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BASIC AREAS
Article

Influence of seasonal variation on the chemical composition of *Piper amalago* essential oils and their phytocytogenotoxic activity in model plants and weeds

Loren Cristina Vasconcelos[1], Aline dos Santos Bergamim[1], Geisiele Silva Martins[1], Gustavo Fernandes Mariano[2], Luiza Alves Mendes[3], Tatiana Tavares Carrijo[1], Milene Miranda Praça-Fontes[1,*]

1. Universidade Federal do Espírito Santo – Programa de Pós-graduação em Genética e Melhoramento. – Alegre (ES), Brazil.
2. Universidade Federal do Espírito Santo – Programa de Pós-graduação em Biologia Vegetal - Vitória (ES), Brazil.
3. Universidade Federal de Viçosa – Programa de Pós-graduação em Agroquímica - Viçosa (MG), Brazil.

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***Corresponding author:** milenemiranda@yahoo.com.br

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ABSTRACT: The essential oil of *Piper amalago* L. is recognized for its bioactive compounds with phytotoxic potential against invasive plants. However, little is known about the role of seasonal variation in the action of these compounds. This study aimed to investigate the impact of dry and rainy seasons on the chemical composition, phytotoxic, and cytogenotoxic activity of *P. amalago* essential oil. Analysis of the chemical composition revealed qualitative and quantitative variations, highlighting β-elemene, germacrene A, linalool, and β-caryophyllene as major compounds. The essential oil from the rainy season showed higher yield compared to that of the dry season. In pre-emergence tests against *Bidens pilosa* (invasive plant) and *Lactuca sativa* (non-target plant), negative effects on germination and root and shoot growth were observed, with these effects being more pronounced at higher concentrations, resembling the herbicide glyphosate. The essential oil from the dry season exhibited greater phytotoxic activity on the germination and development of the aerial part of *B. pilosa*, associated with higher concentrations of linalool and caryophyllene oxide. Additionally, the essential oils of *P. amalago* induced changes in the mitotic index and aneugenic alterations in *L. sativa* meristematic cells. These results underscore the bioherbicidal potential of *P. amalago* essential oil, highlighting its greater efficacy against *B. pilosa* during the dry season, possibly due to higher levels of linalool and caryophyllene oxide.

Key words: natural herbicide; weeds; cellular cycle; bioassays.

INTRODUCTION

Bidens pilosa L. is an annual weed species of Asteraceae, native of tropical America, that is posing challenges to approximately 30 critical economic crops, such as corn, sugarcane, sorghum, and rice (Chauhan et al. 2019). Glyphosate is commonly used to control *B. pilosa*, but the excessive use has led to resistance, evident in Mexican populations (Alcantara-De La Cruz et al. 2016). The allelopathic activity of essential oils (EOs) has been explored as an alternative to synthetic herbicides (Han et al. 2021). However, the role of the sazonal fluctuations in phytochemical compound production by specialized metabolism is poorly known.

EOs are complex mixtures of bioactive compounds, including monoterpenes, sesquiterpenes, and phenylpropanoids. The chemical composition of EOs is influenced by genetic, biotic, and environmental factors (Dhifi et al. 2016). EOs, structurally diverse bioactive compound mixtures, offer new modes of action, reducing the risk of resistance development. Moreover, EOs are considered environmentally friendly herbicides due to their biodegradability and relatively low toxicity to non-target organisms (Dayan and Duke 2014).

Seasonal variations, such as changes in temperature and water availability, can induce fluctuations in phytochemical compound production by specialized metabolism (e.g., Luz et al. 2020, Santos et al. 2023). This alteration in the chemical profile can impact the biological activity of EOs extracted from plants collected at different times of the year (Luz et al. 2020).

Among the aromatic species, *Piper amalago* L. (Piperaceae), commonly known as "jaborandi-manso" or "jamaica pepper," stands out for its economic and medicinal importance. This species is widely used in traditional medicine to treat various illnesses. Studies validating the biological properties of its EO have reported antimicrobial properties and insecticidal activity (Araujo Baptista et al. 2019, Gonçalves et al. 2022). However, there are no reports on the phytotoxic or herbicidal potential of *P. amalago* EO.

This study aimed to assess the impact of the rainy and dry seasons on the chemical composition of *P. amalago* leaf EO, and to investigate its phytotoxic activity on the seed germination and early growth of the eudicots, *B. pilosa* and *L. sativa* (non-target). Additionally, the study aimed to explore changes in the cell cycle of *L. sativa*, to understand the mechanism of action of EOs as a bioherbicide. This plant is a model organism commonly used for cytotoxicity and genotoxicity analyses due to its sensitivity to external factors, easily visualized chromosomes, and rapid cell proliferation (Silveira et al. 2017). Furthermore, the effects found in *L. sativa* can be correlated to those found in *B. pilosa*, since both species are eudicots.

MATERIAL AND METHODS

Plant material and study area

Seeds of *B. pilosa* were collected from a radius of 400 m^2 in a *Coffea arabica* L. field located in the municipality of Iúna, Espírito Santo, Brazil. The seeds of the *L. sativa* cultivar Grandes Lagos Americana (Isla seeds) were obtained from agricultural input stores.

Leaves of *P. amalago* without signs of disease or herbivory were collected in an area of Atlantic Forest located in a forest fragment located in Castelo, Espírito Santo, Brazil. Sample collections were carried out between 9 a.m. and 12 p.m. on January 31st and September 1st, 2022, dates that correspond to the rainy and dry periods, respectively, in the region. During the rainy season collection, individuals of *P. amalago* were in the reproductive period and presented inflorescences on spikes with an intense green hue in the initial fruiting phase (Fig. 1). The studied specimen was previously examined under the taxonomic approach of Christ et al. (2016), and a voucher specimen (J. A. Christ; F. Torres-Leite; M. Zanetti 42, CAP000005) is housed in the Herbarium CAP (Thiers, 2024). Data on edaphoclimatic factors at the study collection site are presented in Table 1.

Figure 1. Individual of *Piper amalago* in the study area. (a) Appearance during the rainy season and (b) dry season.

Table 1. Climate data for average temperature, precipitation, relative humidity, and cloudiness for January 31 and September 1, 2022.

Month (year/day)	Temperature (°C)	Precipitation (mm)	Relative humidity (%)	Cloud (%)
Jan. 2022/31	27.79	3.30	84.00	66.00
Sep. 2022/01	18.39	0.00	70.00	27.00

Source: Data on abiotic factors of the local site and collection time of the study were obtained from the Agrometeorological Monitoring System (https://www.agritempo.gov.br) for the meteorological station (TRMM.1591) and from the World Weather Online repository (https://www.worldweatheronline.com).

The rainy period comprises the months of October to April, with an average rainfall of 1,111.4 mm, accounting for 84.9% of the accumulated annual total, while the dry period comprises May to September, with an average rainfall of 196.9 mm, representing 15.1% of the annual precipitation (Incaper 2020).

Extraction and chemical characterization of essential oils

The fresh leaves collected were carefully stored in paper bags and dried in an oven with air circulation at 40 °C until they reached constant mass. Around 270 g of dry *P. amalago* leaves were subjected to the hydrodistillation process in a Clevenger-type device for 4 h following the methodology recommended by the Brazilian Pharmacopoeia for the extraction of volatile oils (Anvisa 2022). The EO was extracted and subsequently stored in a freezer at 4 °C until further analysis. The yield of EO was calculated as a percentage, through the ratio between the mass of EO and the mass of dry plant material, expressed in % (m/m).

The chemical composition of EO from *P. amalago* leaves was analyzed by gas chromatography with flame ionization detection (GC-FID, Shimadzu QP2010SE, Kyoto, Japan) and gas chromatography coupled to mass spectrometry (GC-MS, Shimadzu QP2010SE, Japan). Identification of the components was done according to the methodology described by Mendes et al. (2017).

Phytotoxic activity

The phytotoxic effect of *P. amalago* EO in *B. pilosa* and *L. sativa* was determined by pre-emergence Petri dish assays. *Bidens pilosa* seeds were disinfected with sodium hypochlorite (NaClO, 0.5%) for 5 minutes and washed multiple times with distilled water. Five concentrations of EO (1,500; 750; 375; 187.5 and 0 $\mu g \cdot mL^{-1}$) were prepared by diluting them in solvent [acetone (2%) and tween 80 (0.05%)]. The solvent (1,500 $\mu g \cdot mL^{-1}$) served as an analysis white (0.0 $\mu g \cdot mL^{-1}$) of the EO. Distilled water was used as a negative control and the herbicide glyphosate (1 $mL \cdot L^{-1}$) as a positive control.

To carry out the tests, 25 seeds from each plant were placed on filter paper moistened with 3 mL of the concentrations. To prevent evaporation of the solution, the plates were wrapped in plastic film. The plates were then placed in a germination chamber set at (24 ± 2) °C with a 16-hour light/8-hour dark photoperiod. After seven days, the germination rate and the length of the root and shoot (coleoptile) were determined for *B. pilosa*. In *L. sativa*, the germination rate and root length were measured after two days of exposure, and the shoot length was measured after five days (Mendes et al. 2023).

Cytogenotoxic activity

The cytogenotoxicity of *P. amalago* EO was evaluated using *L. sativa*. Seeds of *L. sativa* were subjected to the same experimental conditions as the pre-emergence test. Distilled water was used as a negative control. After 48 h of exposure to EO concentrations (1,500; 750; 375; 187.5 and 0 $\mu g \cdot mL^{-1}$), root tips measuring 2–3 cm in length were fixed in a solution of Carnoy (ethanol: acetic acid, 3:1 v/v) and stored at -18 °C. To study the cell cycle and chromosomes, meristematic slides were prepared using the crushing technique and were stained with 2% acetic orcein. The slides were observed under an optical microscope to determine the different phases of mitosis. A total of 1,000 cells per slide was evaluated, totaling

5,000 cells per concentration. Cytotoxicity was assessed by the mitotic index (MI), and genotoxicity was determined by the frequencies of cells with chromosomal alterations (CAs) (Mendes et al. 2023).

Statistical analysis

Phytotoxicity and cytogenotoxicity tests were carried out in a completely randomized design with five replications per concentration. Phytotoxicity tests were carried out in a double factorial scheme and involved two factors: EO and the different concentrations used. The normality of errors and homogeneity of variances were verified using the Shapiro-Wilk's and Bartlett's tests, respectively. All data were subjected to analysis of variance, and mean values were compared using the Tukey's test ($p \leq 0.05$). Statistical analyses were performed using the ExpDes.pt package within R software version 4.3.2 (R Core Team 2023).

RESULTS

Chemical composition and yield

Piper amalago EO exhibited varying colors based on the collection period (Fig. 2a). The EO from the rainy season (PAEOR) exhibited a light green color, while the EO from the dry period (PAEOD) exhibited a dark blue-green hue. The productivity of *P. amalago* EO also showed significant differences considering seasonality, with higher productivity in the rainy period, at 0.075% (m/m), compared to the dry period, which was at 0.063% (m/m) (Fig. 2b).

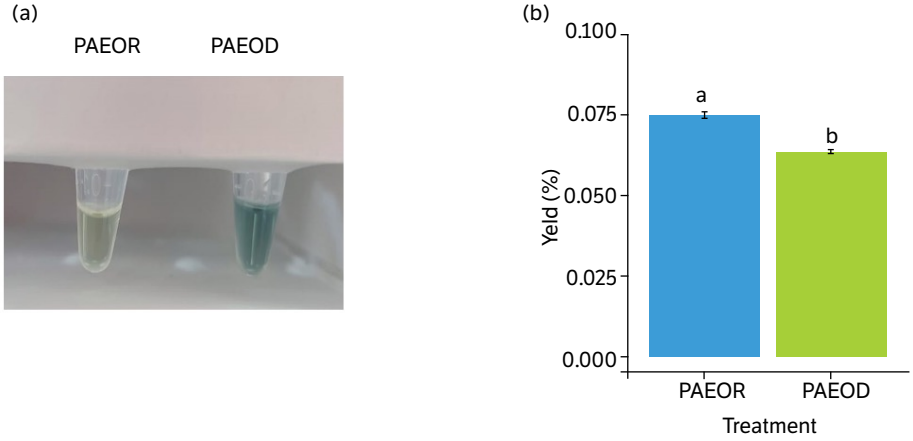

Figure 2. The yield of *Piper amalago* essential oils obtained in the rainy (PAEOR) and dry (PAEOD) seasons. Equal letters do not differ by Tukey's test ($p > 0.05$).

Chemical analysis identified a total of 15 different compounds, including two monoterpenes and 13 sesquiterpenes, at the collection stations, representing 86–91.1% of the oils (Table 2). Sesquiterpene hydrocarbons presented a greater relative area in both EO, with a higher concentration during the rainy season (73.8%) compared to the dry season (63.8%). The predominant compound among the EOs studied was β-elemene, followed by germacrene A, linalool, and β-caryophyllene. Although the main compounds remained consistent in the EO, their relative concentrations varied depending on the harvest time. During the rainy season, hydrocarbon sesquiterpenes such as β-elemene, β-caryophyllene, and germacrene A were more abundant, while the oxygenated monoterpene linalool was more abundant during the dry season. The compounds α-cubene, α-copaene, and caryophyllene oxide were produced exclusively during the dry season, while allo-aromadendrene and valencene were identified exclusively during the rainy season.

Table 2. Phytochemical profiling of *Piper amalago* essential oils obtained in the rainy (PAEOR) and dry (PAEOD) seasons.

Compounds[a]	RIcal[b]	RItab[c]	Relative area (%)[d]	
			PAEOD	PAEOR
α-pinene	929	932	4.3	6.8
Linalool	1,101	1,095	15.5	10.5
δ-elemene	1,335	1,335	4.1	5.0
α-cubebene	1,346	1,345	2.2	-
α-copaene	1,371	1,374	2.5	-
β-elemene	1,391	1,389	17.0	17.8
β-caryophyllene	1,416	1,417	11.5	14.3
α-humulene	1,449	1,452	2.5	3.1
allo-aromadendrene	1,457	1,458	-	2.6
β-selinene	1,482	1,489	2.7	3.0
Bicyclogermacrene	1,489	1,500	3.0	3.3
Valencene	1,491	1,496	-	4.2
germacrene A	1,503	1,508	15.2	17.8
δ-cadinene	1,521	1,522	3.1	2.7
caryophyllene oxide	1,578	1,582	2.4	-
Hydrocarbon monoterpene			4,3	6.8
Oxygenated monoterpene			15.5	10.5
Hydrocarbon sesquiterpene			63.8	73. 8
Oxygenated sesquiterpene			2.4	-
Total			86.0	91.1

[a]Compounds listed in order of elution using the Rtx®-5MS column; [b]calculated retention index using data obtained from a sample of saturated n-alkanes (C_7-C_{40}); [c]tabulated retention index (Adams 2007, El-Sayed 2014, NIST 2011); [d]compounds with a relative area > 2% were identified.

Phytotoxic effects

The analysis of variance found a significant interaction between the sources of variation, that is, the harvest time (rainy period and dry period), and the concentrations of EO for the variables germination percentage and shoot length of *B. pilosa* ($p \leq 0.001$ by F test). In *L. sativa*, the interaction between factors was also significant for root length ($p \leq 0.05$ by F test). This indicates that both factors have a joint effect on these variables, as shown in Table 3.

Table 3. Analysis of variance of phytotoxic effects on germination and initial growth of *Bidens pilosa* subjected to *Piper amalago* essential oil from the dry and rainy season and its different concentrations.

Source of variation	*Df*	*Bidens pilosa*			*Lactuca sativa*		
		Germination rate (%)	Root length (mm)	Shoot length (mm)	Germination rate (%)	Root length (mm)	Shoot length (mm)
Harvest season (A)	1	531.4*	94.7*	225.19***	119ns	0.53^{ns}	1.242^{ns}
Concentration (B)	4	2,903.3***	811.75***	1,727.76***	50,181***	631.13***	87.398***
A X B	4	4,364.7***	98.39ns	272.03***	409^{ns}	38.48*	6.484ns
Residue	40	3,074.4	648.65	328.26	3,333	127.67	38.730
Coefficient of variation		10.64%	24.99%	17.06%	13.68%	28.81%	14.59%

df: Degrees of freedom; number of repetitions (n): 5; ns: not significant; *significant at the 5% probability level ($p \leq 0.05$); **significant at the 1% probability level ($p \leq 0.01$); ***significant at the 0.1% probability level ($p \leq 0.001$).

Considering the effects of each EO on *B. pilosa*, the concentration of 1,500 μg·mL^{-1} of PAEOD inhibited germination by 50%, surpassing the effect of PAEOR by 48.3% (Fig. 3a). Furthermore, all PAEOD concentrations, except for 375 μg·mL^{-1}, resulted in shorter shoot lengths than corresponding PAEOR concentrations. The concentration of 1,500 μg·mL^{-1} of PAEOD completely inhibited the development of the aerial part, overcoming the effect of the herbicide glyphosate. All PAEOR concentrations also resulted in shorter shoot lengths compared to the negative control, with the 1,500 μg·mL^{-1} concentration showing a similar effect to glyphosate (Fig. 3c). PAEOD and PAEOD had the same effect on the root length of *B. pilosa*, showing no statistical difference. Both PAEOD and PAEOR reduced root length compared to water. Concentrations of 1,500 μg·mL^{-1} of PAEOR and PAEOD demonstrated the same effect as glyphosate, suppressing root length by 61.83 and 76.19%, respectively (Fig. 3b).

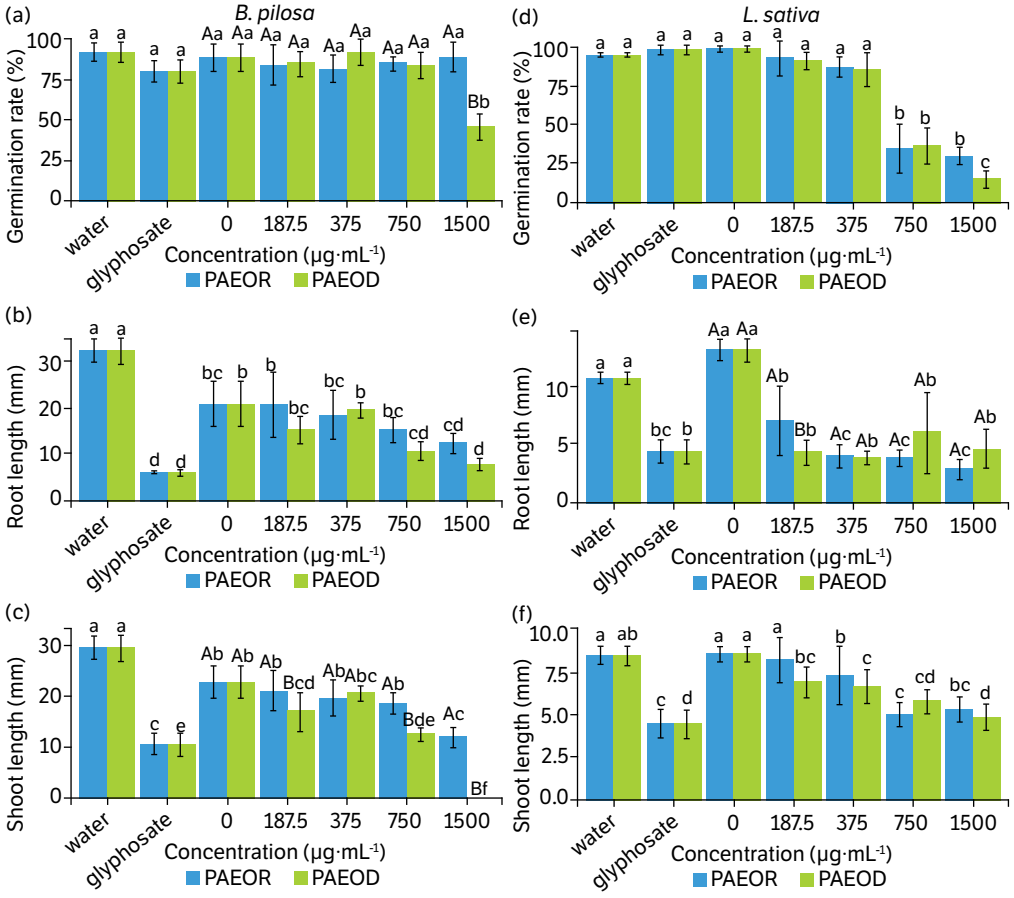

Figure 3. Phytotoxic effects of *Piper amalago* essential oils obtained in the rainy (PAEOR) and dry (PAEOD) seasons on germination rate (a,d), root length (b,e) and shoot length (c, f) of *Bidens pilosa* and *Lactuca sativa*. Bars (mean ± standard error; n = 5) with equal letters (uppercase between essential oils and lowercase for concentrations and controls within each essential oil) do not differ by Tukey's test ($p > 0.05$).

For *L. sativa*, the concentration of 187.5 μg·mL^{-1} of PAEOD resulted in the lowest root length compared to the same concentration of PAEOR. All concentrations of PAEOD and PAEOR reduced root length compared to water, showing similarities to glyphosate (Fig. 3e). Regarding germination rate, the two highest concentrations of PAEOD and PAEOR significantly reduced this variable compared to controls (Fig. 3d).

PAEOD and PAEOR had the same effect on the germination and shoot length of *L. sativa*, showing no statistical difference. Concentrations of 750 and 1,500 μg·mL^{-1} of PAEOD inhibited germination by 44.71 and 57.81%, respectively, while the same concentrations of PAEOR exhibited higher inhibition rates of 64.92 and 74.36% (Figs. 3d and 3f). In terms of shoot length, concentrations of 375, 750, and 1,500 μg·mL^{-1} of PAEOR and PAEOD resulted in shorter lengths compared to water, with higher concentrations responsible for greater inhibitions, similar to glyphosate (Fig. 3f).

Cytogenotoxic effects

The cytogenotoxicity of *P. amalago* EO was assessed by analyzing changes in MI and CAs in *L. sativa* root tip meristematic cells (Tables 4 and 5). PAEOR exhibited dose-dependent effects, reducing MI at concentrations equal to or greater than 187.5 μg·mL^{-1} and inducing CAs at concentrations equal to or greater than 375 μg·mL^{-1}. The concentration of 1,500 μg·mL^{-1} resulted in an 84.05% increase in CAs to the negative control. Increased interphase frequencies were observed at concentrations equal to or greater than 187.5 μg·mL^{-1}, while the prophase frequency decreased. The metaphase frequency also increased with concentrations equal to or greater than 375 μg·mL^{-1}. PAEOD also negatively affected the MI and increased the frequency of interphases at concentrations equal to or greater than 375 μg·mL^{-1}. Concentrations of 750 and 1,500 μg·mL^{-1} induced a 49.27% increase in CAs to the negative control. At the concentration of 1,500 μg·mL^{-1}, the metaphase frequency increased significantly (58.88%), while the prophase frequency decreased (31.32%) compared to the negative control.

Table 4. Effect of *Piper amalago* essential oil obtained from the rainy season (PAEOR) on the mitotic and phase index in meristematic cells of the root of *Lactuca sativa**.

Treatments (μg·mL^{-1})	MI (%)	CA (%)	Interphase	Phase Index (%)			
				Prophase	Metaphase	Anaphase	Telophase
water	8.24 ± 0.27a	1.38 ± 0.08b	91.76 ± 0.27c	42.01 ± 1.85a	30.50 ± 2.14d	21.14 ± 2.01a	6.34 ± 1.63a
0.0	7.78 ± 0.22ab	1.32 ± 0.16b	92.22 ± 0.22bc	33.72 ± 3.54ab	35.01 ± 1.69cd	22.58 ± 1.27a	8.67 ± 2.47a
187.5	6.76 ± 0.48bc	1.44 ± 0.11b	93.22 ± 0.46ab	32.91 ± 2.25b	36.34 ± 2.40bcd	24.02 ± 2.50a	6.71 ± 2.91a
375	6.38 ± 0.10c	2.04 ± 0.09a	93.62 ± 0.10a	26.68 ± 1.15b	42.42 ± 2.95abc	23.09 ± 2.08a	7.79 ± 1.26a
750	7.00 ± 0.30bc	2.38 ± 0.15a	93.0 ± 0.30ab	28.18 ± 1.18b	44.67 ± 1.37ab	19.30 ± 1.32a	7.83 ± 1.54a
1500	6.82 ± 0.08bc	2.54 ± 0.13a	93.18 ± 0.08ab	27.56 ± 0.81b	47.84 ± 1.51a	17.58 ± 1.37a	7.01 ± 1.38a

*Data represented as mean ± standard error (n = 5). Means with the same letters do not differ from each other using the Tukey's test ($p > 0.05$); MI: mitotic index; CA: chromosomal abnormalities.

Table 5. Effect of *Piper amalago* essential oil obtained from the dry season (PAEOD) on the mitotic and phase index in meristematic cells of the root of *Lactuca sativa**.

Treatments (μg·mL^{-1})	MI (%)	CA (%)	Interphase	Phase Index (%)			
				Prophase	Metaphase	Anaphase	Telophase
water	8.24 ± 0.27a	1.38 ± 0.08b	91.76 ± 0.27b	42.01 ± 1.85a	30.50 ± 2.14b	21.14 ± 2.01a	6.34 ± 1.63ab
0.0	7.78 ± 0.22a	1.32 ± 0.16b	92.22 ± 0.22b	33.72 ± 3.54ab	35.01 ± 1.69b	22.58 ± 1.27a	8.67 ± 2.47ab
187.5	8.38 ± 0.24a	1.50 ± 0.24ab	91.50 ± 0.21b	40.24 ± 2.29a	34.43 ± 1.86b	17.91 ± 1.55a	7.40 ± 1.67ab
375	6.02 ± 0.27b	1.20 ± 0.10b	93.98 ± 0.27a	31.90 ± 3.13ab	36.06 ± 3.65b	19.03 ± 2.72a	12.99 ± 1.98a
750	5.96 ± 0.26 b	2.06 ± 0.08a	94.04 ± 0.26a	36.29 ± 2.27ab	37.75 ± 1.82ab	20.84 ± 2.21a	5.10 ± 0.85b
1500	6.08 ± 0.18b	2.06 ± 0.20a	93.84 ± 0.19a	26.85 ± 1.32b	48.46 ± 3.23[a]	18.92 ± 2.57a	5.75 ± 1.47ab

*Data represented as mean ± standard error (n = 5). Means with the same letters do not differ from each other using the Tukey's test (p > 0.05); MI: mitotic index; CA: chromosomal abnormalities.

About individual chromosomal changes, aneugenic changes (action on the mitotic spindle), chromosomal adhesion, c-metaphases, chromosomal loss, and anaphase delays were found (Fig. 4). PAEOR significantly increased the frequency of c-metaphases from the concentration of 375 μg·mL^{-1} to water. The concentration of 1,500 μg·mL^{-1} tripled the frequency of this change. PAEOD also affected the frequency of c-metaphases, doubling it to water at the concentration of 1,500 μg·mL^{-1}. The frequency of chromosomal adhesion was tripled in cells exposed to the concentration of 187.5 μg·mL^{-1} of PAEOD (Fig. 5).

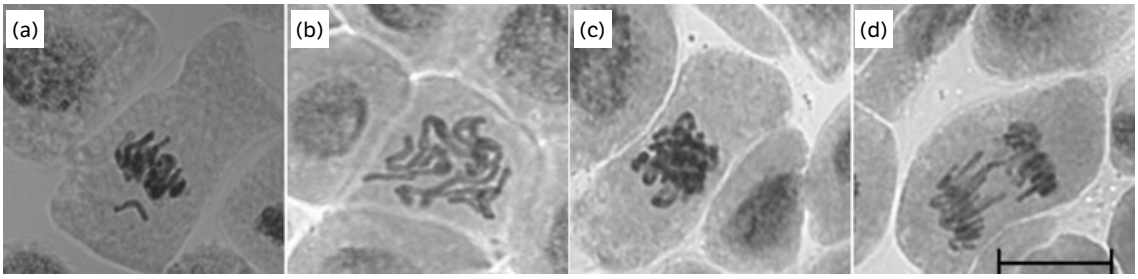

Figure 4. Chromosomal abnormalities observed in meristematic cells of *Lactuca sativa* roots exposed to *Piper amalago* essential oils. (a) Metaphase with chromosome loss, (b) c-metaphase, (c) metaphase with chromosome adhesion, (d) anaphase delay. Scale bar = 20 µm.

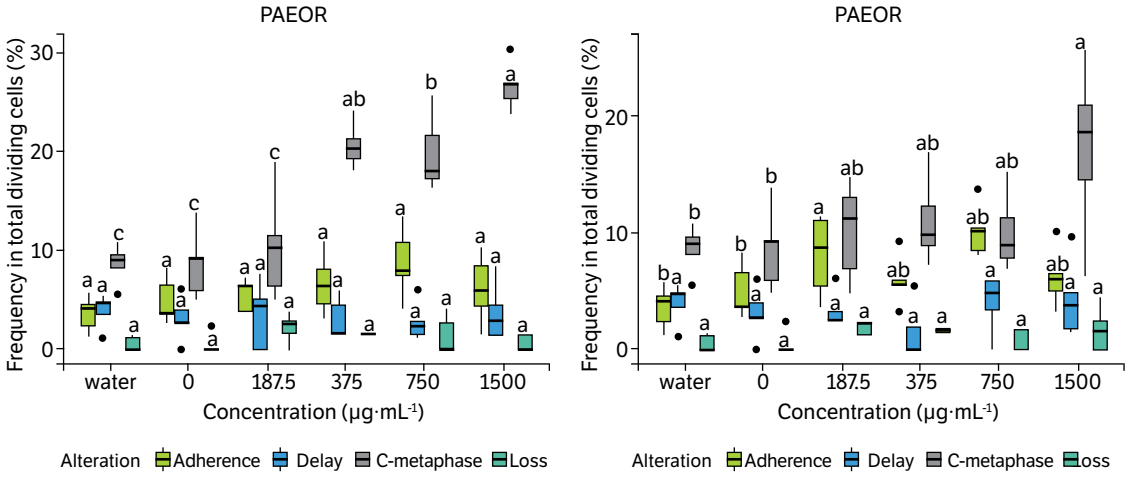

Figure 5. Frequency of chromosomal abnormalities observed in meristematic cells of *Lactuca sativa* roots exposed to *Piper amalago* essential oils obtained from the rainy (PAEOR) and dry (PAEOD) seasons. The lowercase letters above the boxplots indicate a significant difference between the treatments by Tukey's test ($p < 0.05$).

DISCUSSION

The present study observed that the EO of *P. amalago* had a higher yield during the rainy season, contradicting previous results suggesting that seasonality does not significantly impact the production of EO in this species (Perigo et al. 2016). The reduction in EO production during the dry period appears to be related to the scarcity of water and the high incidence of light, characteristics of the dry period. Water stress disrupts metabolic processes, altering metabolic pathways (Ciarmiello et al. 2011). Solar exposure intensified by the lack of clouds and limited rainfall may have affected the accumulation of photoassimilates. This could lead to the generation of reactive oxygen species and, consequently, the production of EO (Oliveira et al. 2016). Studies involving other *Piper* species have revealed variable EO productivity in response to solar intensity and shading (Mattana et al. 2010, Ramos et al. 2021). This diversity in EO production in response to solar intensity highlights the importance of understanding the specificities of each *Piper* species.

Considering the relative area of the compounds, we observed that hydrocarbon sesquiterpenes were more abundant in the rainy season, with higher concentrations of the major compounds: β-elemene, β-caryophyllene, and germacrene. Prolonged exposure to high temperatures during the dry season may have led to the degradation of sesquiterpenes (An et al. 2016), explaining the lower concentration of hydrocarbon sesquiterpenes, as well as the lower EO yield.

The highest EO productivity of *P. amalago* was observed during the flowering and early fruiting phases (rainy period), following a trend consistent with other *Piper* species (Ramos et al. 2020, Ramos et al. 2021). The growth of reproductive organs induces stress in several plant species due to the reallocation of energy reserves for the growth of flowers/inflorescences and defense mechanisms. Consequently, plants direct their metabolic activities towards the biosynthesis of primary and

secondary metabolites. Given this evidence, it is possible to anticipate variations in EO content during plant growth, and the ideal phase for maximum accumulation may differ between species (Farhadi et al. 2020).

When analyzing the phytotoxic effects of EO, it is important to consider that these effects result from the interaction between their different components. Phytotoxic activities have been reported in EO containing germacrene A, β-caryophyllene, β-elemene, and linalool (Dutra et al. 2020, Jiang et al. 2021, Mendes et al. 2023, Veryer and Bozok 2023), main compounds of *P. amalago* EO. Based on this, the phytotoxic activity of *P. amalago* EO can be attributed to these compounds, although it was not possible to determine whether the major components acted alone or in synergism/antagonism with other components.

In the present study, *P. amalago* EO showed different phytotoxic effects on the plants studied. For development of *L. sativa*, PAEOD and PAEOR had a similar effect. On the other hand, PAEOD caused more significant phytotoxic effects on *B. pilosa* compared to PAEOR. This suggests that plant responses to EO may be influenced by species-specific factors such as metabolism, seed coat permeability, absorption, and translocation mechanisms (Mendes et al. 2022).

PAEOD showed a greater relative area of linalool and a presence of caryophyllene oxide. Linalool was identified as the main active phytotoxic compound in the EO of *Artemisia absinthium* L., being reported to suppress root elongation and completely inhibit germination of *Amaranthus retroflexus* L., *Medicago sativa* L., *Triticum aestivum* L., and *Poa annua*. L. Scanning electron microscopy image analyses further revealed that linalool inhibited root hair formation and metaxylem development (Jiang et al. 2021). Caryophyllene oxide was one of the main components of EO reported to cause phytotoxic effects, such as reducing germination and root length of weeds (Moraes et al. 2023) such as *B. pilosa* (El-Gawad et al. 2019). Based on this, the higher concentrations of these compounds may have contributed to the greater phytotoxicity of PAEOD, especially the monoterpene oxygenated linalool. Monoterpenes are potent inhibitors of plant growth and germination (Galán-Pérez et al. 2022). They also affect chlorophyll content, cellular respiration, DNA synthesis, cell proliferation, and the activity of enzymes involved in glycolysis. Additionally, they can cause oxidative damage (Kaur et al. 2010, Macías et al. 2007, Nishida et al. 2005).

Phytotoxic effects, such as inhibition or abnormal growth, can result from the cytotoxic action of a compound or substance on the cell cycle. This happens because plant tissue growth depends on cell division and elongation during differentiation and development (Aragão et al. 2015, Singh et al. 2020). In this study, *P. amalago* EO reduced the MI of *L. sativa*. The MI, a key cytotoxic parameter, reflects the frequency of cell division and is crucial for determining root growth rate (Silveira et al. 2017). A decrease in MI indicates a disturbance in the cell cycle (Fiskesjo 1997), which may result from a prolonged S phase and the inhibition of DNA and nucleoprotein synthesis. This leads to a blockade of the G1 and G2 phases and inhibition of microtubule formation (Türkoğlu 2012). The observed increase in cells in interphase and the decrease in cells in prophase, marking the start of mitotic division, show a slowdown in cell progression in *L. sativa*, explaining the reduction in root growth. Similarly, a decrease in MI in *B. pilosa* could also reduce root growth. Zomba et al. (2024) reported similar results, noting a reduction in the MI of *Allium cepa* L. (another plant model for cytotoxicity and genotoxicity analyses) and in the growth of *B. pilosa* treated with EO of *Allardia tridactylites* (Kar. & Kir.) Sch. Beep. Other studies also link reduced root growth to a decline in the MI in meristematic cells (Parveen et al. 2024, Pinheiro et al. 2024).

Piper amalago EO also increased the frequency of CAs. CAs are changes in the structure or number of chromosomes, resulting from the action of genotoxic agents. These agents can have two mechanisms of action: clastogenic, leading to damage, breakage, or exchange of chromosomal materials in the DNA molecule, or aneugenic, characterized by the absence or poor formation of the mitotic spindle (Silveira et al. 2017).

Among the chromosomal changes observed, PAEOR and PAEOD significantly induced cells with aneugenic c-metaphase and chromosomal adhesion changes. C-metaphases occur when the chromosome centromeres are not linked to the mitotic spindle, due to their malfunction or inactivation. This abnormality compromises the cell cycle, paralyzing it in metaphase (Freitas et al. 2016).

Chromosome adhesion is characterized by chromosomes surrounded by a viscous matrix of chromatin rather than DNA. This change is due to damage to chromatin organization due to the denaturing action on its proteins or its inadequate folding (El-Ghamery et al. 2003). Chromosomal adhesion is irreversible and can lead to death and consequently to the decline of MI (Zhang et al. 2014). Silveira et al. (2017) suggested that the high frequency of chromosomal adhesion may have activated the cell death mechanisms of *L. sativa* exposed to cadmium, causing reduction in root growth. In this sense,

this change may have prevented *L. sativa* and *B. pilosa* cells from completing the mitotic cycle, leading to the observed reduction in root growth.

The increase in the frequency of c-metaphases and chromosome adhesion explains why the frequency of metaphase cells exposed to PAEOD and PAEOR increases. Given these results, the cytotoxic and genotoxic effects caused by the aneugenic mechanism of action of *P. amalago* EO on cell division and formation of the mitotic spindle of *L. sativa* can elucidate the phytotoxic effects observed in *B. pilosa*.

CONCLUSION

The EO from *P. amalago* has promising potential in controlling weeds. Higher yields were obtained with leaves collected in the rainy season, but leaf collection in the dry season is recommended due to the greater phytotoxic effect on the germination rate, root length, and aerial part length of *B. pilosa* and *L. sativa*. Cytogenotoxic analyses showed that *P. amalago* EO interfere with cell division and the formation of the mitotic spindle through aneugenic mechanisms of action.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Vasconcelos, L. C. and Praça-Fontes, M. M.; **Methodology:** Vasconcelos, L. C., Bergamin, A. S., Martins, G. S., Mariano, G. F. and Mendes, L. A.; **Investigation:** Vasconcelos, L. C., Carrijo, T. T. and Praça-Fontes, M. M.; **Writing – Original Draft:** Vasconcelos, L. C.; **Writing – Review and Editing:** Vasconcelos, L. C., Carrijo, T. T. and Praça-Fontes, M. M.; **Supervision:** Praça-Fontes, M. M.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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