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Suppression of herbaceous weed germination and growth by extracts of Acacia dealbata

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Short title: *Acacia dealbata* weed suppression

Suppression of herbaceous weed germination and growth by extracts of *Acacia dealbata*

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Abstract

Weeds are major constraints to agricultural production, and increasing concerns associated with intensive herbicide use and herbicide resistance highlight the need to identify new sources of compounds for weed management. In this context, invasive plants may provide bioactive metabolites with phytotoxic properties. In this study, the effects of methanolic extracts from the stems, leaves, flowers, and pods of silver wattle (*Acacia dealbata* Link) on the seed germination and early growth of herbaceous weeds were evaluated. The extracts significantly inhibited germination, radicle elongation, and hypocotyl growth, although the responses varied according to weed species and donor organ. Common lambsquarters (*Chenopodium album* L.) showed the highest germination percentage under extract exposure, whereas large crabgrass (*Digitaria sanguinalis* (L.) Scop.) was the most sensitive species. Jimsonweed (*Datura stramonium* L.), spurred anoda (*Anoda cristata* (L.) Schltdl.), and yellow foxtail (*Setaria pumila* (Poir.) Roem. & Schult.) also showed substantial inhibition depending on the extract applied. Radicle development was particularly affected, indicating that roots are a major target of silver wattle phytotoxins. Anatomical analyses of *D. stramonium* and *A. cristata* radicles revealed reduced or inhibited root hair formation, rhizodermal and cortical damage, root apex alterations, and disorganization of the vascular cylinder. Overall, the species- and organ-dependent phytotoxicity demonstrated here highlights silver wattle aerial biomass as a promising source of bioactive compounds for further development and evaluation in weed management strategies.

Keywords: bioherbicides; phytotoxicity; invasive species; root anatomy; secondary metabolites; silver wattle aerial

Introduction

Globally, pests represent among the main constraints to agricultural and forestry production, causing yield losses of up to 70% (Skendžić et al. 2021; Gullino et al. 2022). Among them, weeds are responsible for the greatest losses, accounting for approximately 30% and reaching even higher values in certain agroecosystems (Gordon 2022). For this reason, weeds are considered the most detrimental pest to agroforestry production (Gharde et al. 2018). These species are defined as plants that grow in competition with crops for limited resources such as water, light, soil nutrients, and space (Sabzi et al. 2020). Weeds include approximately 30,000 described taxa, 250 of which significantly impact global agriculture because of their high competitive ability, adaptability, and tolerance to different control methods (WeedScience 2025).

In Chile, weed diversity ranges from 120 species in the Atacama Region to more than 400 in the Valparaíso and Metropolitan Regions (Matthei 1995). A total of 743 introduced species have been reported in the country (90.4% herbaceous) (Fuentes et al. 2013; 2020). Among these, several cosmopolitan annual weeds of global importance—including common lambsquarters (*Chenopodium album* L., Chenopodiaceae), large crabgrass (*Digitaria sanguinalis* (L.) Scop., Poaceae), and jimsonweed (*Datura stramonium* L., Solanaceae)—are widely distributed and represent major constraints for crop production.

Chenopodium album is a highly competitive broadleaf species that infests cereals, oilseeds, and horticultural crops, with the potential to cause severe yield losses (>90%) because of its prolific seed production, persistent seed bank, and wide germination niche, which are further aggravated by the allelopathy suppression of neighboring crops (Tang et al. 2022; Al-Andal et al. 2025). *Digitaria sanguinalis* is among the most troublesome C4 grasses in maize, soybean, and vegetable

systems, combining strong competitive ability with broad environmental tolerance and a documented capacity to evolve resistance to ALS-inhibiting and glyphosate herbicides, which threatens the efficacy of current weed management programs (Oreja et al. 2025). *Datura stramonium*, a noxious invasive broadleaf weed of arable fields and field margins, not only competes with crops but also poses serious food and food safety risks because of its high content of toxic tropane alkaloids, with numerous poisoning cases linked to the contamination of harvested crops and forage (Kızıl et al. 2024; Aćimović 2025). Although less studied, *S. pumila* and *A. cristata* also act as summer annual weeds in row crops, contributing to persistent seed banks and requiring integrated management strategies (Puricelli et al. 2002; Zhang et al. 2021).

The effects of weeds on Chilean agricultural yields are consistent with international reports, and in the forestry sector, a reduction of up to 60% in *Pinus radiata* D. Don (Pinaceae) biomass during the first year has been attributed to weed competition (Kogan and Alister 2010). Subsequent studies confirmed that in *P. radiata* and *Eucalyptus globulus Labill (Myrtaceae)*, weed management is the most critical factor during early establishment (Kogan et al. 2002; Rubilar et al. 2013, 2023; Albaugh et al. 2015; Vargas et al. 2018, 2022). Currently, 298 herbicides are registered in Chile (SAG 2025), and 16 resistant species have been reported (WeedScience 2025), including *Avena fatua* L. (Poaceae), *Lolium multiflorum* Lam. (Poaceae), and *Conyza bonariensis* (L.) Cronquist (Asteraceae), which are major concerns for agricultural crops (Heap 2024).

Reports on the sale of agricultural pesticides in Chile (SAG 2025) indicate that some of the most commercialized herbicides, on the basis of their active ingredients, are those containing glyphosate. Glyphosate dependency is particularly evident in forest plantations, where it is applied alone or in combination with other herbicides (Kogan and Alister 2010). This intensive use also

92 extends to the control of highly problematic weed species in Chile (Heap 2024). Such intensive
93 use of herbicides raises not only ecological risks but also public health concerns. The World Health
94 Organization (WHO) estimates that approximately 385 million cases of unintentional acute
95 pesticide poisoning occur annually, resulting in 11,000 deaths (WHO 2019). In Chile, 17.63% of
96 agricultural workers are poisoned (Boedeker et al. 2020). Moreover, these figures likely
97 underestimate the problem by excluding chronic diseases associated with pesticide exposure, such
98 as cancer, neurological disorders, and reproductive impairments (Boedeker et al. 2020; Ghosh et
99 al. 2024).

100 In this context, there is an urgent need to explore sustainable alternatives for weed control. One
101 of the most promising strategies is the development of bioherbicides, defined as products derived
102 from living organisms or their secondary metabolites that are capable of suppressing weeds
103 without causing adverse environmental impacts (Scavo and Mauromicale 2021). These
104 compounds can be formulated from microorganisms or from plant extracts with phytotoxic
105 properties (Cordeau et al. 2016; Kumar et al. 2021). In particular, allelopathy has become relevant
106 as a natural mechanism in weed regulation (Hoang Anh et al. 2021) since plant-released
107 metabolites can affect key processes such as seed germination, early growth, photosynthesis, and
108 soil microbial dynamics (Kostina-Bednarz et al. 2023).

109 Within this framework, invasive plants represent a key reservoir of bioactive compounds. Silver
110 wattle (*Acacia dealbata* Link, Fabaceae), one of the most aggressive invasive Fabaceae worldwide
111 (Rodríguez et al. 2017), has been shown to release bioactive compounds (allelochemicals) with
112 negative effects on native plants and soil microorganisms (Fuentes-Ramírez et al. 2010; Aguilera
113 et al. 2015a). Among the metabolites identified in its leaves, flowers, pods, and bark are resorcinol,

lupanine, and stigmasterol, all of which have recognized phytotoxic activity (Aguilera et al. 2015a). Previous studies have shown that *A. dealbata* extracts induce morphological alterations in the radicle of model species such as *Lactuca sativa* L. (Asteraceae), *Quillaja saponaria* Molina (Quillajaceae), and *Helenium aromaticum* (Hook.) L.H. Bailey (Asteraceae) (Aguilera et al. 2015b, c). These traits position *A. dealbata* as a promising candidate for the development of new bioherbicides to support sustainable agriculture and reduce its dependence on synthetic herbicides. On the basis of this evidence, a central question emerges: How do natural extracts obtained from *A. dealbata* affect the germination and early development of herbaceous weeds? Accordingly, the aim of this study was to evaluate the effects of methanolic extracts obtained from the stems, leaves, flowers, and pods of silver wattle on the seed germination and early growth of herbaceous weeds. We hypothesized that extracts derived from the aerial organs of silver wattle would inhibit weed germination and early seedling growth, supporting their potential as a source of bioactive compounds for weed management.

Materials and Methods

Plant material (leaves, stems, flowers, and pods) was obtained from randomly selected *A. dealbata* trees (n = 10) growing in monoculture patches located on the “La Cantera” and “El Guindo” (36°50’05"S, 73°01’51"W; 62 m a.s.l.) farms in the vicinity of the Concepción Campus of the University of Concepción, Bíobio, Chile.

Prospection and collection of weed seeds were carried out at the “Los Placeres,” Santa Clara, Ñuble Region (36°49’33.2” S, 72°19’11.4” W) farm. Species selection was guided by local farmers’ assessments of the weeds causing the most significant crop damage in the area (Table 1).

Whole plants were collected to verify the taxonomic identification by an herbarium specialist at the Herbarium of the University of Concepción (CONC).

Disinfection and scarification assays

The collected seeds were subjected to different disinfection and scarification protocols (Table 2) to assess their germination rates and dynamics. Treated seeds (n = 30 per species) were evenly distributed on Whatman No. 1 filter paper discs placed in Petri dishes (9 cm diameter). Each dish received 3 mL of distilled water, was covered, and was sealed with Parafilm®. Three replicates per treatment and species were established (n = 60 seeds) and randomly arranged in a growth chamber set at 22 °C, 60% relative humidity, a light intensity of ~100 μmol m^{-2} s^{-1}, and a photoperiod of 16 h light/8 h dark. The seeds remained under these conditions for 13 to 28 days, depending on the germination rate of each species. At the end of the assay, the treatment with the best results (germination percentage) was selected and used in subsequent bioassays (Table 3).

Methanolic extracts of *A. dealbata* aerial organs

Samples of leaves, stems, flowers, and pods from ten plants were cut with pruning scissors and macerated in methanol under dark conditions to avoid the degradation of photosensitive compounds. Extracts were prepared at a ratio of 50 g of aerial biomass (each organ separately) per 500 mL of methanol. After 7 days, the extracts were filtered through Whatman No. 1 filter paper and stored in amber bottles.

Bioassays in Petri dishes

Four treatments were considered (methanolic extracts of stems, leaves, flowers, and pods), and two controls were used: distilled water and methanol. Independent assays were performed for each weed species (Table 1). Each treatment consisted of six Petri dishes (n = 6 per species). On each Whatman No. 1 filter paper disc, 3 mL of methanolic extract was added and left under a fume hood until complete solvent evaporation occurred. In the control treatments, 3 mL of distilled water or 2 mL of methanol was applied. Thirty seeds of each weed were subsequently evenly distributed per dish. The dishes were sealed with Parafilm® and placed in the growth chamber under the same conditions described above. The evaluation period ranged from 7 to 14 days depending on the germination rate of each species, with all measurements taken 7 days after seed germination. The variables assessed included the germination percentage (%), hypocotyl length (cm), radicle length (cm), number of leaves, and degree of root necrosis (NR); the latter was evaluated using a standardized scale (Table 3) (Aguilera et al. 2015a).

Anatomical study of roots

At the end of the bioassays, 3-mm segments were collected from the maturation, elongation, and root cap zones of *D. stramonium* and *A. cristata* (n = 6 per species and root zone). In some treatments, the root cap zone could not be sampled because of severe damage caused by the extracts. For the anatomical study, the radicles of Poaceae species could not be collected because they were too thin and fragile. The segments were fixed in 2.5% glutaraldehyde in sodium phosphate buffer (0.1 M; pH 7.2) at 4 °C. After being prewashed in phosphate buffer (0.1 M) twice for 10 min, the samples were fixed in 1% osmium tetroxide in phosphate buffer (0.1 M) for 2 h at 4 °C and subsequently washed again. Transverse sections (200 μm) were made using a rotary microtome HM 550 (MICROM International GmbH, Walldorf). The samples were dehydrated in

176 a graded ethanol series (30–100%) and subsequently dried using critical-point CO_2 drying (Balzers
177 Union FL-9496). Sections and whole roots were mounted on aluminum stubs coated with a carbon
178 film and sputter-coated with gold in an Edwards S150 Sputter Coater (USA) for 3 min at 30 mA,
179 producing a thickness of ~400 Å. Samples were examined under a scanning electron microscope
180 (JEOL JSM-6380 LV, Japan) and compared with controls.

181 **Statistical analysis**

182 Data normality and homogeneity of variance were tested using Kolmogorov–Smirnov and
183 Levene's tests, respectively. When the assumptions were met, the data were subjected to one-way
184 ANOVA with a significance level of 0.05, and Tukey's test was applied for multiple mean
185 comparisons. When assumptions were not met, the data were log-transformed [log (n + 1)]. The
186 nonparametric Kruskal–Wallis test was used to determine the degree of necrosis. All the statistical
187 analyses were performed using STATISTICA 7.0 (StatSoft Inc.) for Windows.

188 **Results and Discussion**

189 *Phytotoxic effects of* Acacia dealbata *aerial organ extracts are species dependent*

190 Methanolic extracts obtained from the aerial organs of *A. dealbata* significantly affected seed
191 germination and early seedling development, although the magnitude and nature of the response
192 depended on both the weed species and the donor organ (Figs. 1–4). Under control conditions, the
193 evaluated weeds exhibited relatively low germination percentages (40–70%) (Figure 1).
194 Nevertheless, exposure to extracts from stems, leaves, flowers, and pods significantly reduced
195 germination in all the species, revealing marked differences in sensitivity among the weeds (Figure
196 1). Stem extracts reduced germination across all species, whereas leaf extracts completely

197 inhibited germination in *D. stramonium* and significantly decreased it in *Di. sanguinalis*, *A.*
198 *cristata*, and *S. pumila* while stimulating germination in *C. album* (Figure 1). Flower extracts
199 completely inhibited germination in *A. cristata* and *Di. sanguinalis* and reduced germination in
200 the remaining species (Figure 1). Similarly, the pod extracts negatively affected the germination
201 of all the species except *C. album* (Figure 1).

202 The contrasting responses among the weeds indicate that the phytotoxic effects of *A. dealbata*
203 cannot be generalized solely to broad taxonomic groups. *Digitaria sanguinalis* was the most
204 sensitive species overall, with germination reduced to approximately 1% under extract exposure,
205 whereas the other monocot, *S. pumila*, exhibited approximately 6% germination. In contrast, the
206 dicot *C. album* was relatively tolerant, with germination ranging from 21 to 49%. In this species,
207 the effects of the leaf extracts on germination did not significantly differ from those of the controls,
208 and only the stem extracts significantly reduced germination (Figure 1). The other dicots also
209 showed high sensitivity, with germination ranging from 0 to 36% in *A. cristata* and from 0 to 5%
210 in *D. stramonium*, depending on the donor organ.

211 This pattern contrasts with the general tendency reported for conventional and alternative weed-
212 control methods, in which dicot weeds are often more susceptible than monocots, whereas grasses
213 may require higher doses, specific modes of action, or integrated approaches for effective
214 suppression (McSteen 2010; Song 2014). For instance, birch-based pyrolysis liquids and acid-
215 based herbicides strongly inhibited dicot weeds but showed limited efficacy against monocots such
216 as *Elymus repens* (L.) Gould (Poaceae), indicating the need for further optimization of products
217 targeting grassy weeds (Hagner et al. 2023). Similarly, pelargonic acid achieved up to 91% control
218 of dicots but only up to 71% control of monocots (Muñoz et al. 2022). The pronounced sensitivity

219 of *Di. sanguinalis* and, to a lesser extent, *S. pumila* to *A. dealbata* extracts, therefore suggesting
220 that some of the compounds present in this invasive species may be particularly effective against
221 certain grassy weeds.

222 The inhibitory effects of *A. dealbata* were not restricted to germination. Early seedling growth
223 was also affected in a species- and organ-dependent manner (Figures 2, 3). Compared with
224 germination responses, hypocotyl responses were more variable. In *D. stramonium*, none of the
225 extracts significantly altered hypocotyl length (Figure 2). In *C. album*, the pod extracts stimulated
226 hypocotyl elongation, whereas the leaf and flower extracts had no significant effect, and the stem
227 extracts significantly reduced hypocotyl growth (Figure 2). In *A. cristata*, the leaf and flower
228 extracts inhibited hypocotyl elongation, the pod extract significantly reduced it, and the stem
229 extract did not significantly differ from the control (Figure 2). In *S. pumila*, compared with that in
230 the control treatment, hypocotyl length in the treatment group was reduced following exposure to
231 the leaf, flower, and stem extracts, although these reductions were not individually significant,
232 whereas the pod extract had no effect (Figure 2).

233 Compared with hypocotyl growth, radicle elongation was more consistently affected, indicating
234 greater sensitivity of the belowground organ to *A. dealbata* phytotoxins (Figure 3). The length of
235 the radicles differed significantly among the treatments for all the evaluated species. In *D.*
236 *stramonium*, the stem and pod extracts markedly reduced radicle length, whereas the flower
237 extracts did not significantly affect length compared with that in the control despite the significant
238 overall treatment effect (Figure 3). In *C. album* and *S. pumila*, all four organ extracts significantly
239 reduced radicle length compared with that of the controls, with no significant differences among

240 the extracts (Figure 3). In *A. cristata*, stem and pod extracts significantly reduced radicle length,
241 whereas leaf extracts completely inhibited radicle formation (Figure 3).

242 Importantly, successful germination did not necessarily result in normal seedling development.
243 Even when radicle emergence occurred, severe necrosis was frequently observed (Figure 4). In *D.*
244 *stramonium*, 40–55% of the emerged radicles exhibited necrosis grades 3 and 4 following exposure
245 to stem and pod extracts, whereas flower extracts predominantly induced grade 2 necrosis (Figure
246 4). In *C. album*, stem extracts induced grade 4 necrosis in 100% of the emerged radicles. Moreover,
247 30–47% of the radicles exposed to the leaf, flower, and pod extracts exhibited grade 4 necrosis,
248 whereas 55.3% of the radicles exposed to the pod extracts exhibited grade 3 necrosis (Figure 4).
249 In *A. cristata*, all the extracts induced grade 4 necrosis, except for the pod extract, which caused
250 grade 3 necrosis in 2.9% of the radicles (Figure 4). A similarly severe response was observed in *S.*
251 *pumila*, where grade 4 necrosis ranged from 80 to 90% following stem, flower, and pod extract
252 exposure and reached 100% under leaf extract exposure. The remaining radicles exhibited grade 2
253 necrosis following flower extract treatment or grade 3 necrosis following stem and pod extracts
254 (Figure 4).

255 Taken together, these findings indicate that the phytotoxicity of *A. dealbata* involves more than
256 the inhibition of seed germination. In some species, seeds retain the ability to germinate but
257 subsequently develop severely shortened or necrotic radicles (Choopayak et al. 2022; Wang et al.
258 2022). This is particularly relevant from a weed management perspective because suppression of
259 early root development may compromise successful seedling establishment even when
260 germination itself is not completely prevented. The greater and more consistent sensitivity of

radicle elongation and integrity compared with that of hypocotyl growth further indicates that the root is a major target of phytotoxic compounds (Šoln et al. 2022), as in *A. dealbata* extracts.

Previous studies support the phytotoxic potential observed here. Species of *Acacia* are chemically diverse and contain numerous bioactive secondary metabolites, particularly phenolic compounds, flavonoids, terpenoids, phytosterols, and alkaloids (Madjid et al. 2020; Batiha et al. 2022; Tiwari et al. 2023). Several of these compounds have been associated with phytotoxic activity in *Acacia* species, including phenolic acids and flavonoids in *A. confusa* and *A. melanoxylon* (Chou et al. 1998; Hussain et al. 2011) and triterpenoid derivatives in *A. cyclops* (Jelassi et al. 2016). These findings indicate that the chemical diversity of the genus provides a broad range of compounds capable of interfering with seed germination and early plant growth.

Evidence specific to *A. dealbata* further supports this phytotoxic potential. Methyl cinnamate isolated from this species inhibits the germination and early growth of both monocot and dicot weeds (Lorenzo et al. 2019), while aqueous bark extracts and fresh biomass also reduce weed germination and radicle elongation, although the efficacy of these methods may decrease under soil conditions (Quintela-Sabarís et al. 2022; Duarte et al. 2023). In Chile, methanolic extracts and residues from *A. dealbata* aerial organs inhibited root growth and induced radicle necrosis and structural damage in several herbaceous and woody species, including inhibition of root hair formation, rhizodermal disruption, and damage to the vascular cylinder (Aguilera et al. 2015a, b, c). The present findings extend this evidence to agriculturally relevant herbaceous weeds, demonstrating that the phytotoxic effects of *A. dealbata* involve not only inhibition of germination but also severe postgerminative effects that may compromise early seedling establishment.

The marked differences among the stem, leaf, flower, and pod extracts may be related to the differences in the chemical profiles of these organs. Under the Mediterranean climatic conditions of the coastal Biobío Region of Chile, where the present study was conducted, gas chromatography–mass spectrometry revealed differences in the compounds and relative peak areas detected among *A. dealbata* aerial organs (Aguilera et al. 2015a). Leaves are rich in triterpenoids and phenolic compounds, including the triterpenoid lupenone and the phenol resorcinol, together with compounds such as benzophenone and maculosin. Flowers predominantly contain phenolic esters and aromatic compounds, including methyl *p*-hydroxycinnamate, *p*-anisyl alcohol, and anisal. Pods are characterized by sterols, tocopherol derivatives, and fatty acid esters, including stigmasterol, d-α-tocopherolquinone, and methyl hexadecanoate. Stems, which remain the least studied aerial organ, have recently been reported to contain phytol, squalene, α-tocopherol, lupeol, and lupenone (lup-20(29)-en-3-one), with these compounds showing some of the highest relative peak areas in chemical profiling (Correia et al. 2022). However, the chemical composition of *A. dealbata* stems from Chilean ecosystems has not yet been characterized.

The abundance of triterpenoids may be particularly relevant to the strong response observed in monocot weeds. Triterpenoids have previously been associated with phytotoxic activity against monocot species (Macías et al. 1997, 2019; Kong et al. 2007). For example, lupeol isolated from the leaves and branches of *Machaerium eriocarpum* and *M. hirtum* (Fabaceae) strongly inhibited seed germination, root elongation, and lateral root formation in *Sorghum bicolor* (Poaceae) (Tahira et al. 2021). Thus, the triterpenoid-rich composition of leaves and stems could partly explain the pronounced sensitivity of *Di. sanguinalis* and *S. pumila*, although direct causal relationships cannot be established from the present experiments.

Phenolic compounds may also contribute substantially to the observed phytotoxicity. Phenolic acids and flavonoids are among the most widely reported phytotoxic secondary metabolites, and cinnamic acid ester derivatives are particularly noteworthy because of their inhibitory activity against standard target species such as lettuce (Macías et al. 2000; DellaGreca 2007). Methyl *p*-hydroxycinnamate, detected in *A. dealbata* flowers, belongs to this group. Similarly, resorcinol and other aromatic compounds detected in *A. dealbata* aerial organs may contribute to their biological activity. The phytotoxicity of aromatic compounds depends not only on the presence of the aromatic ring but also on its substitutions, functional groups, and lipophilic–hydrophilic balance, which influence its interaction with plant tissues (Bravo et al. 2013). Consequently, differences in chemical composition among donor organs may account, at least in part, for the contrasting responses observed among extracts.

The effects of plant extracts, however, cannot necessarily be attributed to individual compounds. Each organ contains mixtures of metabolites that may exert additive, synergistic, or antagonistic effects, and the sensitivity to these mixtures differs among recipient species (Scognamiglio & Schneider 2020; Pardo-Muras et al. 2022; Cheng et al. 2025). Moreover, sensitivity to these complex mixtures varies among recipient species, resulting in responses ranging from stimulation or tolerance to strong inhibition (Scavo et al. 2019; Thiébaut et al. 2019; Akter et al. 2024). This donor organ × recipient species interaction provides a plausible explanation for the lack of a uniform ranking of extract toxicity among the evaluated weeds. For example, *C. album* tolerated or even responded positively to some extracts but was strongly affected by the stem extract, whereas *A. cristata* and the two Poaceae were sensitive to extracts from several aerial organs. Therefore, the present results indicate that the phytotoxic activity of *A. dealbata* is determined by the interaction between organ-specific chemistry and recipient-species sensitivity.

The root is a primary structural target of Acacia dealbata *phytotoxicity*

The pronounced inhibition of radicle elongation and the high incidence of necrosis observed across weed species indicate that roots are particularly sensitive to the phytotoxic compounds present in *A. dealbata* extracts (Figures 3, 4). This sensitivity was further supported by the morphological and anatomical alterations observed in *D. stramonium* and *A. cristata* radicles (Figures 5, 6). The effects extended from the rhizodermis and root hairs to the cortex, endodermis, vascular cylinder, and root apex, demonstrating that *A. dealbata* phytotoxicity compromises several tissues essential for root growth and function.

Compared with aerial organs, roots are commonly considered primary targets of phytotoxic compounds because they are directly exposed to bioactive substances in the growth medium and frequently exhibit stronger growth inhibition (Cheng et al. 2016; Zhang et al. 2018; Yan and Wang 2020; Šoln and Koce 2021; Šoln et al. 2022). Most studies reviewed by Staszek et al. (2021) and Šoln et al. (2022), regardless of the origin or chemical nature of the phytotoxins, report root growth inhibition associated with increased production of reactive oxygen species (ROS), particularly hydrogen peroxide, sometimes accompanied by cell death. Excessive ROS accumulation can increase membrane permeability, alter mitochondrial ultrastructure, decrease ATP production, and impair cell division, ultimately leading to programmed cell death. These disturbances can also reduce DNA, RNA, and protein synthesis and compromise water and mineral acquisition, collectively restricting root growth. Such effects may be particularly pronounced in the root cap (Pereira et al. 2017; Burgos et al. 2024) and apical meristem (Soltys et al. 2011, 2012; Araniti et al. 2017).

Under control conditions (water and methanol), *D. stramonium* radicles showed the typical organization of a developing root. The maturation zone exhibited numerous elongated root hairs (Figure 5a, b), whereas the root apex was conical, with a well-organized root cap, smooth surface, and elongated epidermal cells clearly distinguishable in frontal view (Figure 5c). In cross section, the maturation zone was well organized, with a diarch vascular cylinder containing at least five polygonal xylem elements and delimited by an endodermis composed of moderately elongated cells. The cortex consisted of at least five layers of large polyhedral cells, whereas the rhizodermis was formed by relatively small rectangular cells (Figure 5d).

Exposure to *A. dealbata* extracts progressively disrupted this organization. The stem extract did not inhibit root hair formation in *D. stramonium* (Figure 5e) but induced an abrupt transition between the elongation zone and the root apex (Figure 5f), resulting in the loss of its characteristic conical morphology and a disorganized shape (Figure 5g). In cross section, the maturation zone retained its general organization but showed an apparent reduction in the vascular cylinder area (Figure 5h), although this apparent reduction was not confirmed by histometric measurements. The effects of the flower and pod extracts were considerably more severe. Both completely inhibited root hair formation (Figure 5j, m). Following flower extract exposure, the rhizodermis became smooth and hairless, while the apex was shortened and truncated (Figure 5i, j). The vascular cylinder appeared reduced, vessel elements showed collapsed walls, the endodermis was almost indistinguishable, cortical cells became rounded, and rhizodermal cells collapsed (Figure 5k). At the root apex, the internal structure completely collapsed, with poorly differentiated and compacted tissues (Figure 5l).

Pod extracts similarly caused severe structural disruption in *D. stramonium* (Figure 5m–p). The rhizodermis of the maturation zone became disorganized and scaly (Figure 5m), whereas the root apex developed an amorphous termination with an irregular and desquamated surface (Figure 5n). Internally, the vascular cylinder was disorganized, cortical cells became rounded, and the outer cortical layer contained large radially elongated cells. Compared with control radicles, rhizodermal cells were larger (Figure 5o). At the root apex, the xylem elements exhibited thickened cell walls, the cortical cells were large and polyhedral and occupied most of the tissue area, and the rhizodermis collapsed and was barely distinguishable (Figure 5p).

A comparable but extract-specific pattern occurred in *A. cristata*. Under the water and methanol control conditions, the elongation zone was relatively short, and the maturation zone exhibited numerous elongated root hairs (Figure 6a, b). The apex was short and conical, with a well-organized root cap and smooth surface (Figure 6b). In cross section, the maturation zone contained a well-organized tetrarch vascular cylinder with several xylem elements characterized by circular lumina. The endodermis consisted of polyhedral cells, the cortex contained several layers of large polyhedral cells, and the rhizodermis was composed of relatively small rectangular cells (Figure 6c).

Stem extract exposure did not completely inhibit root hair formation in *A. cristata*, although the hairs became sparse and short (Figure 6d). The apex maintained its conical morphology, but the root cap cells developed an irregular and rough appearance (Figure 6e). Internally, the vascular cylinder became disorganized, endodermal cells were enlarged, and the stele lost its characteristic tetrarch arrangement, while the rhizodermal cells appeared collapsed (Figure 6f). Leaf extracts also altered root organization. The root hairs remained present but were sparse and elongated

(Figure 6g), whereas the root cap cells retained a relatively normal elongated appearance (Figure 6h). Nevertheless, cross-sections of the maturation zone revealed substantial vascular disorganization, with loss of the tetrarch pattern and xylem elements scattered throughout the stele (Figure 6i). Pod extracts induced additional alterations in *A. cristata*. Few root hairs were present; these were very short, and the rhizodermal cells exhibited an abnormally rough appearance (Figure 6j). At the root cap, the surface became irregular, and the cells appeared slightly raised (Figure 6k). Cross-sections of the maturation zone revealed a compacted vascular cylinder, with vascular cells lacking a defined distribution pattern and xylem elements exhibiting thickened cell walls. Although the endodermis remained recognizable, the cortical cells were large and exhibited sinuous walls, whereas the outer cortical layers adjacent to the rhizodermis consisted of small, flattened cells with little intercellular space. Rhizodermal cells were also small and exhibited undulated contours (Figure 6l).

Among these alterations, the reduction or complete inhibition of root hair formation represents an important functional response. Similar morphological damage has previously been observed in radicles exposed to phytotoxic extracts from invasive Fabaceae in Chile (Aguilera et al. 2015a, b, c; Aguilera et al. 2023; Rodríguez-Cerda et al. 2023; Guedes et al. 2024, 2025). In particular, direct contact with the leaves, pods, and seeds of *A. dealbata* caused severe radicle damage to *L. sativa* and *Q. saponaria*. In *L. sativa*, these alterations included inhibition of root hair formation and progressive destruction of the rhizodermis, particularly following leaf exposure, whereas pods and seeds also induced extensive root hair-free areas (Aguilera et al. 2015c). Root hairs substantially increase the absorptive surface of young roots and contribute to water and nutrient acquisition (Bertin et al. 2003; Chuah et al. 2016). Their differentiation from rhizodermal cells is regulated by developmental and hormonal signaling, particularly that of auxins (Kohli et al. 2022), and previous

studies have associated reductions in root hair length and density with the disruption of auxin transport (Lupini et al. 2014; Šoln et al. 2025). Therefore, the reduction or complete suppression of root hairs by *A. dealbata* extracts could substantially impair resource acquisition during early seedling establishment.

The structural alterations observed in the cortex, endodermis, and vascular cylinder of both weed species provide further evidence that *A. dealbata* extracts compromise root functionality. Cortical cells showed deformation, enlargement, and, in severely affected tissues, collapse, which is consistent with previous observations in *Q. saponaria* radicles exposed to *A. dealbata* (Aguilera et al. 2015b). Because the cortex contributes to water storage and the endodermis regulates the radial movement of water and solutes toward the stele, disruption of these tissues may impair root water relations and transport processes (Gatti et al. 2010; Burgos et al. 2004). Moreover, alteration of the characteristic vascular organization of both weed species, accompanied by xylem disorganization and vessel wall modifications, may further compromise their transport capacity. Similar changes in vascular organization under stress have been associated with impaired cell differentiation and root function (Niu et al. 2014; Miki et al. 2024).

Damage to the root cap and apex was also particularly severe, especially in *D. stramonium*, including necrosis, surface desquamation, loss of the characteristic conical morphology, and, following flower extract exposure, collapse of inner tissues (Figure 5). Because the root cap protects the underlying meristematic region and is essential for normal root development (Kumpf and Nowack 2015; Berhin et al. 2019), these alterations may compromise continued cell division and root elongation. Together, damage to cortical, endodermal, vascular, and apical tissues provides a structural basis for the pronounced inhibition of radicle growth observed in the

bioassays (Figure 3) and indicates that *A. dealbata* extracts simultaneously affect tissues involved in water relations, transport, and root growth.

Overall, the present findings demonstrate that methanolic extracts from the aerial organs of *A. dealbata* strongly phytotoxicly affect herbaceous weeds, although their efficacy depends on both the donor organ and the recipient species. The effects extended beyond germination inhibition, with radicle growth and integrity being particularly affected. The severe alterations observed in the rhizodermis, root hairs, cortex, endodermis, vascular cylinder, and root apex provide a structural basis for the reduced radicle elongation and high incidence of necrosis, identifying the root as a primary target of *A. dealbata* phytotoxicity. Consequently, even when germination is not completely inhibited, damage to tissues involved in water and nutrient acquisition, transport, and root growth may compromise subsequent seedling establishment. Collectively, these findings support *A. dealbata* aerial biomass as a promising source of natural phytotoxic compounds for weed management. However, identification of the bioactive compounds and their interactions, determination of effective concentrations, and validation of their efficacy under soil and field conditions are essential steps toward assessing their feasibility for the development of bioherbicidal formulations.

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Data availability

The data that support our findings of this study are available from the first author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Tables

Table 1. Selected weed species for evaluation

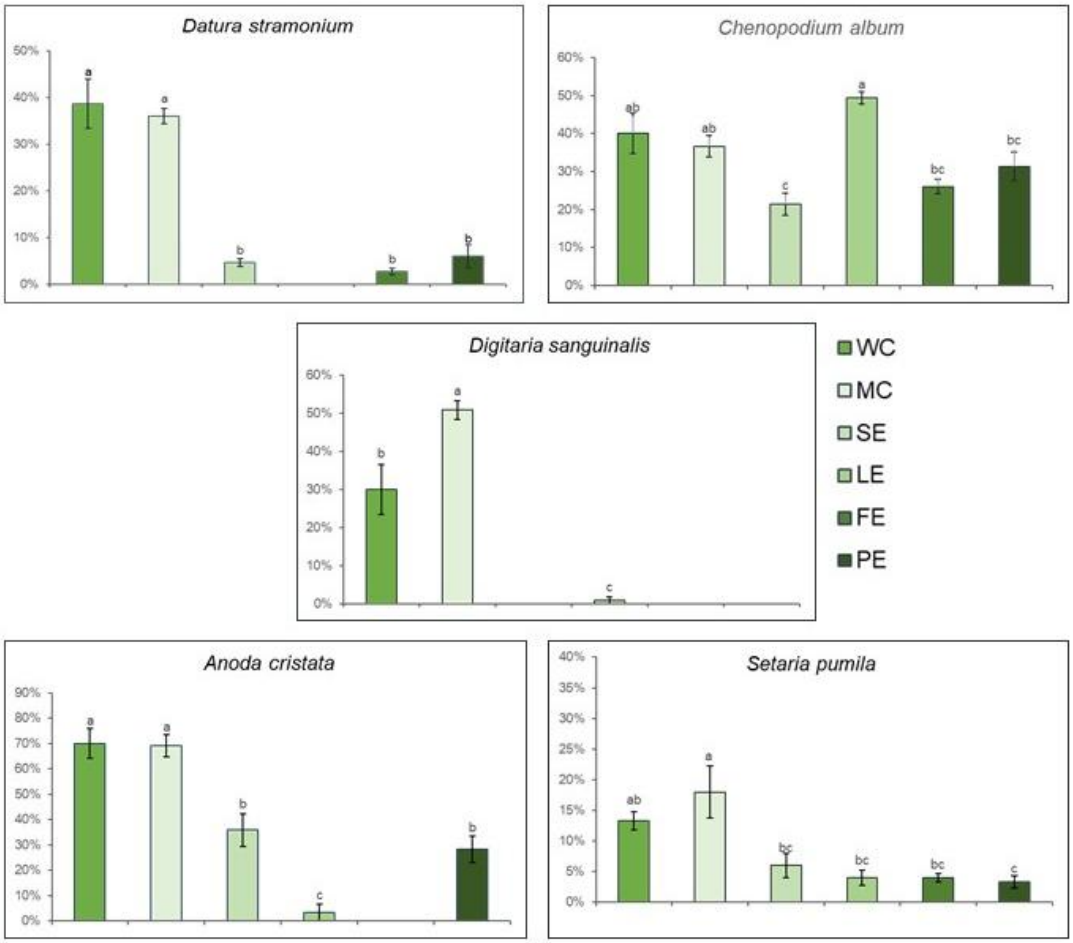
Common Name (Chile)	Common Name (English)	Scientific Name	Family
Chamico	Jimsonweed	*Datura stramonium* L.	Solanaceae
Quinhuilla	Lamb's quarters	*Chenopodium album* L.	Chenopodiaceae
Pata de gallina	Crabgrass	*Digitaria sanguinalis* (L.) Scop	Poaceae
Malva	Mallow	*Anoda cristata* (L.) Schltdl	Malvaceae
Setaria	Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult	Poaceae

Table 2. Effectiveness of disinfection treatments applied to weed seeds. The "Effective plant species" column indicates the species for which each treatment was successful. A dash (–) indicates that the treatment was not effective for any of the tested species.

Treatment	Description	Effective plant species
Control 1	Tap water rinse, rinsed with distilled water 3 times	*Digitaria sanguinalis*
Control 2	Tap water rinse	*Datura stramonium*, *Chenopodium album*
Treatment 1	Tap water rinse, ethanol (2 min), rinsed with distilled water 3 times	–
Treatment 2	Tap water rinse, ethanol (1 min), rinsed with distilled water 3 times	–
Treatment 3	Tap water rinse, ethanol (1 min), rinsed with distilled water 3 times; tap water rinse, immersed in distilled water at 70 °C for 10 min	*Anoda cristata*, *Setaria pumila*

Table 3. Degree of radicle necrosis (Aguilera et al. 2015a)

Necrosis Degree	Description
0	Radicle without discoloration and with abundant root hairs
1	Radicle light brown with reduction of root hairs up to 50% of their length
2	Radicle brown with 5–10% necrosis; root hairs absent
3	Radicle dark brown with approximately 50% necrosis; root hairs absent
4	Radicle dark brown with more than 75% necrosis; root hairs absent



518

519 **Fig. 1** Germination percentage of weed species exposed to stem extracts (SE), leaf extracts (LE),
520 flower extracts (FE), and pod extracts (PE) of *Acacia dealbata* Link. The data are presented as the
521 means ± standard errors. Means were compared using one-way ANOVA with water (WC) and
522 methanol (MC) controls. Different letters indicate significant differences among treatments within
523 the same species according to Tukey's test ($p < 0.05$)

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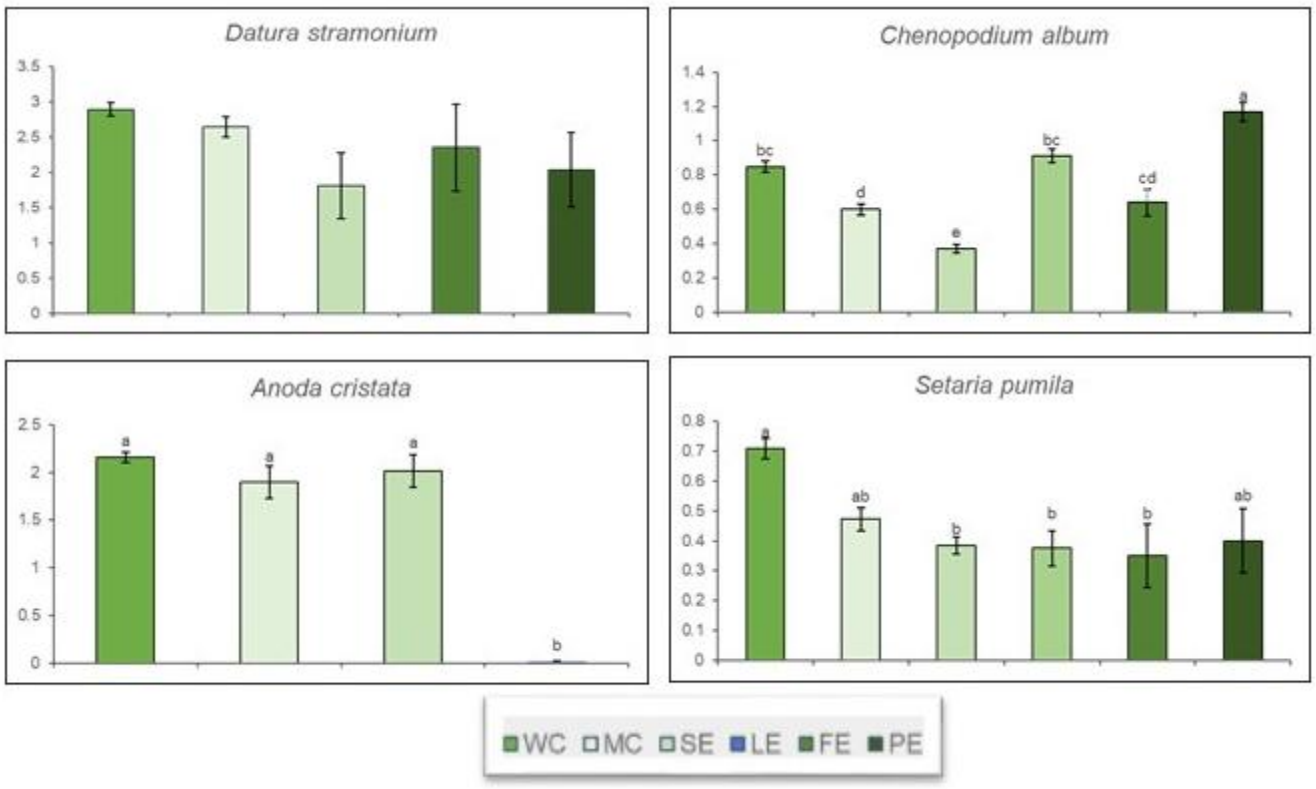
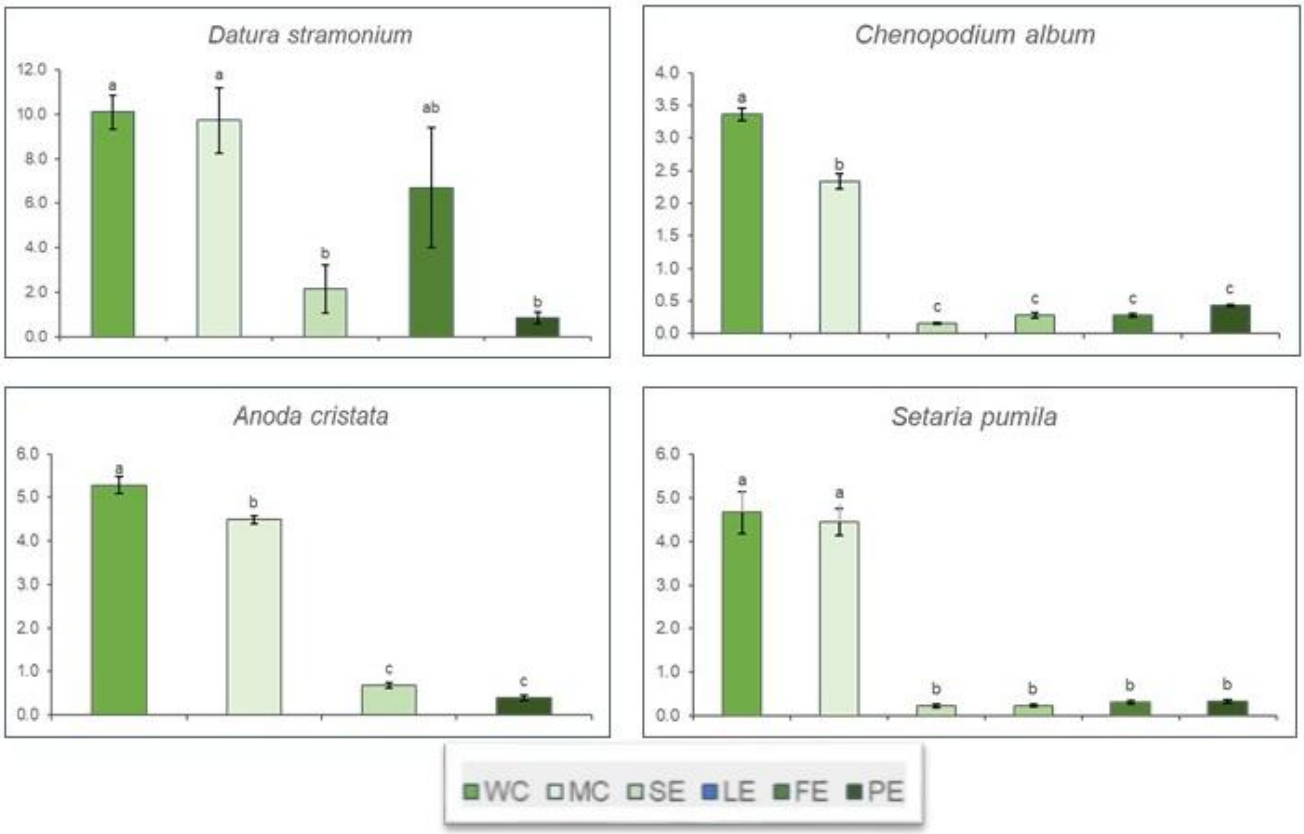


Fig. 2 Hypocotyl length (cm) of weed species exposed to stem extracts (SE), leaf extracts (LE), flower extracts (FE), and pod extracts (PE) of *Acacia dealbata* Link. The data are presented as the means ± standard errors. Means were compared using one-way ANOVA with water (WC) and methanol (MC) controls. Different letters indicate significant differences among treatments within the same species according to Tukey's test ($p < 0.05$)



536

537 **Fig. 3** Radicle length (cm) of weed species exposed to stem extracts (SE), leaf extracts (LE), flower
538 extracts (FE), and pod extracts (PE) of *Acacia dealbata* Link. The data are presented as the means
539 ± standard errors. Means were compared using one-way ANOVA with water (WC) and methanol
540 (MC) controls. Different letters indicate significant differences among treatments within the same
541 species according to Tukey's test ($p < 0.05$)

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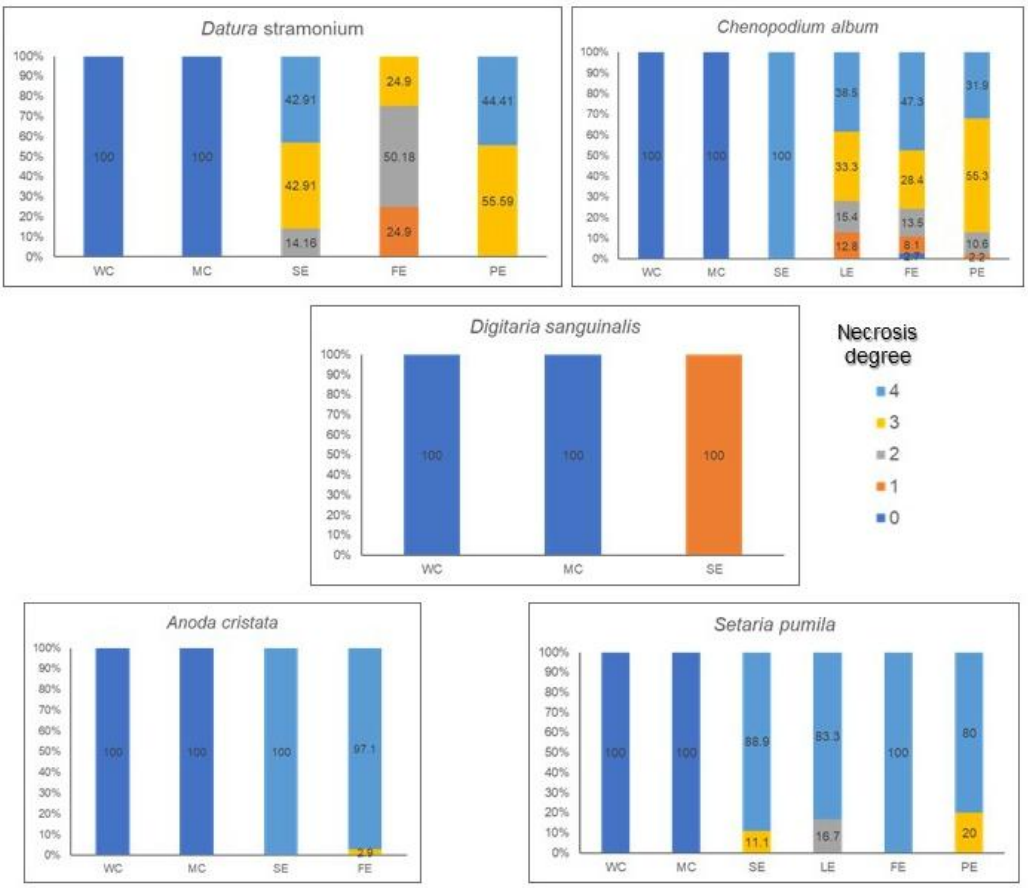

548

549 **Fig. 4** Percentage of plants of each weed species showing different degrees of radicle necrosis
550 (Table 3) after exposure to stem extracts (SE), leaf extracts (LE), flower extracts (FE), and pod
551 extracts (PE) of *Acacia dealbata*. Water (WC) and methanol (MC) were used as controls. Necrosis
552 levels were categorized and analyzed using the nonparametric Kruskal-Wallis test. Data are
553 expressed as the percentage of plants in each necrosis category

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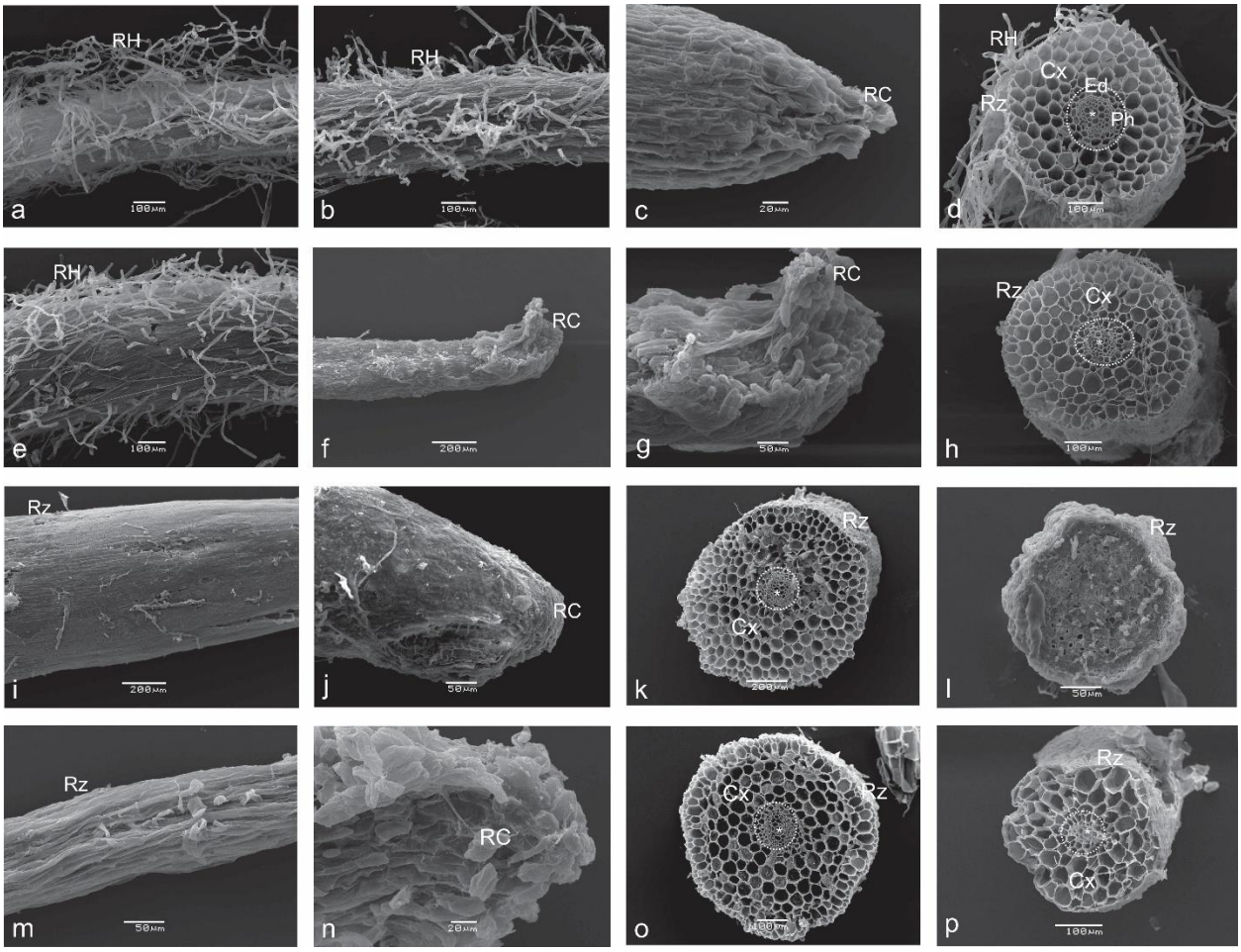

560

561 **Fig. 5** Scanning electron micrographs of radicles of *Datura stramonium* grown in water **(a, d)**,
562 methanol **(b, c)**, and methanolic extracts of stems **(e-h)**, flowers **(i-l)**, and pods **(m–p)** of *Acacia*
563 *dealbata*. Frontal view of the maturation zone **(a, b, e, i, m)** and the root cap zone **(c, f, g, j, n)**.
564 Transverse sections of the maturation **(d, h, k, o)** and root cap **(l, p)** zones. The dashed white circle
565 outlines the vascular cylinder. White asterisks indicate xylem elements. Abbreviations: Cx, cortex;
566 Ed, endodermis; HR, root hairs; Ph, phloem; RC, root cap; RZ, rhizodermis

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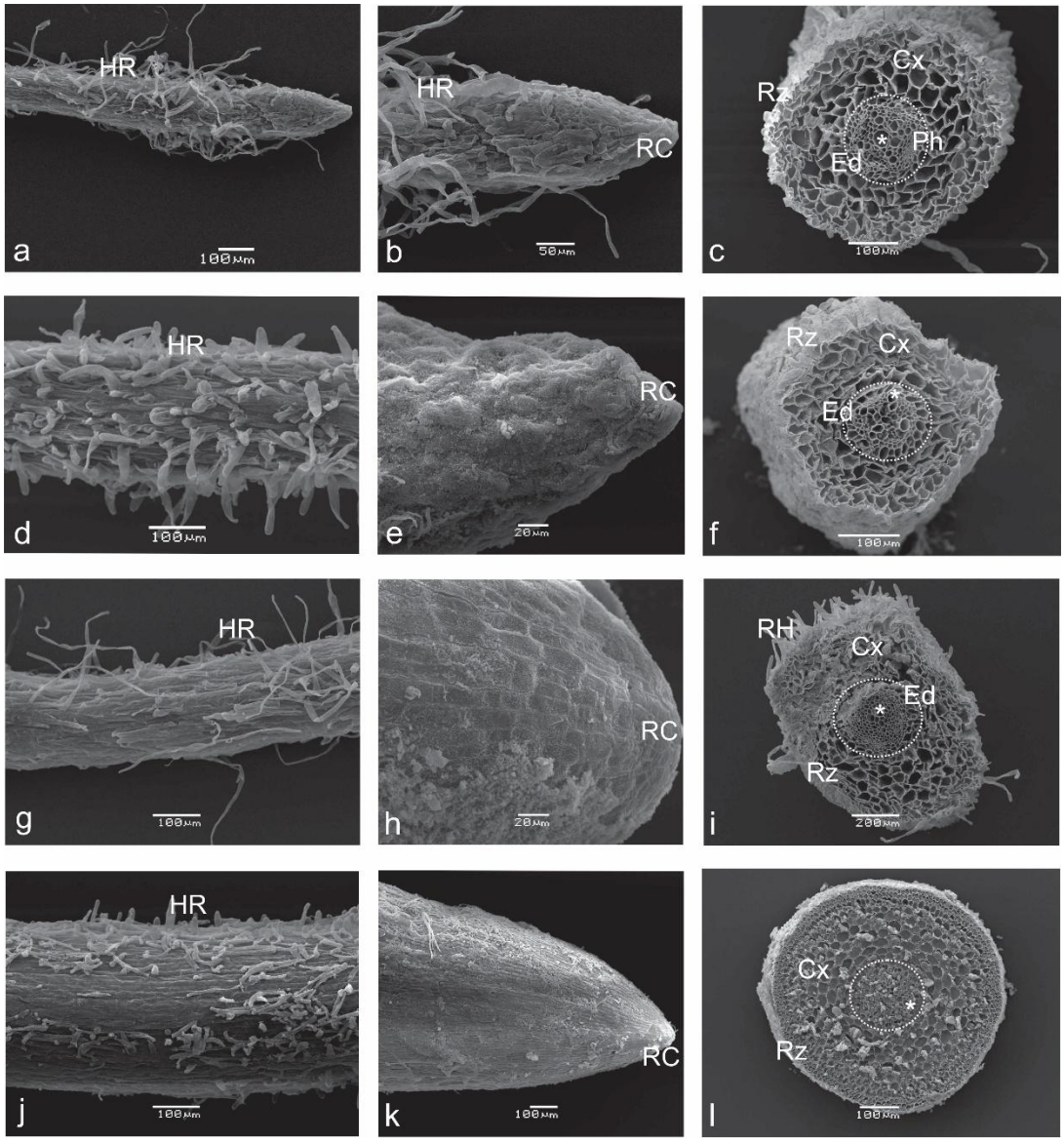

572

573 **Fig. 6** Scanning electron micrographs of the radicles of *Anoda cristata* grown in water **(a–b)**,
574 methanol **(c)**, and methanolic extracts of the stems **(d–f)**, leaves **(g–i)**, and pods **(j–l)** of *Acacia*
575 *dealbata*. Frontal view of the maturation zone **(a, d, g, j)** and the root cap zone **(b, e, h, k)**.
576 Transverse sections of the maturation zone **(c, f, i, l)**. The dashed white circle outlines the vascular
577 cylinder. White asterisks indicate xylem elements. Abbreviations: Cx, cortex; Ed, endodermis;
578 HR, root hairs; Ph, phloem; RC, root cap; RZ, rhizodermis

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Common Name (Chile)	Common Name (English)	Scientific Name	Family
Chamico	Jimsonweed	*Datura stramonium* L.	Solanaceae
Quinhuilla	Lamb's quarters	*Chenopodium album* L.	Chenopodiaceae
Pata de gallina	Crabgrass	*Digitaria sanguinalis* (L.) Scop	Poaceae
Malva	Mallow	*Anoda cristata* (L.) Schltdl	Malvaceae
Setaria	Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult	Poaceae

831 **Table**s

832 Table 1. Selected weed species for evaluation

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Table 2. Effectiveness of disinfection treatments applied to weed seeds. The "Effective plant species" column indicates the species for which each treatment was successful. A dash (–) indicates that the treatment was not effective for any of the tested species.

Treatment	Description	Effective plant species
Control 1	Tap water rinse, rinsed with distilled water 3 times	*Digitaria sanguinalis*
Control 2	Tap water rinse	*Datura stramonium*, *Chenopodium album*
Treatment 1	Tap water rinse, ethanol (2 min), rinsed with distilled water 3 times	–
Treatment 2	Tap water rinse, ethanol (1 min), rinsed with distilled water 3 times	–
Treatment 3	Tap water rinse, ethanol (1 min), rinsed with distilled water 3 times; tap water rinse, immersed in distilled water at 70 °C for 10 min	*Anoda cristata*, *Setaria pumila*

Table 3. Degree of radicle necrosis (Aguiler et al. 2015a)

Necrosis Degree	Description
0	Radicle without discoloration and with abundant root hairs
1	Radicle light brown with reduction of root hairs up to 50% of their length
2	Radicle brown with 5–10% necrosis; root hairs absent
3	Radicle dark brown with approximately 50% necrosis; root hairs absent
4	Radicle dark brown with more than 75% necrosis; root hairs absent

Figures

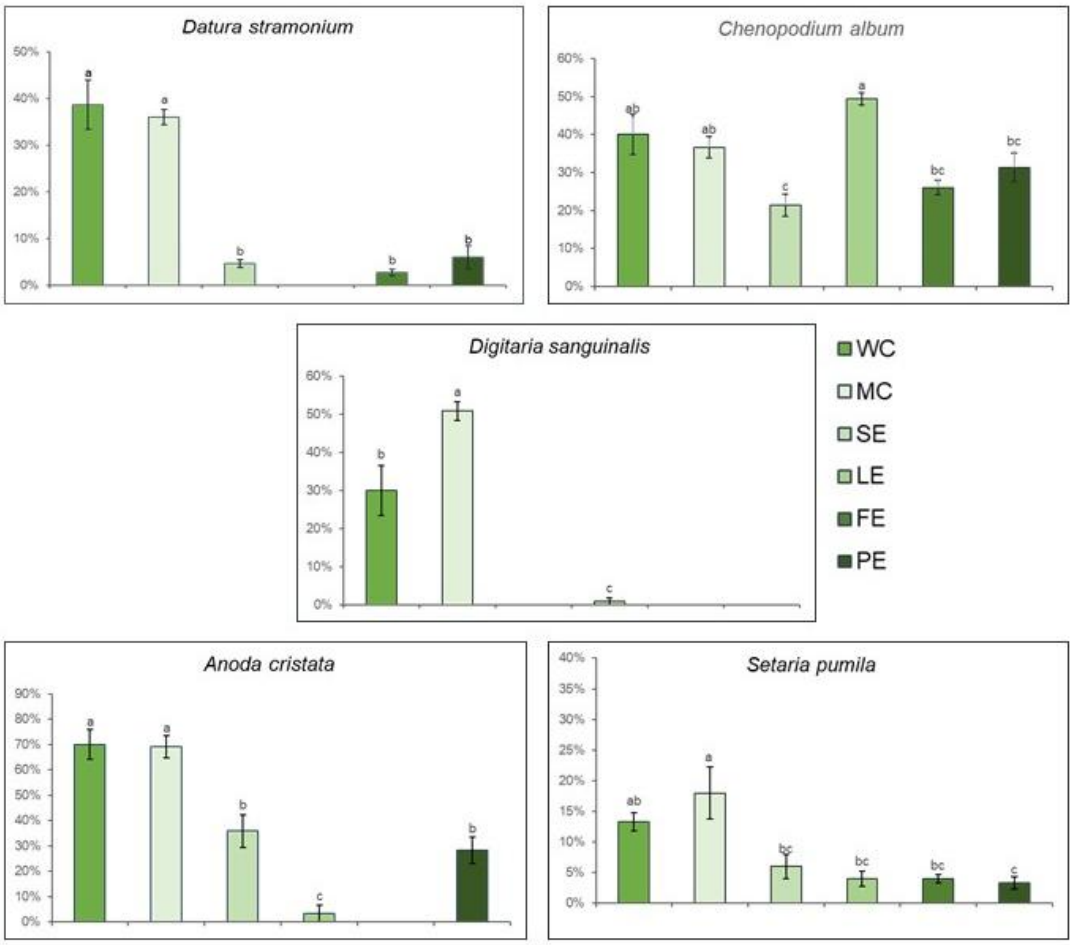


Figure 1. Germination percentage of weed species exposed to stem extracts (SE), leaf extracts (LE), flower extracts (FE), and pod extracts (PE) of *Acacia dealbata* Link. The data are presented as the means ± standard errors. Means were compared using one-way ANOVA with water (WC) and methanol (MC) controls. Different letters indicate significant differences among treatments within the same species according to Tukey's test ($P < 0.05$).

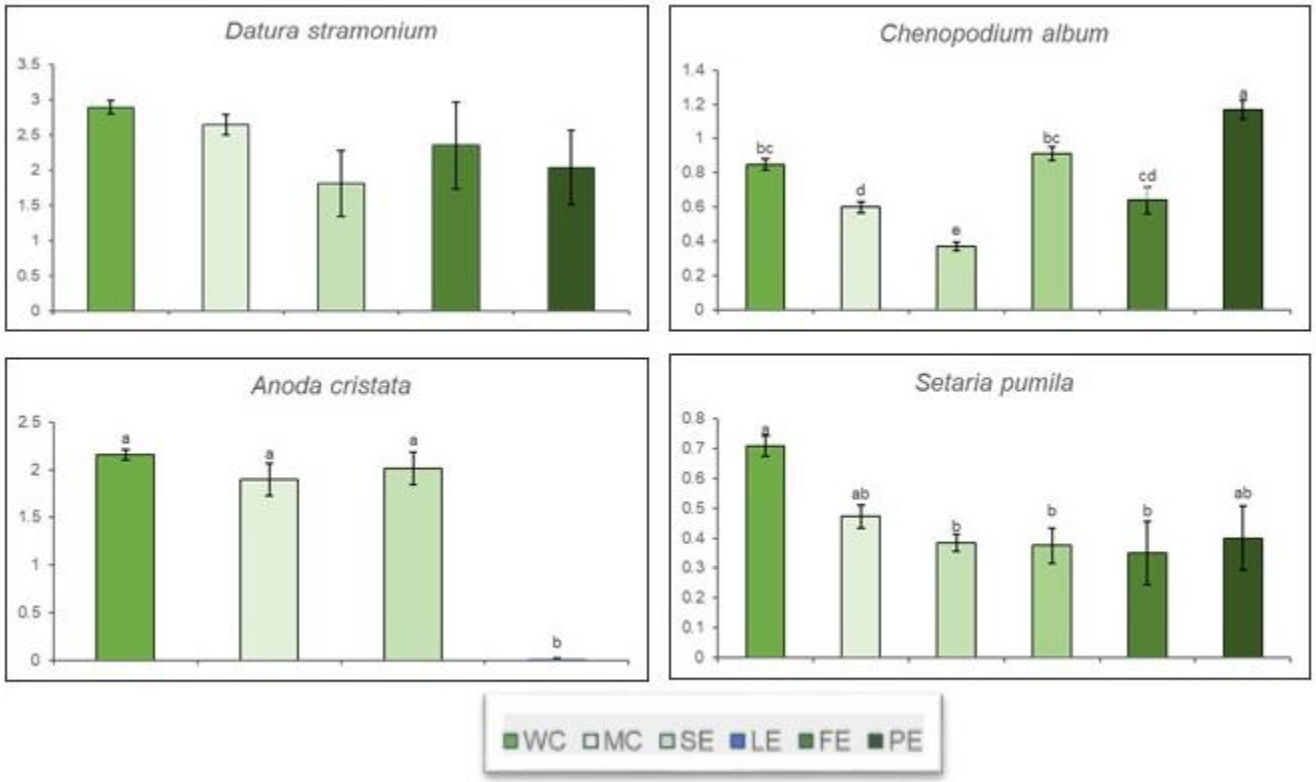
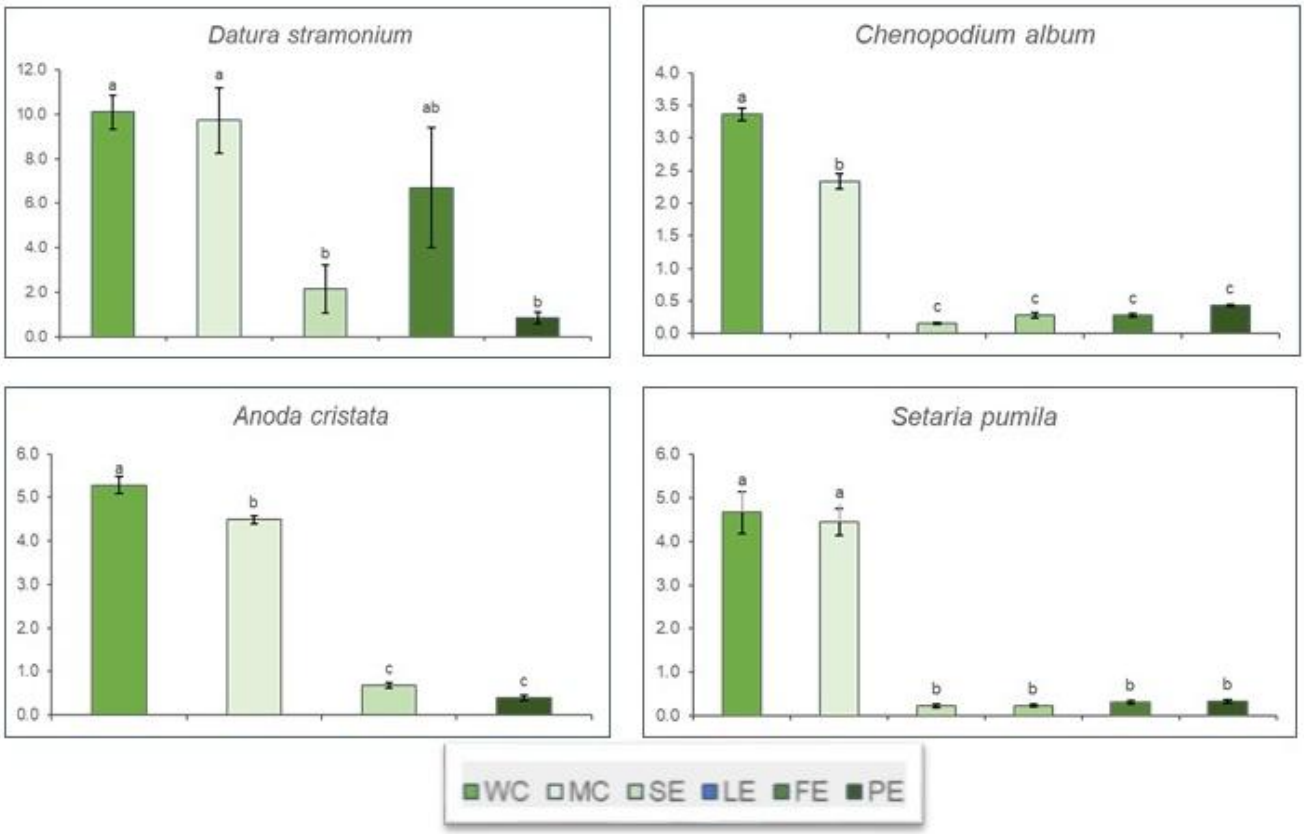


Figure 2. Hypocotyl length (cm) of weed species exposed to stem extracts (SE), leaf extracts (LE), flower extracts (FE), and pod extracts (PE) of *Acacia dealbata* Link. The data are presented as the means ± standard errors. Means were compared using one-way ANOVA with water (WC) and methanol (MC) controls. Different letters indicate significant differences among treatments within the same species according to Tukey's test ($P < 0.05$).



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934 **Figure 3.** Radicle length (cm) of weed species exposed to stem extracts (SE), leaf extracts (LE),
935 flower extracts (FE), and pod extracts (PE) of *Acacia dealbata* Link. The data are presented as the
936 means ± standard errors. Means were compared using one-way ANOVA with water (WC) and
937 methanol (MC) controls. Different letters indicate significant differences among treatments within
938 the same species according to Tukey's test ($P < 0.05$).

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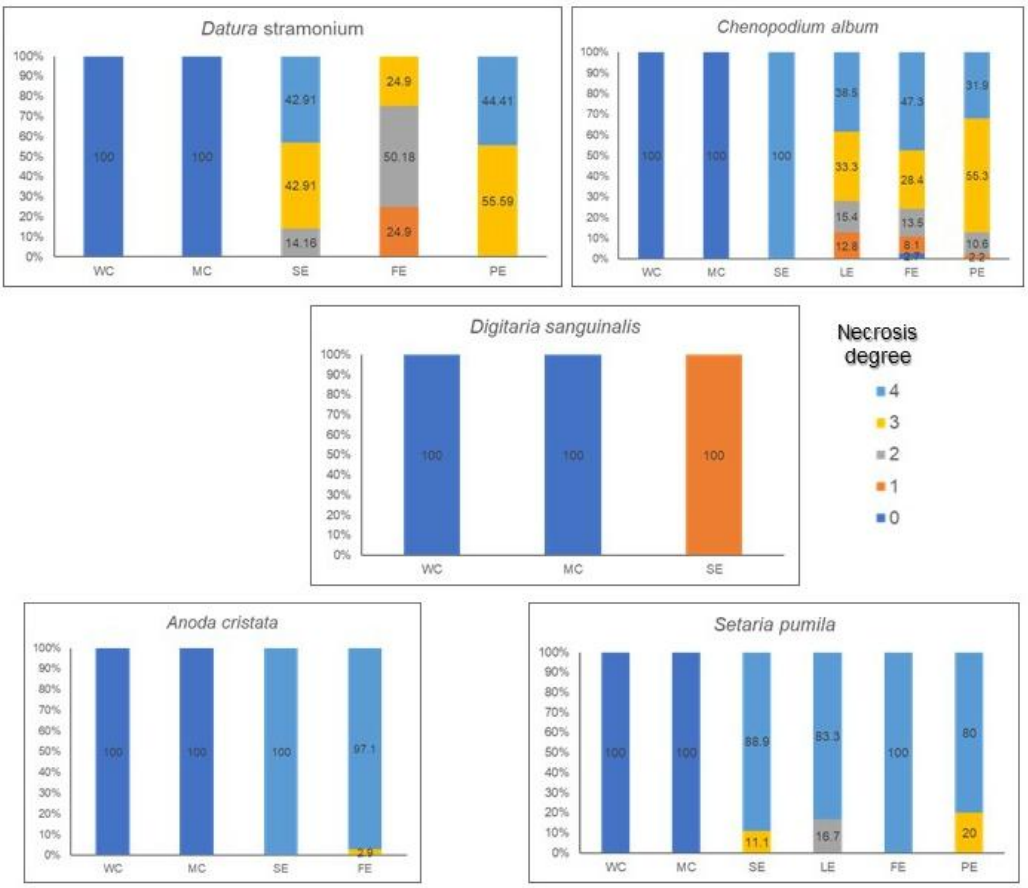


Figure 4. Frequencies (%) of plants per species showing different degrees of necrosis (Table 3) after exposure to *Acacia dealbata* Link extracts: stem extracts (SE), leaf extracts (LE), flower extracts (FE), and pod extracts (PE). Frequencies were compared with those of water (WC) and methanol (MC) controls. Necrosis levels were categorized and analyzed using the nonparametric Kruskal–Wallis test. The data are expressed as the percentage of plants in each necrosis category.

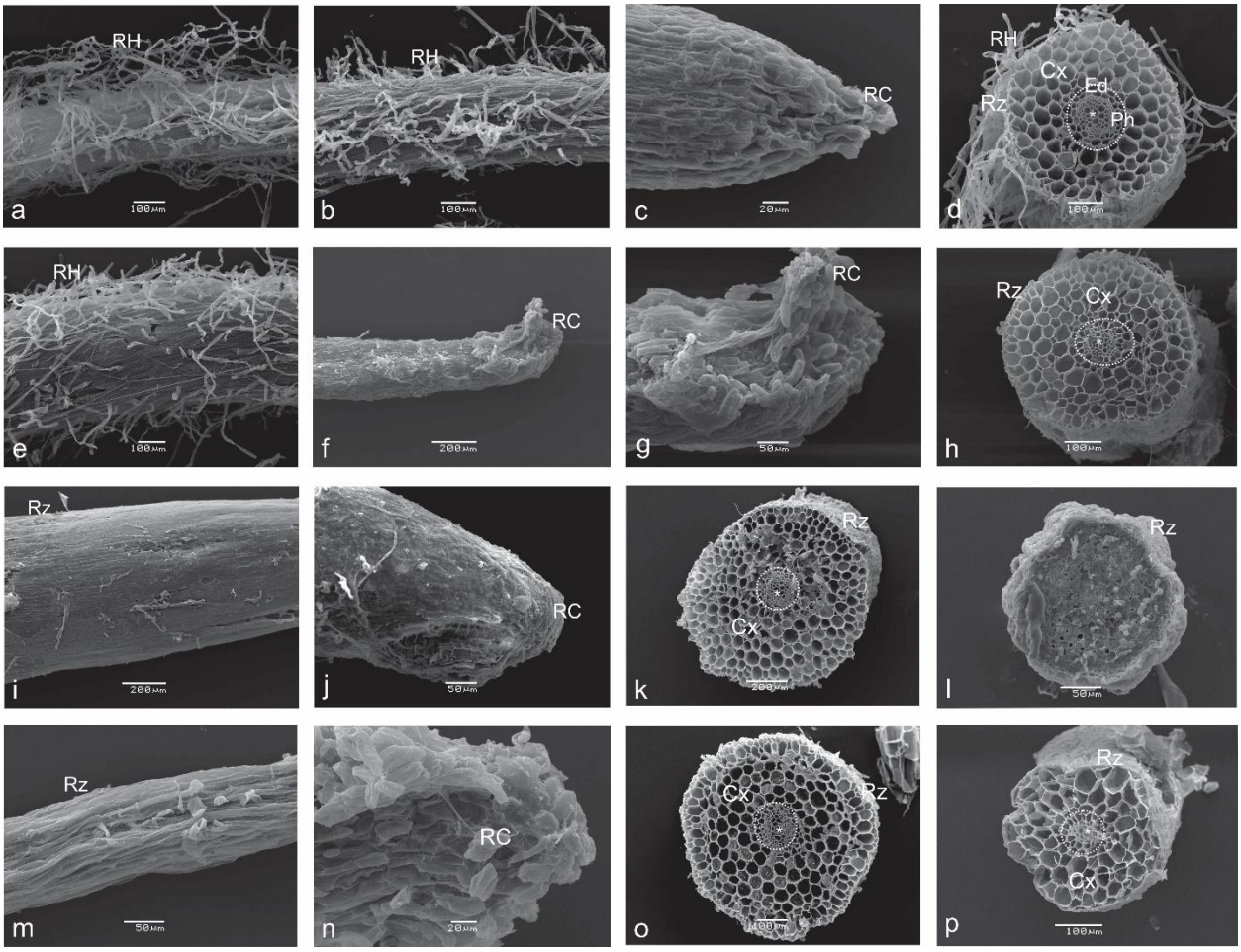
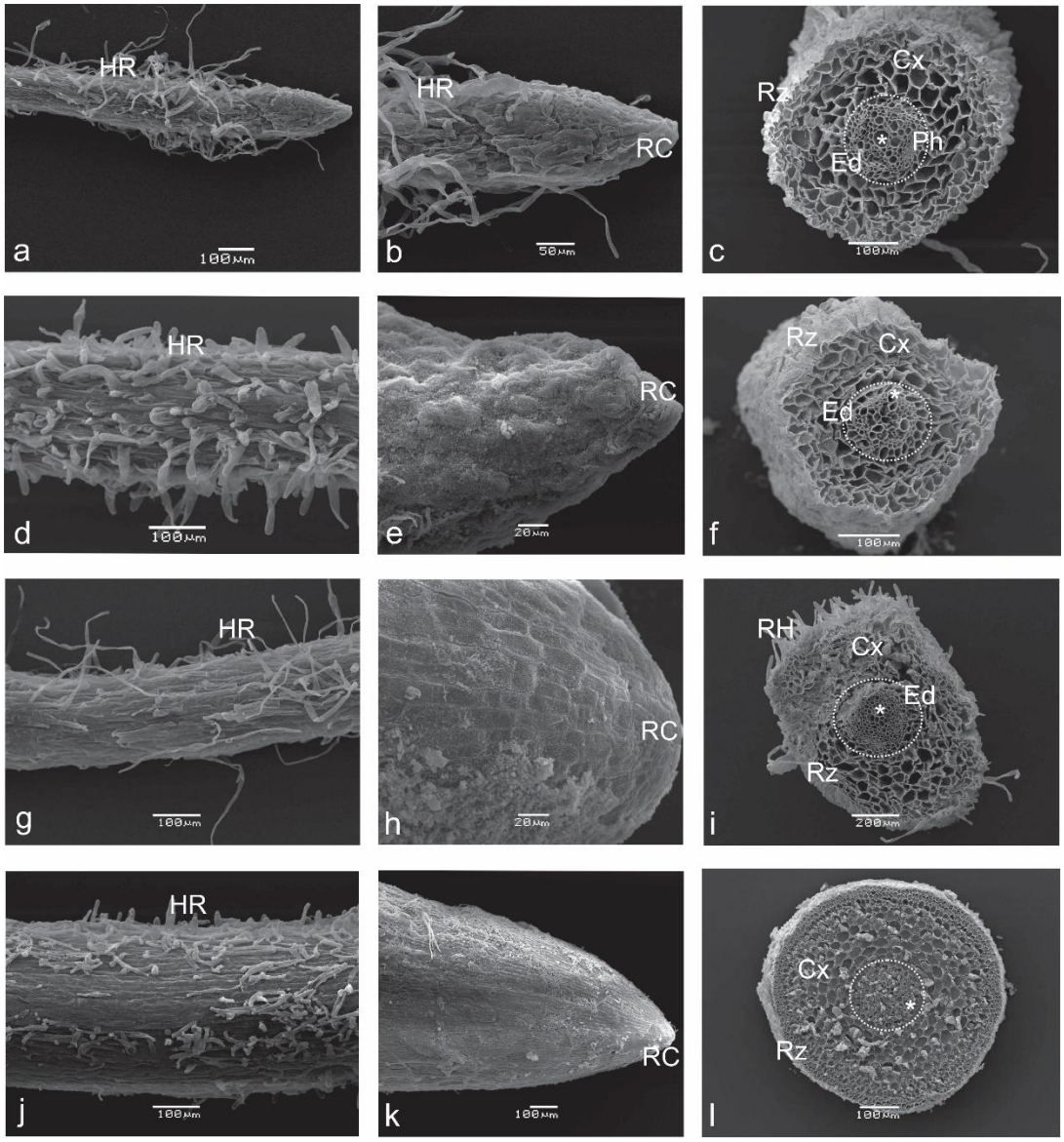


Figure 5. Scanning electron micrographs of radicles of *Datura stramonium* grown in water **(a, d)**, methanol **(b, c)**, and methanolic extracts of stems **(e-h)**, flowers **(i-l)**, and pods **(m–p)** of *Acacia dealbata*. Frontal view of the maturation zone **(a, b, e, j, m)** and the root cap zone **(c, f, g, j. n)**. Transverse sections of the maturation **(d, h, k, o)** and root cap **(l, p)** zones. The dashed white circle outlines the vascular cylinder. White asterisks indicate xylem elements. Abbreviations: Cx, cortex; Ed, endodermis; HR, root hairs; Ph, phloem; RC, root cap; RZ, rhizodermis.



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970 **Figure 6**. Scanning electron micrographs of the radicles of *Anoda cristata* grown in water **(a–b)**,
971 methanol **(c)**, and methanolic extracts of the stems **(d–f)**, leaves **(g–i)**, and pods **(j–l)** of *Acacia*
972 *dealbata*. Frontal view of the maturation zone **(a, d, g, j)** and the root cap zone **(b, e, h, k)**.
973 Transverse sections of the maturation zone **(c, f, i, l)**. The dashed white circle outlines the vascular
974 cylinder. White asterisks indicate xylem elements. Abbreviations: Cx, cortex; Ed, endodermis;
975 HR, root hairs; Ph, phloem; RC, root cap; RZ, rhizodermis.

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