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Essential Oil Potential of *Echinophora cinerea* Boiss: An Alternative to Systemic Herbicides in Agroecosystems

Ali Nasiri[1], Sina Fallah[*1], Amir Sadeghpour[2]

[1]*Department of Agronomy, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran*
[2]*Department of Plant, Soil, and Agricultural Systems, School of Agricultural Sciences, Southern Illinois University, Carbondale, USA*

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ABSTRACT

Weed management in agricultural ecosystems often relies on synthetic herbicides, which, despite their effectiveness, incur high costs and contribute to environmental pollution. The development and industrial production of plant-based herbicides could address these issues. In this context, the effects of varying concentrations of essential oil from Fedaleh (*Echinophora cinerea* Boiss) and glyphosate herbicides on *Chenopodium album* (L.) were examined through a factorial experiment conducted within a completely randomized design. Analysis of variance revealed that antioxidant enzyme activity, membrane lipid peroxidation, hydrogen peroxide and proline content, photosynthetic pigment concentrations, and seedling growth were influenced by the type and concentration of the inhibitor. A significant interaction between the type of inhibitor and its concentration was observed regarding the activities of catalase and superoxide dismutase enzymes, membrane lipid peroxidation, hydrogen peroxide and proline content. The accumulation of hydrogen peroxide and alterations in the levels of chlorophyll-b, carotenoids, as well as the enzymes catalase, superoxide dismutase, and ascorbate peroxidase, and to a certain extent, proline content in plants treated with the essential oil from the Fedaleh flower were comparable to those observed with glyphosate. These effects resulted in a similar reduction in the weight of the *C. album* seedlings treated with the essential oil from the Fedaleh flower, analogous to the glyphosate treatment. Additionally, the levels of chlorophyll-a and seedling length demonstrated an inverse linear relationship with the concentration of the essential oil from the Fedaleh flower. Taken together, the essential oil from the Fedaleh flower shows significant potential as an inducer of oxidative stress and disruptor of the plant defense system, effectively suppressing the growth of the *C. album*. Hence, its comparable efficacy to glyphosate suggests promising prospects for its industrial production as a plant-based herbicide.

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1. Introduction

Food security and safety are critical components of human health. The global population, projected to grow from the current 7.7 billion to 8.9 billion by 2050, places the agricultural sector in the pivotal role of fulfilling the food demands of this increasing population (Hernandez-Tenorio *et al*., 2022). Crops significantly contribute to agricultural production and global nutrition, providing approximately 90% of energy and 80% of protein required by humans (Berners-Lee *et al*., 2018). Therefore, addressing factors that negatively impact crop production is of paramount importance (Nasiri *et al*., 2024a).

Weeds represent a critical damaging factor in agricultural production across developing and developed nations, markedly decreasing crop yields more than pathogens (Madadi *et al*., 2022; 2023). Beyond competing with crops, weeds serve as hosts for crop pests and pathogens (Jabran *et al*., 2015), and their uncontrolled proliferation can lead to substantial yield losses (Horvath *et al*., 2023). Globally, weed damage accounts for approximately 34% of the harm caused by significant crop pests (Jabran and Farooq, 2012). *C. album* (L.) is among the most significant weed species affecting agricultural systems, particularly in summer crops such as maize (*Zea mays* L.), soybean (*Glycine*

* Corresponding author.
E-mail addresses: falah1357@yahoo.com; fallah-s@sku.ac.ir

max (L.) Merr.) and potato (*Solanum tuberosum* L.) (Bajwa *et al*., 2019), and ranks as one of the top ten weed species in soybean and maize cultivation (Fried *et al*., 2019). This weed can produce around 500,000 seeds, with a seed lifespan in soil estimated at 30 to 40 years (Bajwa *et al*., 2019).

Synthetic herbicide application is a commonly employed technique for weed control in agricultural fields (Avila *et al*., 2023). However, excessive and indiscriminate use of these chemicals has raised environmental and human health concerns (Madadi *et al*., 2022; 2023), and a significant rise in herbicide-resistant weed ecotypes poses a serious threat to agriculture (Hulme, 2023). The highest instances of herbicide-resistant weed species across various crops have been observed in the following order: wheat > maize > rice > soybean > spring barley > rapeseed > cotton (Heap, 2023).

Among alternative methods, using natural plant products has gained considerable attention due to their lack of residual effects (Madadi *et al*., 2022; 2023). Certain plants possess allelochemical compounds, such as phenols and flavonoids, which can influence the germination, growth, and development of target plants, acting as natural herbicides (Elisante *et al*., 2013).

Research indicates that many aromatic plants, due to their substantial production of essential oils, can deter grazers and pests and inhibit the growth of competing neighbor plants through their allelopathic effects (Aungtikun *et al*., 2021; Han *et al*., 2021). Essential oils and their components, primarily terpenoids, are promising candidates for formulating new bioherbicides, whether as pure natural compounds or in mixtures, owing to their structural diversity and notable phytotoxicity (De Mastro *et al*., 2021). Essential oils and terpenoids have been extensively studied for their phytotoxic properties, and their modes of action are well-documented (Verdeguer *et al*., 2020). Successful commercial herbicides, such as Burnout II (Ahuja *et al*., 2015) and Ecotrol Plus (Zhou *et al*., 2021), derived from plant essential oils, have already been introduced to the market.

Hazrati et al. (2018) examined the essential oil composition of three rosemary species (*Rosmarinus officinalis* L., *Satureja hortensis* L., and *Laurus nobilis*) on *Amaranthus retroflexus* (L.) and *Bromus tectorum* (L.). These essential oils significantly inhibited the germination and seedling growth of the tested species. Post-emergence application of essential oil from *Artemisia scoparia* six weeks after treatment on *Echinochloa crus-galli*, *Ageratum conyzoides*, *Parthenium hysterophorus*, *Achyranthes aspera*, and *Cassia occidentalis* resulted in symptoms ranging from chlorosis to complete necrosis within one to seven days. The most notable effects were observed on *E. crus-galli* and *P. hysterophorus*. The essential oil disrupted both photosynthesis and chlorophyll content in the weeds, affecting their respiratory metabolism (Kaur *et al*., 2010). A study by Verma et al. (2017) on the essential oil of *Achillea millefolium* (L.) indicated that the allelopathic effects reduced root and shoot lengths in *Lactuca sativa* seedlings. Research by Sabzi Nojadeh et al. (2021) on the chemical composition of *Foeniculum vulgare* (Mill.) essential oil and its phytotoxic effects on *Convolvulus arvensis* (L.) identified trans-anethole, estragole, fenchone, and limonene as the main components. The phytotoxic activity of this oil significantly inhibited germination, early growth, root length, seedling length, and fresh weight of ivy seedlings compared to controls. Sharma et al. (2019) investigated the essential oil of *Hyptis suaveolens* on *E. crus-galli*, finding that it primarily contained monoterpenoids such as α-phellandrene, α-pinene, and limonene. Biological tests demonstrated that these compounds completely inhibited the growth, germination, and seedling development of *E. crus-galli*, leading to reduced chlorophyll content and cell viability, ultimately resulting in complete wilting.

E. cinerea, known as Fedaleh, grows in the Zagros, Alborz, and Azerbaijan mountains and possesses aromatic and medicinal qualities (Mozaffarian, 2012). This perennial species reaches heights of 30 to 100 cm, features cylindrical, glabrous stems, alternate needle-shaped leaves, small yellow flowers arranged in umbellate inflorescences, straight conical roots, and hazelnut-like fruit containing tiny seeds. It flourishes on the slopes of the Zagros mountains, and its foliage serves as a food additive, holding significant value in botany, medicine, and food science due to its aroma (Nasiri *et al*., 2024b). The essential oil and extract derived from *E. cinerea* are primarily composed of monoterpenoids, including α-phellandrene, α-pinene, β-phellandrene, and p-cymene, with sesquiterpenoids, phenolic compounds, and flavonoids as secondary components (Ghasemi Pirbalouti and Gholipour, 2016). In light of the need to reduce chemical herbicide

use for sustainable agriculture, alternative weed control methods are essential. Plant-derived herbicides could effectively decrease reliance on chemical herbicides. *C. album* is one of the major weeds in the Faryab agricultural system, making the success of *E. cinerea* essential oil in controlling this weed vital for managing summer crop weeds. Consequently, various concentrations of Fedaleh essential oil were evaluated against glyphosate herbicide for controlling *C. album* seedlings.

2. Materials and methods

2.1. Plant collection, seed and essential oil preparation

Various parts (stems, leaves, and flowers) of the Fedaleh plant were harvested in July 2019 from the Zagros Mountains (latitude 32◦13'34'' N, longitude 50◦20'21'' E; altitude 2587 m.a.s.l.). The collected plant materials were dried in a shaded area with adequate ventilation, and the dried parts were stored in a paper bag in the refrigerator until essential oil extraction. Before oil extraction, the dried plant parts were thoroughly crushed using an electric grinder. Essential oil was extracted using a Clevenger apparatus according to the British Pharmacopoeia (1988). For each extraction, 400 grams of the desired Fedaleh were mixed with one liter of distilled water in the Clevenger device, and the essential oil was extracted over three hours. The essential oil was collected with sodium sulfate at a dehydration rate of 15 grams, and its composition was analyzed using GC-MS (Nasiri *et al*., 2024b). Seeds of *C. album* plants from potato fields in Kiar County were collected and stored in a dedicated bag.

2.2. Experimental setup

The experiment was designed as a factorial experiment in a completely randomized arrangement with three replications, conducted in the greenhouse of the Faculty of Agriculture at Shahrekord University in 2022. The treatments included glyphosate herbicide at four concentrations (0.5, 1, 2, and 5 µl ml^{-1}) and essential oils from various parts of the plant (stem, leaf, and flower) at four concentrations (0.5, 1, 2, and 5 µl ml^{-1}). An appropriate surfactant (Tween-20; 2.5% vol/vol) was added to all concentrations, with control treatment (distilled water) included, each with three replications. On May 31, 2022, 350 grams of sterilized soil were placed in a plastic pot, and 20 *C. album* seeds were sown in each pot. The pots were watered regularly, and after 20 days, the seeds germinated. Uniform seedlings were maintained in each pot. Glyphosate herbicide and essential oils from different Fedaleh plant parts were added to 100 ml according to the designated treatments and sprayed on the seedlings in each pot on June 20 and June 25, 2022 (Sharma *et al*., 2019). Ten days post-spraying (July 5, 2022), various parameters were measured.

2.3. Measurement of traits

2.3.1. Seedling length

At the conclusion of the experiment, the seedling length (from the surface of the pot to the top of the seedling) was measured and recorded with a ruler.

2.3.2. Seedling weight

The seedlings that received the treatment were cut at the soil surface with scissors. Their fresh weight was measured, and the seedlings were then placed in an oven until the moisture content stabilized, after which their dry weight was recorded using a precise scale.

2.3.3. Concentration of photosynthetic pigments

To determine the concentrations of chlorophyll-a, chlorophyll-b, and carotenoids, 0.1 g of homogenized seedling leaves was centrifuged at 10,000 rpm. The absorbance of the resulting solution was measured with a UV-VIS spectrophotometer at wavelengths 440, 645, and 663 nm (Equations 1-3) (Lichtenthaler and Wellburn, 1983).

(1) $\text{Chlorophyll-a} = 12.7_{A663} - 2.69_{A645}$

(2) $\text{Chlorophyll-b} = 22.9_{A645} - 4.68_{A663}$

(3) $\text{Carotenoids} = 4.7_{A440} - (1.38_{A663} + 5.48_{A645})$

A indicates the quantity of light absorbed by the extract at specific wavelengths.

2.3.4. Determination of antioxidant enzyme activity

To extract enzymes and proteins, 0.1 g of *C. album* leaves was homogenized in 2 ml of phosphate buffer (50 mM, pH 7) and 150 mg of polyvinylpolypyrrolidone (PVPP) on ice using a mortar and pestle, then centrifuged at 10,000 rpm for 10 minutes. The supernatant served as the source for antioxidant enzymes and total soluble proteins, with total protein quantities assessed as described by Bradford (1976).

Catalase activity (CAT) was measured by tracking the reduction of hydrogen peroxide (H_2O_2) at 240 nm for 3 minutes using a spectrophotometer. The reaction mixture consisted of 600 µl of phosphate buffer (83.5 mM, pH 7), 300 µl of H_2O_2 (33 mM), and 100 µl of enzyme extract (Chance and Maehly, 1954).

Peroxidase activity (POD) was evaluated by transforming guaiacol to tetraguaiacol at 470 nm for 3 minutes using a spectrophotometer. The final mixture included 350 µl of phosphate buffer (28.57 mM, pH 7), 300 µl of H_2O_2 (16.6 mM), 300 µl of guaiacol, and 50 µl of enzyme extract (Chance and Maehly, 1954).

Ascorbate peroxidase (APX) activity was determined by measuring the reduction of ascorbic acid at 290 nm for 3 minutes using a spectrophotometer. The final reaction mixture included 600 µl of phosphate buffer (50 mM, pH 7), 400 µl of 0.2 mM ethylene diamine tetraacetic acid tetrasodium salt dihydrate (EDTA), 400 µl of 0.5 mM ascorbic acid, 100 µl of 0.1 mM H_2O_2, and 100 µl of enzyme extract (Scandalios, 1997).

Superoxide dismutase (SOD) activity was assessed based on the inhibition of nitro blue tetrazolium (NBT) protection against diformazan, produced by the superoxide radicals generated through riboflavin photolysis. The reaction mixture consisted of 2. 65 ml of phosphate buffer (0.067 mM, pH 7.8), 200 µl of 0.1 mM EDTA containing sodium cyanide (0.3 mM NaCN), 100 µl of 1.5 mM NBT, and 50 µl of extract. After 5 minutes, 50 µl of 0.12 mM riboflavin was added to the mixture, and the solution was incubated under 40 W light for 12 minutes. The absorbance was recorded at 560 nm. SOD activity is defined as the amount of enzyme required to inhibit 50% of NBT protection (Winterbourne *et al*., 1975).

2.3.5. Lipid peroxidation

This parameter was quantified by measuring malondialdehyde following the method of Boominathan and Doran (2002). Fresh leaf samples were ground in a mortar with 0.1% trichloroacetic acid and then centrifuged on ice at 10,000 rpm for 5 minutes.350 µl of the supernatant was combined with 1400 µl of 20% TCA containing 5% Thiobarbituric acid (TBA) and heated at 96°C for 30 minutes. The mixture was promptly transferred to an ice-water bath and centrifuged at 10,000 rpm for 15 minutes. The absorbance of the solvent was measured at 532 nm, and malondialdehyde levels were calculated using a standard curve prepared with 3,1,1,3-tetraethoxypropane.

2.3.6. Hydrogen peroxide content

The measurement of hydrogen peroxide was conducted following the protocol established by Loreto and Velikova (2001). Fresh leaf samples were ground with 0.1% trichloroacetic acid in a mortar, followed by centrifugation at 10,000 rpm for 5 minutes. A volume of 150 µl of the supernatant was combined with 650 µl of potassium phosphate buffer (10 mM, pH 7) and 200 µl of potassium iodide (1 mM KI). The absorbance at 390 nm was recorded after one hour. The concentration of hydrogen peroxide was determined using a standard curve generated from various hydrogen peroxide concentrations, expressed in µmol g^{-1} FW.

2.3.7. Proline content

Proline content was determined using the method described by Bates et al. (1973). Approximately 100 mg of stem and root samples from weed seedlings were placed in 3 ml of sulfosalicylic acid (3% w/v) and centrifuged at 2000 rpm for 10 minutes. The resulting supernatant (1 ml) was mixed with ninhydrin acid and frozen acetic acid in a 1:1:1 ratio, incubated in boiling water for one hour, and then cooled in an ice-water bath for 10 minutes. To this mixture, 4 ml of toluene was added, and the solution was mixed to separate the organic and inorganic phases. The organic phase was analyzed spectroscopically at 520 nm. Proline content was quantified using a calibration curve prepared with pure proline.

2.4. Statistical analysis

All data presented are averaged across three replicates for each treatment. Statistical analyses, including two-way analysis of variance (ANOVA) and post hoc multiple comparisons (least significant difference, LSD test), were performed using the software package SAS (version 9.1). The threshold for statistical significance was set at $p<0.05$. Data are presented as mean ± standard deviation (SD) and were visualized using Microsoft Excel 2016.

3. Results and discussion

The analysis of variance (ANOVA) revealed that peroxidase enzyme activity, membrane lipid

peroxidation, hydrogen peroxide levels, proline content, photosynthetic pigments concentration, seedling length, fresh weight, and dry weight were significantly affected by the type of inhibitor at the 1% probability level. Additionally, catalase, superoxide dismutase, and ascorbate peroxidase enzyme activities were significantly influenced by the inhibitor type at the 5% probability level. The concentration of the inhibitor also had a significant effect on all traits mentioned above at the 1% probability level. Furthermore, the interaction between inhibitor type and concentration was significant for catalase and superoxide dismutase enzyme activities and hydrogen peroxide content at the 1% probability level, and for membrane lipid peroxidation and proline content at the 5% probability level (Tables 1-4).

Table 1. Analysis of variance (mean square) of catalase (CAT), superoxide dismutase (SOD), Peroxidase (POX), and ascorbate peroxidase (APX) activity in *C. album* under the influence of different concentrations of Fedaleh essential oil and glyphosate herbicide.

S.O.V.	DF	CAT	SOD	POX	APX
Inhibitor type (IT)	3	30767*	485*	6.4**	72.7*
Inhibitor concentration (IC)	4	162497**	51690**	10.1**	194**
IT × IC	12	89166**	3270**	1.3^{ns}	34.0^{ns}
Error	40	7777	155	0.72	22.6
C.V. (%)		12.8	7.7	24.6	13.8

ns = not significant, * = significant at the 5% statistical probability level, ** = significant at the 1% statistical probability level.

Table 2. Analysis of variance (mean square) of hydrogen peroxide, malondialdehyde and proline content in *C. album* under the influence of different concentrations of Fedaleh essential oil and glyphosate herbicide.

S.O.V.	DF	Hydrogen peroxide	Membrane lipid peroxidation	Proline content
Inhibitor type (IT)	3	5591**	0.39**	4343**
Inhibitor concentration (IC)	4	12302**	0.90**	37712**
IT × IC	12	376**	0.03*	362*
Error	40	108	0.01	139
C.V. (%)		5.5	7.7	5.0

* = significant at the 5% statistical probability level, ** = significant at the 1% statistical probability level.

Table 3. Analysis of variance (mean square) of photosynthesis pigments in *C. album* under the influence of different concentrations of Fedaleh essential oil and glyphosate herbicide.

S.O.V.	DF	Chlorophyll-*a*	Chlorophyll-*b*	Carotenoids
Inhibitor type (IT)	3	48034**	2947**	920**
Inhibitor concentration (IC)	4	546738**	42346**	20468**
IT × IC	12	4206^{ns}	590^{ns}	209^{ns}
Error	40	3547	329	173
C.V. (%)		14.4	8.3	9.0

ns = not significant, ** = significant at the 1% statistical probability level.

Table 4. Analysis of variance (mean square) of seedling length, seedling fresh weight, seedling dry weight in *C. album* under the influence of different concentrations of Fedaleh essential oil and glyphosate herbicide.

S.O.V.	DF	Seedling length	Seedling fresh weight	Seedling dry weight
Inhibitor type (IT)	3	249**	0.73**	0.0003**
Inhibitor concentration (IC)	4	2537**	4.4**	0.005**
IT × IC	12	26.2*	0.06*	0.00003^{ns}
Error	40	11.8	0.03	0.00004
C.V. (%)		10.2	13.2	21.2

ns = not significant, ** = significant at the 1% statistical probability level.

3.1. Catalase enzyme (CAT)

In Fig. 1a, it is shown that increasing the concentration of herbicide and essential oil up to 1 µl ml^{-1} enhanced CAT activity in *C. album*, followed by a decrease (P<0.05). CAT activity levels between leaf and stem essential oils were similar across all concentrations. The peak CAT activity occurred at a concentration of 1 µl ml^{-1} for glyphosate and flower essential oil (Fig. 1a). There was no significant difference in CAT activity between 1 and 2 µl ml^{-1} for Fedaleh flower essential oil and glyphosate. However, at concentrations ≥ 2 µl ml^{-1}, CAT activity levels were significantly lower than the control (P<0.05; Fig. 1a).

3.2. Superoxide dismutase enzyme (SOD)

The pattern of SOD activity changes in response to the application of essential oils from various plant parts and glyphosate at 0.5 and 1 µl ml^{-1} was similar to that of CAT, but differed at 2 and 5 µl ml^{-1} (Fig. 1b). At a concentration of 2 µl ml^{-1} for leaf and flower essential oils, SOD activity exceeded the control, while glyphosate and flower essential oils significantly inhibited SOD activity. At 5 µl ml^{-1}, all essential oils and glyphosate significantly reduced SOD activity levels (P<0.05; Fig. 1b).

3.3. Peroxidase enzyme (POX)

There were no significant differences in POX levels between the stem and leaf essential oils of Fedaleh; however, glyphosate and flower essential oils significantly increased POX levels (Fig. 2a). Relative to the control, POD enzyme activity rose by 45%, 76%, and 80% at concentrations of 0.5, 1, and 2 µl ml^{-1} of the inhibitors (P<0.05). The concentration of 5 µl ml^{-1} did not show a significant difference from the control (Fig. 2b).

3.4. Ascorbate peroxidase enzyme (APX)

The APX enzyme activity in *C. album* treated with Fedaleh flower essential oils was not significantly different from that induced by glyphosate (P>0.05, Fig. 2c), whereas the APX activity induced by glyphosate was significantly different from that of the essential oils of the stem and leaves of Fedaleh (P<0.05; Fig. 2c). The activity reduction of the APX enzyme at inhibitor concentrations of 1 and 2 µl ml^{-1} was 21% and 18% higher, respectively, compared to the control (P<0.05; Fig. 2d). Conversely, no significant differences from the control were observed at concentrations of 0.5 and 5 µl ml^{-1} (P>0.05; Fig. 2d).

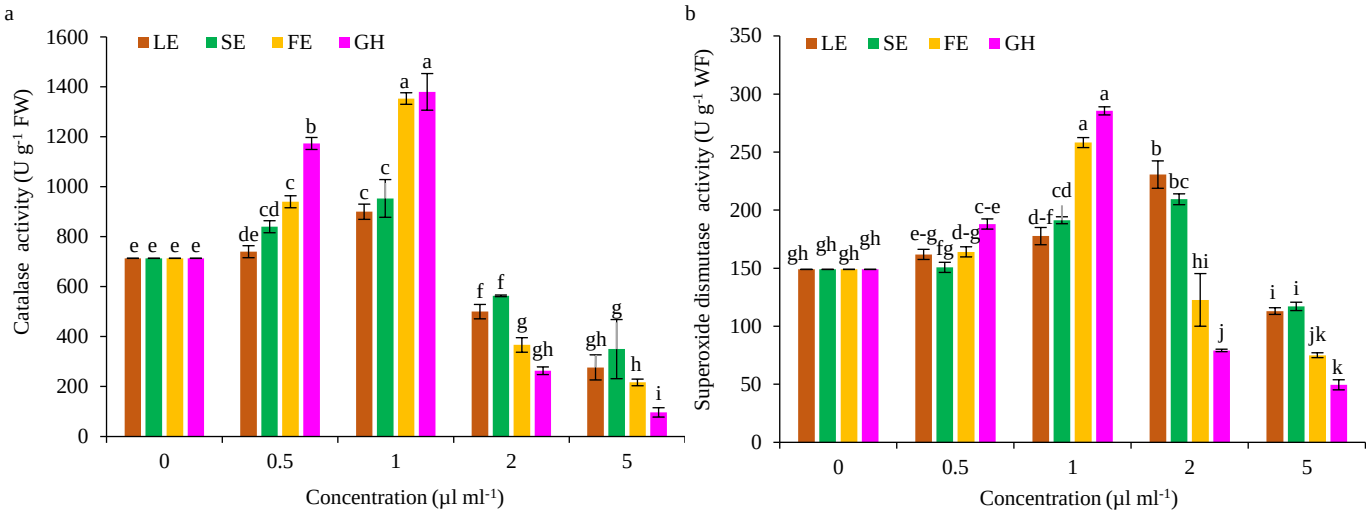

Figure 1. Interaction effect of type and concentration of growth inhibitor for catalase (a) and superoxide dismutase enzymes (b) activity in *C. album* weed. Means with different letters are statistically significant based on LSD test at 5% probability level. LE, SE, FE and GH represent essential oil of leaves, stems and flowers of Fedaleh and herbicide glyphosate, respectively. Bars indicate standard deviation (±SD).

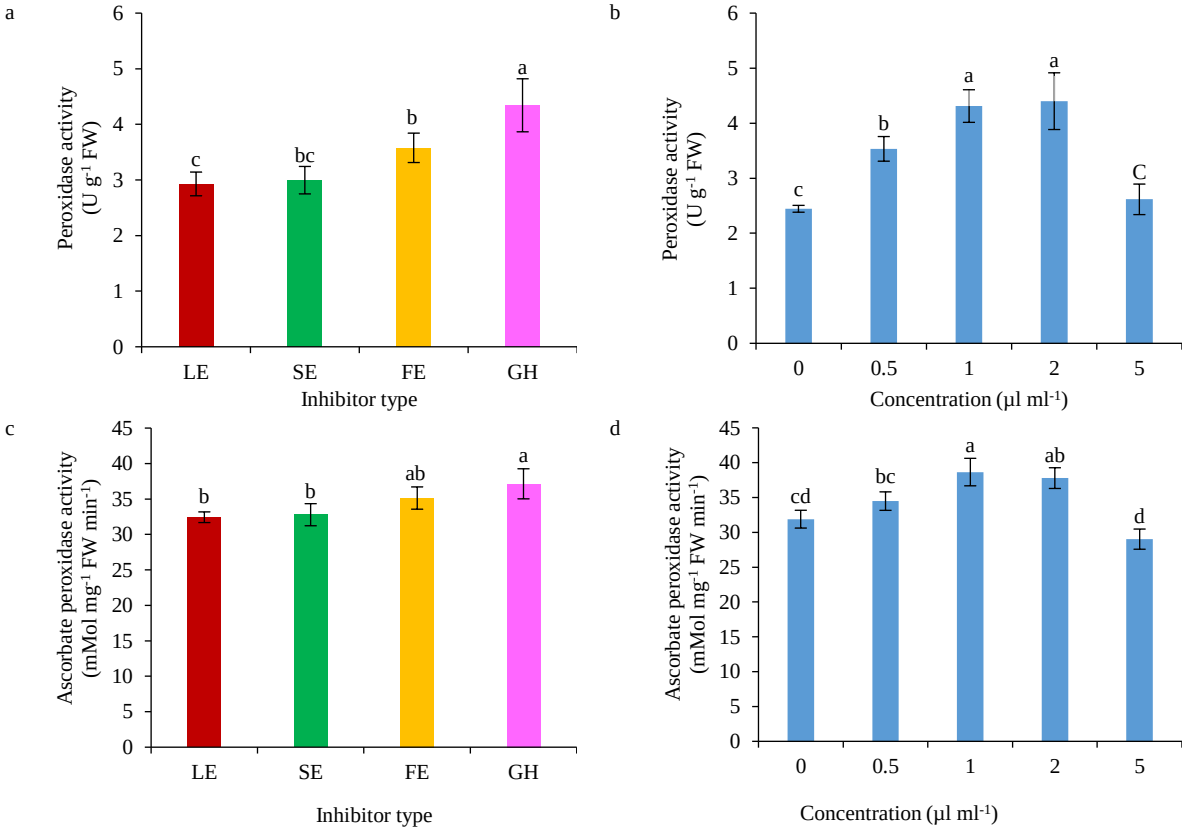

Figure 2. Effect of type and concentration of growth inhibitor on peroxidase (a,b) and ascorbate peroxidase (c,d) activity in *C. album* weed. Means with different letters are statistically significant based on LSD test at 5% probability level. LE, SE, FE and GH represent essential oil of leaves, stems and flowers of Fedaleh and herbicide glyphosate, respectively. Bars indicate standard deviation (±SD).

3.5. Hydrogen peroxide

The threshold for increasing H_2O_2 in *C. album* seedlings treated with glyphosate herbicide and essential oil from Fedaleh flowers was 0.5 µl ml^{-1}, while for the essential oils of Fedaleh leaves and stems, it was 1 µl ml^{-1}. Additionally, the increase in H_2O_2 in response to flower essential oil was significantly higher than that from leaf and stem essential oils; at concentrations of 2 and 5 µl ml^{-1}, flower essential oil elevated H_2O_2 levels comparably to glyphosate (P>0.05; Fig. 3a). Compared to the control, *C. album* exposed to 2-5 µl ml^{-1} of leaf, stem, and flower essential oils, as well as glyphosate herbicide, demonstrated increases in H_2O_2 of 26-43%, 29-43%, 53-68%, and 48-74%, respectively (P<0.05; Fig. 3a).

3.6. Membrane lipid peroxidation

At every concentration, the level of membrane lipid peroxidation in *C. album* treated with leaf and stem essential oils was comparable, with an increase threshold beginning at 1 µl ml^{-1}. The threshold for membrane lipid peroxidation in *C. album* treated with Fedaleh flower essential oil was lower than that of stem and leaf essential oils (Fig. 3b). Compared to the control, the levels of membrane lipid peroxidation in *C. album* exposed to 0.5-5 µl ml^{-1} of leaf, stem, flower essential oils, and glyphosate herbicide increased by 9-49%, 2-50%, 25-74%, and 44-91%, respectively (Fig. 3b).

3.7. Proline content

C. album exposed to 0.5-5 µl ml^{-1} of leaf, stem, flower essential oils, and glyphosate herbicide showed increases in proline of 1-69%, 1-66%, 10-82%, and 19-94%, respectively, compared to the control (Fig. 3c). Proline levels in *C. album* treated with 0.5, 1, and 2 µl ml^{-1} of Fedaleh flower essential oil were similar to those treated with glyphosate (P<0.05; Fig. 3c), while at a concentration of 5 µl ml^{-1}, proline levels were lower than those in glyphosate-treated samples (Fig. 3c).

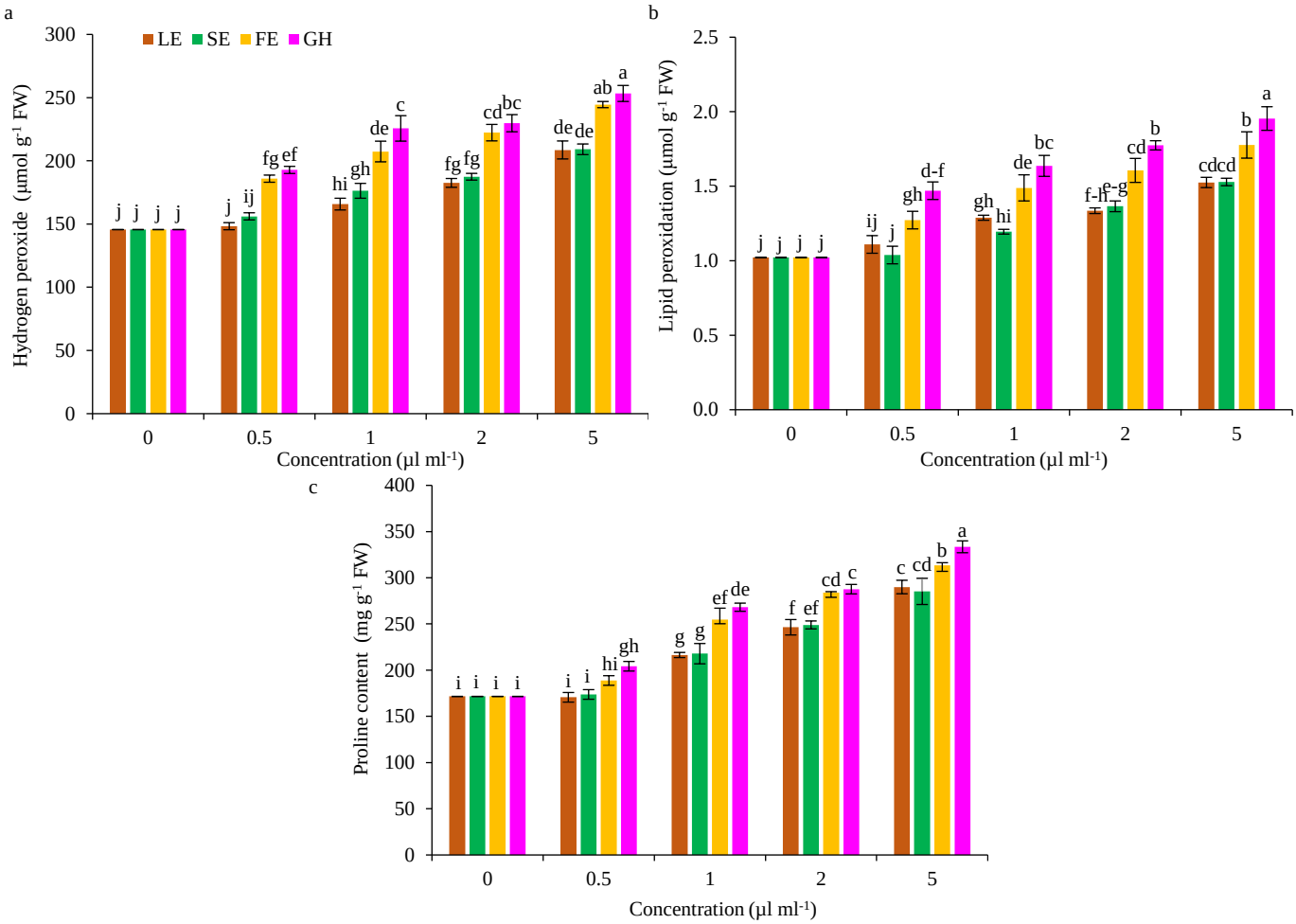

Figure 3. Interaction effect of type and concentration of growth inhibitor for hydrogen peroxide (a), membrane lipid peroxidation (b), and proline content (c) in *C. album* weed. Means with different letters are statistically significant based on LSD test at 5% probability level. LE, SE, FE and GH represent essential oil of leaves, stems and flowers of Fedaleh and herbicide glyphosate, respectively. Bars indicate standard deviation (±SD).

3.8. Chlorophyll-a and Chlorophyll-b

The reduction rate of chlorophyll-a in *C. album* treated with flower essential oil was significantly lower than that observed with leaf and stem essential oils, yet still higher than that of glyphosate (P<0.05; Fig. 4a). A dose-dependent inverse linear trend was noted for chlorophyll-a (Table S1). Compared to the control, reductions in chlorophyll a at inhibitor concentrations of 0.5, 1, 2, and 5 µl ml^{-1} were 21%, 45%, 59%, and 77%, respectively (P<0.05; Fig. 4b). The chlorophyll-b content in *C. album* exposed to the essential oil from the Fedaleh flower was comparable to that affected by the herbicide glyphosate (P>0.05, Fig. 4c). However, the reduction in chlorophyll-b content was significantly different compared to the essential oils from the leaves and stems of Fedaleh (P<0.05; Fig. 4c). Variations in chlorophyll-b levels were observed at different concentrations of inhibitors. Relative to the control, chlorophyll-b reduction at 0.5, 1, 2, and 5 µl ml^{-1} concentrations of the inhibitors was 11%, 27%, 40%, and 49%, respectively (P<0.05; Fig. 4d).

3.9. Carotenoids

The carotenoid levels in *C. album* treated with the essential oil of the Fedaleh flower showed no significant difference from those treated with glyphosate. In contrast, the carotenoid content in seedlings treated with essential oil from the leaves and stems of Fedaleh remained consistent (P>0.05, Fig. 4e). Compared to the control, the carotenoid reduction rates at inhibitor concentrations of 0.5, 1, 2, and 5 µl ml^{-1} were 19%, 32%, 40%, and 52% (P<0.05; Fig. 4f).

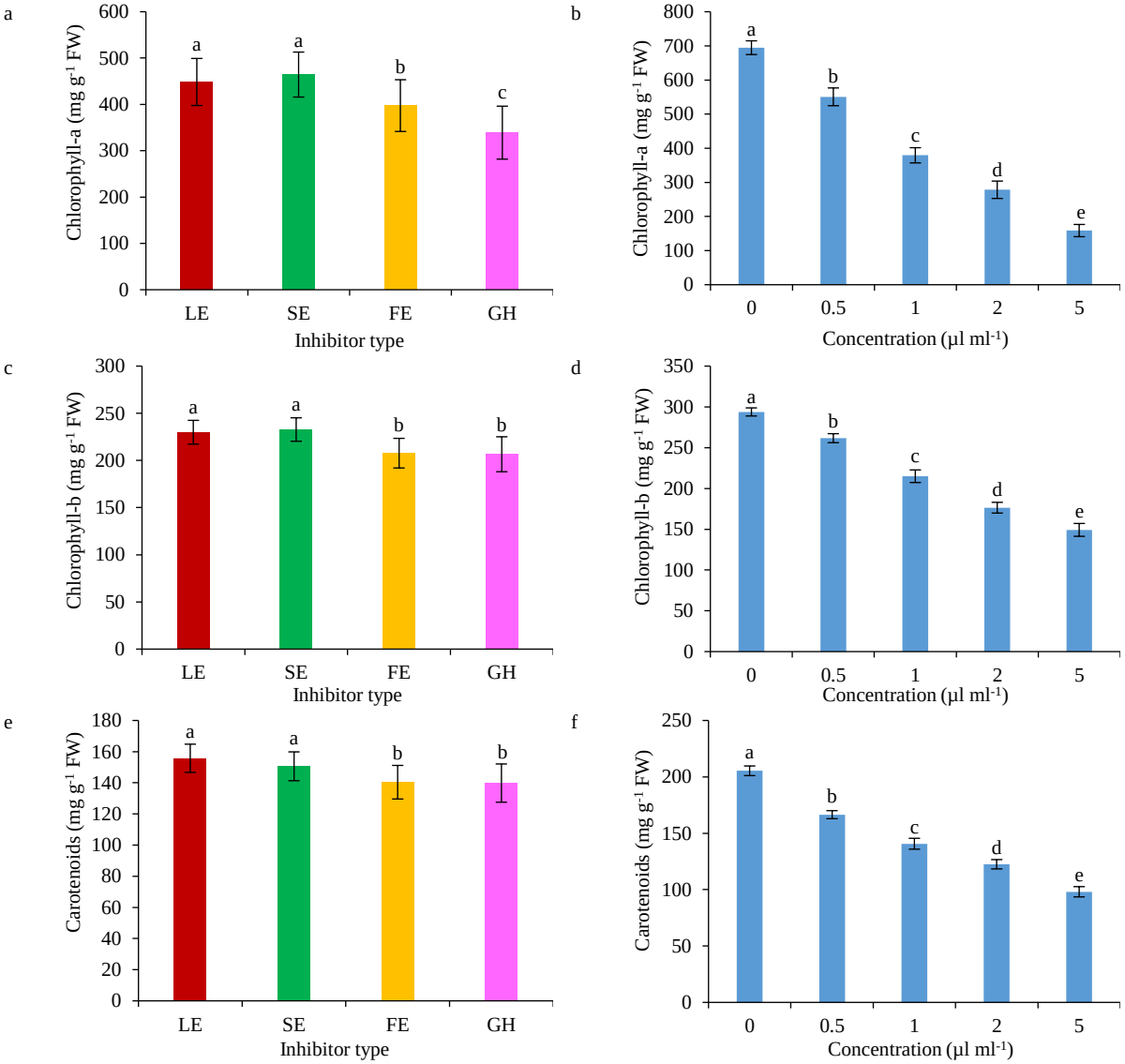

Figure 4. Effect of type and concentration of growth inhibitor on chlorophyll-a (a,b), chlorophyll-b (c,d), and carotenoids (e,f) in *C. album* weed. Means with different letters are statistically significant based on LSD test at 5% probability level. LE, SE, FE and GH represent essential oil of leaves, stems and flowers of Fedaleh and herbicide glyphosate, respectively. Bars indicate standard deviation (±SD).

3.10. Seedling length

The essential oil from Fedaleh flowers significantly curtailed the seedling length of *C. album* compared to the oils from leaves and stems (P<0.05; Fig. 5a). Nevertheless, glyphosate treatment resulted in the shortest seedling length, which was 5% less than that of Fedaleh flower essential oil (P<0.05; Fig. 5a). Fig. 5b indicates that concentrations of ≥ 0.5 µL ml^{-1} of essential oil from various plant parts and glyphosate herbicide substantially reduced seedling length compared to the control (P<0.05). The seedling length diminished by 29%, 40%, 50%, and 63%, respectively, with increasing concentrations of essential oil and glyphosate herbicide compared to the control (Fig. 5b).

3.11. Seedling fresh weight

C. album seedlings treated with flower and stem essential oils experienced a significant reduction in fresh weight compared to those treated with leaf essential oil from the Fedaleh plant (P<0.05; Fig. 5c). No significant difference was found in the fresh weight of *C. album* seedlings treated with flower essential oil relative to glyphosate (P>0.05, Fig. 5c). An inverse linear relationship was observed between seedling fresh weight and inhibitor concentration (Table S1). The lowest fresh weight for *C. album* was recorded at a concentration of 5 µl ml^{-1}, 73% lower than the control (Fig. 5d).

3.12. Seedling dry weight

The response of *C. album* dry weight to spraying essential oil from the leaves and stems of the plant was similar. The reduction effect on dry weight in *C. album* sprayed with the essential oils of the leaves and stems, as well as with glyphosate, was significantly greater than that observed with the oils alone (P<0.05; Fig. 5e). However, no significant difference was noted between the essential oils of leaves and stems and glyphosate (P>0.05; Fig. 5e). As the concentration of inhibitory substances increased, a significant decrease in *C. album* dry weight was recorded; at 5 µl ml^{-1}, the reduction compared to the control was 76% (P<0.05; Fig. 5f).

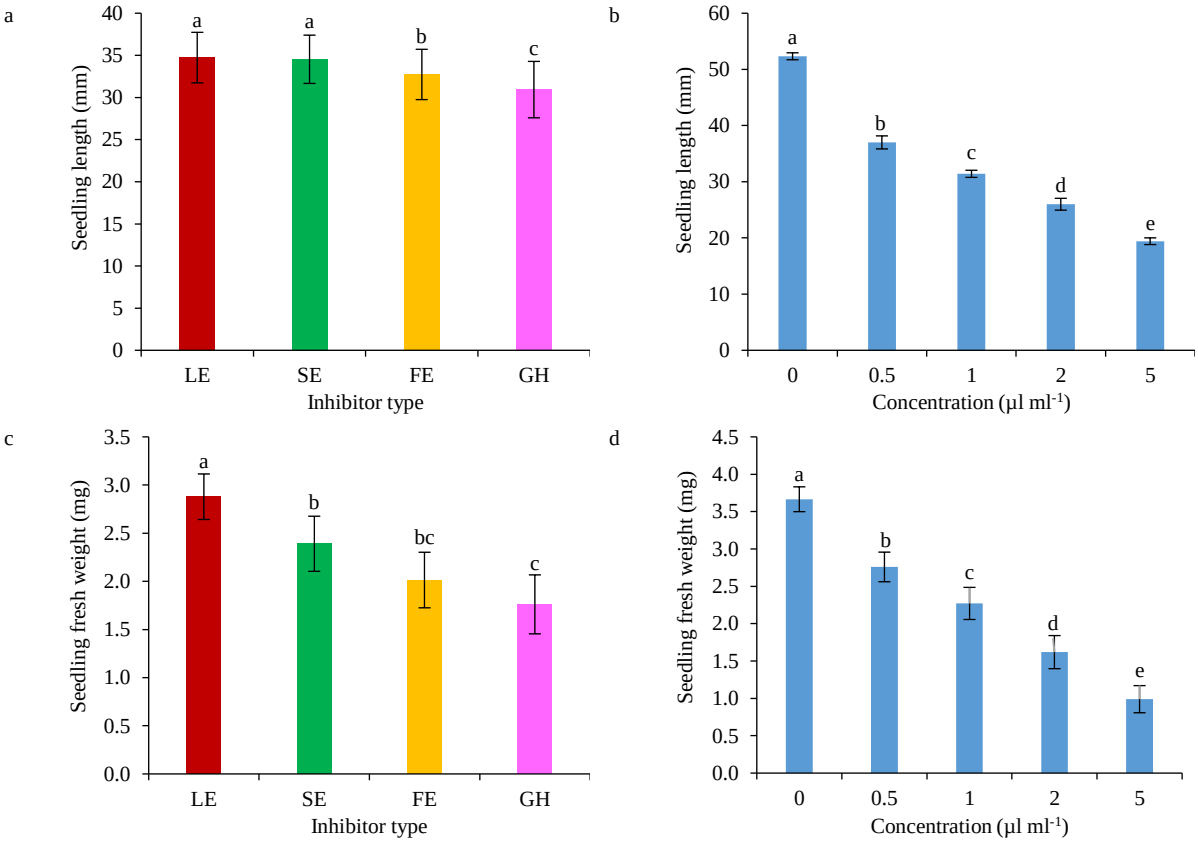

Figure 5. Effect of type and concentration of growth inhibitor on seedling length (a,b), and seedling fresh weight (c,d) in *C. album* weed. Means with different letters are statistically significant based on LSD test at 5% probability level. LE, SE, FE and GH represent essential oil of leaves, stems and flowers of Fedaleh and herbicide glyphosate, respectively. Bars indicate standard deviation (±SD).

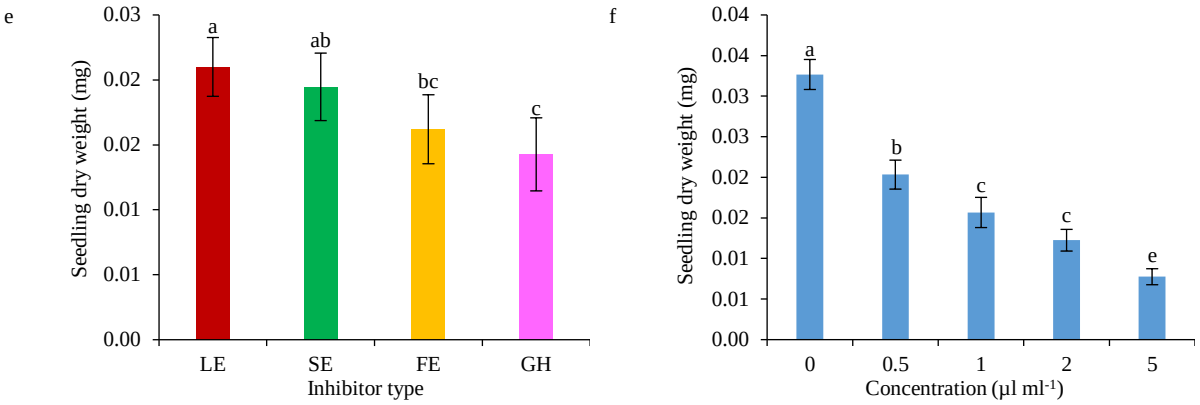

Figure 5 (continued). Effect of type and concentration of growth inhibitor on seedling dry weight (e,f) in *C. album* weed. Means with different letters are statistically significant based on LSD test at 5% probability level. LE, SE, FE and GH represent essential oil of leaves, stems and flowers of Fedaleh and herbicide glyphosate, respectively. Bars indicate standard deviation (±SD).

The pronounced inhibition of antioxidant enzymes, including catalase, superoxide dismutase, and ascorbate peroxidase, observed when applying essential oil from flowers at the same concentration as glyphosate herbicide can be attributed to the presence of compounds such as linalool, camphene, α-pinene, caryophyllene oxide, β-pinene, β-myrcene, α-phellandrene, β-phellandrene, sabinol, verbenol, and (-)-spathulenol in the essential oil (Nasiri *et al*., 2024b). Other studies indicate that the catalase enzyme activity in *Lolium perenne* plants, influenced by *Litsea pungens* essential oil containing α-pinene, β-pinene, β-myrcene, linalool, and eucalyptol at a concentration of 0.25 mg ml^{-1}, first increased and then decreased, hitting a minimum at 2 mg ml^{-1} (Kong *et al*., 2021). Additionally, compounds such as germacrene D, α-pinene, β-pinene, β-myrcene, caryophyllene oxide, and caryophyllene in *Ambrosia artemisiifolia* (L.) essential oil initially enhanced SOD enzyme activity in *Poa annua*, *Setaria viridis*, and *Amaranthus retroflexus* at concentrations of 0.25 and 0.5 mg ml^{-1}, but at higher concentrations of 2.5 mg ml^{-1}, this activity diminished or dropped to zero (Han *et al*., 2021). Furthermore, the presence of geranyl acetate, geranial, neral, and geraniol in *Dracocephalum moldavica* (L.) essential oil increased POD enzyme activity by 37% in *Bromus tectorum* at a concentration of 0.25 µl ml^{-1}. In comparison, a higher concentration of 1 µl ml^{-1} decreased this activity by 82% (Pouresmaeil *et al*., 2022). The APX enzyme activity in *Convolvulus arvensis* was significantly elevated by 27% with 1% *Artemisia fragrans* essential oil, containing α-thujone, camphor, 1,8-cineole, and β-thujone, but decreased by 36% at a concentration of 4% (Pouresmaeil *et al*., 2020). Antioxidant enzymes are crucial in eliminating reactive oxygen species (ROS) in plant cells. Consequently, excessive inhibition of these enzymes by monoterpenoids results in the accumulation of ROS, adversely affecting plant cell viability (Mutlu *et al*., 2011). The APX enzyme is located in chloroplasts, cytosol, mitochondria, peroxisomes, and the apoplast. In contrast, the CAT enzyme is primarily found in peroxisomes, giving APX a greater affinity for H_2O_2 than CAT. Thus, APX regulates H_2O_2 levels precisely, while CAT is mainly responsible for H_2O_2 removal during oxidative stress (Mutlu *et al*., 2011). The findings of this study indicated that H_2O_2 production from Fedaleh essential oil increased in a concentration-dependent manner. Conversely, the activities of CAT, SOD, and APX enzymes in *C. album* initially rose at low concentrations of glyphosate herbicide and Fedaleh essential oil but then significantly decreased at higher concentrations. Insufficient activity of scavenging enzymes hinders the effective removal of free radicals, including H_2O_2, leading to an accumulation of ROS, oxidative stress, and the eradication of *C. album*.

The impact of flower essential oil on the increase of H_2O_2 in *C. album*, alongside its interaction with glyphosate, underscores that oxidative stress may significantly inhibit the essential oil of *C. album*. A separate study indicated that compounds like geranyl acetate, geranial, neral, and geraniol in the essential oil of *Dracocephalum moldavica* (L.) at a concentration of 1 µl ml^{-1} elevated H_2O_2 levels in the *Bromus tectorum* (L.) by 77% (Pouresmaeil *et al*., 2022). The overproduction of ROS, including H_2O_2, singlet oxygen ($^1O_2$), hydroxyl radical ($HO^{\bullet}$), and superoxide (O_2^{-}), occurs in plant cells during photosynthesis and respiration in chloroplasts, mitochondria, and

peroxisomes under stress (Tripathy and Oelmüller, 2012). Concurrently, plants activate their antioxidant systems extensively, including both enzymatic and non-enzymatic mechanisms, to eliminate ROS (Dumanović *et al*., 2021). In this study, the levels of antioxidant enzymes such as CAT, POD, APX, and SOD increased at low glyphosate and Fedaleh essential oil concentrations. Still, at high concentrations, these levels alongside carotenoids significantly decreased, indicating a disruption in the biosynthesis of defense substances in *C. album*, impairing the plant's ability to scavenge free radicals. Monoterpenoids have been shown to disrupt the photosynthetic system in plants, potentially leading to ROS production and oxidative stress (Pouresmaeil *et al*., 2020). This stress further contributes to lipid peroxidation and increased plant membrane permeability (Sharma *et al*., 2012). The lipid peroxidation observed in plants treated with Fedaleh essential oil suggests that this essential oil can damage cell membranes through growth-inhibitory compounds (Nasiri *et al*., 2024a). A related study demonstrated that the application of *Pogostemon benghalensis* essential oil, due to the presence of compounds such as β-pinene, trans-β-ocimene, and trans-caryophyllene, caused oxidative stress and compromised membrane integrity in *Avena fatua* (L.) due to excessive ROS (Dahiya *et al*., 2020), which aligns with findings from this study. ROS are unavoidable byproducts of oxidative metabolism and are engaged in various cellular organelles, contributing to ion leakage (Das *et al*., 2015). Chemical compounds can disrupt the balance between ROS levels and antioxidant systems, amplify membrane lipid peroxidation, and enhance plant membrane permeability. In this study, the defense mechanisms of various antioxidants were insufficient to detoxify the excess H_2O_2 production, potentially leading to increased membrane lipid peroxidation.

Increased proline production in *C. album* seedlings treated with inhibitors, along with the similarity of the essential oil of Fedaleh to glyphosate at concentrations of 0.5 to 2 µl ml^{-1}, indicated the presence of stress in the treated seedlings. However, this proline production did not effectively protect the seedlings. The essential oil of Fedaleh is rich in a variety of compounds including monoterpenes, such as hydrocarbon monoterpenes (α-thujene, α-pinene, camphene, sabinene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, β-phellandrene, ɣ-terpinene, and terpinolene), alcoholic monoterpenes (linalool, terpin-4-ol, α-terpineol), ketone monoterpenes (camphor and sis-jasmone), aldehyde monoterpenes (citronellal and geranial), phenolic monoterpenes (thymol and carvacrol), and phenylpropene monoterpenes (methyl eugenol), which can induce stress and promote proline production. Another study reported increased proline accumulation in *Phalaris canariensis* and *Sinapis arvensis* following the application of *Eucalyptus maculata* essential oil, attributed to the presence of compounds such as α-thujene, α-pinene, sabinene, β-pinene, β-myrcene, linalool, and 1,8-cineole (Marwa *et al*., 2023), aligning with the findings of this study. The compound α-pinene, a monoterpene hydrocarbon recognized for its herbicidal properties (De Martino *et al*., 2010), induces oxidative stress, alters membrane integrity, generates ROS, and ultimately inhibits ATP synthesis (Xie *et al*., 2021). Proline acts as a non-enzymatic antioxidant, increasing in response to cellular disruption or damage caused by elevated ROS levels in various plant tissues (Dar *et al*., 2016; Czarnocka and Karpiński, 2018), significantly reducing membrane lipid peroxidation related to double bonds under stress conditions (Jurkonienė *et al*., 2023). Additionally, proline activates levels of POD and APX enzymes in plants while reducing H_2O_2 levels (Ozden *et al*., 2009). The prevention of significant lipid peroxidation in seedlings treated with glyphosate or Fedaleh essential oil may be linked to the high proline accumulation; however, proline did not effectively lower hydrogen peroxide levels.

Any alteration in the biosynthesis of photosynthetic reactions influences the growth of treated plants. In this study, following the application of glyphosate herbicide, the essential oil extracted from Fedaleh flowers significantly lowered the levels of chlorophyll-a. In contrast, the suppression of chlorophyll-b and carotenoid levels by the Fedaleh essential oil mirrored the effects of glyphosate. These findings indicate that the biosynthesis inhibitors or photosynthetic pigment disruptors present in Fedaleh essential oil possess a high efficacy comparable to glyphosate herbicide. Volatile allelochemicals have been shown to diminish the formation of photosynthetic pigments (Xie *et al*., 2021). Other research demonstrates that compounds such as α-thujone, camphor, 1,8-cineole, and β-thujone in the essential oil of *Artemisia fragrans* resulted in a

reduction of chlorophyll-a in the *Convolvulus arvensis* at concentrations of 1-4% (Pouresmaeil *et al*., 2020). Moreover, compounds including geranyl acetate, geranial, neral, and geraniol in the essential oil of *Dracocephalum moldavica* (L.) at a concentration of 1 µl/ml decreased the amount of chlorophyll-b in the *Bromus tectorum* (L.) (Pouresmaeil *et al*., 2022). The presence of compounds like α-Pinene, β-pinene, linalool, camphene, β-Myrcene, and caryophyllene oxide in the essential oil of *Litsea pungens*, is consistent with the findings of this experiment and the common compounds found in the essential oil of Fedaleh at a concentration of 2 mg ml^{-1}, which lowered photosynthetic pigments in *Lolium perenne* and *Bidens pilosa* plants (Kong *et al*., 2021). Consequently, inhibitory compounds may impact physiological processes such as cell viability, enzymatic activity, and chlorophyll synthesis, leading to a reduction in organelles due to membrane rupture (Fagodia *et al*., 2017; Mahdavikia *et al*., 2017). Additionally, allelochemicals can decrease light absorption by lowering photosynthetic pigment levels, thereby inhibiting plant growth (Uyun *et al*., 2024).

Although the reduction in seedling length treated with essential oils from various parts of Fedaleh did not mirror the effects of glyphosate, essential oils from Fedaleh flowers significantly decreased seedling length, likely due to the specific compounds within the essential oil (Nasiri *et al*., 2024a). Previous studies have indicated that an increase in certain compounds can lead to toxicity, allelopathic effects, and inhibition of seedling growth. For instance, the presence of significant compounds such as caryophyllene, β-pinene, and germacrene D in the essential oil of *Ambrosia artemisiifolia* negatively affected the growth and seedling length of the *Amaranthus retroflexus*, *Setaria viridis*, and *Poa annua* (Han *et al*., 2021). Research indicates that essential oils and their constituents, particularly terpenes, exhibit phytotoxic effects (De Oliveira *et al*., 2021). The observed reduction in seedling weight of *C. album* due to flower essential oil, which shows no significant difference from the herbicide glyphosate, likely stems from compounds such as α-pinene, β-pinene, β-myrcene, α-phellandrene, β-phellandrene, and (-)-spathulenol present in the essential oil of Fedaleh flower, along with the physiological and anatomical changes they induce. Since the composition and quantity of the primary constituents of the essential oil from *E. cinerea* in this study were comparable to seven wild populations distributed in various microclimates of Zagros Mountain (Jahantab *et al*., 2022), the efficacy of the essential oil from other ecotypes of this species in inhibiting weed growth is somewhat assured. Research indicates that α-phellandrene exerts toxic and inhibitory effects on the growth of weeds like *Raphanus sativus* and *Lepidium sativum* (Chowhan *et al*., 2011). Similarly, β-pinene, β-myrcene, α-terpinene, and α-terpineol found in the essential oil of *Daviesia tortuosa* have demonstrated potential toxicity and inhibition (Chowhan *et al*., 2011; De Martino *et al*., 2010). It has been reported that terpenoids can alter cell membrane permeability, enzymatic activities, DNA transcription, and RNA translation in plants, leading to reduced growth (Hoshino, 2024). Terpenoids are principal components of the plant's essential oil (Nasiri *et al*., 2024b), and this study highlights their potent toxicity. Another study indicated that the essential oil of *Litsea pungens* negatively impacted the growth of weed seedlings *Lolium perenne* and *Bidens pilosa* at high concentrations (0.5-2 mg ml^{-1}), resulting in pale yellow leaves and stunted growth (Kong *et al*., 2021), consistent with findings on the essential oil's effects in *C. album*. Furthermore, some researchers have noted that phytochemicals can directly suppress antioxidant enzyme activity within cells, increasing active oxygen levels, which leads to oxidative stress and inhibits seedling growth (Xie *et al*., 2021). In the present study, the inhibitory effects of compounds in the essential oil of the Fedaleh flower on H_2O_2 accumulation and the plant's energy usage for proline synthesis, alongside decreased activities of enzymes such as catalase, superoxide dismutase, and ascorbate peroxidase, as well as diminished photosynthetic capacity, contributed to the reduced growth of the *C. album*.

4. Conclusion

The biochemical analysis of weed seedlings in this experiment indicated that the essential oil of the plant induced oxidative stress due to the presence of various allelochemical compounds, including α-pinene, α-phellandrene, γ-terpinene, linalool, β-myrcene, neric acid, β-phellandrene, isobornyl formate, carvacrol, thymol, and spathulenol. The observed accumulation of hydrogen peroxide and alterations in the levels of chlorophyll-b and carotenoids, as well as the enzymes

catalase, superoxide dismutase, and ascorbate peroxidase, alongside variations in proline content in plants treated with the Fedaleh essential oil, closely mirrored the effects of the herbicide glyphosate. Consequently, the weight of *C. album* seedlings treated with the Fedaleh essential oil decreased in a manner akin to that induced by glyphosate. Furthermore, chlorophyll-a levels and seedling length exhibited an inverse linear relationship with the concentration of the essential oil. Taken together, the essential oil from the Fedaleh demonstrates considerable potential as an inducer of oxidative stress and disruptor of plant defense mechanisms, as well as an inhibitor of the growth of the *C. album*. Its comparable efficacy to glyphosate suggests promising prospects for its industrial production as a plant-based herbicide. However, subsequent research on the impact on non-target species, including crops, as well as its duration in aquatic and terrestrial environments, is advised for future investigation.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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