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Article

Can pruning enhance the allelopathic effect? A study with Golden-Dewdrop

A poda pode aumentar o efeito alelopático? Um estudo com pingo-de-ouro

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Abstract: Golden-Dewdrop (*Duranta erecta* L.), known for its landscaping applications, undergoes successive pruning to maintain its ornamental structure, a practice known to induce numerous physiological and chemical responses in the plant. Therefore, this study aimed to evaluate the influence of pruning and different extraction methods on the allelopathic potential of Golden-Dewdrop on the germination and initial growth of lettuce. Leaf extracts were obtained by decoction (hot) and maceration (cold) at concentrations of 20%, 40%, 60%, 80%, and 100%, with distilled water as a negative control. Phytochemical screening of the extracts was performed for phenolic compounds, flavonoids, anthocyanidins, tannins, cardioactive heterosides, saponins, alkaloids, and terpenes. For the bioassay, germinative and morphological parameters were measured and characterized. The data were subjected to analysis of variance (ANOVA), and for quantitative data, regression models were adjusted, while for qualitative data, means were compared using the Scott-Knott test with 5% significance. Significant differences were observed between extraction methods and management practices, with the hot aqueous extract from pruned plants significantly reducing germination at higher concentrations. The initial growth of lettuce was severely affected, presenting numerous morphological abnormalities, with severe damage to the root system at all concentrations. This study suggests that pruning practices have a modulating effect on the allelopathic activity of Golden-Dewdrop, providing valuable information for landscaping projects and botanical studies considering its evaluation and production of phytotoxic phytochemicals.
Keywords: allelopathy effect index, bioassay, *Duranta erecta*, *Lactuca sativa,* ornamental plants.

Resumo: Pingo-de-ouro (*Duranta erecta* L.), conhecida por suas aplicações paisagísticas, é frequentemente submetida a sucessivas podas para manter sua estrutura ornamental, sabendo-se que essa prática gera inúmeras respostas fisiológicas e químicas na planta. Portanto, este estudo teve como objetivo avaliar a influência da poda e de diferentes métodos de extração sobre o potencial alelopático da Pingo-de-ouro na germinação e crescimento inicial da alface. Os extratos das folhas foram obtidos por decocção (quente) e maceração (frio) em concentrações de 20%, 40%, 60%, 80% e 100%, tendo água destilada como controle negativo. Foi realizada a triagem fitoquímica dos extratos para identificar compostos fenólicos, flavonoides, antocianidinas, taninos, heterosídeos cardiotônicos, saponinas, alcaloides e terpenos. Para o bioensaio, foram mensurados e caracterizados parâmetros germinativos e morfológicos. Os dados foram submetidos à análise de variância (ANOVA) e, para os dados quantitativos, foram ajustados modelos de regressão, enquanto para os dados qualitativos, as médias foram comparadas pelo teste de Scott-Knott com 5% de significância. Foram observadas diferenças significativas entre os métodos de extração e as práticas de manejo, sendo que o extrato aquoso quente das plantas podadas reduziu significativamente a germinação em concentrações mais elevadas. O crescimento inicial da alface foi gravemente prejudicado, apresentando inúmeras anormalidades morfológicas, com danos severos ao sistema radicular em todas as concentrações. Este estudo sugere que as práticas de poda têm um efeito modulador na atividade alelopática da Pingo-de-ouro, fornecendo informações valiosas para projetos paisagísticos e estudos botânicos, considerando sua avaliação e produção de fitoquímicos fitotóxicos.
Palavras-chave: bioensaio, *Duranta erecta*, índice de efeito alelopático, *Lactuca sativa*, plantas ornamentais.

Introduction

Belonging to the Verbenaceae family, the genus *Duranta* spp. comprises approximately 35 species, and *Duranta erecta* L. (Golden-Dewdrop) shows promising research in different areas (Calvelli et al., 2023; Srivastava and Shanker, 2022). Commonly referred to as Golden Dewdrop, this woody shrub is characterized by leaves displaying a yellow-golden shade and an alternating or opposite arrangement. It is recognized for its production of violet flowers and orange-colored fruits, making it a popular choice in landscaping projects (Song et al., 2021). Golden-Dewdrop is extensively cultivated in tropical and subtropical regions and is native from Mexico, South America, and the Caribbean (Khanal and Patil, 2022).

Due to its fast growth and widespread presence in landscaping projects, regular maintenance pruning is required. However, this practice of shaping the plant to meet human interests can be considered an act of aggression that induces stress on the organism, leading to alterations in its basic structures and functions. Additionally, pruning may also inhibit or stimulate the plant's defense mechanisms against natural enemies (Nakada-Freitas et al., 2021). As a result, the plant develops mechanisms to defend itself against pruning stress, as competition for nutrients between reproductive and vegetative organs can occur, and certain stimuli can induce flowering or the production of chemical compounds (Kruidhof et al., 2014).

Genotypic conditions, along with edaphoclimatic and cultural management conditions, can also influence the biosynthesis and accumulation of bioactive molecules in plants (Sharma et al., 2021). Because these factors directly interfere with the final quality of landscaping projects and commercial production of bioactives, understanding the influence of pruning as a management technique is crucial.

As a consequence of their metabolic processes, plants are capable of producing chemical substances that can have both beneficial and detrimental effects on the development of other organisms in their surrounding environment (Calvelli et al., 2023). This phenomenon, known as allelopathy, involves complex interactions between different species that are mediated by compounds from various categories, typically grouped into phenolics, terpenoids, glucosinolates, alkaloids, and hydroxamic acids (Mrid et al., 2021). Such substances can be present

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in several organs of different plant species, including leaves, flowers and fruits (Nogueira et al., 2021).

The *Duranta* genus is renowned for its diverse secondary metabolites, which have been isolated and identified as various classes of compounds, including alkaloids, acetophenones, coumarins, cinnamic acid, diterpenes, flavonoids, steroids, phenylpropanoid glycosides, triterpenes, saponins, and iridoids (Calvelli et al., 2023; Srivastava and Shanker, 2022). These compounds can exhibit allelopathic effects on surrounding organisms and, therefore, should be considered when designing plant communities or landscaping projects. By understanding the chemical nature of these compounds and their effects on other species, it is possible to optimize the use of allelopathy in plant management, promoting healthy growth while minimizing negative interactions with other organisms (Palanivel et al., 2021).

Therefore, the objective of this study was to evaluate whether aqueous extracts from hot and cold extraction processes of leaves of Golden-Dewdrop associated with pruning practice influence the allelopathic potential. Does the practice of pruning in the cultivation of Golden-Dewdrop alter its allelopathic potential? The extractive method potentiates the allelopathic effect of Golden-Dewdrop plants?

Material and methods

Sample collection and preparation

Golden-Dewdrop leaves were collected in populations located in the respective coordinates: specimen 1 (with pruning) S21º25"09.7" HO45º56'55.8"; and specimen 2 (without pruning) S21º30'22" HO46º11'45.2".

Extract preparation

The leaves were dried in an air circulation oven at 45 °C until constant weight. They were pulverized in a knife mill (Skymsen-TA04) and the granulometry was determined in an electromagnetic sieve shaker (Bertel®); the obtained powder was classified as coarse. The hot aqueous extract was obtained by the decoction method at 20%. The cold aqueous extract was obtained by turbolysis, at 20%. Subsequently, the extracts were filtered on filter paper and distilled water was added to complete and obtain the stock solution (20%) (Brasil, 2019).

From the stock solutions (hot and cold), solutions diluted at concentrations of 20%, 40%, 60%, 80% and 100% were obtained for the conduction of phytotoxicity bioassays (Amâncio et al., 2021). The pH and osmotic potential of all extracts were evaluated (Pinto and Kolb, 2015) in order to eliminate interference in the bioassay.

Phytochemical screening

Phytochemical screening of the extracts was carried out through qualitative chemical reactions in triplicate, according to the methodologies described by Cardoso (2009). The following compounds were evaluated: phenolic compounds, flavonoids, anthocyanidins, tannins, cardioactive heterosides, saponins, alkaloids and terpenes.

Phytotoxicity assays

The bioassays were conducted in 70-mm Petri dishes, containing two sheets of Germitest® paper moistened with 3 mL of solution, at the different extract concentrations (20%, 40%, 60%, 80%, and 100%); distilled water was used as a negative control. For each replication, 30 lettuce samples (*Lactuca sativa* L.) cv. Babá-de-Verão, purchased in the local commerce, were evenly distributed. The dishes were kept in a BOD (Biochemical Oxigen Demand) germination chamber at 25 °C, with a 12-hour photoperiod.

The physiologic germination, characterized by root protrusion, was counted in 24 hours while the germination percentage was evaluated on the 4th and 7th day after the plot of the experiment, and germination speed index (GSI) was calculated from analyses performed every 6 hours until 48 hours, with 24-hour intervals until the 7th day, adapted from (Govêa et al., 2020). On the seventh day of evaluation, the number of normal seedlings (NS) was determined when root, stem and leaves were developed. All material in the dishes was weighed, comprising the fresh biomass (FB). Root length (RL) and shoot length (SL) were measured from 10 randomly selected seedlings on the seventh day of germination, with the aid of a digital caliper (DIGIMESS® 150mm). From the germination data, the allelopathic effect index (RI) was calculated (Calvelli et al., 2023).

Statistical analysis

The experimental design was completely randomized in a factorial scheme (2x2x6), with two management methods (with and without pruning), two types of extraction (hot and cold) and six concentrations (0, 20%, 40%, 60%, 80%, and 100%), with three replicates of 30 seeds each. The data were submitted to variance analysis (ANOVA) and for the quantitative data were adjusted regression models and for the qualitative data the means were compared by the Scott-Knott test with 5% significance using the SISVAR statistical program (Ferreira, 2019).

Results

Phytochemical screening

The phytochemical profile presented in the study was identical for both management and extraction methods. Only cardioactive heterosides were absent.

Table 1. Phytochemical analysis of *Duranta erecta* L. extracts, where the results show the presence (+) or absence (-) of metabolites.

Phytochemical tests	With pruning		Unpruned	
	Hot	Cold	Hot	Cold
Alkaloids	+	+	+	+
Anthocyanidins	+	+	+	+
Cardioactive Heterosides	-	-	-	-
Flavonoids	+	+	+	+
Phenolic Compounds	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Terpenes	+	+	+	+

Phytotoxicity assays

All Golden-Dewdrop leaf extracts showed a significant phytotoxic effect on all germination parameters. The phytotoxic effect varied according to the extraction method and pruning practice. In root protrusion (Rp), the pruning management (Fig. 1A) and the hot extraction method (Fig. 1B) showed a more evident effect, with a marked inhibition of the initial germination processes at the highest concentrations of the hot extract.

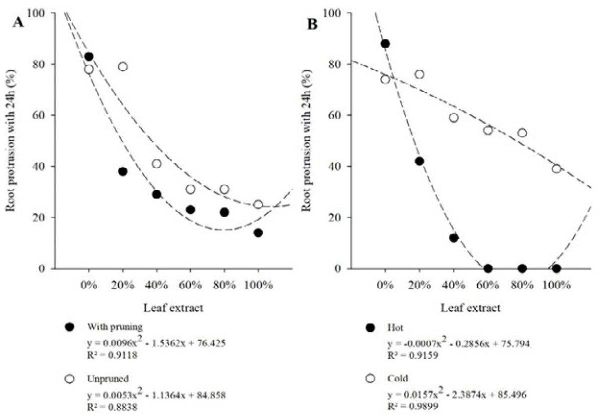

Fig. 1. Root protrusion of *Lactuca sativa* L. after 24-hour exposure to different management methods and extraction of *Duranta erecta* L. leaves (A) difference between management methods with and without pruning; (B) difference between hot and cold extraction methods.

The germination count (Fig. 2) between the first (4th day) and the last (7th day) evaluation showed a similar tendency, where the germination capacity of *L. sativa* was totally impaired from the fourth day (Fig. 2A), not showing physiological recovery of the bioassay, emphasizing that pruning and the hot extraction method were intensifiers of the phytotoxic effect. It is also notable that hot extracts are highly superior in inhibiting the final germination of *L. sativa.*

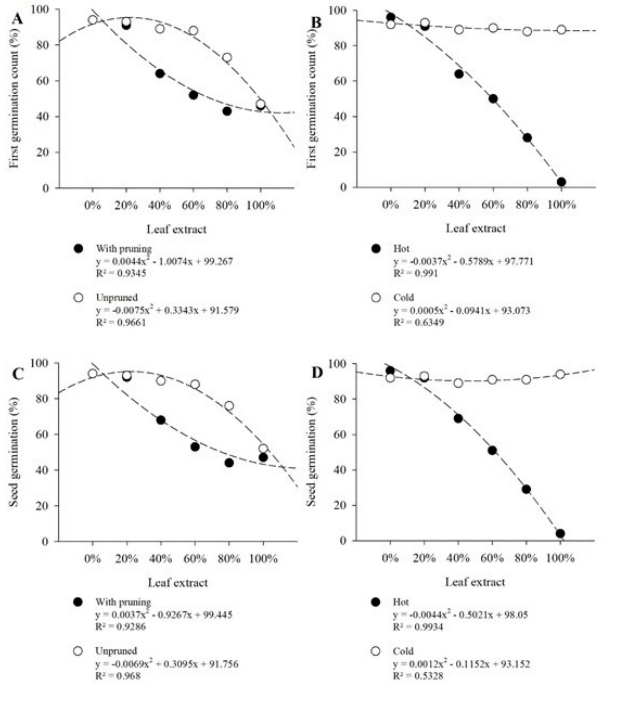

Fig. 2. (A) first germination count of *Lactuca sativa* L. at 4 days in relation to management with or without pruning and (B) in relation to hot or cold aqueous extraction method; (C) germination percentage of *Lactuca sativa* L. with 7 days in relation to the management with or without pruning and (D) in relation to the hot or cold aqueous extraction method.

Although there is a difference in the tendency of the allelopathic effect between the forms of management for final germination, it can be observed in Figure 3 that the germination speed index (GSI) remains similar between the practice or not of pruning. Although showing no difference in the final germination within concentrations, the cold extraction method caused a delay in the germination of *L. sativa*.

No interaction was observed between concentrations and management (Fig.3 A) for the allopathic effect index (RI), since the effect of the extracts was negative on *L. sativa* seeds, with greater inhibition caused by the extracts of plants under pruning. The form of extraction, on the other hand, presented a concentration-dependent interaction, with smaller inhibitions; -0.8 was verified from a concentration of 60% for hot extracts.

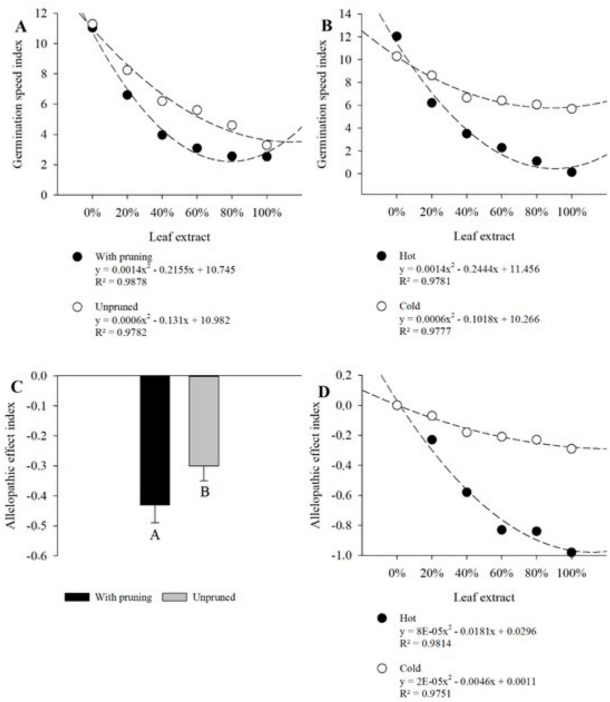

Fig. 3. (A) germination speed indices of *Lactuca sativa* L. between management methods with and without pruning and (B) between hot and cold extraction methods; (C) allelopathic effect index in *Lactuca sativa* L. between management methods with and without pruning and (D) between hot and cold extraction methods.

More intense phytotoxic effects of the extracts were observed on the morphology of *L. sativa* (Fig. 4), mainly on root elongation, which had its development totally affected in all treatments (Fig. 4A and B), resulting in the absence of normal seedlings (Fig. 5). Shoot development showed no interaction between management and concentrations, with greater effect for plant extracts under pruning (Fig. 4C), since the hot extract reduced the shoot growth of *L. sativa* seedlings (Fig. 5B and D), while the management method did not show dependence on concentrations. The inhibition of the initial germination processes, as well as the predictive parameters of germination, the germination speed index and the allelopathic effect index, were determinant in all treatments, which indicate the reduction in germination vigor of the bioassay.

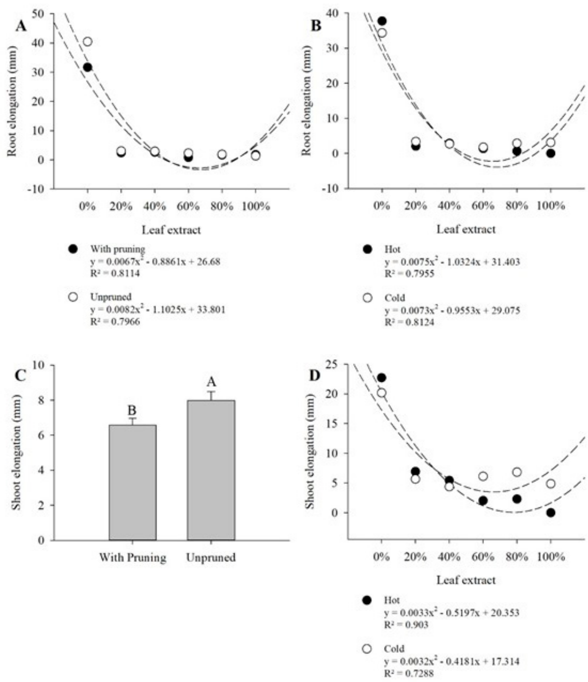

Fig. 4. (A) root elongation of *Lactuca sativa* L. subjected to extracts comparing management methods with and without pruning of *Duranta erecta* L. and (B) between hot and cold extraction methods; (C) shoot elongation of *Lactuca sativa* L. subjected to extracts comparing management methods with and without pruning of *Duranta erecta* L. and (D) between hot and cold extraction methods.

The decrease in seed vitality and failures in seedling development was observed (Fig. 5) and, for hot extracts, even with root protrusion, abnormal seedling development and even death of individuals occurred.

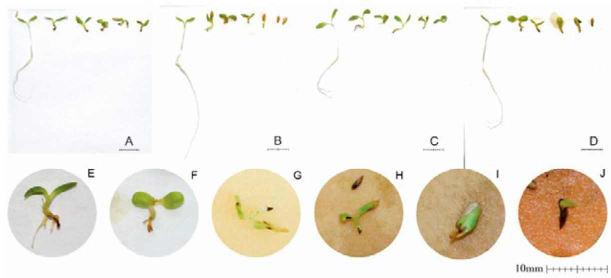

Fig. 5. *L. sativa* L. seedlings exposed to *D. erecta* L. extracts on the 7th day. A) Cold extract without pruning; B) Hot extract without pruning; C) Cold extract with pruning; D) Hot extract with pruning; E and F) Secondary root emission; G and H) Darkening at edges and medial portions; I and J) Absence of roots. Bar represents 10 mm.

Due to the impairment caused by the different Golden-Dewdrop treatments on the growth of *L. sativa* seedlings, it is possible to observe that the fresh mass presents the same decrease relation with concentrations (Fig. 6A and B). Although the dry mass (Fig. 6C and D) did not show significant differences for the concentrations in the management or extraction method interactions, plant extracts under pruning management and hot extraction presented higher average reduction.

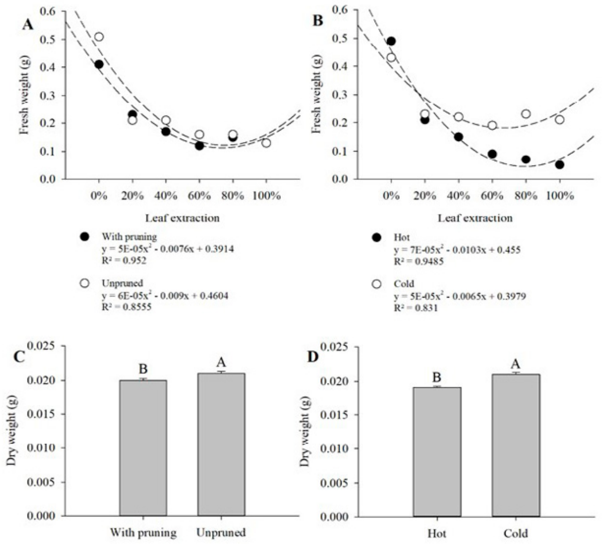

Fig. 6. Fresh (FW) and dry (DW) biomass of *Lactuca sativa* L. seedlings exposed to different management methods and extraction of *Duranta erecta* L. leaves. A – Comparison of FW between management methods with and without pruning; B - Comparison of FW between hot and cold extraction methods; C – DW comparison between management methods with and without pruning; D - Comparison of DW between hot and cold extraction methods.

Discussion

Studies conducted by Bricchi et al. (2010) have contributed to the understanding of the effect of mechanical damage on plants. By mechanically simulating damage caused by caterpillars in *Phaseolus lunatus*, a close relationship between the level of damage and the accumulation of volatile compounds was established.It is known that many chemical groups that are active in defense against herbivores also function as allelopathic compounds (Kruidhof et al., 2014).

Comparing the effect of caterpillar herbivory and artificial damage (mechanical stress), an increase in the production of phenolics was observed in leaves damaged by both stress agents (Hartley and Firn, 1989).Thus, in response to natural or artificial damage, plants modify their metabolite production, which directly impacts palatability and can function as detergents or toxins (Sharma et al., 2021). In this manner, mechanical stress can lead to an increase in the production of compounds that act either individually or synergistically, enhancing the allelopathic effect (Palanivel et al., 2021). These compounds are known to interfere with various physiological processes and enzymes in plants related to hormonal activity, membrane permeability, photosynthesis, respiration, and the synthesis of organic compounds (Sharma et al., 2021).

Other factors are recognized as amplifiers of allelopathic effect. Kong et al. (2002) describe that *Ageratum conyzoides* produces more volatile allelochemicals under conditions of nutritional deficiency, competition with *Bidens pilosa*, infection by *E. cichoracearum*, and *A. gossypii*. Yu et al. (2023) reports an increase in the allelopathic effect of *Solidago canadensis* and *Conyza canadenses* under saline stress. Kruidhof et al. (2014) emphasize that cover crops under mechanical damage are more effective in inhibiting weed germination and initial growth.

Thus, the present study demonstrates the broad capacity of invasive plants to modulate allelopathic potential and outcompete the biotic community, ensuring a successful invasion. Previous findings have also shown that an unfavorable environment can enhance the allelopathy of invasive plants Yu et al. (2023).

The variation in effects observed between the management methods suggests that the quantitative content of secondary metabolites involved in allelopathic and phytotoxic activities can be modulated through pruning. The decoction extraction method showed higher inhibitory effects, indicating greater extractive capacity due to the application of heat, which enhances solubility and consequently facilitates extraction of the bioactive compounds of interest.

According to Lopes et al. (2022), the main substances responsible for allelopathy are grouped into: organic acids; simple lactones; fatty acids; quinones; phenolics; cinnamic acid; coumarins; flavonoids; tannins; steroids and terpenoids. Thus, the metabolites present in the samples of this study are constituents described as potentially related to allelopathic activity, as already reported for Golden-Dewdrop (Srivastava and Shanker, 2022). Calvelli et al. (2023) described twelve new compounds found in Golden-Dewdrop leaves with possible phytotoxic actions, causing damage to the root system.

Therefore, regardless of the extraction method and management, the species presents a wide diversity of phytochemicals capable of acting in allelopathic interactions in the environment. Hiradate et al. (1999), the presence of saponins, called Durantin I, II and III, are involved in the inhibition of root growth of *B. Juncea* seedlings. Ahmed et al. (2009) proved that triterpene saponins, Durantins 2 and 7 found in Golden-Dewdrop, were significantly cytotoxic against a HepG2 cell line. Tur et al. (2010) reported that *D. repens* leaves reduce the development of *L. sativa* and *Lycopersicum esculentum*, while the fruit of the same species stimulates their growth.

The initial growth of plants is a crucial parameter for allelopathic studies, with roots showing greater sensitivity to allelochemicals present in extracts compared to germination processes (Amâncio et al., 2021; Cunha Neto et al., 2023; Nogueira et al., 2021).These substances can affect enzymes that depolarize the membrane of root cells, increasing their permeability and blocking nutrient absorption, which reduces root size, inhibits growth, and decreases metabolic rates (Kumar and Garkoti, 2022; Silva et al., 2019). The allelopathic effect on the initial germination process, between root protrusion and seedling formation, occurs at the cellular and molecular levels, possibly damaging the lipid membrane, altering DNA transcription and translation, and modifying enzymatic activity and cellular respiration (Calvelli et al., 2023). The delay in germination rate, resulting in reduced cell elongation and division, leads to less vigorous, abnormal, and disease-susceptible seedlings, which can incapacitate the recipient species from developing (Silva et al., 2019).

The intensity of this effect is demonstrated by the allelopathic effect index, which allows verifying whether there is an inhibitory or stimulatory effect.This index has proven to be a useful tool for evaluating the intensity of this effect promoted by different extracts (Calvelli et al., 2023; Jin et al., 2019; Wang et al., 2021). In summary, allelopathic studies should take into account not only germination but also evaluate the initial growth of plants, especially the roots, as well as the indices of germination speed and allelopathic effect, which have been shown to be the main events of allelopathy.Providing important insights into the complex and dynamic interactions of ecosystems, it can contribute to the development of more sustainable and environmentally-friendly agricultural practices.

Conclusions

Although the use of different management methods did not significantly affect the phytochemical profile, pruning practice in conjunction with the decoction extraction method exhibited greater inhibitory effects, thus modulating allelopathic activity. It can be stated that plants subjected to pruning stress increase the phytotoxic effect of Golden-Dewdrop, which is crucial information to be considered in the development of landscaping projects and in the production of bioactive compounds.

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Author Contribution

LLM: conceptualization, data curation, formal analysis, investigation, project administration, writing - original draft. **JVBC:** conceptualization, data curation, formal analysis, investigation, project administration, writing – original draft, writing - review & editing. **ARCN:** formal analysis, investigation, writing - review & editing; **pia:** formal analysis, investigation. **RGB:** formal analysis, investigation. **LORT:** writing - original draft; formal analysis, investigation. **GAS:** conceptualization, funding acquisition, project administration, resources, supervision, writing –- review & editing. **SB:** conceptualization, funding acquisition, project administration, resources, supervision, writing - review & editing.

Conflict of interest

All authors have no conflict of interest to declare

Data Availability Statement

Data will be available on request.

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