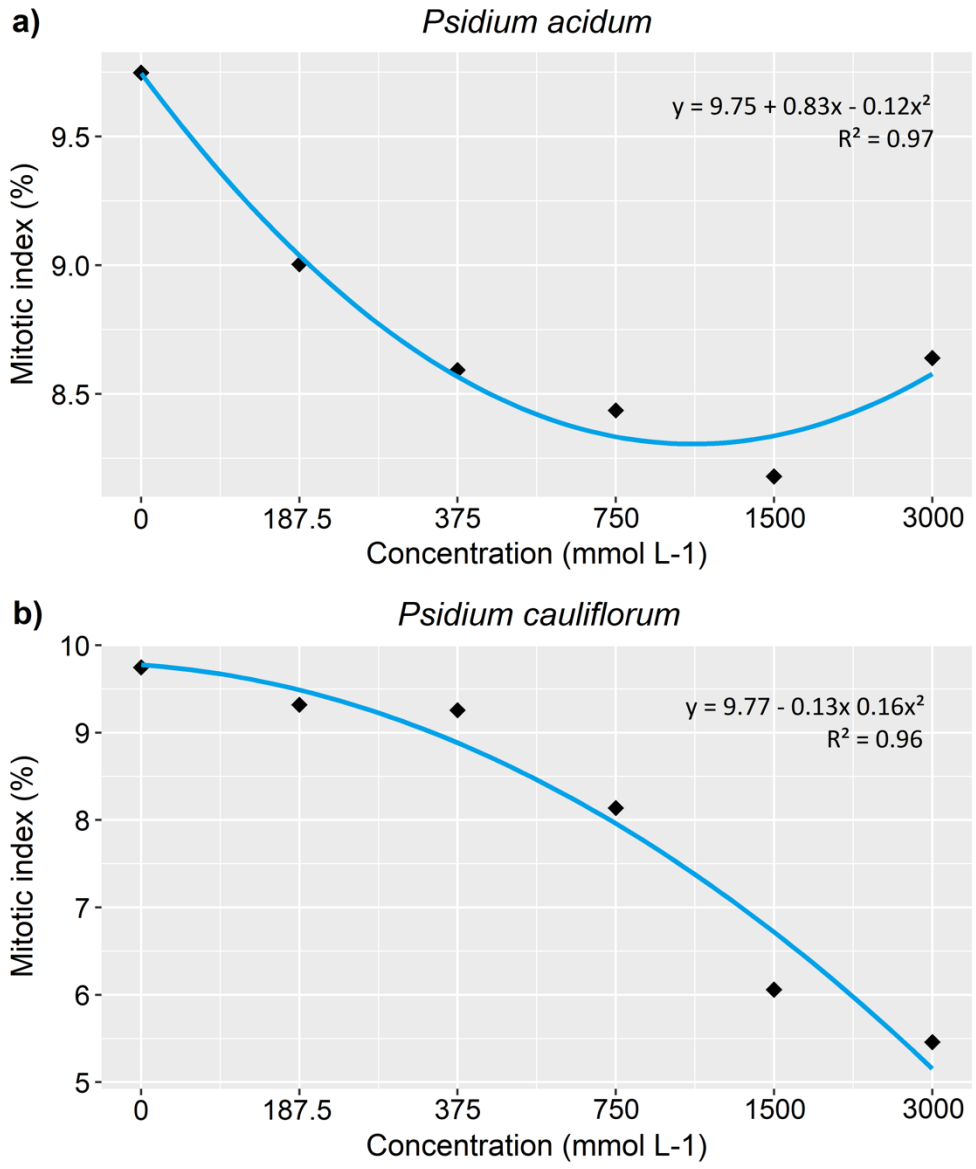

Figure 5. Graphs of MI (mitotic index) for *Lactuca sativa* treated with solutions of *Psidium acidum* and *Psidium cauliflorum* essential oils.

The NA increased, when compared to the negative controls, in all concentrations of

P. cauliflorum and in concentrations 1500, 750 and 187.5 mg mL^{-1} of *P. acidum* (Figure 6). However, all tested concentrations were less toxic than the positive control, demonstrating that the treatments determine less environmental harmfulness, when compared to the commercial herbicide.

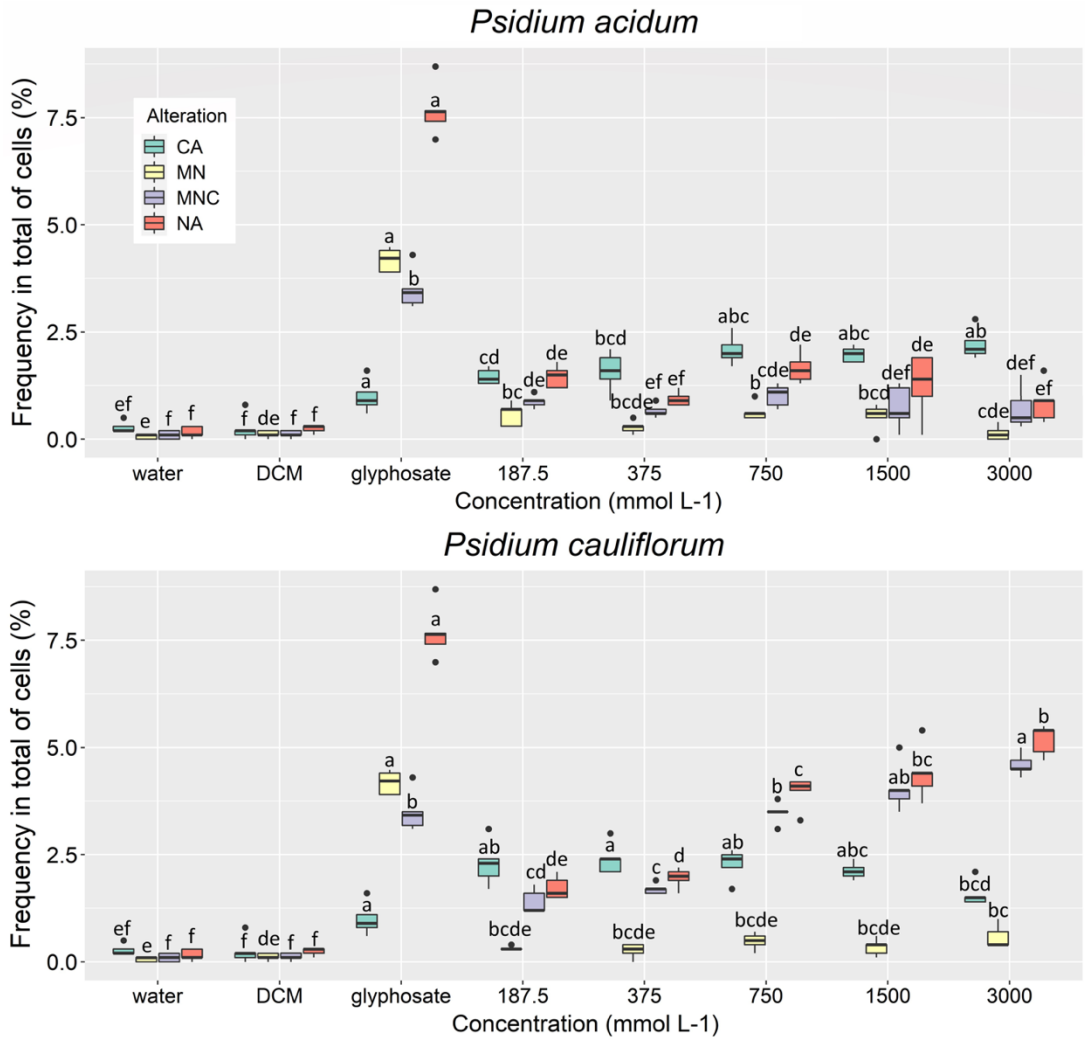

Figure 6. Nuclear and chromosomal alterations in root meristems of lettuce treated with the essential oils of *Psidium acidum* and *Psidium cauliflorum* at the concentrations of 3000, 1500, 750, 375 and 187.5µg L^{-1}. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$). The abbreviations represent: CA - chromosomal alterations, NA - nuclear alterations, MNC - micronucleus, NC - condensed nucleus.

The alterations occurring in the cell nucleus were observed in the present work in

the form of MNC and NC. NCs were significant, but less frequent than in C+, in the highest concentration of *P. cauliflorum* and in concentrations 750 and 187.5 mg mL^{-1} of *P. acidum* (Figure 6). The presence of MNC was significant in all treatments with the essetial oil of *P. cauliflorum* and at concentrations 750 and 187.5 mg mL^{-1} of *P. acidum* (Figure 6).

The CA showed a significant increase in all studied treatments (Figure 6). The CA c-metaphase showed a significant increase in treatment with the essential oil of *P. cauliflorum* at a concentration of 1500 mg mL^{-1} (Figure 7). The CA fragment also showed a significant increase, when compared to the negative and positive controls, in *P. cauliflorum* at a concentration of 750 mg mL^{-1} (Figure 7). Chromosomal adherence was observed significantly in all treatments with the essential oil of *P. cauliflorum* and at concentrations of 1500 and 750 mg mL^{-1} of *P. acidum* (Figure 7).

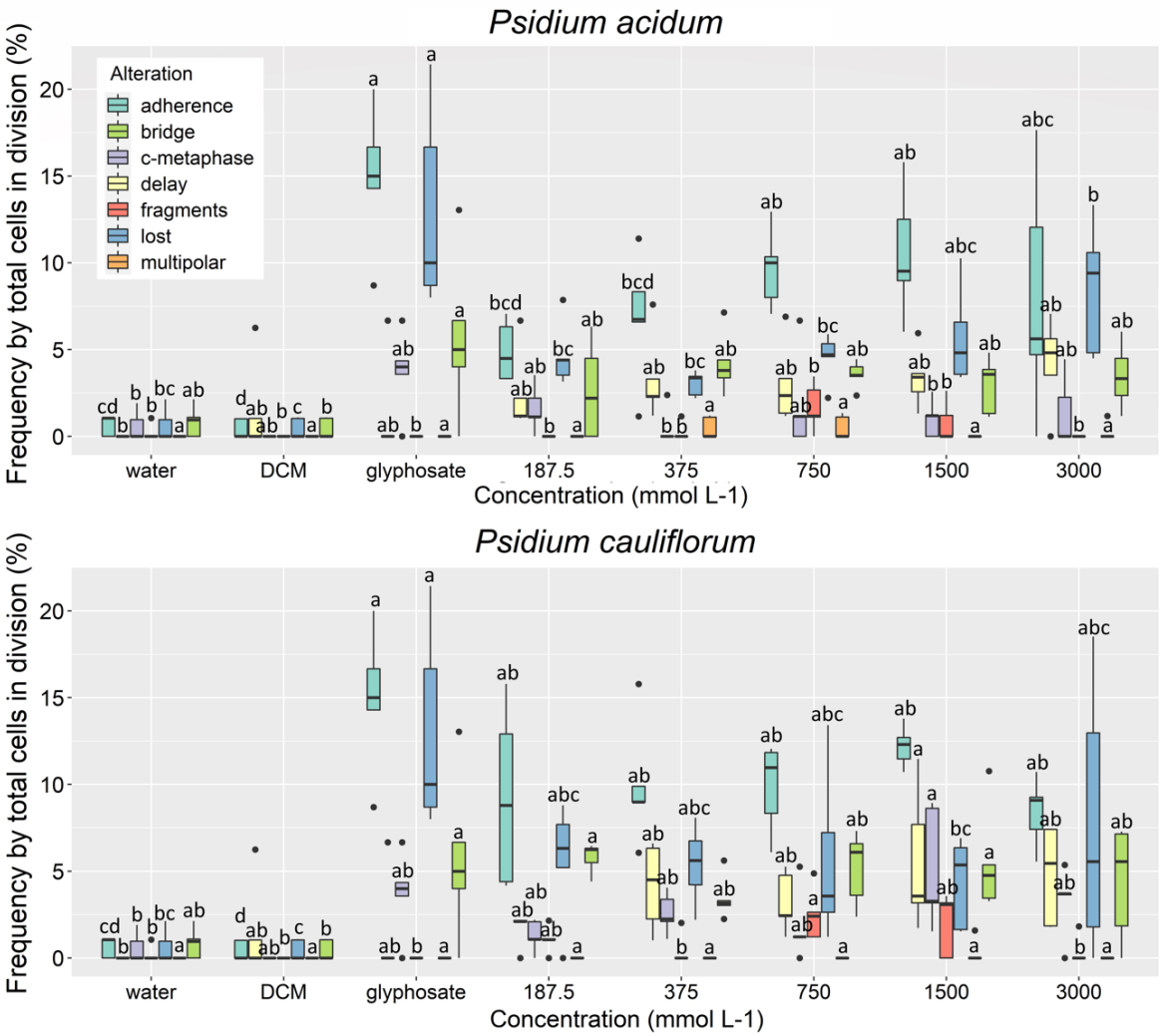

Figure 7. Frequency of each CA observed in meristematic cells of lettuce treated with essential oils of *Psidium acidum* and of the *Psidium cauliflorum* at the concentrations of 3000, 1500, 750, 375 and 187.5 μg L^{-1}. The small letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

4. Discussion

Essential oils of different species belonging to the same genus may have different phytotoxicity, due to their different chemical compositions[2]. In addition, essential oils can interfere in different ways in the different evaluation parameters and/or model organisms[12].

The chemical composition of essential oils is influenced by several variables such as plant age, nutritional condition, water availability, genetic predisposition, organ in which the oil is extracted, climatic conditions, time of collection and the season of the year collection was carried out, as well as the extraction method[18-19].

This variation in chemical composition can be observed by comparing the chemical characterization of *P. acidum* essential oils from leaves collected in the Atlantic Forest (present study) and from thin branches (leaves and thin stems) in the Amazon Forest[20]. In previous research, the essential oil obtained from the mixture of leaves and fine stems, collected in the Brazilian Amazon region, was studied and the major compounds obtained, differently the current research, were α-pinene (14.8%), 1,8-cineole (12.9 %) and β-pinene (10.1%)[20].

The main components of essential oils are monoterpenes, which enter the Earth's atmosphere through leaf volatilization of vegetation, by leaching of leaf residues and because of root exudation. Among the compounds belonging to this class, the most abundant in the atmosphere surrounding different forest areas in the world, are α-pinene and its isomer β-pinene[21].

The α-pinene, a major part of the foliar essential oil of *P. cauliflorum* and present in low concentration in *P. acidum*, is recognized in different studies, for its biological activities[22-25]. Such fact may be related to the potential non-selective bioherbida presented by the essential oil of *P. cauliflorum*, which inhibited both mono and eudicot. The essential oil of *P. acidum* did not show high phytotoxicity compared to the initial development of model plants and showed a low amount of α-pinene.

The phytotoxic effect of essential oils whose α-pinene is the majority, has been

previously documented. *Rosmarinus officinalis* L., whose majority is α-pinene (25.8-27.7%), promoted the inhibition of the initial development of *Lactuca serriolada* L. and *Rhaphanus sativus* L. treated with concentrations of 300, 600, 900, 1200, 1500 and 1800 µL L^{-1}, such inhibition being verified in GP, GSI, RG and SL[26]. In addition, it has been reported that the essential oil of *R. officinalis* (25.85% α-pinene) inhibited the germination of weeds *Amaranthus retroflexus* L. and *Bromus tectorum* L. treated with the concentration of 400 µL L^{-1} [27].

The phytotoxicity of α-pinene in the initial development of the weed *Cassia occidentalis* was related to the generation of reactive oxygen species, membrane degradation and ion leakage. These free radicals (hydrogen peroxide, superoxide radical and hydroxyl radical) can cause damage to cell membranes, slowing down the growth of the organism and increasing cell death[28]. This performance is explained by the high lipophilia of the monoterpenes, which unbalance the production of cellular energy through the uncoordination of oxidative photophosphorylation and the suppression of the cellular respiration[29].

The inhibition of PG and GSI and the allelopathic potential of *Eucalyptus globulus* L. against the initial development of *Brassica campestris* L., *Brassica oleracea* L. cv. capitata, *Brassica oleracea* L. cv. italica, *Brassica pekinensis* (Lour.) Rupr., *L. sativa*, *Lycopersicum esculentum* Mill., *Brassica rapa* L., *Eruca sativa* Mill. and *R. sativus* were attributed to the presence of monotorpenes in their composition[30],

The negative influence of volatile compounds on plant energy metabolism has been related to the process of cellular respiration[31]. Such authors reported that volatile terpenes have the potential to damage mitochondria, resulting in an unfavorable effect on the process of cellular respiration. Thus, monoterpenoids can interfere with germination and initial plant development, due to morphoanatomical and physiological changes such as chlorophyll synthesis, photosynthesis, accumulation of lipids in the cytoplasm and reduction of organelles due to membrane rupture[32]. These changes also compromise the individual's cell dynamics, being its study important.

The MI expresses the amount of cells in mitotic division[33]. Thus, the reduction of this variable represents an inhibition of mitosis, and consequently, the increase of cells in interphase. The cytotoxicity of a test agent can be expressed by reducing and/or increasing of the MI[12]. However, the treatments evaluated were not effectively toxic, considering that the really toxic test agent is the one that reduces over 50% the MI[34] and this level of

reduction was only observed in cells treated with the commercial herbicide glyphosate used in this study as a positive control.

The reduction of MI in practical terms, represents less cells in mitotic division, due to the changes promoted by the interactions between the chemical compounds found in the test compound and the target organism[35]. Such decrease is related to the macroscopic parameter RG, which was, in general, the most affected in the present study. Since the reduction of RG indicates the inhibition of cell division and cell death, in addition to the reduction of nutrient absorption and cell extension[36]. Thus, the reduction in MI can be expressed macroscopically by reducing the RG, as occurred in the present study.

Cell death is related to the decline in MI and the reduction of RG, is an NA. It is evaluated through the presence of condensed nuclei, which is the cytological expression of cell death[37-38]. This genetically programmed cell death has the function of maintaining tissue homeostasis and eliminating cells with DNA alterations[39].

Another NA observed is the presence of MNC, these are formed with the function of incorporating and eliminating portions of DNA present in the cell cytoplasm. Such DNA fractions can come from unaligned whole chromosomes and incorporated into the nuclei of daughter cells during cell division or from fragments of acentric chromosomes[40-42]. Thus, such NA is related to previous CA, such as chromosomal lost and fragmentation.

The CA are observed through alterations in the total quantity and structure of chromosomes. These changes occur spontaneously, less frequently, and can also occur as a result of exposure to toxic agents[43]. The CA are important tools in elucidating the mechanism of cellular action of the test agent. Since the types of CA present in cells indicate the type of interaction that is taking place between the test agent and the target organism[3].

Chromosomal alterations resulting from structural changes in chromatin/DNA, causing anomaly in the DNA sequence are considered clastogenic. Chromosomal fragmentation is a type of CA that indicates this action mechanism. These fragments are acentric, if the fragmentation occurs in the telomeres, enabling the formation of bridges between chromosomes. Thus, the spindle fibers cannot link to the chromosome during division[13, 15, 41].

The CA can also indicate aneugenic action mechanism, when they determine genomic instability, which may result in mutations. Such action mechanism causes failure in the functionality of the machinery of the mitotic spindle, being able to determine failures in both the polymerization and the depolymerization of the microtubules, causing

the elimination or increase of the genetic material in the daughter cells[41]. An example of aneugenic CA is the c-metaphase which originates in the complete inactivation of the mitotic spindle, due to the non-polymerization of the microtubules during the metaphase[41]. This change determines when the division stops at this stage of the cell division[36].

The CA of the type of adherence, on the other hand, indicates both aneugenic and clastogenic action mechanisms, in addition, they can also indicate epigenetic changes. This alteration is due to the irreversible union of chromosomes, which can result in cell death. It is an indicator of epigenetic changes due to alters in the pattern of the phosphorylation of serine 10 of the histone 3[13, 36, 42].

Thus, the essential oil of *P. cauliflorum* proved to be aneugenic and clastogenic in terms of its cellular action mechanism. Since it showed an increase in the frequency of c-metaphases (biggest percent) and chromosomal fragmentation (smaller percent). In addition, both oils evaluated determine epigenetic alteration, observed by the increase in chromosomal adherence. The understand of the cellular action mechanism of test agents is important for comprehend the pathways of changes that determine the observed physiological characteristics. Thus, the cellular and molecular effects of the compounds help us to understand the observed phytotoxicity.

5. Conclusion

The essential oil of *P. acidum* was phytotoxic for the RG of sorghum and for the SL of lettuce, whereas the oil of *P. cauliflorum* was toxic for all variables evaluated in both models. Thus, *P. cauliflorum* oil proved to be more phytotoxic than that of *P. acidum*, regardless of the test model. Regarding the level of phytotoxicity, it was observed that the sorghum model was more sensitive to *P. cauliflorum* oil, since it was inhibited even at the lowest concentrations.

The cytotoxic test demonstrated that both essential oils promoted epigenetic effects, observed by the presence of chromosomal adherence. This test also demonstrated that the essential oil of *P. cauliflorum* has clastogenic and aneugenic action mechanism.

The essential oil of *P. cauliflorum* has bioherbicidal potential as it demonstrates the same inihibition rate of glyphosate that was used as a positive control. In addition, cytotoxic analysis has shown that the oil of this same species has a lower nuclear harmfulness than the commercial herbicide.

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References

1. de Assis Alves, T.; Fontes Pinheiro, P.; Miranda Praça-Fontes, M.; Fonseca Andrade-Vieira, L.; Barelo Corrêa, K.; de Assis Alves, T.; da Cruz, F. A.; Lacerda Júnior, V.; Ferreira, A.; Bastos Soares, T. C., Toxicity of thymol, carvacrol and their respective phenoxyacetic acids in Lactuca sativa and Sorghum bicolor. *Industrial Crops and Products* **2018,** *114*, 59-67.

2. Vasconcelos, L. C.; de Souza Santos, E.; de Oliveira Bernardes, C.; da Silva Ferreira, M. F.; Ferreira, A.; Tuler, A. C.; Carvalho, J. A. M.; Pinheiro, P. F.; Praça-Fontes, M. M., Phytochemical analysis and effect of the essential oil of Psidium L. species on the initial development and mitotic activity of plants. *Environmental Science and Pollution Research* **2019,** *26* (25), 26216-26228.

3. Quezia Pains Dutra, J. A. C., Tatiana T avares C arrijo,; Thayllon de Assis Alves, T. d. A. A., Luiza Alves Mendes,; Praça-Fontes, M. M., Phytocytotoxicity of volatile constituents of essential oils from *Sparattanthelium* Mart. species (Hernandiaceae). *Scientific Reports* **2020**.

4. Silva, J. M. d.; Novato-Silva, E.; Faria, H. P.; Pinheiro, T. M. M., Agrotóxico e trabalho: uma combinação perigosa para a saúde do trabalhador rural. *Ciência & saúde coletiva* **2005,** *10*, 891-903.

5. Souza Filho, A.; Santos, R.; Santos, L.; Guilhon, G.; Santos, A.; Arruda, M.; Muller, A.; Arruda, A., Potencial alelopático de Myrcia guianensis. *Planta daninha* **2006,** *24* (4), 649-656.

6. Zhang, S. Z.; Li, Y. H.; Kong, C. H.; Xu, X. H., Interference of allelopathic wheat with different weeds. *Pest management science* **2016,** *72* (1), 172-178.

7. de Jesus Jatoba, L.; Varela, R. M.; Molinillo, J. M. G.; Ud Din, Z.; Juliano Gualtieri, S. C.; Rodrigues-Filho, E.; Macías, F. A., Allelopathy of bracken fern (Pteridium arachnoideum): new evidence from green fronds, litter, and soil. *PloS one* **2016,** *11* (8), e0161670.

8. Głąb, L.; Sowiński, J.; Bough, R.; Dayan, F. E., Allelopathic potential of sorghum (Sorghum bicolor (L.) Moench) in weed control: a comprehensive review. *Advances in agronomy* **2017,** *145*, 43-95.

9. Landrum, L. R.; Sobral, M., Psidium cauliflorum (Myrtaceae), a new species from Bahia, Brazil. *SIDA, Contributions to Botany* **2006**, 927-929.

10. Popenoe, W., *Manual of tropical and subtropical fruits: excluding the banana, coconut, pineapple, citrus fruits, olive, and fig*. Macmillan: 1920.

11. Landrum, L. R., Re-evaluation of Psidium acutangulum (Myrtaceae) and a new combination in Psidium. *Brittonia* **2016,** *68* (4), 409-417.

12. Pinheiro, P. F.; Costa, A. V.; Alves, T. d. A.; Galter, I. N.; Pinheiro, C. A.; Pereira, A. F.; Oliveira, C. M. R.; Fontes, M. M. P., Phytotoxicity and Cytotoxicity of Essential Oil from Leaves of Plectranthus amboinicus, Carvacrol, and Thymol in Plant Bioassays. *Journal of Agricultural and Food Chemistry* **2015,** *63* (41), 8981-8990.

13. Silveira, G. L.; Lima, M. G. F.; Reis, G. B. d.; Palmieri, M. J.; Andrade-Vieria, L. F., Toxic effects of environmental pollutants: Comparative investigation using Allium cepa L. and Lactuca sativa L. *Chemosphere* **2017,** *178*, 359-367.

14. Alves, T. A.; Roberto, C. E. O.; Pinheiro, P. F.; Alves, T. A.; Henrique, M. K.; Ferreira, A.; Clarindo, W. R.; Praça-Fontes, M. M., Searching an auxinic herbicide to use as positive control in toxicity assays. *Anais da Academia Brasileira de Ciências* **2021,** *93* (1).

15. Aragão, F.; Palmieri, M.; Ferreira, A.; Costa, A.; Queiroz, V.; Pinheiro, P.; Andrade-Vieira, L., Phytotoxic and cytotoxic effects of Eucalyptus essential oil on lettuce (Lactuca sativa L.). *Allelopathy Journal* **2015,** *35* (2), 259-272.

16. Cruz, C. D., GENES: a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy* **2013,** *35*, 271-276.

17. Core Team, R., R: A language and environment for statistical computing (Computer software). Vienna, Austria. Retrieved from https://www. R-project. org: 2020.

18. Mendes, L. A.; Martins, G. F.; Valbon, W. R.; de Souza, T. d. S.; Menini, L.; Ferreira, A.; da Silva Ferreira, M. F., Larvicidal effect of essential oils from Brazilian cultivars of guava on Aedes aegypti L. *Industrial Crops and Products* **2017,** *108*, 684-689.

19. de Souza, T. d. S.; da Silva Ferreira, M. F.; Menini, L.; de Lima Souza, J. R. C.; Parreira, L. A.; Cecon, P. R.; Ferreira, A., Essential oil of Psidium guajava: Influence of genotypes and environment. *Scientia Horticulturae* **2017,** *216*, 38-44.

20. da Silva, J. D.; Luz, A. I. R.; da Silva, M. H. L.; Andrade, E. H. A.; Zoghbi, M. d. G. B.; Maia, J. G. S., Essential oils of the leaves and stems of four Psidium spp. *Flavour and Fragrance Journal* **2003,** *18* (3), 240-243.

21. Chowhan, N.; Singh, H. P.; Batish, D. R.; Kohli, R. K., Phytotoxic effects of β-pinene on early growth and associated biochemical changes in rice. *Acta Physiologiae Plantarum* **2011,** *33* (6), 2369-2376.

22. Leite, A. M.; Lima, E. d. O.; Souza, E. L. d.; Diniz, M. d. F. F. M.; Trajano, V. N.; Medeiros, I. A. d., Inhibitory effect of beta-pinene, alpha-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria. *Revista Brasileira de Ciências Farmacêuticas* **2007,** *43* (1), 121-126.

23. Zamyad, M.; Abasnejad, M.; Esmaeili-Mahani, S.; Mostafavi, A., Alpha-pinene as the main component of Ducrosia anethifolia (Boiss) essential oil is responsible for its effect on locomotor activity in rats. *Avicenna Journal of Neuro Psycho Physiology* **2016,** *3* (2), 29-34.

24. Bouzenna, H.; Hfaiedh, N.; Giroux-Metges, M.-A.; Elfeki, A.; Talarmin, H., Potential protective effects of alpha-pinene against cytotoxicity caused by aspirin in the IEC-6 cells. *Biomedicine & Pharmacotherapy* **2017,** *93*, 961-968.

25. Zamyad, M.; Abbasnejad, M.; Esmaeili-Mahani, S.; Mostafavi, A.; Sheibani, V., The anticonvulsant effects of Ducrosia anethifolia (Boiss) essential oil are produced by its main component alpha-pinene in rats. *Arquivos de neuro-psiquiatria* **2019,** *77* (2), 106-114.

26. Alipour, M.; Saharkhiz, M. J., Phytotoxic activity and variation in essential oil content and composition of Rosemary (Rosmarinus officinalis L.) during different phenological growth stages. *Biocatalysis and agricultural biotechnology* **2016,** *7*, 271-278.

27. Hazrati, H.; Saharkhiz, M. J.; Moein, M.; Khoshghalb, H., Phytotoxic effects of several essential oils on two weed species and tomato. *Biocatalysis and agricultural biotechnology* **2018,** *13*, 204-212.

28. Singh, H. P.; Batish, D. R.; Kaur, S.; Arora, K.; Kohli, R. K., α-Pinene inhibits growth and induces oxidative stress in roots. *Annals of Botany* **2006,** *98* (6), 1261-1269.

29. Abrahim, D.; Braguini, W. L.; Kelmer-Bracht, A. M.; Ishii-Iwamoto, E. L., Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. *Journal of chemical ecology* **2000,** *26* (3), 611-624.

30. Yamagushi, M. Q.; Gusman, G. S.; Vestena, S., Efeito alelopático de extratos aquosos de Eucalyptus globulus Labill. e de Casearia sylvestris Sw. sobre espécies cultivadas. *Semina: Ciências Agrárias* **2011,** *32* (4), 1361-1374.

31. Lorber, P.; Muller, W. H., Volatile growth inhibitors produced by Salvia leucophylla: effects on seedling root tip ultrastructure. *American Journal of Botany* **1976,** *63* (2), 196-200.

32. Grosso, C.; Coelho, J. A.; Urieta, J. S.; Palavra, A. M.; Barroso, J. G., Herbicidal activity of volatiles from coriander, winter savory, cotton lavender, and thyme isolated by hydrodistillation and supercritical fluid extraction. *Journal of agricultural and food chemistry* **2010,** *58* (20), 11007-11013.

33. ANDRADE-VIEIRA, L. F.; BOTELHO, C. M.; LAVIOLA, B. G.; PALMIERI, M. J.; PRAÇA-FONTES, M. M., Effects of Jatropha curcas oil inLactuca sativa root tip bioassays. *Anais da Academia Brasileira de Ciências* **2014,** *86*, 373-382.

34. Fiskesjö, G., The Allium test—a potential standard for the assessment of environmental toxicity. In *Environmental Toxicology and Risk Assessment: Second Volume*, ASTM International: 1993.

35. Barroso Aragão, F.; Tebaldi Queiroz, V.; Ferreira, A.; Vidal Costa, A.; Fontes Pinheiro, P.; Tavares Carrijo, T.; Fonseca Andrade-Vieira, L., Phytotoxicity and cytotoxicity of Lepidaploa rufogrisea (Asteraceae) extracts in the plant model Lactuca sativa (Asteraceae). *Revista de Biología Tropical* **2017,** *65*, 435-443.

36. Freitas, A. S.; Cunha, I. M. F.; Andrade-Vieira, L. F.; Techio, V. H., Effect of SPL (Spent Pot Liner) and its main components on root growth, mitotic activity and phosphorylation of Histone H3 in Lactuca sativa L. *Ecotoxicology and environmental safety* **2016,** *124*, 426-434.

37. Andrade-Vieira, L.; Gedraite, L.; Campos, J.; Davide, L., Spent Pot Liner (SPL) induced DNA damage and nuclear alterations in root tip cells of Allium cepa as a consequence of programmed cell death. *Ecotoxicology and environmental safety* **2011,** *74* (4), 882-888.

38. Andrade-Vieira, L. F.; de Campos, J. M. S.; Davide, L. C., Effects of Spent Pot Liner on mitotic activity and nuclear DNA content in meristematic cells of Allium cepa. *Journal of environmental management* **2012,** *107*, 140-146.

39. Silva, R. C.; Neris, M. A.; Lopes, M. A.; Cerqueira, E. M. M.; Meireles, J. R. C., Danos cromossômicos e alterações nucleares em células esfoliadas do epitélio gengival

de indivíduos com periodontite crônica moderada. *Revista de Ciências Médicas e Biológicas* **2017,** *16* (1), 19-24.

40. Dietz, J.; Diehl, A.; Prolla, J.; Furtado, C.; Furtado, A., Pesquisa de micronúcleos na mucosa esofágica e sua relação com fatores de risco ao câncer de esôfago. *Revista da Associação Médica Brasileira* **2000,** *46* (3), 207-211.

41. Fernandes, T. C. C.; Mazzeo, D. E. C.; Marin-Morales, M. A., Origin of nuclear and chromosomal alterations derived from the action of an aneugenic agent—Trifluralin herbicide. *Ecotoxicology and environmental safety* **2009,** *72* (6), 1680-1686.

42. dos Santos, F. E.; Carvalho, M. S. S.; Silveira, G. L.; Correa, F. F.; das Graças Cardoso, M.; Andrade-Vieira, L. F.; Vilela, L. R., Phytotoxicity and cytogenotoxicity of hydroalcoholic extracts from Solanum muricatum Ait. and Solanum betaceum Cav.(Solanaceae) in the plant model Lactuca sativa. *Environmental Science and Pollution Research* **2018**, 1-11.

43. Leme, D. M.; Marin-Morales, M. A., Allium cepa test in environmental monitoring: a review on its application. *Mutation Research/Reviews in Mutation Research* **2009,** *682* (1), 71-81.