https://doi.org/10.1590/1678-4499.20230180

BASIC AREAS
Article

Chemical composition, phytotoxic, and cytogenotoxic properties of essential oils from *Psidium cauliflorum* and *P. acidum* (Myrtaceae)

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Received: Aug. 21, 2023 | **Accepted:** Oct. 16, 2023

Section Editor: Gabriel Constantino Blain

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How to cite: Alves, T. A., Venancio, A. N., Alves, T. A., Vasconcelos, L. C., Tuler, A. C., Silva, M. A., Radael, L. A. N., Menini, L., Ferreira, M. F. S., and Praça-Fontes, M. M. (2024). Chemical composition, phytotoxic, and cytogenotoxic properties of essential oils from *Psidium cauliflorum* and *P. acidum* (Myrtaceae). Bragantia, 83, e20230180. https://doi.org/10.1590/1678-4499.20230180

ABSTRACT: The search for bioherbicides has been encouraged, and plants used in food or with bioactivity have been studied. Therefore, this article aimed to investigate the bioherbicidal potential of essential oils from *Psidium cauliflorum* and *P. acidum* through a plant toxicity bioassay using *Lactuca sativa* and *Sorghum bicolor*. The seeds were treated with essential oils of both species, along with control groups treated with distilled water, dichloromethane, and glyphosate. 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 major compound of the essential oil of *P. cauliflorum* was α-pinene, and of *P. acidum* were trans-caryophyllene, β-elemene, germacrene A, and α-copaene. The essential oils from both species exhibited phytotoxic effects. *P. acidum* oil inhibited sorghum RG and lettuce SL, while *P. cauliflorum* oil reduced GP, GSI, RG, and SL in both plants, indicating higher phytotoxicity than *P. acidum* and non-selective behavior. Cytotoxic investigations showed that both oils inhibited the MI. CA analysis revealed that *P. cauliflorum* oil exhibited aneugenic and clastogenic action mechanisms. The results demonstrate the bioherbicidal potential of *P. cauliflorum* essential oil, in addition to being non-selective and displaying a similar inhibition rate to glyphosate.

Key words: bioherbicides, cytogenetics, plant bioassay, secondary metabolites.

INTRODUCTION

The increasing demand for food has been a driving force behind the modernization of agricultural techniques, machinery, and products (Alves et al. 2018). In this context, herbicides have gained prominence as the most widely used class of agrochemicals in the field, primarily for weed control in agricultural crops. Failure to effectively control weeds, which are considered invasive in crops, can significantly impact production profits (Vasconcelos et al. 2019; Dutra et al. 2020). However, there have been reports of plants developing resistance to synthetic commercial herbicides. The use of these products has compromised food quality, led to environmental contamination, and raised concerns regarding human health risks. As a result, alternative methods for weed control have been extensively studied (Silva et al. 2005; Souza Filho 2006).

One of the current methods under investigation involves the application of secondary metabolites, such as bioherbicides, which are considered less harmful to humans and the environment. These compounds can be used either by developing products containing natural compounds as their base or by directly applying the secondary metabolites to crops (Alves et al. 2018). Secondary metabolites are naturally produced by plants and can be found, among other sources, in essential oils. Their utilization is recognized as an environmentally friendly and recommended alternative (Jatoba et al. 2016; Głąb et al. 2017).

Myrtaceae is a family of angiosperms that stands out among other plant groups due to the presence of essential oils in its representatives. Additionally, fruits from various species belonging to the genus *Psidium* are utilized in human food. These culinary applications, coupled with the substantial production of essential oils in the leaves, contribute to the commercial significance of this genus. Regular pruning of the plants for fruit production generates a substantial amount of leaf residues. Hence, studies have been conducted to explore the applications of essential oils derived from *Psidium* species, focusing on the utilization of these pruning residues (Vasconcelos et al. 2019).

The genus *Psidium* encompasses several species, including *Psidium cauliflorum* Landrum & Sobral and *Psidium acidum* (DC.) Landrum. *Psidium cauliflorum* is primarily found in the Atlantic Forest and is noteworthy for being the first species within the genus to be described with cauliflorous inflorescence. However, since its description, in 2006, there have been limited studies conducted on this species (Landrum and Sobral 2006). *Psidium acidum* is native to Brazil and is cultivated in various parts of the country (Popenoe 1934; Landrum 2016). This species is valued for its fruits, which are used in human food applications (Landrum 2016).

One approach to assess the bioherbicidal potential of products derived from secondary plant metabolism is through plant bioassays. These assays can be conducted in laboratories, focusing on macroscopic variables related to seedling germination and initial growth, as well as microscopic parameters like the mitotic index and nuclear and chromosomal alterations. For these tests, seeds of well-established plants such as *Lactuca sativa* and *Sorghum bicolor* are commonly employed due to their small seed size, rapid growth, and easy availability in agricultural stores (Alves et al. 2018; Pinheiro et al. 2015).

Based on the information provided, the present study aimed to investigate the potential bioherbicidal effects of the essential oils derived from the leaves of *P. cauliflorum* and *P. acidum*. Specifically, the study aimed to examine the impact of these essential oils on the initial growth and development of *S. bicolor* and *L. sativa*, as well as to assess their effects on the mitotic cycle of *L. sativa*'s meristematic cells. The main objective was to elucidate the phytotoxic and cytogenotoxic potential of these essential oils.

MATERIAL AND METHODS

Plant material

The essential oils (test agents) studied were obtained from leaves of *P. cauliflorum* Landrum and Sobral (2006) collected in Cachoeiro de Itapemirim, Espírito Santo, Brazil, location: 20°75'19.09"S / 41°23'16.39"W (A. C. Tuler 480, RB00887251); and *P. acidum* DC. collected in Alegre, Espírito Santo, Brazil, location: 20°45'37.8"S / 41°27'24.8"W (A. C. Tuler 524). Young leaves were collected from adult individuals at a height of 1.30 m, in the month of February (summer), in the morning period.

Seeds of eudicotyledonous *L. sativa* L. (lettuce) 'Crespa Grand Rapids' (Isla Pak) and monocotyledonous *S. bicolor* L. Moench (sorghum) 'AL Precioso' (BR Seeds) were used as model plants (Pinheiro et al. 2015).

Essential oils extraction and yield

The fresh leaves were dried in a drying chamber at 40°C for 12 hours and then frozen at -20°C until use (three days), weighed, and then crushed with water. The resulting material was transferred to a Clevenger apparatus for hydrodistillation extraction, which lasted for 5 hours (Pinheiro et al. 2015). After the extraction process, the obtained oil was weighed. The oil yield was determined by calculating the ratio of 100 times the weight of the oil to the weight of the leaves (Pinheiro et al. 2015). The essential oil was subsequently stored in a freezer at -20°C, protected from light until its use and/or characterizations.

Essential oils characterization

The oil samples were subjected to analysis using gas chromatography coupled to mass spectrometry instrument (GC-MS) model QP2010 Plus (Shimadzu, Tokyo, Japan) and gas chromatography equipped with a flame ionization detection ionstrument (GC-FID) model GC-2010 (Shimadzu, Tokyo, Japan). The samples were analyzed, and the compounds present in the samples were identified following the protocol described by Pinheiro et al. (2015).

Phytotoxicity assays

The experiment was conducted using a completely randomized design with five replications, with each replication consisting of 25 seeds. The treatments included different concentrations of the essential oils from *P. acidum* and *P. cauliflorum*, namely 3,000, 1,500, 750, 375, and 187.5 $mg \cdot mL^{-1}$. The positive control treatment consisted of the commercial herbicide glyphosate at the concentration of 1 $mg \cdot mL^{-1}$. Distilled water and dichloromethane (DCM), which was used as the solvent to dilute the essential oils, served as negative controls (Pinheiro et al. 2015).

The seeds were evenly distributed in Petri dishes with 9 cm diameter that had been cleaned beforehand and containing filter paper. The plates were then treated with 2 mL of the respective treatments, sealed with film paper, and placed inside a germination chamber (biochemical oxygen demand). The germination chamber had a 12-hour photoperiod and maintained the temperature of 25°C ± 2°C (Alves et al. 2018).

The germination process was monitored at 8-hour intervals for the initial 48 hours to calculate the seed germination speed index (GSI). After 48 hours of exposure to the test agents, the percentage of seed germination (GP) and root growth (RG) were evaluated. The Petri dishes were then returned to the germination chamber and kept until 120 hours, at which point the shoot length (SL) of the seedlings was measured (Alves et al. 2018).

Cytotoxicity assay

Lactuca sativa seeds were chosen for this assay due to their suitability for microscopic analysis, which allows for the evaluation of toxicity in meristematic cells. After being treated, the seeds were exposed to the test agents for a period of 48 hours. Subsequently, the emerged roots were carefully collected and fixed in a solution of ethyl alcohol and acetic acid (3:1). Two changes of the fixing solution were performed: the first change was made 10 minutes after fixation, and the second change was made 24 hours after fixation. After a minimum of 24 hours of fixation, slides were prepared for further analysis (Alves et al. 2021).

For each slide preparation, two root meristems were utilized. The roots were subjected to three consecutive baths of 10 minutes each in distilled water. Following this, hydrolysis was performed by treating the roots with 5N HCl for 18 minutes at the temperature of 25°C. The slides were prepared by gently squashing the root tips and then staining them with a 2% solution of acetic orcein (Alves et al. 2018).

A total of 1,000 cells per slide was evaluated, resulting in 5,000 cells assessed per treatment. The observed mitotic phases were recorded, and any possible chromosomal alterations (CA) and nuclear alterations (NA) were noted. The mitotic index (MI) was calculated by determining the proportion of cells in interphase and cells in mitosis. The frequencies of CA, such as c-metaphase (c-met), lost chromosomes, chromosomal adherence, and bridge formations, were determined by calculating the ratio of the number of cells with alterations to the total number of dividing cells. Additionally, the frequencies of micronuclei (MNC) and condensed nuclei (CN) were determined. The frequencies of CA and NA were obtained by calculating the ratio of the number of cells with alterations to the total number of cells evaluated (Aragão et al. 2015).

Statistic

The results obtained were subjected to analysis of variance (ANOVA), and the means were subsequently tested using the Tukey's test ($p < 0.05$) with the Genes program as the statistical tool (Cruz 2013). Regression analysis was employed

to evaluate the MI. Polynomial regression models were adjusted based on the significance of the ANOVA F-test, and the quality of the models was assessed using the coefficient of determination (R^2). The regression analysis and the creation of regression and boxplot graphs were conducted within the R computing environment (R Core Team 2020). All graphs were made using the ggplot2 package (Wickham 2016).

RESULTS

Yield and chemical characterization of essential oils

The essential oil from *P. cauliflorum* showed an extraction yield of 79% and *P. acidum* of 85% ($m \cdot m^{-1}$). The major compound in the essential oil of *P. cauliflorum* was α-pinene, accounting for 49.16% (Table 1). In the essential oil of *P. acidum*, four major compounds were identified: trans-caryophyllene (18.43%), β-elemene (18.36%), germacrene A (16.83%), and α-copaene (11.67%) (Table 2). Both oils contained α-copaene and Δ-cadinene in equal proportions (Tables 1 and 2), but there was a significant difference in the concentration of α-pinene, with 49% in *P. cauliflorum* and 4% in *P. acidum* (Tables 1 and 2).

Table 1. Identification of the compounds present in *Psidium cauliflorum* essential oil using the linear temperature programmed retention indexes and gas chromatography-mass spectrometry[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	1,027	1,024
β-Ocimene	13.434	3.19	1,049	1,044
Borneol	18.765	4.24	1,165	1,165
α-Terpineol	19.966	7.45	1,191	1,186
α-Copaene	28.107	11.39	1,374	1,374
α-Bisabolene	33.644	3.05	1,508	1,506
Δ-Cadinene	34.205	3.65	1,523	1,522
(E)-Nerolidol	35.841	13.05	1,565	1,561

[a]The compounds were identified by the LTPRI index (GC / FID) and by mass spectrometry using a column Rtx®-5MS; [b]tabulated retention index (Adams, 2007; NIST, 2011; El-Sayed, 2016); [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 the compounds present in *Psidium acidum* essential oil using the linear temperature programmed retention indexes and gas chromatography-mass spectrometry[a].

Compound[b]	Retention time (min)	Relative area (%)c	Calculated retention indexd	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	1,049	1,044
α-Copaene	28.124	11.67	1,374	1,374
β-Elemene	28.871	18.36	1,391	1,389
trans-Caryophyllene	29.943	18.43	1,417	1,417
α-Humulene	31.305	2.56	1,451	1,452
γ-Muurolene	32.456	2.83	1,479	1,478
β-Selinene	32.649	2.82	1,483	1,489
Δ-selinene	33.036	4.22	1,493	1,492

Continue...

Table 2 . Continuation...

Compound[b]	Retention time (min)	Relative area (%)c	Calculated retention indexd	Literature retention index
germacrene A	33.467	16.83	1,504	1,508
α-Farnesene	33.679	2.00	1,509	1,505
Δ-Cadinene	34.215	3.66	1,523	1,522
Caryophyllene oxide	36.420	2.52	1,580	1,582
NI[e]	39.175	5.68	1,654	-

[a]The compounds were identified by the LTPRI index (GC / FID) and by mass spectrometry using a column Rtx®-5MS; [b]tabulated retention index (Adams, 2007; NIST, 2011; El-Sayed, 2016); [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.

Phytotoxicity assay

The essential oil of *P. acidum* did not significantly inhibit the germination percentage in either of the two plants studied (Fig. 1a). However, the essential oil of *P. cauliflorum* at concentrations of 3,000 and 1,500 mg·mL^{-1} resulted in a 19.33 and 15.97% inhibition of lettuce germination percentage, respectively (Fig. 1a). Additionally, at concentrations of 3,000, 1,500, and 750 mg·mL^{-1}, the essential oil of *P. cauliflorum* inhibited sorghum germination percentage by 35.34, 25.86, and 18.10%, respectively (Fig. 1a).

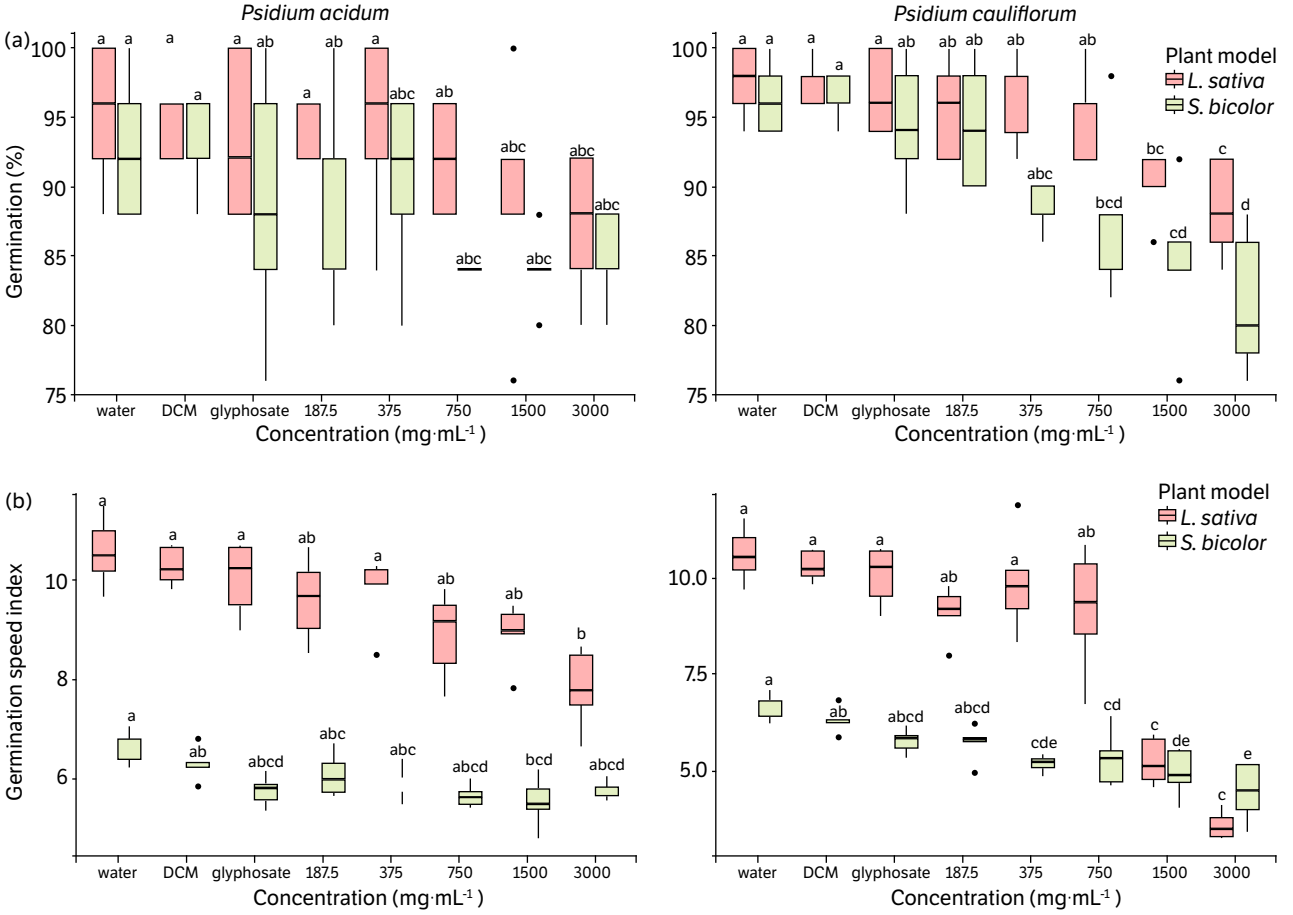


Figure 1. Effect of the essential oil of *Psidium acidum* and *Psidium cauliflorum* on the (a) germination percentage and on the (b) germination speed index of *Lactuca sativa* and *Sorghum bicolor*. The different lowercase letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

The essential oil of *P. acidum* had no significant effect on the sorghum GSI, and the highest tested concentration inhibited the lettuce GSI by 25.87% (Fig. 1b). On the other hand, the essential oil of *P. cauliflorum* at concentrations of 3,000 and 1,500 mg·mL^{-1} inhibited 65.95 and 50.36% of the lettuce GSI, respectively. Furthermore, at concentrations of 3,000, 1,500, 750, and 375 mg·mL^{-1}, the essential oil of *P. cauliflorum* inhibited the sorghum GSI by 36.05, 24.95, 19.31, and 21.41%, respectively (Fig. 1b).

The essential oil of *P. cauliflorum* demonstrated higher toxicity in both germination parameters evaluated, in comparison to the two plants used (Fig. 1).

The sorghum RG was inhibited by 42.32% with the highest concentration of *P. acidum* oil, while no significant changes were observed in the lettuce (Fig. 2a). On the other hand, the highest and second highest concentrations of *P. cauliflorum* oil inhibited over 70 and 50% of RG in both plants, respectively (Fig. 2a). In the sorghum, inhibition was observed with all four highest concentrations tested, while in the lettuce inhibition was observed with the two highest concentrations (Fig. 2a).

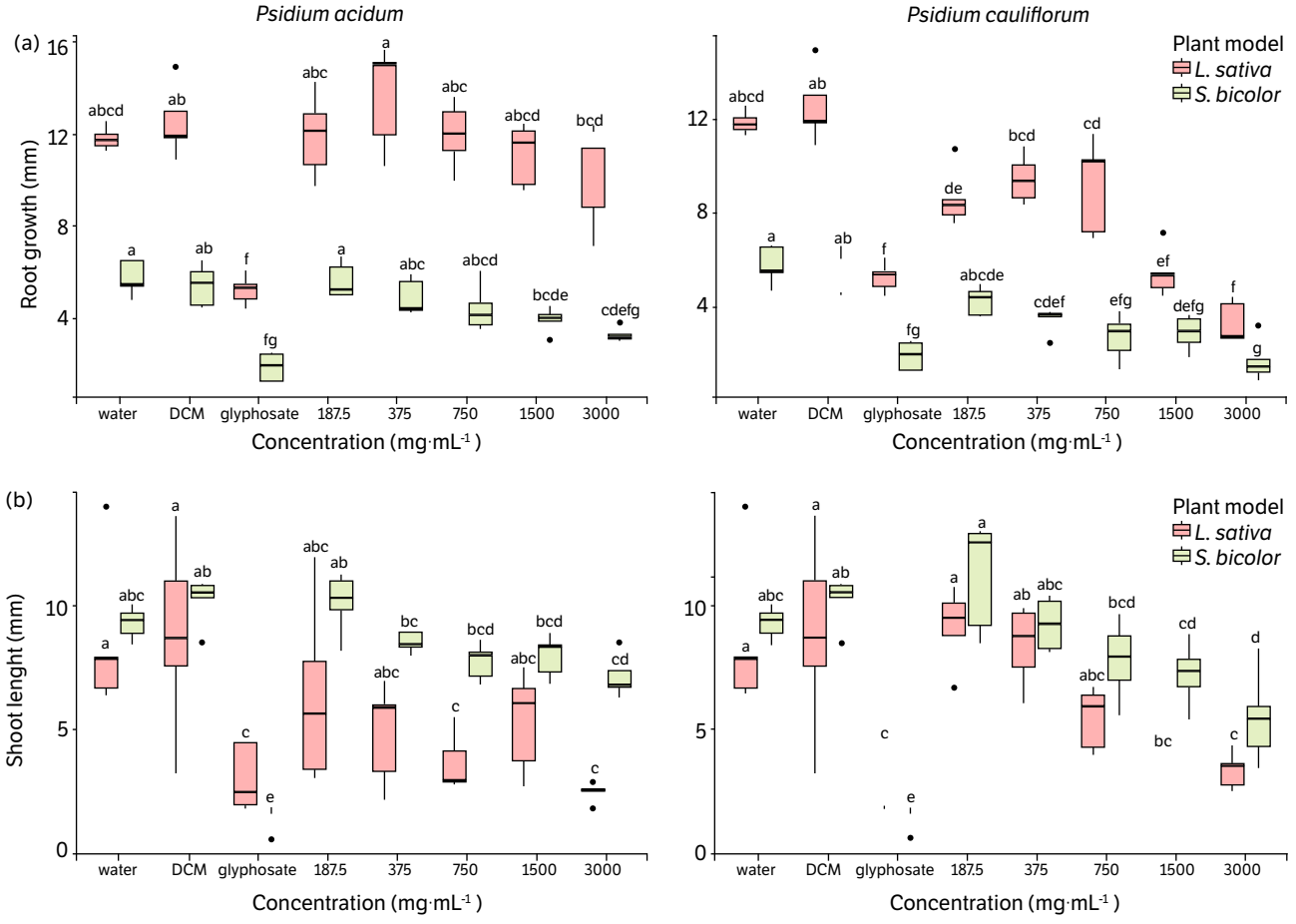


Figure 2. Effect of the essential oil of *Psidium acidum* and *Psidium cauliflorum* on the (a) root growth and on the (b) shoot length of *Lactuca sativa* and *Sorghum bicolor*. The different lowercase letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

The sorghum showed 41.14% inhibition in SL with the highest concentration of *P. cauliflorum* oil (Fig. 2b). In the lettuce, the SL was inhibited by 61.17 and 54.20% with the highest concentrations of *P. cauliflorum* oil. Furthermore, the oil of *P. acidum* at concentrations of 3,000 and 750 mg·mL^{-1} inhibited the lettuce SL by 71.44 and 57.72%, respectively (Fig. 2b).

Cytotoxicity assay

The MI of the root meristems treated with the three highest concentrations of the essential oil of *P. cauliflorum* and with concentrations of 1,500, 750, and 375 mg·mL^{-1} of *P. acidum* was significantly decreased compared to the negative controls. However, no treatment reduced the MI by more than 50% (Fig. 3).

The NA increased in all concentrations of *P. cauliflorum* and in concentrations of 1,500, 750, and 187.5 mg·mL^{-1} of *P. acidum* when compared to the negative controls (Fig. 4). However, all tested concentrations were less toxic than the positive control, indicating that the treatments resulted in lower environmental harm compared to the commercial herbicide.

The alterations occurring in the cell nucleus were observed in the present work in the form of MNC and CN. CNs were significant but less frequent than in the positive control (C+) in the highest concentration of *P. cauliflorum* and in concentrations of 750 and 187.5 mg·mL^{-1} of *P. acidum* (Fig. 4). The presence of MNC was significant in all treatments with the essential oil of *P. cauliflorum* and at concentrations of 750 and 187.5 mg·mL^{-1} of *P. acidum* (Fig. 4).

The incidence of CA showed a significant increase in all studied treatments (Fig. 4). Specifically, the CA in c-metaphase exhibited a significant increase in the treatment with the essential oil of *P. cauliflorum* at the concentration of 1,500 m·mL^{-1} (Fig. 5). Additionally, the CA fragment also demonstrated a significant increase, compared to both the negative and positive controls, in *P. cauliflorum* at the concentration of 750 mg·mL^{-1} (Fig. 5). Notably, chromosomal adherence was significantly observed in all treatments with the essential oil of *P. cauliflorum* and at concentrations of 1,500 and 750 mg·mL^{-1} of *P. acidum* (Fig. 5).

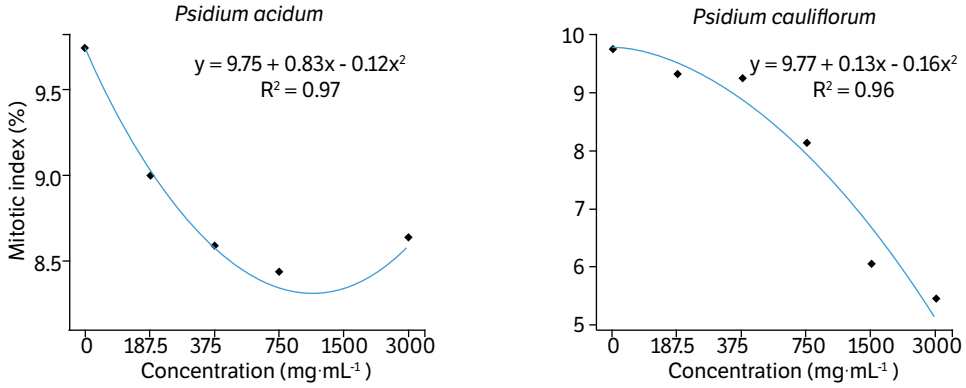
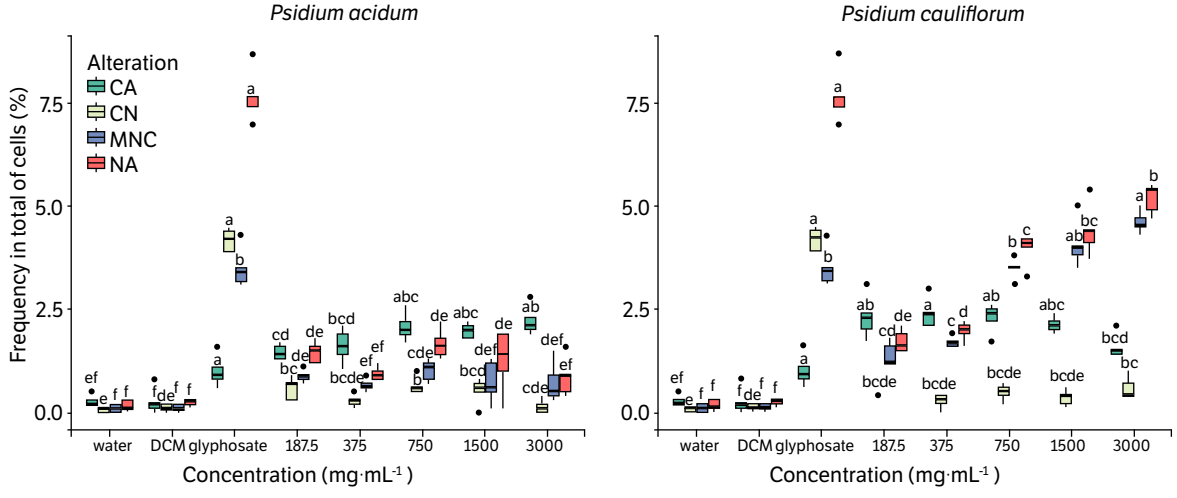


Figure 3. Mitotic index for *Lactuca sativa* treated with solutions of *Psidium acidum* and *Psidium cauliflorum* essential oils.



CA: chromosomal alterations; NA: nuclear alterations; MNC: micronucleus; CN: condensed nucleus.

Figure 4. Nuclear and chromosomal alterations were observed in the root meristems of lettuce treated with the essential oils of *Psidium acidum* and *Psidium cauliflorum* at concentrations of 3,000, 1,500, 750, 375, and 187.5 mg·mL^{-1}. The different lowercase letters above the boxplots indicate significant differences between the treatments, as determined by Tukey's test ($p < 0.05$).

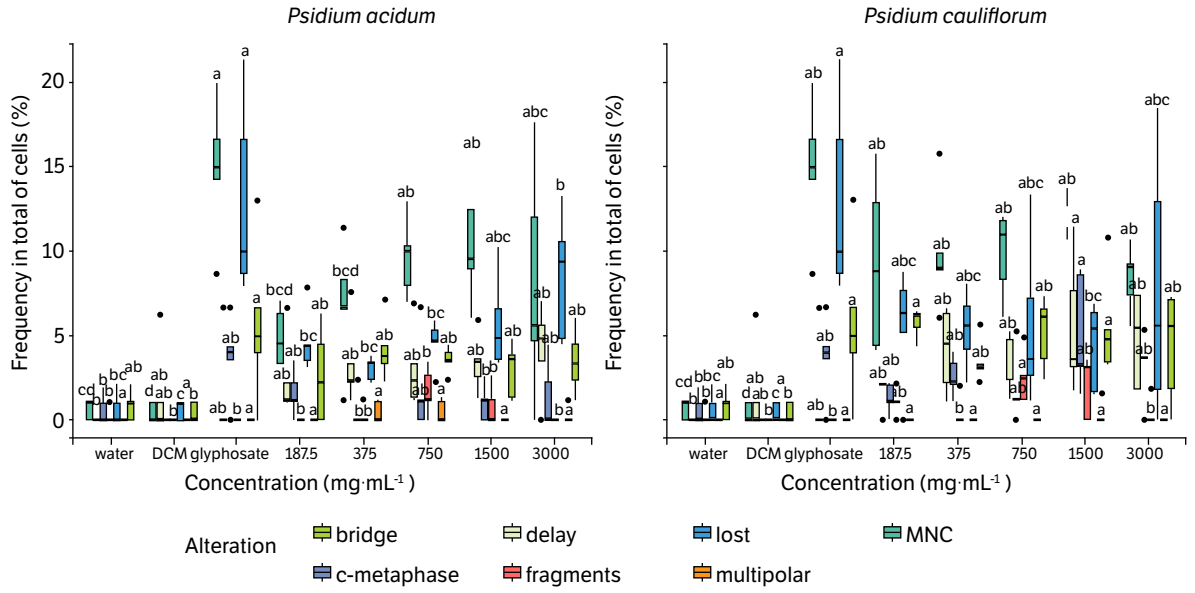


Figure 5. Frequency of each chromosomal alterations observed in meristematic cells of lettuce treated with essential oils of *Psidium acidum* and of the *Psidium cauliflorum* at the concentrations of 3,000, 1,500, 750, 375 and 187.5 mg·mL^{-1}. The different lowercase letters above the boxplots indicate significant difference between the treatments by Tukey's test ($p < 0.05$).

DISCUSSION

Essential oils of different species belonging to the same genus may exhibit varying degrees of phytotoxicity due to their distinct chemical compositions (Vasconcelos et al. 2019). Furthermore, essential oils can interfere with different evaluation parameters and/or organisms in diverse ways (Pinheiro et al. 2015).

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. 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, unlike the current research, were α-pinene (14.8%), 1,8-cineole (12.9%), and β-pinene (10.1%) (da Silva et al. 2003).

α-Pinene, a major component of the foliar essential oil of *P. cauliflorum* and present in low concentrations in *P. acidum*, is recognized for its biological activities in various studies (Leite et al. 2007; Bouzenna et al. 2017; Zamyad et al. 2019). This fact may be related to the potential non-selective bioherbicidal effect exhibited by the essential oil of *P. cauliflorum*, which inhibited both mono- and dicotyledonous plants. On the other hand, the essential oil of *P. acidum* did not demonstrate high phytotoxicity during the initial development of plants and contained a lower amount of α-pinene.

The phytotoxic effect of essential oils containing a high proportion of α-pinene has been previously documented. For instance, *Rosmarinus officinalis* L., which is predominantly composed of α-pinene (25.8–27.7%), has been found to inhibit the initial development of *Lactuca serriola* L. and *Rhaphanus sativus* L. when treated with concentrations of 300, 600, 900, 1,200, 1,500, and 1,800 μL·L^{-1}. This inhibition was observed in terms of GP, GSI, RG, and SL (Alipour and Saharkhiz 2016).

Furthermore, it has been reported that the essential oil of *R. officinalis* (containing 25.85% α-pinene) inhibited the germination of weeds *Amaranthus retroflexus* L. and *Bromus tectorum* L. when treated with the concentration of 400 μL·L^{-1} (Hazrati et al. 2018).

The negative impact of volatile compounds on plant energy metabolism has been linked to the cellular respiration process (Lorber and Muller 1976). These authors reported that volatile terpenes have the potential to harm mitochondria, leading to adverse effects on cellular respiration. As a result, monoterpene compounds can interfere with germination and initial plant development, causing morphoanatomical and physiological changes such as reduced chlorophyll synthesis,

impaired photosynthesis, cytoplasmic lipid accumulation, and organelle reduction due to membrane rupture (Grosso et al. 2010). These changes also disrupt cellular dynamics, making their study essential.

The MI indicates the number of cells undergoing mitotic division (Andrade-Vieira et al. 2014). Therefore, a reduction in this variable signifies an inhibition of mitosis and consequently an increase in cells in the interphase. The cytotoxicity of a test substance can be determined by observing a decrease and/or increase in the MI (Pinheiro et al. 2015). However, the treatments evaluated in this study did not exhibit significant toxicity since a truly toxic test substance is defined as one that reduces the MI by more than 50% (Fiskesjo 1993). Such a level of reduction was only observed in cells treated with the commercial herbicide glyphosate, which was used as a positive control in this study.

Cell death is associated with the decrease in the MI, and the reduction of RG is an important indicator. It is assessed by the presence of condensed nuclei, which is a cytological manifestation of cell death (Andrade-Vieira et al. 2011; Andrade-Vieira et al. 2012). This genetically programmed cell death serves the purposes of maintaining tissue homeostasis and eliminating cells with DNA alterations (Silva et al. 2017).

Another NA observed is the presence of MNC, which are formed with the purposes of incorporating and eliminating portions of DNA found in the cell cytoplasm. These DNA fragments can originate from unaligned intact chromosomes that are incorporated into the nuclei of daughter cells during cell division, or from fragments of acentric chromosomes (Dietz et al. 2000; Fernandes et al. 2009; dos Santos et al. 2019). Therefore, this particular abnormality is associated with preceding CAs, such as chromosome loss and fragmentation.

CAs are identified by alterations in the total number and structure of chromosomes. These changes can occur spontaneously, albeit infrequently, or as a result of exposure to toxic agents (Leme and Marin-Morales 2009). The types of CAs observed in cells provide insight into the specific interactions occurring between the test substance and the target organism (Dutra et al. 2020).

Chromosomal alterations that arise from structural changes in chromatin/DNA, leading to anomalies in the DNA sequence, are referred to as clastogenic. Chromosomal fragmentation is a specific type of CA that indicates this mechanism of action. These fragmented segments are considered acentric when the fragmentation occurs at the telomeres, resulting in the formation of bridges between chromosomes. As a consequence, the spindle fibers are unable to properly attach to the affected chromosome during cell division (Fernandes et al. 2009; Aragão et al. 2015; Silveira et al. 2017).

CAs can also indicate an aneugenic mechanism of action, characterized by genomic instability that may lead to mutations. This mechanism disrupts the functionality of the mitotic spindle machinery, affecting both microtubule polymerization and depolymerization, consequently causing the loss or gain of genetic material in daughter cells (Fernandes et al. 2009). An example of aneugenic CA is the presence of c-metaphase, which arises from the complete inactivation of the mitotic spindle due to the failure of microtubule polymerization during metaphase (Fernandes et al. 2009). This alteration leads to cell division arrest at this stage (Freitas et al. 2016).

On the other hand, adherence-type CA indicate both aneugenic and clastogenic mechanisms of action, and they can also be an indicative of epigenetic changes. This alteration occurs due to the irreversible fusion of chromosomes, which can result in cell death. It is an indicator of epigenetic changes due to alterations in the pattern of serine 10 phosphorylation of histone 3 (Freitas et al. 2016; Silveira et al. 2017; dos Santos et al. 2019).

Thus, the essential oil of *P. cauliflorum* has demonstrated an aneugenic and clastogenic cellular action mechanism. This is evidenced by the increased frequency of c-metaphases (higher percentage) and chromosomal fragmentation (lower percentage). Furthermore, both oils evaluated have been found to induce epigenetic alterations, as indicated by the increased chromosomal adherence. Understanding the cellular action mechanism of test agents is crucial for comprehending the pathways of changes that contribute to the observed physiological characteristics.

CONCLUSION

The essential oil of *P. acidum* exhibited phytotoxicity towards the RG of sorghum and the SL of lettuce, while the oil of *P. cauliflorum* was toxic to all variables assessed in both plants. Therefore, regardless of the test, the oil of *P. cauliflorum*

proved to be more phytotoxic than that of *P. acidum*. In terms of phytotoxicity levels, the sorghum demonstrated greater sensitivity to *P. cauliflorum* oil, as even the lowest concentrations inhibited its growth. The cytotoxic test revealed that the essential oils induced epigenetic effects, and operates through clastogenic and aneugenic action mechanisms.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Alves, T. A. and Praça-Fontes, M. M.; **Methodology:** Alves, T. A., Venancio, A. N., Alves, T. A., Vasconcelos, L. C., Tuler, A. C., Silva, M. A. and Radael, L. A. N. **Investigation:** Alves, T. A., Vasconcelos, L. C., Tuler, A. C., Silva, M. A., Menini, L., Ferreira, M. F. S. and Praça-Fontes, M. M.; **Writing – Original Draft:** Alves, T. A. and Alves, T. A.; **Writing – Review and Editing:** Vasconcelos, L. C., Tuler, A. C., Menini, L., Ferreira, M. F. S. and Praça-Fontes, M. M.; **Supervision:** Ferreira, M. F. S. and Praça-Fontes, M. M.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico
Grants No: PQ no. 310504/2021-0 and 312497/2022-9

Fundação de Amparo à Pesquisa do Estado do Espírito Santo
Grant no. 180 /2017 and 484/2021

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
Finance Code 001

ACKNOWLEDGMENTS

Not applicable.

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