

Original Article

Allelopathic potential of *Leucaena leucocephala* (Lam.) de Wit leaf extracts on native species

Potencial alelopático de extratos de folhas de *Leucaena leucocephala* (Lam.) de Wit sobre espécies nativas

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Abstract

Allelopathy is seen as one of the possible factors that favor the invasion of exotic plants in the environment, as these species, by releasing allelochemicals, can negatively interfere with the establishment of native plants, facilitating the growth and dissemination of invasive exotic plants. This study aimed to verify the possible allelopathic effects of *Leucaena leucocephala* (Lam.) de Wit (leucaena) on native tree species *Pterogyne nitens* Tul. and *Peltophorum dubium* (Spreng.) Taub., via laboratory bioassays. We used Petri dishes containing seeds of native species and aqueous extract of leucaena leaves with 2, 4, 8, 10 and 20% concentrations (m/v) for germination tests and estimated the percentage, mean time, and germination speed index at the end of the germination period. For initial growth, we kept the Petri dishes containing aqueous leucaena extract and germinated seeds of native species in a germination chamber at 25 °C, and after 48 hours we obtained the length of the primary root, hypocotyl, and fresh and dry biomass of the seedlings. We obtained the *a*, *b*, and total chlorophyll and carotenoid content of seedling leaves kept in the aqueous extract at different concentrations. We verified the content of total phenolic compounds in mg/L from the aqueous leucaena extracts. Both native species showed a reduction in the percentage and germination speed index when in contact with the aqueous leucaena extracts at the highest concentrations. The initial growth of native species was also negatively affected, and *P. nitens* showed a reduction of all growth parameters analyzed in all concentrations of the aqueous extract. *P. dubium* showed a reduction in growth, especially at the highest concentrations. The effects can be associated with phenolic compounds present in leucaena extracts, and we found the highest total phenolic content in the extract with the highest concentration. The results show the allelopathic potential of leucaena, which may contribute to this plant ability to settle in natural areas.

Keywords: total phenolics, seedlings growth, germination, *Pterogyne nitens*, *Peltophorum dubium*.

Resumo

A alelopatia é vista como um dos possíveis fatores que favorecem a invasão de plantas exóticas no ambiente, pois essas espécies ao liberarem aleloquímicos, podem interferir, negativamente, no estabelecimento de plantas nativas, facilitando o crescimento e disseminação das plantas exóticas invasoras. O objetivo desse trabalho foi verificar os possíveis efeitos alelopáticos de *Leucaena leucocephala* (Lam.) de Wit (leucena) sobre espécies arbóreas nativas, *Pterogyne nitens* Tul. e *Peltophorum dubium* (Spreng.) Taub., através de bioensaios laboratoriais. Para os testes de germinação, utilizou-se placas de Petri, contendo sementes das espécies nativas e extrato aquoso de folhas de leucena, nas concentrações de 2, 4, 8, 10 e 20%. (m/v) e ao final do período germinativo foram calculados a porcentagem, o tempo médio e o índice de velocidade de germinação. Para o crescimento inicial, placas de Petri, contendo extrato aquoso de leucena e sementes germinadas das espécies nativas, foram mantidas em câmara de germinação a 25 °C, e após 48 horas foi obtido o comprimento da raiz primária, do hipocótilo e a biomassa fresca e seca das plântulas. Obteve-se o teor de clorofila *a*, *b*, total e carotenoides das folhas de plântulas mantidas no extrato aquoso, nas diferentes concentrações. Dos extratos aquosos de leucena verificou-se o teor de compostos fenólicos totais em mg/L. Ambas as espécies nativas apresentaram redução da porcentagem e do índice de velocidade de germinação quando em contato com os extratos aquosos de leucena, nas maiores concentrações. O crescimento inicial das espécies nativas também foi afetado negativamente, sendo que *P. nitens* apresentou redução de todos os parâmetros de crescimento, em todas as concentrações do extrato aquoso, enquanto *P. dubium* apresentou redução do crescimento, principalmente nas maiores concentrações. Os efeitos podem ser associados aos compostos fenólicos presentes nos extratos de leucena, sendo que o maior teor de fenólicos totais foi encontrado no extrato de maior concentração. Os resultados obtidos revelam o potencial alelopático de leucena, o que pode contribuir para a capacidade desta planta em se estabelecer em áreas naturais.

Palavras chave: fenólicos totais, crescimento da plântula, germinação, *Pterogyne nitens*, *Peltophorum dubium*.

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1. Introduction

The invasion of exotic plants is one of the greatest threats to global biodiversity and may become one of the major environmental problems faced in the conservation of natural areas due to its role in changing the ecosystem (Bravo-Monasterio et al., 2016). Invasive exotic species can not only survive and adapt to the new environment but also exercise dominance over native biodiversity (Portz et al., 2011). Exotic tree species with invasive potential used in the landscaping of urban parks threaten the conservation of the remaining forest areas (Biondi and Muller, 2013). Moreover, invasive plants can affect soil quality by releasing natural compounds called allelochemicals (Lorenzo et al., 2013).

Allelopathy is defined as the influence a plant or microorganism can exert on another by releasing chemical compounds known as secondary metabolites or allelochemicals. Allelochemicals may cause several effects on the development of other plants, such as the reduction of germination percentage, effects on initial growth, photosynthetic activity, root system, and chlorosis (Oliveira et al., 2020; Turnes et al., 2014). Furthermore, allelopathy has been considered an important factor that influences the invasion and dissemination of exotic plants (Chengxu et al., 2011; Kumar and Garkoti, 2022).

Leucaena leucocephala (Lam.) de Wit (leucaena) is a small tree species belonging to the Fabaceae family and native to Central America (Oliveira et al., 2023b). The species was used in Brazil for the reforestation of degraded areas, animal feeding, and green manure (Prates et al., 2000). Its fruits are characterized as vegetables that produce 15 to 30 seeds with high viability. Its initial growth may be slow, but when it grows vigorously established, remaining green throughout the year. Leucaena can withstand dry periods and its root system is deep and can absorb water and nutrients from the lower layers of the soil (Torres, 2019). Since the introduction processes began, leucaena has been presenting problems worldwide, including in Brazil (Silva and Silva-Forsberg, 2015). The species overlap with native species, presenting rapid development and high reproduction capacity (Oliveira et al., 2023b), including successive regrowths after cutting (Costa and Durigan, 2010). Considering that leucaena has become invasive of fragments of native areas, the study of this species' allelopathic effects on native tree plants becomes essential. Furthermore, allelochemicals can be released into the environment in different ways, such as leaf leaching, volatilization, root exudation, and decomposition, so the use of aqueous extracts is a way to verify the allelopathic action, being widely used in several studies on the topic, such as the studies carried out by Oliveira et al. (2023a); Nogueira et al. (2021); Scrivanti and Anton (2021).

Studies showed the allelopathic effect of leucaena in weeds and cultivated plants; however, given the evidence that its introduction has contributed to the loss of species in several environments, studies that test these effects in native species should be conducted. Research verified that the aqueous leucaena extract altered the

germination of *Peltophorum dubium* (Spreng.) Taub. (Canafístula), but the aqueous extract only showed a negative effect at higher concentrations (Scherer et al., 2005). Other experiments also verified the effect of aqueous leucaena extracts on the growth of lettuce and weeds roots (Mauli et al., 2009). Pires et al. (2001) observed inhibition of root growth and reduction of the mitotic index in corn seedlings with the increase in the concentration of leucaena leaf extract.

Therefore, this study aims to verify the possible allelopathic effects of aqueous extracts of *Leucaena leucocephala* (Lam.) de Wit leaves on germination and initial growth of native tree species, *Peltophorum dubium* (Spreng) Taub. and *Pterogyne nitens* Tul.

2. Materials and Methods

2.1. Collection of leaves, seeds, and production of extracts

Native species, *Peltophorum dubium* (Spreng) Taub. and *Pterogyne nitens* Tul. were selected for bioassays, both belonging to the Fabaceae family. *P. nitens* seeds and leucaena leaves were collected in forest fragments in northwestern Paraná, Brazil. *P. dubium* seeds were obtained from the campus of the State University of Maringá (UEM), in Paraná.

Before the bioassays, the *P. dubium* and *P. nitens* seeds were scarified using sandpaper 80 and after disinfestation with commercial sodium hypochlorite solution for 10 minutes, they were washed with running water.

To obtain the aqueous *L. leucocephala* extracts, the leaves were collected in their reproductive state and dried in a dry heat oven at 60 °C for 72 hours. Afterward, samples were obtained with a dry mass of 2, 4, 8, 10, and 20g. Then, the leaves were crushed with a mortar and pestle, filtered, and diluted to the volume of 100 mL of distilled water, obtaining the extracts at 2, 4, 8, 10, and 20% concentrations (m/v).

2.2. Germination tests

Germination bioassays were conducted in the research and analysis laboratory of the University of the State of Paraná, Paranavaí. The scarified and disinfested seeds of the native species were distributed in previously sterilized Petri dishes. The bioassays were separately performed for each species and concentration of the extract. Each Petri dish contained two germitex® paper discs and 10 seeds of the target species, totaling five replicates per treatment (extract concentration) (Ahmed and El-Darier, 2024). The petri plates were moistened with 6 mL of the aqueous extracts, which were then covered with plastic film to avoid evaporation and loss of extracts (Silva et al., 2022). The procedure was performed for each concentration of aqueous extract. Only distilled water was used for control. The dishes were kept in a germination chamber at 25 °C and a photoperiod of 12 hours (light/dark). Germination counting was performed for four days, every 24 hours from the installation of the experiment. Germination percentage (GP) data were obtained with the formula $GP (\%) = (ni / n \times 100)$, in which "ni" is the number of

germinated seeds and “n” is the total number of seeds (Maguire, 1962), Germination speed index (GSI) data were obtained with the formula $GSI = \sum (n_i/t_i)$, in which n_i is equivalent to the number of seeds germinated at time “i” and “ t_i ” is equivalent to incubation time (Maguire, 1962). To obtain the mean germination time (MGT), the data were subjected to the formula $MGT = (\sum n_i \cdot t_i) / \sum n_i$ (Maguire, 1962).

2.3. Initial growth analysis

For initial growth analysis, the seeds of the native species were first germinated in distilled water (Oliveira et al., 2023a). Then, the seedlings (with 0.5 radicle length) were transferred to Gerbox boxes containing the aqueous extract, as performed for the germination bioassays (Oliveira et al., 2023a; Silva et al., 2022). The Gerbox boxes remained for 48 hours in the germination chamber at 25 °C and photoperiod of 12 hours (light/dark), with 10 seedlings per Gerbox box and five replicates per treatment (extract concentration). After 48 hours, the length of the root and shoot of the seedlings was obtained with graph paper and fresh biomass was determined immediately after incubation. The dry biomass was estimated after oven-drying at 70 °C for 48 hours.

2.4. Chlorophyll and carotenoid content

The preparation for the analyses of chlorophyll and carotenoid content occurred according to the initial growth preparation pattern, in which the seeds of the native species were germinated in distilled water, and after the seedlings were transferred to gerbox dishes containing the extracts of *L. leucocephala*, incubated for five days. After this period, 0.1g leaves were weighed, crushed with 80% acetone solution, filtered, and their absorbance was determined in a spectrophotometer at 663, 645, 652, and 470 nm. The results were expressed in milligram (mg) of chlorophyll per gram of fresh leaf tissue weight according to the equation proposed by Whitham et al. (1971), in which Chlorophyll $a = [(12.7 \times A_{663} - 2.69 \times A_{645}) \times V] / 1000 \times W$, Chlorophyll $b = [(22.9 \times A_{645} - 4.68 \times A_{663}) \times V] / 1000 \times W$, Total Chlorophyll $=[(A_{652} \times 1000) \times (V / 1000 \times W)] \times 34.5$ and Carotenoids $=[(1000 \times A_{470} - 3.27 \times \text{Chl } a - 104 \times \text{Chl } b) / 229] / 1000 \times W$. “A” corresponds to the absorbance value, “V” to the final volume of the extract expressed in mL and “W” corresponds to the fresh matter of the plant material expressed in g (grams).

2.5. Total phenolic content

The quantification of the total phenolic content was carried out from the aqueous extract used for the allelopathic tests on germination and initial growth. The colorimetric method of Folin-Ciocalteu (Singleton et al., 1999) was used for dosing the total phenolic content. For the reaction in test tubes, 0.25 mL of leucaena extract diluted 20 times, 2.75 mL of 3% Folin-Ciocalteu solution, and 0.25 mL of 10% sodium carbonate were added. The tubes were at rest for 60 minutes at room temperature and in the absence of light. After this period, absorbance was determined to 765 nm. The total phenol contents were

expressed in an equivalent gallic acid concentration (mg/L) according to a standard curve.

2.6. Statistical analysis

The statistical analyses in this study followed the precepts of the completely randomized design. The data obtained in the germination and initial growth tests were subjected to ANOVA and Dunnett's test, with 5% significance, using the statistical program GraphPad Prism 7.0. The results without homogeneity and normality were analyzed via nonparametric tests (Kruskal-Wallis and Dunn).

3. Results

3.1. Germination

The seeds of the native species *P. nitens* had their germination affected at 10% and 20% concentrations of the extract, with a lower percentage and germination speed index compared to the control group (Figure 1). Still, the seeds maintained in these concentrations showed a longer mean germination time.

Figure 2 shows the reduction of germination of *P. dubium* seeds at 20% concentration. Germination speed was affected at 8, 10, and 20% concentrations, with no difference in the mean germination time, except for the 20% extract.

3.2. Initial growth

Regarding the initial growth of *P. nitens*, we observed a significant reduction in the patterns of root length, hypocotyl length, and fresh and dry biomass in all extract concentrations compared to the control group (Figure 3).

Regarding the initial growth of *P. dubium*, the root length was shorter at all extract concentrations (Figure 4). Hypocotyl length was impaired at 8, 10, and 20% concentrations. Regarding biomass, *P. dubium* seedlings only had a significant difference in fresh biomass at 20% concentration. We observed a reduction in dry biomass at 8, 10, and 20% concentrations (Figure 4).

3.3. Chlorophyll and carotenoid content

The species *P. nitens* showed a reduction in the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids in seedlings maintained in the aqueous leucaena extract, from 4% the concentration (Figure 5). The content of photosynthetic pigments showed a higher reduction in seedlings maintained at 20% concentration compared to the control group.

P. dubium seedlings exposed to leucaena extract had their *a* and total chlorophyll contents reduced at 4, 8, 10, and 20% concentrations compared to the control group, whereas chlorophyll *b* and carotenoids were lower at 8%, 10%, and 20% concentrations (Figure 6).

3.4. Total phenolic content

The results of the total phenolic content analyses found in the *L. leucocephala* (values correspond to the average of three replications, each performed in duplicate) leaf

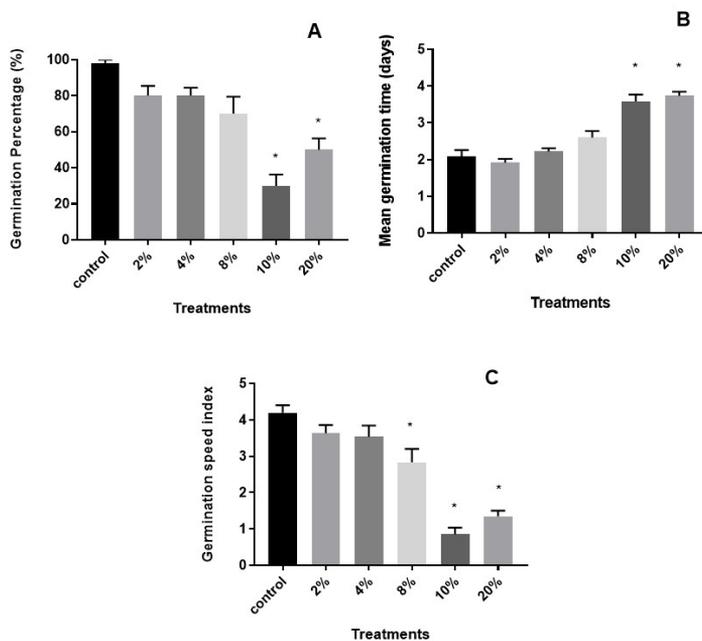


Figure 1. Germination percentage (A), Mean germination time (B) and Germination speed index (C) of *Pterogyne nitens* Tul. seeds, maintained in aqueous *Leucaena leucocephala* (Lam.) de Wit extracts. *Treatments compared to the control group, via the Kruskal-Wallis and Dunn test, $p < 0.05$.

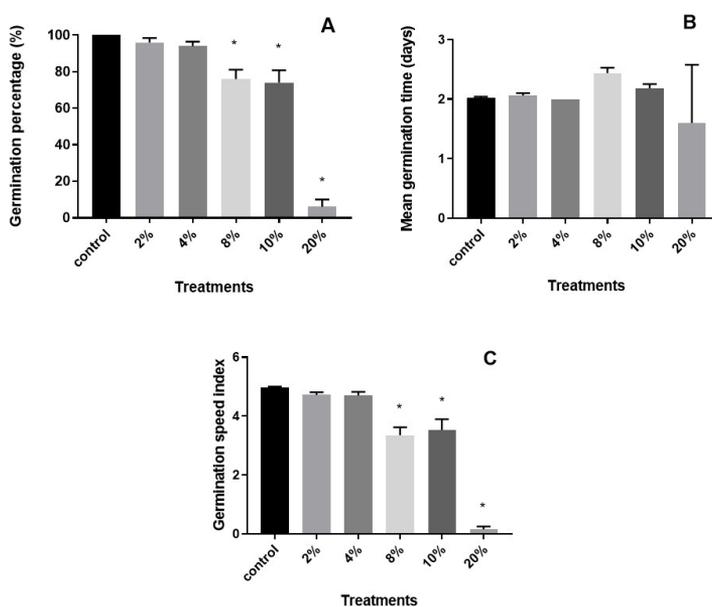


Figure 2. Germination percentage (A), Mean germination time (B) and Germination speed index (C) of *Peltophorum dubium* (Spreng) aqueous *Leucaena leucocephala* (Lam.) de Wit extracts. *Treatments compared to the control group, via the Kruskal-Wallis and Dunn test, $p < 0.05$.

extracts showed that phenolic compounds increased significantly at the highest concentrations of the extract, mainly at 20%, in which 142.19 mg/L of total phenolic

compounds were found (Table 1). Gallic acid was used as the reference standard and the calibration curve showed an angular coefficient of 0.9945.

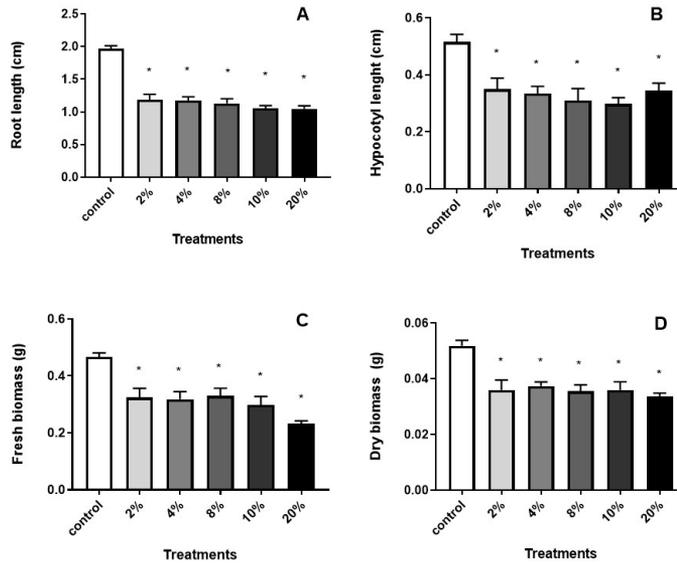


Figure 3. Initial growth of *Pterogyne nitens* Tul. seedlings, maintained in aqueous *Leucaena leucocephala* (Lam.) de Wit extracts. A- Root length; B- Hypocotyl length; C- Fresh biomass; D- Dry biomass. *Data subjected to ANOVA and compared to the control group via Dunnett's test, $p < 0.05$.

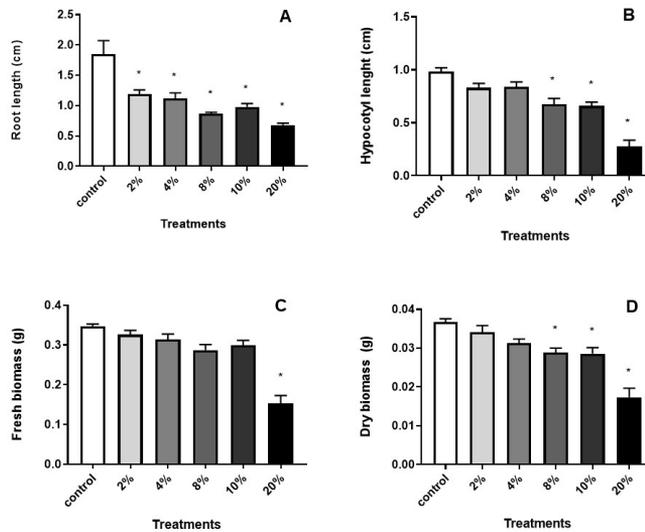


Figure 4. Growth of *Peltophorum dubium* (Spreng) Taub seedlings under aqueous *Leucaena leucocephala* (Lam.) de Wit extracts. A- Root length; B- Hypocotyl length; C- Fresh biomass; D- Dry biomass. *Data submitted to ANOVA and compared to the control group via Dunnett's test, $p < 0.05$.

Table 1. Total phenolic compounds content of *Leucaena leucocephala* (Lam.) de Wit leaf extract at different concentrations.

Treatments	2%	4%	8%	10%	20%
Total phenolics	24.99 mg/L a*	55.76 mg/L b	72.72 mg/Lb	83.65 mg/L c	142.19 mg/L d

*Non-different equal letters by Dunnett's test, with 5% significance.

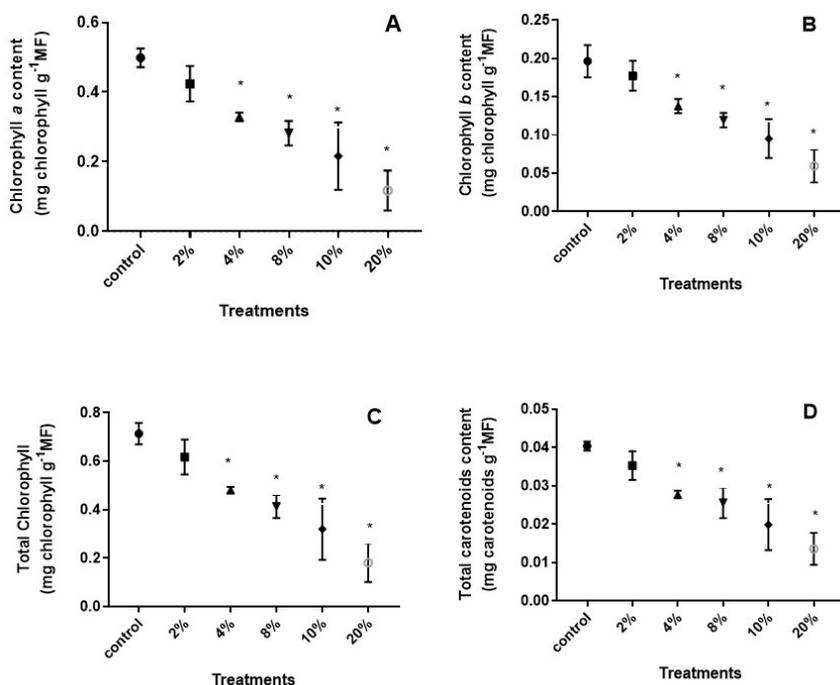


Figure 5. A- Chlorophyll a, B- chlorophyll b, C- total chlorophyll, and D- carotenoids of *Pterogyne nitens* Tul. under aqueous *Leucaena leucocephala* (Lam.) de Wit extracts. *Data submitted to ANOVA and compared to the control group via Dunnett's test, $p < 0.05$.

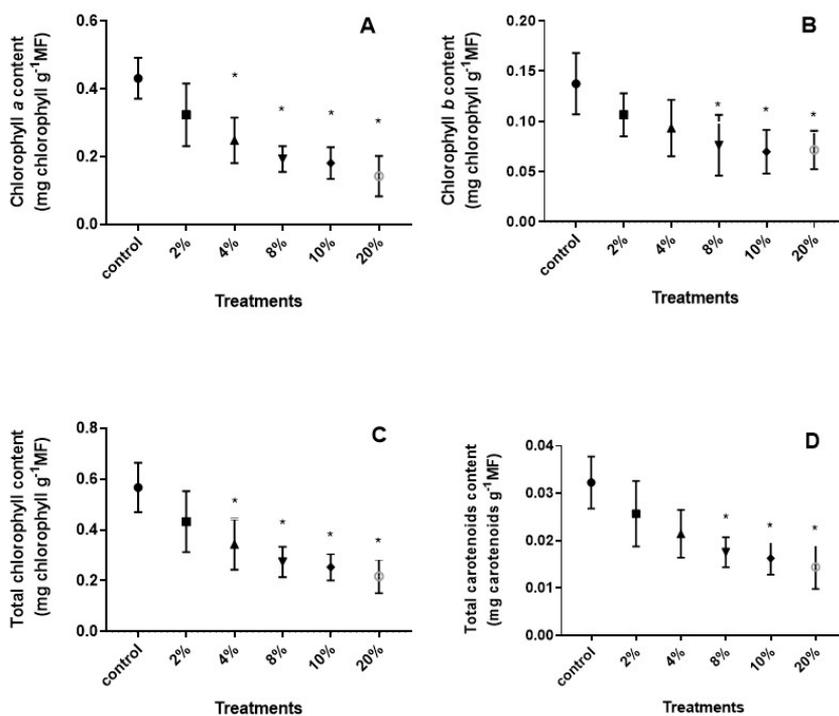


Figure 6. A- Chlorophyll a, B- chlorophyll b, C- total chlorophyll, and D- carotenoid content of *Peltophorum dubium* (Spreng) Taub. under aqueous *Leucaena leucocephala* (Lam.) de Wit extracts. *Data subjected to ANOVA and compared to the control group via Dunnett's test, $p < 0.05$.

4. Discussion

Germination bioassays showed a reduction of germination of native species exposed to higher concentrations of the aqueous leucaena extract. Although some studies found that the reduction of the germination process is the least observed effect in allelopathic tests (Oliveira et al., 2023a), the *P. dubium* and *P. nitens* seeds showed a reduction of germination parameters when under the action of leucaena extracts. Considering the several modes of action of phenolic compounds, we can attribute the presence of phenolic compounds in leucaena extracts to the negative effect on the germination of native species, especially in extracts with higher concentrations, in which we observed a higher total phenolic content. The inhibitory effect of phenolic acids on plant growth and germination is related to the concentration (Kato-Noguchi and Kurniadie, 2022).

Studies have revealed that phenolic compounds can inhibit germination and growth of target plants. These allelochemicals have different modes of action, changing the permeability of the cell membrane, interacting with phytohormones and enzymes of metabolic routes, in addition to affecting cell division (Bubna et al., 2011; Ladhari et al., 2020). These actions of phenolic compounds can be associated with the reduction of the germination percentage and the germination speed index of native seeds. The longer mean germination time, observed in seeds kept in the highest concentrations of leucaena extract, could cause a disadvantage in the establishment of *P. dubium* seedlings, which proved to be more sensitive to aqueous extracts.

Phenolic compounds constitute a diverse group of secondary metabolites present in most plants (Macías et al., 2019). They are associated with the defense strategies of plants, improving the phytochemical composition of seeds, as well as their antioxidant properties (Rasera and Castro, 2020). They come from the metabolism of shikimic acid in plants and are also associated with allelopathic interactions, causing ecological and economic problems, such as reduced crop productivity and difficulties in the regeneration of natural forests (Li et al., 2010).

Regarding their negative effects, phenolic compounds can affect many biological activities, such as cellular functioning and metabolic pathways. They can induce oxidative stress, inhibit cell division and root elongation, affect cell permeability and thus nutrient absorption, inhibit energy metabolism such as photosynthetic processes and respiration, affect the formation of nucleic acids, and suppress protein biosynthesis (Almeida et al., 2008; Anh et al., 2021; Bubna et al., 2011; Ferrarese et al., 2000; Santos and Rezende, 2008).

Research has revealed that the allelopathic action of leucaena can be linked to allelochemicals such as phenolic acids and mimosine. However, information on phenolic compounds in leucaena is still limited (Kato-Noguchi and Kurniadie, 2022). The content of total phenolic compounds was higher in the aqueous extracts of leucaena with the highest concentration (20%), with a progressive increase in the concentration of phenolic compounds being observed as the concentration of the extracts increased. The value

found in the highest concentration was 142.19 mg/L or 0.142 mg/g, being lower than the estimated value of 1.3 to 2.8 mg/g MS obtained by Sharma and Chaurasia (2014). The lower content of phenolic compounds compared to other studies can be associated with the use of mature leaves in our work. The concentration of phenolic acids in young leaves can be 2--fold greater than in mature ones (Kato-Noguchi and Kurniadie, 2022).

The germination of native species reduced from the concentration of 4%, whereas all growth parameters of *P. nitens* were reduced in all concentrations tested and for *P. dubium*, the reduction of shoot growth and dry biomass occurred from the 8% concentration. Our results differed from those obtained by Scherer et al. (2005), who verified a reduction in the growth of the *P. dubium* root in the aqueous leucaena extract at 200g/L concentration, being 2.5 times higher than that used in our study.

We observed a reduction in the initial growth of the target species, which can also be associated with phenolic compounds quantified in the leucaena extracts. Similar results were observed by Bubna et al. (2011) and Ladhari et al. (2020), in which allelopathic action on target plants was attributed to phenolic compounds identified in donor plants.

P. nitens seedlings were more sensitive to the allelochemicals of the leucaena aqueous extracts, in all concentrations, observing a reduction in the growth of the root, aerial part and fresh and dry biomass. The degree of inhibition or impact of the phytotoxic effect may be dependent on the target species (Ladhari et al., 2020; Oliveira et al., 2023a). This justifies the differences found between native species in response to leucaena extracts. In *P. dubium*, a reduction in shoot growth and dry biomass was observed for seedlings maintained at the highest concentrations (8, 10 and 20%). However, seedlings of both native species showed reduced root growth when maintained at all concentrations of leucaena aqueous extract. The greater sensitivity of the roots may be an indicator of the phytotoxic effect of allelochemicals (Ladhari et al., 2020) present in the leucaena aqueous extract. However, direct contact of roots with allelochemicals can induce a greater reduction in growth (Silva et al., 2022). Moreover, the reduction in root growth, observed in seedlings under leucaena extract, can compromise the establishment of seedlings, also causing a disadvantage in the acquisition of nutrients and water from the environment.

The results of the analysis of chlorophyll and carotenoid contents showed a reduction in chlorophyll *a* and *b*, total chlorophyll, and carotenoids in *P. nitens* and *P. dubium*. These results show the allelopathic action of leucaena extracts on native species, affecting the performance of photosynthetic pigments. The reduction in chlorophyll content results in inhibition of photosynthesis (Cândido et al., 2022), causing reduced growth. Liu et al. (2017) observed a reduction in chlorophyll content in wheat might be due to the decrease in chlorophyll synthesis and the disruption of the metabolism by allelochemicals present in *Juniperus rigida* litter aqueous extract as phenolic compounds.

The harmful effects found in target species may have been caused by the allelopathic action of leucaena,

associated with phenolic compounds present in the aqueous extracts, causing negative effects on the growth and metabolism of seeds and seedlings of native species. Considering allelopathy as a biochemical interaction process between plants, causing effects on the germination and growth of target plants, associated with allelochemicals released by the donor plant, this gives leucaena allelopathic potential, which can contribute to its efficiency in the invasion and establishment in natural areas.

5. Conclusion

The aqueous extracts affected the germination, initial growth, and chlorophyll and carotenoid contents of the native species *P. nitens* and *P. dubium*, showing the allelopathic potential of *L. leucocephala* leaves. The allelopathic effect is associated with the presence of allelochemicals such as phenolic compounds, which increased in content with extract concentration.

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