Allelopathic potential of *Artemisia absinthium* L. on seed germination and seedling growth of various plant species

Potencial alelopático de *Artemisia absinthium* L. sobre la germinación de semillas y el crecimiento de plántulas de varias especies de plantas

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ABSTRACT

In plant-plant interactions, the emission of secondary metabolites can have significant effects, potentially serving as a tool for weed management. The study of plant-derived substances offers an environmental alternative to traditional production processes. The aim of the research was to evaluate the allelopathic potential of the aqueous extract of *Artemisia absinthium* L. on the germination of seeds of five species: *Calendula officinalis* L., *Taraxacum officinale* L., *Bidens pilosa* L., *Senecio vulgaris* L., and *Brassica juncea* L. The experiment involved a factorial design with five species, five extract concentrations and five replicates, for a total of 125 experimental units (EU). The aqueous extract of *A. absinthium* was prepared from dried foliage at a concentration of 1/50 (w/v) and applied in varying proportions (0, 25, 50, 75, and 100%) according to the respective treatments. In each EU, 20 seeds of the corresponding species were placed and grown under controlled conditions for 21 d, during which germination was monitored. The results indicate that *A. absinthium* has significant potential as an inhibitor of seed germination and seedling growth in *T. officinale* and *B. pilosa*. In *S. vulgaris* and *B. juncea,* the response was highly dose-dependent. In *C. officinalis*, no inhibition was observed in the evaluated parameters. The results indicate that *A. absinthium* extract offers a sustainable alternative to weed management.

Key words: allelopathy, weed management, secondary metabolites, plant-plant interactions, bioactive compounds.

RESUMEN

En las interacciones planta-planta, la emisión de metabolitos secundarios puede tener efectos significativos, los cuales son herramientas potenciales para el manejo de malezas, por lo que el estudio de estas sustancias de origen vegetal ofrece una alternativa medioambiental a los procesos de producción tradicionales. La investigación tuvo como objetivo evaluar el potencial alelopático del extracto acuoso de *Artemisia absinthium* L. sobre la germinación de semillas de cinco especies: *Calendula officinalis* L., *Taraxacum officinale* L., *Bidens pilosa* L., *Senecio vulgaris* L. y *Brassica juncea* L. El experimento tuvo un diseño factorial con cinco especies, cinco concentraciones de extracto y cinco repeticiones, para un total de 125 unidades experimentales (UE). El extracto acuoso de *A. absinthium* se obtuvo a partir de follaje seco a una concentración de 1/50 (p/v), y se aplicó en proporciones variables (0, 25, 50, 75 y 100%) según los respectivos tratamientos. En cada UE se colocaron 20 semillas de la especie correspondiente, que se cultivaron en condiciones controladas durante 21 d durante los cuales se monitoreó la germinación. Los resultados indican que *A. absinthium* tiene un potencial significativo como inhibidor de la germinación de semillas y el crecimiento de plántulas en *T. officinale* y *B. pilosa*. Para el caso de *S. vulgaris* y *B. juncea*, la respuesta fue altamente dependiente de la dosis y en *C. officinalis* no se observó inhibición en los parámetros evaluados. Los resultados señalan al extracto de *A. absinthium* como una alternativa sostenible en el manejo de malezas.

Palabras clave: alelopatía, manejo de malezas, metabolitos secundarios, interacciones planta-planta, compuestos bioactivos.

Introduction

In plant-plant relationships, an interaction between the individual plants develops, a process by which the plants respond to a limiting condition. The main components of this interference are competition and allelopathy (Weidenhamer *et al*., 2023). Competition arises from the need to acquire limiting resources such as water, light, and nutrients (Craine & Dybzinski, 2013), whereas allelopathy involves the production or emission of biochemicals by a plant or its parts which influence the germination, growth, or reproduction of other plant species. This emission can harm sensitive species and plays a crucial role in ecological succession and dominance, contributing to

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distinct vegetation patterns (Latif *et al*., 2017; Schandry & Becker, 2020).

In crop production, due to the limited resources available to plants, weed management is crucial because weeds can decrease productivity and quality (Radhakrishnan *et al*., 2018). Various strategies exist for weed control, with chemical control using herbicides being the most widespread; thus, there is a need for more sustainable alternatives for weed management (Dayan *et al.,* 2009; Green & Owen, 2011). Therefore, there is increasing interest in identifying compounds that do not remain active in the environment for long periods, capable of controlling weeds and reducing the selection pressure that leads to herbicide-resistant weeds (Hasan *et al*., 2021).

Among the alternatives, the use of plant extracts emerges as a promising option. Bioactive compounds present in these extracts can control weed populations, with some functioning as natural herbicides that offer the advantage of being biodegradable and avoiding long-term contamination (Khamare *et al*., 2022).

The allelopathic effect typically results from the simultaneous action of several compounds, often including diverse metabolites such as phenols, terpenes, and alkaloids (Khamare *et al*., 2022; Zohaib *et al*., 2017). Numerous studies have reported that these bioactive compounds can modify germination patterns (Chenyin *et al*., 2023), affect growth and development (Jabran *et al*., 2015), and reduce plant biomass production (Zhang *et al*., 2021; Zohaib *et al*., 2017). These results vary among plants, as species differ in their sensitivity to these substances, and the types and amounts of these compounds emitted vary according to the species and the plant´s physiological state (Cheng & Cheng, 2015; Radhakrishnan *et al*., 2018).

Artemisia absinthium L., commonly known as wormwood, is a perennial herbaceous species of European origin from the family Asteraceae (Judžentienė, 2016), introduced to the Americas by the Spaniards during the conquest (Beltrán-Rodríguez *et al*., 2017). It has documented uses in medicine and the food industry (Hbika *et al*., 2022; Judžentienė, 2016; Li *et al*., 2021). Extracts of *A. absinthium* have demonstrated antibiotic, antiparasitic, anticancer, and allelopathic properties (Anibogwu *et al*., 2024; Judžentienė, 2016). These extracts can inhibit or delay the germination of sensitive seeds, which is associated with alterations in cell membrane permeability, affecting water and essential nutrient absorption. They can also influence cell division, reduce the synthesis of photosynthetic pigments and photosynthesis, and promote the formation of reactive oxygen species (Bharati *et al*., 2014; Choudhary *et al*., 2023; Hasan *et al*., 2021; Lee *et al*., 2013; Pouresmaeil *et al*., 2020).

Herbaceous plants, *Calendula officinalis* L., *Taraxacum officinale* L., *Senecio vulgaris* L., and *Bidens pilosa* L. from the Asteraceae family and *Brassica juncea* L. from the Brassicaceae family, are dicotyledonous species. *C. officinalis* is a species known for its high production of bioactive compounds, making it commonly used in traditional medicine in various countries of Latin America and Europe (Khalid & Silva, 2012). *T. officinale*, *S. vulgaris*, and *B. pilosa* are characterized by wide distribution in agricultural areas, high seed production per plant, rapid germination, and highly competitive ability (Dotor & Cabezas, 2016; Froese & Van Acker, 2003). These traits enable them to rapidly colonize various agroecosystems and effectively compete for resources, making them problematic weeds in intensive vegetable or fodder crops, such as carrot (Dotor & Cabezas, 2016). As abundant plants in agricultural systems, they are also subject to selection pressures from constant herbicide application (Green & Owen, 2011).

Although the allelopathic effects of *Artemisia* species have been documented, this study provides new insights by focusing on the species-specific responses of five distinct plants. Additionally, this study aims to contribute to the knowledge of non-chemical weed management by evaluating, under laboratory conditions, the effect of the aqueous extract of *A. absinthium* on the germination of seeds of *C. officinalis*, *B. juncea*, *B. pilosa*, *T. officinale,* and *S. vulgaris.*

Materials and methods

Plant material

The plant material of *A. absinthium*, which included old and new leaves and stem, was collected in February from an orchard at Finca San Rafael located in Facatativá, Cundinamarca, Colombia (4°48’17.2” N, 74°16’47.9” W), where it occupied an area of 1.5 m^2 and was mainly surrounded by *Cenchrus clandestinus* (Hochst. ex Chiov.). The material was placed in paper bags and dried for 15 d at room temperature (20±2°C). Seeds of *B. juncea*, *B. pilosa*, *T. officinale, S. vulgaris,* and *C. officinalis* were collected from free-growing plants located in the greenhouse area of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogotá (4°38’12.4” N, 74°05’18.1” W).

Extract of *A. absinthium* leaves

For the preparation of the extract, 10 g of dry foliage (moisture content of 10±5%) of *A. absinthium* was placed in 500 ml of distilled water and heated to 50°C for 3 h in a beaker. The mixture was then filtered and the extract was stored in dark conditions at room temperature (15±2°C) for 3 d.

Identification and quantification of bioactive compounds in *A. absinthium* extract

Aliquots of *A. absinthium* extract were analyzed by LC-MS (Liquid Chromatography-Mass Spectrometry) in a Bruker Impact II system at the Laboratory of Chromatography and Q-TOF Mass Spectrometry of the Universidad Nacional de Colombia, Bogotá campus. The LC used a Bruker Intensity Solo 2 C18 column, with a mobile phase Water-Formic Acid 0.1% and Acetonitrile-Formic Acid 0.1%. The elution was performed with a Gradient 5-95% B in 10 min with a flow rate of 0.250 ml min^{-1}. MS operated in MS/MS scanning mode, in the range of 50 to 1300 m/z, with a spectrum rate of 4 Hz. For the identification of the extract compounds, the chromatogram was run in Bruker MetaboScape® version 6.0.2 (Bruke Daltonics) metabolomics software, using the Bruker HMDB Metabolite Library 2.0, Bruker MetaboBASE® Personal Library 3.0, and MoNA-export-MassBank libraries.

Treatments

A factorial design was used, with seeds of 5 plant species, 5 doses of *A. absinthium* extract, 5 replicates per treatment, and 20 seeds per experimental unit, for a total of 125 experimental units. Fifty ml vials were used, to which were added 25 ml of solutions at 25, 50, 75, and 100% of the stock extract solution (called treatments 25, 50, 75, and 100, respectively) and the control treatment (0% of stock extract solution, only distilled water). A glass fiber was placed inside the vial to allow the seeds to float, on which 20 seeds of the corresponding species were deposited, ensuring their contact with the solution to be evaluated. The experimental units were placed under germination conditions maintained at 20±2°C throughout the entire duration of the experiment, with 12 h of light per day.

Germination variables

The number of germinated seeds was recorded at 4 d intervals; the criterion to consider germination effective was the emission of the radicle with a minimum size of 4 mm (Ranal & Santana, 2006). Based on the information collected, the germination percentage was defined as

$$\mathrm{G}\,(\%) = \Sigma\left(\frac{\mathrm{N}}{\mathrm{N_S}}\right) * 100$$

where N= number of germinated seeds and Ns= total number of seeds (Escobar Escobar & Cardoso, 2015).

The radicle length was determined by measuring the radicle length of 5 seedlings per experimental unit. For this purpose, a graduated caliper was used.

Statistical analysis

The homogeneity of variances and normality were tested using Bartlett's test and the Shapiro-Wilk test. These tests were followed by analysis of variance (ANOVA) and Tukey's mean comparison test ($P \leq 0.05$) using R Studio software version 2023.03.1 and SigmaPlot 12.0 software.

Results and discussion

Characterization of the chemical compounds in the aqueous extract of *A. absinthium*

The preparation of an aqueous extract of *A. absinthium* was proposed as a straightforward and cost-effective method for obtaining plant extracts. However, due to uncertainties regarding the efficacy of this extraction method in isolating bioactive compounds, a metabolomic analysis was conducted using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS).

The HPLC-MS analysis of the extract revealed the presence of 37 components. The bioactive compounds present in the extract suggest a significant level of secondary metabolism in this species, including synthesis of monoterpenes, alcohols, alkaloids, and both protein and non-protein amino acids (Tab. 1). Among these, the compounds artemisinin, limonene, β-myrcene, methyl eugenol or thujone, camphor and 1,8-cineole are found in greater proportions, as indicated by peaks in their characteristic retention times (Tab. 1). Other compounds, such as proline, isoleucine, betaine, leucine, pipecolic acid, phenylalanine, tyrosine, dioctyl phthalate, D-pipecolic acid, dimethyl sulfoxide, 4-hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine, 3-[5-(methoxymethyl)-1,2,4-oxadiazol-3-yl]-N-(2-methylpropyl) pyrrolidine-1-carboxamide, pentaethylene glycol, triethylene glycol, were also identified (Tab. 1).

TABLE 1. Chemical composition of the aqueous extract of *Artemisia absinthium.*

Identification or name of compound	Rt (min)	Molecular formula of the compound	Molecular mass of the compound (g mol^{-1})	Annotation source
Betaine	0.99	$C_5H_{11}NO_2$	117.148	A
L-Proline	1.01	$C_5H_9NO_2$	115.13	B
Pipecolic acid	1.14	$C_6H_{11}NO_2$	129.157	B
D-Pipecolic acid	1.37	$C_6H_{11}NO_2$	129.16	A
4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine	1.42	$C_{11}H_{23}NO_2$	201.309	C
Xanthine	1.44	$C_5H_4N_4O_2$	152.11	C
L-Tyrosine	1.64	$C_9H_{11}NO_3$	181.19	B
L-Isoleucine	1.73	$C_6H_{13}NO_2$	131.17	A
L-Norleucine	1.88	$C_6H_{13}NO_2$	131.17	B
Leucine	1.89	$C_6H_{13}NO_2$	131.17	C
Triethylene glycol	1.98	$C_6H_{14}O_4$	150.175	A
L-Phenylalanine	3.08	$C_9H_{11}NO_2$	165.192	C
Pentaethylene glycol	3.77	$C_{10}H_{22}O_6$	238.281	A
3-[5-(methoxymethyl)-1,2,4-oxadiazol-3-yl]-N-(2-methylpropyl) pyrrolidine-1-carboxamide	4.05	$C_{13}H_{22}N_4O_3$	281.343	A
Artemisinin	4.34	$C_{15}H_{22}O_5$	282.332	C
α-Santonin	4.69	$C_{15}H_{18}O_3$	246.3	A
Leucodin	5.3	$C_{15}H_{18}O_3$	246.306	A
Camphor	6.38	$C_{10}H_{16}O$	152.23	A
1,8-cineole	7.31	$C_{10}H_{18}O$	154.249	A
Limonene	8.94	$C_{10}H_{16}$	136.24	C
Cetrimonium	10.90	$C_{19}H_{41}N$	284.5	A
Dioctyl phthalate	13.03	$C_{24}H_{38}O_4$	390.556	A
Pyridine	13.04	C_5H_5N	79.101	B

RT - Retention time of compounds in the column (min). Annotation source: A: Bruker MetaboBASE Personal Library 3.0; B: Bruker HMDB Metabolite Library _ 2.0; C: MoNA-export-MassBank.

Seed germination

Germination percentage

Figure 1 illustrates that across the various species evaluated at the end of the test, the control treatments (those not exposed to the *A. absinthium* extract) exhibited germination exceeding 90%. The highest proportion of germinated seeds was observed within the first 10 d following the initiation of the trial, with germination (G%) exceeding 60% of the control treatment. These results indicate adequate viability of the seed lots used in the trial and align with findings reported by authors such as Barrera (2015) and Dotor and Cabezas (2015) regarding the high G% of these species.

Seeds treated with the *A. absinthium* extract had a reduction in G%, with varying results depending on the dose and species, with negative correlations for *B. pilosa* (r=-0.89), *T. officinale* (r=-0.75), *S. vulgaris* (r=-0.95) and *B. juncea* (r=-0.97) (Fig. 1). In *B. pilosa* and *T. officinale*, a decrease in G% was observed across all treatments, with significant statistical differences ($P \leq 0.05$) between the control and the other treatments, indicating high sensitivity to extract exposure (Fig. 1). For *S. vulgaris* and *B. juncea*, the decrease in G% was highly dose-dependent (Fig. 1). For both these species, seeds exposed to 25% extract exhibited G% statistically similar to the control during the first 10 d, with germination exceeding 40% in *S. vulgaris* and 60% in *B. juncea*. By the end of the trial, these species recorded germination values of 60±6%, indicating a maximum 20% of the seeds germinated between 11 and 30 d. In the treatments exposed to a 50% extract, *B. juncea* showed rapid germination during the first 10 d, reaching values close to 40% followed by a cessation of germination. *S. vulgaris* stopped germination by 17 d, recording a maximum value of 30±4%. Additionally, the treatments 50 and 75% extract concentrations effectively inhibited germination, maintaining G% values below 10 and 20% in *S. vulgaris* and *B. juncea*, respectively.

In *C. officinalis*, the results indicate an absence of inhibitory effects on germination, as all treatments exhibited germination patterns comparable to the 100% in the control (Fig. 1).

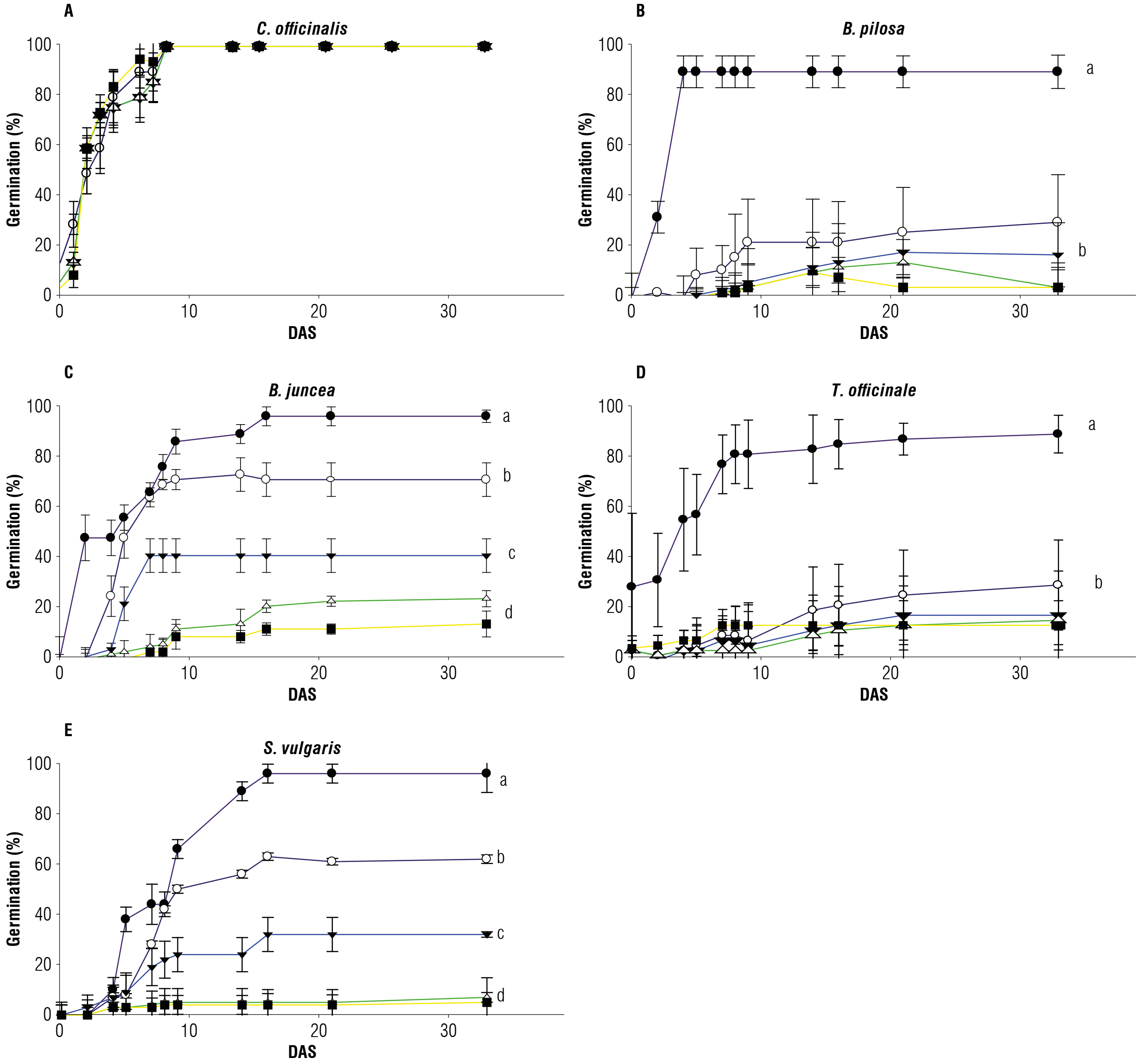

FIGURE 1. Germination (%) in relation to the day after sowing (DAS) in seeds of *Calendula officinalis, Bidens pilosa*, *Senecio vulgaris*, *Taraxacum officinale,* and *Brassica juncea* under different concentrations of *Artemisia absinthium* leaf extract. (----) extract at 0% (control), (----) extract at 25%, (----) extract at 50%, (----) extract at 75%, and (----) 100% extract concentration. The bars correspond to the standard deviation. Different lowercase letters indicate significant differences between treatments according to the Tukey mean test ($P \leq 0.05$).

Root length

Figure 2 displays the average radicle length of the different species, with values expressed as a percentage relative to the control treatment (extract at 0%). This means that the radicle length in the control is set at 100%, and the values for other treatments represent the radicle length as a percentage of that control.

In *B. pilosa*, the greatest reduction was observed in the seeds exposed to 100% of the extract, where the radicle reached only 40% of the control treatment length. Treatments with concentrations of 75% and 50% had lengths of 42% and 50%, respectively. For this species, the 25% treatment did not show statistical differences compared to the control. In *S. vulgaris*, a reduction in radicle length was also observed, with values statistically different from the control for all treatments, with values close to 47% in treatments 50%, 75%, and 100%, and 70% in treatment 25%. A similar statistically significant result was observed in *T. officinale,* with the highest percentage recorded in the 25% treatment

(42%), followed by the 50% treatment (38%), 75% treatment (10%), and 100% treatment (5%). In *B. juncea*, although there is a statistical difference between the control and the treatments, this species exhibited the highest radicle length values in the treatments 25% and 50%, with values of 90% and 85%, respectively. In the treatments 75% and 100%, radicle length was recorded at 40% and 12% compared with control, respectively (Fig. 2). There was also a negative correlation with dose, with values of *r*=-0.84 in *T. officinale*, *r*=-0.83 in *S. vulgaris*, *r*=-0.80 in *B. juncea*, and *r*=-0.79 in *B. pilosa*. The findings suggest that the seed exposure to *A. absinthium* extract influences the germination process, germination speed, and radicle growth.

The results for *C. officinalis* indicate absence of inhibitory effects on the root length, as all treatments exhibited similar results to the 100% in the control treatment (Fig. 2).

The extensive chemical composition of the aqueous extract of *Artemisia* genus has been reported by several authors,

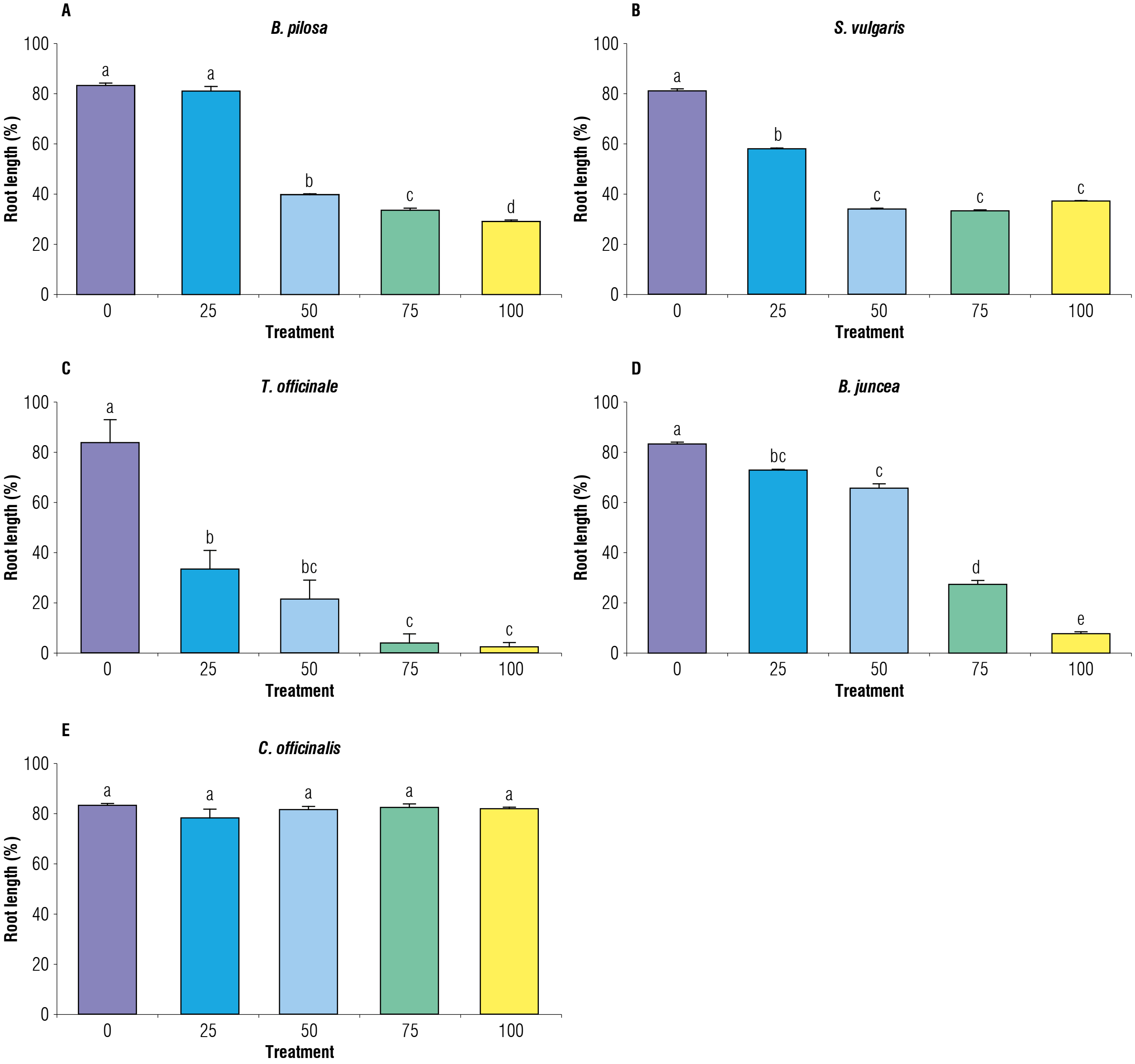

FIGURE 2. Root length expressed as a percentage relative to the control (0% extract) in seedlings of *C. officinalis, B. pilosa, S. vulgaris, T. officinale*, and *B. juncea* under different concentrations of *Artemisia absinthium* leaf extract. (-) extract at 0%, (-) extract at 25%, (-) extract at 50%, (-) extract at 75%, and (-) 100% extract concentration. Different lowercase letters indicate statistically significant differences between treatments according to the Tukey's test (*P*≤0.05), and the bars correspond to standard deviation.

including Basher *et al.* (1997), Li *et al.* (2021), Mirjalili *et al.* (2007), and Singh *et al.* (2009). These compounds have been described by numerous authors as insecticides (Ninkuu *et al.*, 2021), antioxidants (Lee *et al.*, 2013), and antibiotics (Ninkuu *et al.*, 2021), among other functions. The results of the metabolomics analysis also indicate that multiple compounds in the extract have been associated with allelopathic responses, among which stand out artemisinin, limonene, β-myrcene, methyl eugenol or thujone, camphor, and 1,8-cineole (Li *et al.*, 2021; Pouresmaeil *et al.*, 2020).

In relation to the effect of the extract on seed germination, the extracted metabolites affected seed germination and seedling growth in some of the evaluated species (Figs. 1 and 2). The results show different types of responses to the exposure to the *A. absinthium* extract. On one hand, there was an inhibition of germination, with high sensitivity responses observed in *B. pilosa* and *T. officinale*. In contrast, *B. juncea* and *S. vulgaris* showed a dose-dependent sensitivity, while *C. officinalis* showed no sensitivity.

The inhibition in seed germination by allelopathic substances has been studied by numerous researchers, although the mechanisms causing the observed responses are not completely understood. Authors such as Bharati *et al.* (2014), Hasan *et al.* (2021), Pouresmaeil *et al.* (2020), Radhakrishnan *et al.* (2018), and Singh *et al.* (2009) suggested that these effects are the result of synergistic interactions among compounds. These authors report several modes of action that directly affect the germination process, such as inhibition of α-amylase activity, water uptake, alteration of gibberellic acid content, alteration of glycolysis enzyme activity, and interference in respiration. Additionally, exposure to bioactive compounds, such as linalool and cineole alcohols, generates germination inhibition (Cheng & Cheng, 2015; Li *et al.*, 2021). Nikolova *et al.* (2023) reported the inhibitory activity on the germination of *Lolium perenne* seeds exposed to aqueous extracts of *Artemisia lerchiana* and *Artemisia santonicum* in doses 2 µl ml^{-1} and 5 mg ml^{-1}, respectively, which is associated with the presence of 1,8-cineole in the essential oil of the extract. On the other hand, Pouresmaeil *et al.* (2020) indicated the presence of α-thujone, camphor, 1,8-cineole and ß-thujone in the essential oil of *Artemisia fragrans* L., demonstrating that exposure to this essential oil inhibited the germination and growth of *Convolvulus arvensis*.

Regarding plant growth and development, a study conducted by Dayan *et al.* (1999) demonstrated that artemisinin not only inhibits plant growth but also specifically affects root growth in a concentration-dependent manner. The researchers suggest that this result may be attributed to the interference with mitosis, particularly through the disruption of microtubule formation, which consequently hampers cell division. This response was also reported by Verdeguer *et al.* (2020) who found that monoterpenes, such as limonene and pulegone (camphor) affected microtubule assembly in microorganisms. Similarly, Li *et al.* (2021) identified that one of the potential modes of action of the genus *Artemisia* may be attributed to the presence of 1,4-cineole and 1,8-cineole. These compounds have been reported as inhibitors of mitotic activity, leading to the inhibition of root growth (Li *et al.*, 2021). This cellular damage was also noted by Chaimovitsh *et al.* (2017), who indicated that limonene accumulates in the tissues of transgenic *Arabidopsis thaliana* plants. This accumulation is hypothesized to generate stronger anti-microtubule agents and disrupt the normal membrane activities. Pouresmaeil *et al.* (2020) reported the inhibition of pigment biosynthesis, disruption of photosystem II, and induction of oxidative stress in cotyledonal leaves. Shao *et al.* (2018) pointed out that the extracts contain bioactive components such as 1,8-cineole. *Seriphidium terrae-albae* extracts showed phytotoxicity in species such as *Amaranthus retroflexus* and *Poa annua*, reducing the length of both the aerial part and the root, which is consistent with our findings regarding radicle length. Additionally, monoterpenes, such as camphor or 1,8-cineole, can affect cell proliferation and DNA synthesis in apical and root meristems, thereby decreasing root elongation in *Brassica campestris* (Koitabashi *et al.*, 1997; Nishida *et al.*, 2005).

In seeds of *B. juncea* and *S. vulgaris*, the dose-dependent effect of *A. absinthium* could be due to the seed capacity to conjugate or metabolize the bioactive compounds and to contain the oxidative stress that these can generate (Tian & Deng, 2020). This response could be attributed to the antioxidant metabolites present in seeds, which been quantified in multiple species, including *B. juncea* (Tian & Deng, 2020), *Brassica oleracea* (Tarasevičienė, *et al.*, 2018), *S. vulgaris,* and *Senecio inaequidens* plants (Conforti *et al.*, 2006), and *Zizania latifolia* (Chu *et al.*, 2020), among others.

Since this is a metabolic response, the containment of the allelopathic effect depends on the seed capacity to counteract the effects of the substances to which they are exposed (Pouresmaeil *et al.*, 2020). The results indicate that the seeds can partially resist the allelopathic effect of the metabolites (Fig. 1). However, when this inhibition is overcome, higher percentages of the G% inhibition are achieved (Fig. 1). These responses explain the negative correlation between exposure dose and G%. A dose-dependent response for G%

has been reported in studies of the allelopathic effect of the genus *Artemisia* by Li *et al.* (2021) in *Brassica pekinensis*, *Lactuca sativa*, and *Oryza sativa*, and by Pouresmaeil *et al.* (2020) in *C. arvensis*, among others.

The observed response in *C. officinalis* is associated with the presence of anatomical barriers of the seed coats and cell membranes (Victoria *et al.*, 2007), which protect the seed from adverse external conditions (Radchuk *et al.*, 2014). These barriers could limit the penetration of the *A. absinthium* extract into the seeds. Anatomically, *C. officinalis* seeds are larger and heavier compared to the other seeds used in this study, which may result in thicker seed coats (Victoria *et al.*, 2007) that could further restrict the entry of bioactive compounds. Additionally, it is possible that the *C. officinalis* seeds can counteract the effect of the extract, as they contain more than 5% oxygenated fatty acids, which exhibit high antioxidant activity (Avato & Tava, 2022; Badami *et al.*, 1965).

Conclusions

This study provides insights on the potential use of readily available plants in non-chemical weed management.

The extraction method used in this study proved to be efficient, as it had a bioactive effect on the seed germination of the studied species. Therefore, this method represents a replicable field methodology, which could be easily adopted by growers. Further studies on the response of these compounds under field conditions are recommended.

In an agricultural context, understanding the effects of allelopathic substances will enable advances in the development of natural herbicides, including the potential for selectivity by using specific seeds as inhibitors of the allelopathic effect or as protective agents to promote the growth and development of crops, promoting sustainable production practices. Additionally, using natural herbicides derived from plants, such *A. absinthium*, could reduce farmer's reliance on synthetic herbicides, offering environmental benefits by decreasing the amount of potentially harmful chemicals used in agriculture.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

CBC and MDR designed the experiments, CBC and MDR carried out the laboratory experiments, CBC and MDR contributed to the data analysis, CBC and MDR wrote the article. All authors reviewed the final version of the manuscript.

Literature cited

Avato, P., & Tava, A. (2022). Rare fatty acids and lipids in plant oilseeds: Occurrence and bioactivity. *Phytochemistry Reviews, 21,* 401–428. https://doi.org/10.1007/s11101-021-09770-4

Anibogwu, R., Jesus, K. D., Pradhan, S., Van Leuven, S., & Sharma, K. (2024). Sesquiterpene lactones and flavonoid from the leaves of basin big sagebrush (*Artemisia tridentata* subsp. *tridentata*): Isolation, characterization and biological activities. *Molecules*, *29*(4), Article 802. https://doi.org/10.3390/molecules29040802

Badami, R. C., & Morris, L. J. (1965). The oxygenated fatty acid of calendula seed oil. *Journal of the American Oil Chemists' Society, 42*(12 part 2), 1119–1121. https://doi.org/10.1007/bf02636925

Barrera, C. (2015). *Efecto alelopático de malezas leñosas invasoras sobre la germinación de hierbas pratenses* [Undergraduate thesis, Universidad Austral de Chile]. http://cybertesis.uach.cl/tesis/uach/2015/fcb272e/doc/fcb272e.pdf

Basher, K. H. C., Ozek, T., Demirchakmak, B., Nuriddinov, Kh. R., Abduganiev, B. Yo., Aripov, Kh. N., Khodzimatov, K. Kh., Nigmatullaev, O. A., & Shamyanov, E. D. (1997). Essential oils of some *Artemisia* species from Central Asia. *Chemistry of Natural Compounds*, *33*(3), 293–295. https://doi.org/10.1007/BF02234877

Beltrán-Rodríguez, L., García-Madrid, I., & Saynes-Vásquez, A. (2017). Apropiación cultural de una planta europea en la herbolaria tradicional mexicana: el caso del ajenjo *(Artemisia absinthium* L. Asteraceae). *Etnobiología*, *15*(2), 46–67. https://revistaetnobiologia.mx/index.php/etno/article/view/135

Bharati, A., Kar, M., & Sabat, S. C. (2014). Artemisinin inhibits chloroplast electron transport activity: Mode of action. *PLoS ONE*, *7*(6), Article e38942. https://doi.org/10.1371/journal.pone.0038942

Chaimovitsh, D., Shachter, A., Abu-Abied, M., Rubin, B., Sadot, E., & Dudai, N. (2017). Herbicidal activity of monoterpenes is associated with disruption of microtubule functionality and membrane integrity. *Weed Science*, *65*(1), 19–30. https://doi.org/10.1614/WS-D-16-00044.1

Cheng, F., & Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science*, *6*, Article 1020. https://doi.org/10.3389/fpls.2015.01020

Chenyin, P., Yu, W., Fenghou, S., & Yongbao, S. (2023). Review of the current research progress of seed germination inhibitors. *Horticulturae*, *9*(4), Article 462. https://doi.org/10.3390/horticulturae9040462

Choudhary, C. S., Behera, B., Raza, M. B., Mrunalini, K., Bhoi, T. K., Lal, M. K., Nongmaithem, D., Pradhan, S., Song, B., & Das, T. K. (2023). Mechanisms of allelopathic interactions for sustainable weed management. *Rhizosphere, 25*, Article 100667. https://doi.org/10.1016/j.rhisph.2023.100667

Chu, C., Du, Y., Yu, X., Shi, J., Yuan, X., Liu, X., Liu, Y., Zhang, H., Zhang, Z., & Yan, N. (2020). Dynamics of antioxidant activities, metabolites, phenolic acids, flavonoids, and phenolic biosynthetic genes in germinating Chinese wild rice (*Zizania latifolia*). *Food Chemistry*, *318*, Article 126483. https://doi.org/10.1016/j.foodchem.2020.126483

Conforti, F., Loizzo, M. R., Statti, G. A., Houghton, P. J., & Menichini F. (2006). Biological properties of different extracts of two *Senecio* species. *International Journal of Food Science and Nutrition*, *57*(1-2), 1–8. https://doi.org/10.1080/09637480500131236

Craine, J. M., & Dybzinski, R. (2013). Mechanisms of plant competition for nutrients, water and light. *Functional Ecology, 27*(4), 833–840. https://doi.org/10.1111/1365-2435.12081

Dayan, F. E., Cantrell, C. L., & Duke, S. O. (2009). Natural products in crop protection. *Bioorganic & Medicinal Chemistry*, *17*(12), 4022–4034. https://doi.org/10.1016/j.bmc.2009.01.046

Dayan, F. E., Hernández, A., Allen, S. N., Moraes, R. M., Vroman, J. A., Avery, M. A., & Duke, S. O. (1999). Comparative phytotoxicity of artemisinin and several sesquiterpene analogues. *Phytochemistry*, *50*(4), 607–614. https://doi.org/10.1016/S0031-9422(98)00568-8

Dotor, M., & Cabezas, M. (2015). Determinación de la habilidad competitiva entre *Daucus carota* L. y *Senecio vulgaris* L. *Revista U.D.C.A Actualidad & Divulgación Científica*, *18*(1), 81–89. https://doi.org/10.31910/rudca.v18.n1.2015.456

Escobar Escobar, D. F., & Cardoso, V. J. M. (2015). Seed germination and dormancy of *Miconia chartacea* (Melastomataceae) in response to light, temperature, and plant hormones. *Revista de Biología Tropical*, *63*(4), 1169–1184. https://doi.org/10.15517/rbt.v63i4.17955

Froese, N. T., & Van Acker, R. C. (2003). Distribution and interference of dandelion (*Taraxacum officinale*) in spring canola. *Weed Science*, *51*(3), 435–442. http://www.jstor.org/stable/4046681

Green, J. M., & Owen, M. D. K. (2011). Herbicide-resistant crops: Utilities and limitations for herbicide-resistant weed management. *Journal of Agricultural and Food Chemistry*, *59*(11), 5819–5829. https://doi.org/10.1021/jf101286h

Hasan, M., Ahmad-Hamdani, M., Rosli, A. M., & Hamdan, H. (2021). Bioherbicides: An eco-friendly tool for sustainable weed management. *Plants*, *10*(6), Article 1212. https://doi.org/10.3390/plants10061212

Hbika, A., Daoudi, N. E., Bouyanzer, A., Bouhrim, M., Mohti, H., Loukili, E. H., Mechchate, H., Al-Salahi, R., Nasr, F. A., Bnouham, M., & Zaid, A. (2022). *Artemisia absinthium* L. aqueous and ethyl acetate extracts: Antioxidant effect and potential activity *in vitro* and *in vivo* against pancreatic α-amylase and intestinal α-glucosidase. *Pharmaceutics*, *14*(3), Article 481. https://doi.org/10.3390/pharmaceutics14030481

Judžentienė, A. (2016). Wormwood (*Artemisia absinthium* L.) oils. In V. R. Preedy (Ed.), *Essential oils in food preservation, flavor and safety* (pp. 849–856). Academic Press. https://doi.org/10.1016/B978-0-12-416641-7.00097-3

Khalid, K. A., & Silva, J. A. T. (2012). Biology of *Calendula officinalis* Linn.: Focus on pharmacology, biological activities and agronomic practices. *Medicinal and Aromatic Plant Science and Biotechnology, 6*(1), 12–27.

Khamare, Y., Chen, J., & Marble, S. C. (2022). Allelopathy and its application as a weed management tool: A review. *Frontiers in Plant Science*, *13*, Article 1034649. https://doi.org/10.3389/fpls.2022.1034649

Koitabashi, R., Suzuki, T., Kawazu, T., Sakai, A., Kuroiwa, H., & Kuroiwa, T. (1997). 1,8-Cineole inhibits root growth and DNA synthesis in the root apical meristem of *Brassica campestris* L. *Journal of Plant Research*, *110*(1), 1–6. https://doi.org/10.1007/BF02506836

Latif, S., Chiapusio, G., & Weston, L. A. (2017). Allelopathy and the role of allelochemicals in plant defense. In G. Becard (Ed.), *Advances in botanical research* (Vol. 82, pp. 19–54). Academic Press. https://doi.org/10.1016/bs.abr.2016.12.001

Lee, Y.-J., Thiruvengadam, M., Chung, I.-M., & Nagella, P. (2013). Polyphenol composition and antioxidant activity from the vegetable plant *Artemisia absinthium* L. *Australian Journal of Crop Science*, *7*(12), 1921–1926. http://www.cropj.com/november2013.html

Li, J., Chen, L., Chen, Q., Miao, Y., Peng, Z., Huang, B., Guo, L., Liu, D., & Du, H. (2021). Allelopathic effect of *Artemisia argyi* on the germination and growth of various weeds. *Scientific Reports, 11*, Article 4303. https://doi.org/10.1038/s41598-021-83752-6

Mirjalili, M. H., Tabatabaei, S. M. F., Hadian, J., Ebrahimi, S. N., & Sonboli, A. (2007). Phenological variation of the essential oil of *Artemisi*a *scoparia* Waldst. et Kit from Iran. *Journal of Essential Oil Research, 19*(4), 326–329. https://doi.org/10.1080/10412905.2007.9699294

Nikolova, M., Lyubenova, A., Yankova-Tsvetkova, E., Georgiev, B., Berkov, S., Aneva, I., & Trendafilova, A. (2023). *Artemisia santonicum* L. and *Artemisia lerchiana* Web. essential oils and exudates as sources of compounds with pesticidal action. *Plants, 12*(19), Article 3491. https://doi.org/10.3390/plants12193491

Ninkuu, V., Zhang, L., Yan, J., Fu, Z., Yang, T., & Zeng, H. (2021). Biochemistry of terpenes and recent advances in plant protection. *International Journal of Molecular Sciences*, *22*(11), Article 5710. https://doi.org/10.3390/ijms22115710

Nishida, N., Tamotsu, S., Nagata, N., Saito, C., & Sakai, A. (2005). Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: Inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *Journal of Chemical Ecology*, *31*(5), 1187–1203. https://doi.org/10.1007/s10886-005-4256-y

Pouresmaeil, M., Nojadeh, M., Movafeghi, A., & Maggi, F. (2020). Exploring the bio-control efficacy of *Artemisia fragrans* essential oil on the perennial weed *Convolvulus arvensis*: Inhibitory effects on the photosynthetic machinery and induction of oxidative stress. *Industrial Crops and Products*, *155*, Article 112785. https://doi.org/10.1016/j.indcrop.2020.112785

Radchuk, V., & Borisjuk, L. (2014). Physical, metabolic, and developmental functions of the seed coat. *Frontiers in Plant Science, 5*, Article 510. https://doi.org/10.3389/fpls.2014.00510

Radhakrishnan, R., Alqarawi, A. A., & Abd-Allah, E. F. (2018). Bioherbicides: Current knowledge on weed control mechanisms. *Ecotoxicology and Environmental Safety*, *158*, 131–138. https://doi.org/10.1016/j.ecoenv.2018.04.018

Ranal, M. A., & Santana, D. G. (2006). How and why to measure the germination process? *Brazilian Journal of Botany*, *29*, 1–11. https://doi.org/10.1590/S0100-84042006000100002

Schandry, N., & Becker, C. (2020). Allelopathic plants: Models for studying plant–interkingdom interactions. *Trends in Plant Science, 25*(2), 176–185. https://doi.org/10.1016/j.tplants.2019.11.004

Shao, H., Hu, Y., Han, C., Wei, C., Zhou, S., Zhang, C., & Zhang, C. (2018). Chemical composition and phytotoxic activity of *Seriphidium terrae-albae* (Krasch) Poljakov (Compositae) essential oil. *Chemistry & Biodiversity*, *15*(11), Article e1800348. https://doi.org/10.1002/cbdv.201800348

Singh, H. P., Kaur, S., Mittal, S., Batish, D. R., & Kohli, R. K. (2009). Essential oil of *Artemisia scoparia* inhibits plant growth by generating reactive oxygen species and causing oxidative damage. *Journal of Chemical Ecology*, 35, 154–162. https://doi.org/10.1007/s10886-009-9595-7

Tarasevičienė, Ž., Viršilė, A., Danilčenko, H., Duchovskis, P., Paulauskienė, A., & Gajewski, M. (2019). Effects of germination time on the antioxidant properties of edible seeds. *CyTA-Journal of Food*, *17*(1), 447–454. https://doi.org/10.1080/19476337.2018.1553895

Tian, Y., & Deng, F. (2020). Phytochemistry and biological activity of mustard (*Brassica juncea*): A review. *CyTA-Journal of Food*, *18*(1), 704–718. https://doi.org/10.1080/19476337.2020.1833988

Verdeguer, M., Sánchez-Moreiras, A. M., & Araniti, F. (2020). Phytotoxic effects and mechanism of action of essential oils and terpenoids. *Plants*, *9*(11), Article 1571. https://doi.org/10.3390/plants9111571

Victoria, J. A., Bonilla C. R., & Sánchez, M. S. (2007). Morfoanatomía y efecto del secado en la germinación de semillas de caléndula y eneldo. *Acta Agronómica*, *56*(2), 61–68. https://revistas.unal.edu.co/index.php/acta_agronomica/article/view/640

Weidenhamer, J. D., Cipollini, D., Morris, K., Gurusinghe, S., & Weston, L. A. (2023). Ecological realism and rigor in the study of plant-plant allelopathic interactions. *Plant and Soil*, *489*, 1–39. https://doi.org/10.1007/s11104-023-06022-6

Zhang, Z., Liu, Y., Yuan, L., Weber, E., & van Kleunen, M. (2021). Effect of allelopathy on plant performance: A meta-analysis. *Ecology Letters*, *24*(2), 348–362. https://doi.org/10.1111/ele.13627

Zohaib, A., Abbas, T., & Tabassum, T. (2016). Weeds cause losses in field crops through allelopathy. *Notulae Scientia Biologicae*, *8*(1), 47–56. https://doi.org/10.15835/nsb.8.1.9752