Research article

# Assessing the potential of fedaleh (*Echinophora cinerea*) essential oils as a natural herbicide for spring-summer crops

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## ARTICLE INFO

## ABSTRACT

Large quantities of chemical herbicides are used in agroecosystems every year, which not only imposes a significant financial burden on farmers but also leads to environmental pollution. To address this challenge, the present study aimed to replace Treflan with Fedaleh essential oils (EO). Treflan is a commonly used chemical herbicide for spring-summer crops, and *Chenopodium album* has been chosen as a prevalent and abundant weed in many agricultural ecosystems. Four types of inhibitors, including Treflan herbicide and essential oils extracted from the leaf, stem, and flower of Fedaleh, were used in this experiment. These inhibitors were tested at five different concentrations: 0, 0.5, 1, 2, and 5 μL/mL. Based on GC-MS results, the key compounds found in Fedaleh EO were α-pinene, α-phellandrene, γ-terpinene, linalool, sabinol, β-myrcene, neric acid, carvacrol, β-phellandrene, thymol, and spathulenol. A dose-dependent increase in hydrogen peroxide, malondialdehyde, electrolyte leakage, and proline content was observed with Fedaleh EO or Treflan. However, a decline in cell viability, germination, radicle length, radicle weight, plumule length, plumule weight, and seedling vigor index was observed in a dose-dependent manner with Fedaleh EO or Treflan. The weight of the radicle and plumule was noticeably decreased by 27 %, 28 %, and 14 %, 19 % respectively when treated with essential oils derived from the stem and flower of the Fedaleh, as compared to Treflan. Interestingly, at concentrations of 5 μL/mL EO, the germination, radicle length, plumule length, and seedling vigor index significantly decreased by 64–72 %, 56-56 %, 41–44 % and 90–93 % % as compared with control, respectively. The essential oils extracted from different parts of Fedaleh showed no significant difference in terms of hydrogen peroxide, electrolyte leakage, cell viability, and proline content, compared to Treflan ($P > 0.05$). Compared to the control, Treflan did not affect germination ($P > 0.05$), but the EO of Fedaleh's different parts significantly inhibited germination ($P < 0.05$). The growth inhibitors at a concentration of $\geq 1$ μL/mL significantly reduced the length of the radicle and plumule in *Chenopodium album*. Overall, Fedaleh EO has significant potential as a growth inhibitor and oxidative stress inducer to prevent weed interference. This makes it a suitable option for the commercial production of a natural herbicide.

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## 1. Introduction

Weeds play a significant role in causing damage to agricultural production losses in both developing and developed countries [1]. The negative effects of weeds on crop yield exceeds that of plant pathogens [2]. Weeds compete with agricultural crops for essential resources including water, light, nutrients, and physical space, impending crops' normal functioning and surpassing their growth and development [3]. They also serve as hosts for pests and crop pathogens [4]. Failure to effectively control weeds can result in a 100 % decrease in crop yield [5].

In the context of spring-summer crops, the interference of weeds with crops tends to be high as a result of favorable environmental conditions. For instance, *Chenopodium album* (*C. album*), identified as one of the ten significant weeds in soybean (*Glycine max* L.) and corn (*Zea mays* L.) [6], follows a one-year life cycle [7] and can produce up to 500 seeds per plant. It competes with certain crops by germinating at lower temperatures and emerging before them. In addition, the seeds of this plant have a remarkable longevity in the soil, lasting for a period of 30–40 years [8].

The use of chemical herbicides is a common approach for managing weed populations in agricultural ecosystems. These herbicides use diverse mechanisms of action, including the inhibition of photosystem and electron transfer, synthesis of pigments, fatty acids, and amino acids, cell growth through mitosis and cell wall synthesis, regulation of growth, and respiration [9]. However, excessive use of these herbicides has led to environmental and human health risks. Conversely, the emergence of herbicide-resistant weeds poses a significant threat to agriculture, with the number of resistant weed species increasing globally [10] with 500 species of resistant weeds being reported worldwide [11].

Among these alternative methods, the use of natural plant products has attracted the attention of researchers [12,13] and can be a solution to the challenge of resistance to herbicides and environmental pollution [3]. In this method, some plants, due to the presence of toxic gases (cyanogenic glucosides, hydrogen cyanide, amygdalin, etc.), organic acids and aldehydes (malic acid, citric acid, acetaldehyde), aromatic acids (ferotic acid, benzoic acids, etc.), simple unsaturated lactones (patolin, parasorbic acid, etc.), coumarins (coumarin, esculin, scopolin, etc.), quinones (juglone), flavonoids (phlorizin, rotenone), tannins (nitrapyrin), alkaloids (caffeine, quinine, berberine, etc.), terpenoids and steroids (camphene, dipentene, camphor, β-pinene, etc.) and other unknown substances (alcohols, polypeptides, and nucleosides) act as natural herbicides and pesticides [9,14].

A diverse range of primary bioactive compounds, including sesquiterpenoids and monoterpenoids, have been documented in essential oils extracted from plants [15]. These compounds hold the potential to act as natural herbicides [16]. For instance, the essential oil derived from *Pluchea ovalis* (Pers.) DC., predominantly composed of sesquiterpenes (91.3 %) showed significant larvicidal activity against Fall Armyworm insect [17]. Many plant species possess the ability to deter grazers and pests by synthesizing significant of substantial quantities of essential oils, and inhibiting the growth of neighboring competing plants [18,19]. The structural diversity and strong phytotoxicity of essential oils and their constituents have led to their selection as pure natural compounds or mixtures for the development of bioherbicides [20]. So far, successful commercial examples of the main compounds of plants have been introduced as herbicides and pesticides. For example, clove essential oil is the major active component in the BurnOut II herbicide [21]. In addition, the active ingredient of Ecotrol Plus, introduced as an important product in 2003, contains 5 % geraniol, 2 % peppermint EO and 10 % rosemary EO [22].

Fedaleh (*Echinophora cinerea*), which often grows in the mountains of Zagros, Alborz, and Azerbaijan [23], has aromatic and medicinal properties [24]. It has been reported in researches conducted by Amirghofran et al. [25] and Jelodarian et al. [26] that this substance is utilized as a stomach tonic and exhibits anti-cancer properties. The analysis of the aerial parts of Fedaleh revealed that it mainly consists of monoterpenoids (such as β-phellandrene, α-phellandrene, p-cymene, and α-pinene), sesquiterpenoids, flavonoids, and phenolic compounds [27].

Based on field observations, it was found that no plant species were growing around Fedaleh. Additionally, livestock avoided eating this plant. These observations led to the formation of a hypothesis about its potential as a deterrent for the control of *C. album* is considered one of the most widespread weeds in agroecosystems. The hypothesis is as follows: 1) Fedaleh EO can decrease the seed germination, and growth of *C. album* seedling; 2) The inhibitory effect of Fedaleh EO on weed germination and growth is comparable to that of the herbicide Treflan; 3) The mode of action by which Fedaleh EO controls the growth of *C. album* can be determined. In the present study, the toxicity of Fedaleh EO was assessed on *C. album* weed, with particular focus on the induction of oxidative stress during the seedling stages.

## 2. Materials and methods

### 2.1. Plant materials

Fedaleh (*Echinophora cinerea* Boiss.) identity was verified by Dr. H. Shirmardi and Dr. V. Mozafarian, and a voucher specimen (No. D-7062) is currently housed at the RCANR, Chaharmahal & Bakhtiari, Iran. Different components (flowers, leaves, and stems) of Fedaleh were gathered during the peak flowering stage (July 2019) from the Zagros mountains in Iran, situated at an elevation of 2587 m above sea level (latitude 32°13′34″N, longitude 50°20′21″E). The seeds of *C. album* weed were collected from potato fields in the Kiyar region, Iran (latitude 32°04′539″N, longitude 50°47′43″E), and subsequently stored in a designated envelope.

### 2.2. Essential oil extraction

The various components obtained from Fedaleh were collected and subjected to a drying process in a shaded condition with

adequate airflow. Subsequently, they were stored in a paper bag and placed in a refrigerator (4–5 °C) until the essential oil extraction process. The dried plant parts were subsequently crumbed using an electric mill. The essential oils were extracted using a Clevenger apparatus, operating at a temperature range of 250–280 °C. During each essential oil extraction process, 400 g of the specific target part of Fedaleh was combined with 1 L of distilled water in a Clevenger apparatus. After a duration of 3 h, the essential oil extraction was completed. The obtained essential oil was subjected to dehydration using sodium sulfate, with a total quantity of 15 g. The dehydrated oil was then stored in a vial at a temperature of 4 °C in a refrigerator until it was utilized for testing purposes.

## 2.3. Experimental setup

The factorial experiment was conducted in a completely randomized design (CRD) with 3 replications in 2021 at the Faculty of Agriculture, Shahrekord University, Iran. Four types of inhibitors, namely Treflan herbicide (TH), stem essential oil (SE), leaf essential oil (LE), and flower essential oil (FE), were selected as first factor. These inhibitors were tested at five different concentrations (0, 0.5, 1, 2, and 5 μL/mL) as second factor. The concentration levels for this study were determined based on the recommended dose Treflan herbicide of 2 μL/mL. To evaluate the effectiveness of Fedaleh EO, a concentration range from 0.25 to 2.5 times the recommended herbicide dose was considered. Treflan herbicide (TREFLAN 48 % EC) was purchased from AGROXIR Company, Iran. Different concentrations of Fedaleh EO were dissolved in a range of one to five μmol of distilled water and Tween-20 (as surfactant). To mitigate the risk of pathogen contamination, the Petri dishes and weed seeds underwent disinfection by exposure to a 0.5 % NaClO solution for 5 min. To ensure the seed viability of *C. album*, the seeds were tested in a 10 % 2,3,5-TTC. To break dormancy, the seeds were subjected to a treatment of 1000 ppm $KNO_3$ for 24 h, followed by a thorough rinsing with distilled water [28].

Petri dishes measuring 10 cm in diameter were utilized. Two layers of filter paper (Whatman, No. 2) were placed inside the Petri dish and 50 seeds of *C. album* were uniformly placed in each Petri dish. Two mL of each treatment were subsequently added to the samples. Petri dishes were tightly sealed with parafilm and kept for 24 h. After that, the Petri dishes were placed in the growth chamber with 26 ± 2 °C, 75 % relative humidity, 16:8 h light-dark photoperiod, and 150 μmol/m$^2$/s photon flow intensity.

## 2.4. Measurements and calculations

After 7 and 14 days, the percentage of germination (GP), and vigor index of seedling (SVI) were calculated according to the formula of Kalsa and Abbi [29] and Walia et al. [30], respectively.

$$GP\ (\%) = 100 \times \frac{n}{N} \tag{1}$$

$$SVI = GP\ (\%) \times seedling\ fresh\ weight \tag{2}$$

In the first equation, n = the number of germinated seeds after the seventh day and N = the total number of cultivated seeds.

After 14 days, the length and fresh weight of radicles and plumules were measured using methods described by Madadi et al. [12, 13]. Then, the content of malondialdehyde (MDA), hydrogen peroxide, proline, membrane permeability, and cell viability were assayed.

The content of hydrogen peroxide ($H_2O_2$) was measured according to the method of Loreto and Velikova [31]. 100 mg fresh seedlings were crushed with 0.1 % trichloroacetic acid in a mortar and then centrifuged at 10,000 rpm for 5 min 150 μL of the supernatant was mixed with 650 μL of potassium phosphate buffer (10 mM and pH = 7) and 200 μL of potassium iodide or 1 mM KI. The absorbance value at 390 nm was recorded after 1 h. The content of hydrogen peroxide was calculated using a standard curve with different concentrations of hydrogen peroxide and in μmol/g FW.

The content of malondialdehyde (MDA) was measured according to the method of Boominathan and Doran [32]. 1000 mg fresh seedlings were crushed with 0.1 % trichloroacetic acid in a mortar and then centrifuged on ice at 10,000 rpm for 5 min 350 μL of the supernatant was mixed with 1400 μL of 20 % TCA containing 5 % Thiobarbituric acid or TBA and heated at 96 °C for 30 min. The mixture was directly transferred to an ice water bath and then centrifuged at 10,000 rpm for 15 min. Solvent absorbance was recorded at 532 nm and the amount of malondialdehyde was calculated according to the standard curve prepared using 3,1,1, 3-tetraethoxypropane.

Electrolyte leakage (EL) was measured according to the method of Jaballah et al. [33] using an electrical conductivity meter. The initial electrical conductivity ($EC_1$) of the seedling samples in the tube containing 15 mL of distilled water was recorded after 24 h. The final electrical conductivity ($EC_2$) of seedling samples was measured after autoclaving at 121 °C for 20 min. The EL percentage was calculated using the following equation.

$$EL\ (\%) = 100 \times \frac{EC1}{EC2} \tag{3}$$

To determine cell viability, double staining with fluorescein diacetate (FDA) and propidium iodide (PI) was performed [34]. Red and green fluorescence were indicative of dead and live cells, respectively. After treatment with each treatment, the root parts of the seedlings were separated and stained with a mixture of 12.5 μg/mL FDA and 0.5 μg/mL PI for 10 min. After that, the dyed radicles were washed with distilled water and observed using a fluorescence microscope.

Proline content was determined by the method of Bates et al. [35]. About 100 mg fresh seedlings were prepared and placed in 3 mL of sulfosalicylic acid (3 % w/v). Then it was centrifuged at 2000 rpm for 10 min. The supernatant liquid (1 mL) was mixed with

ninhydrin acid and frozen acetic acid in a ratio of 1:1:1 and incubated in boiling water for 1 h and then in an ice water bath for 10 min. Toluene (4 mL) was added and the solution was mixed and the organic and inorganic phases were separated. The organic phase was analyzed by spectrometry at 520 nm. The proline content for the calibration curve was read with pure proline.

## 2.5. Statistical analysis

The data were subjected to analysis (factorial 4 x 5; inhibitor type and inhibitor concentration, respectively) in a completely randomized design (CRD) with 3 replicates using SAS (V. 9.1) software. The comparison of the means of simple effects and interactions was conducted using the Least Significant Difference (LSD) test at a significance level of 5 %. Where the interaction between inhibitor type and concentration was significant, the analysis excluded the examination of simple effects. The non-significance of the effect of the type of inhibitor was considered as the concept of the same effect of Fedaleh essential oil with Treflan herbicide.
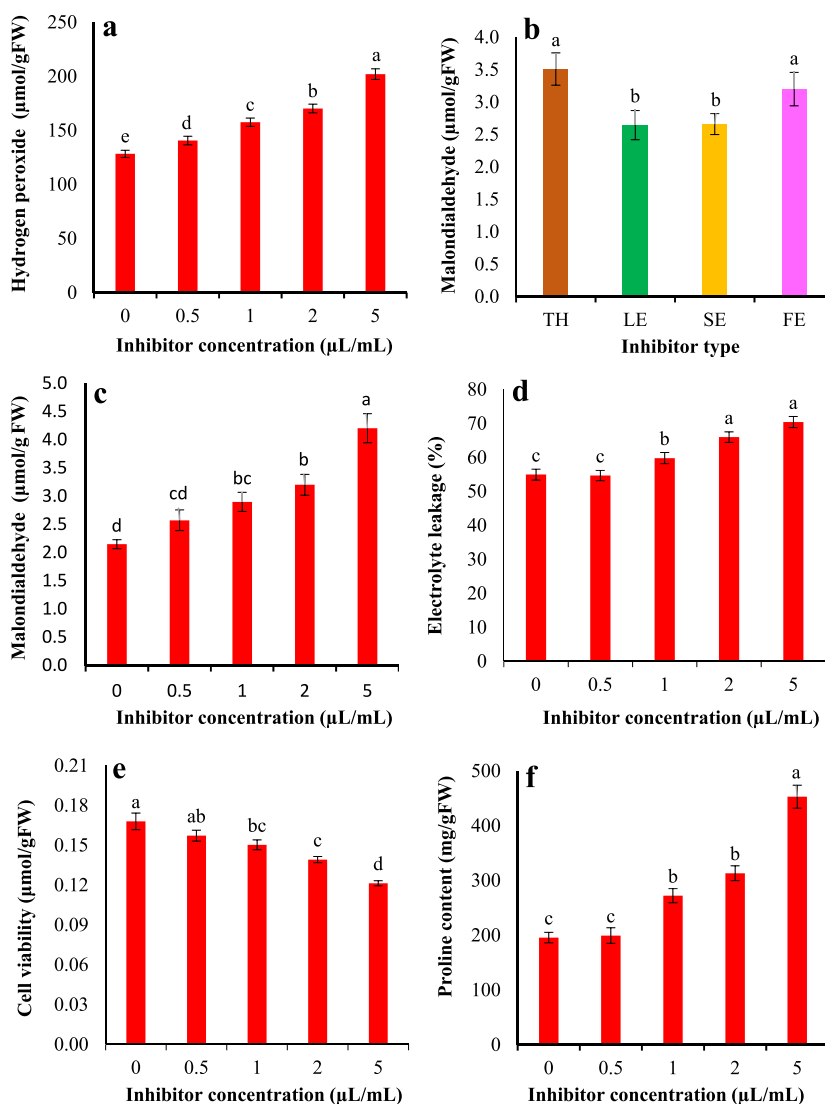


**Fig. 1.** The effect of different concentrations of growth inhibitory substances on hydrogen peroxide (a), malondialdehyde (b, c), electrolyte leakage (d), cell viability (e) and proline content (f) in *Chenopodium album*. Means with different letters have statistically significant differences based on the LSD test (*P* < 0.05). TH, LE, SE, and FE are Treflan herbicide and essential oils of leaf, stem, and flower of *Echinophora cinerea*, respectively. Bars represent ± SE.

## 3. Results

### 3.1. Hydrogen peroxide

The ANOVA results showed that the hydrogen peroxide ($H_2O_2$) content in *C. album* was affected by the concentration of inhibitor substances ($P < 0.01$), but not affected by the type of inhibitor and its interaction with the inhibitor concentration (Table S2). The response of $H_2O_2$ to inhibitor concentration was linear (Fig. la). Compared to the control, the content of $H_2O_2$ increased by 10, 23, 33, and 57 % at concentrations of 0.5, 1, 2, and 5 μL/mL, respectively (Fig. 1a).

### 3.2. Membrane lipid peroxidation

The effect of the concentration of inhibitory substances on the lipid peroxidation (MDA) of the membrane in *C. album* was
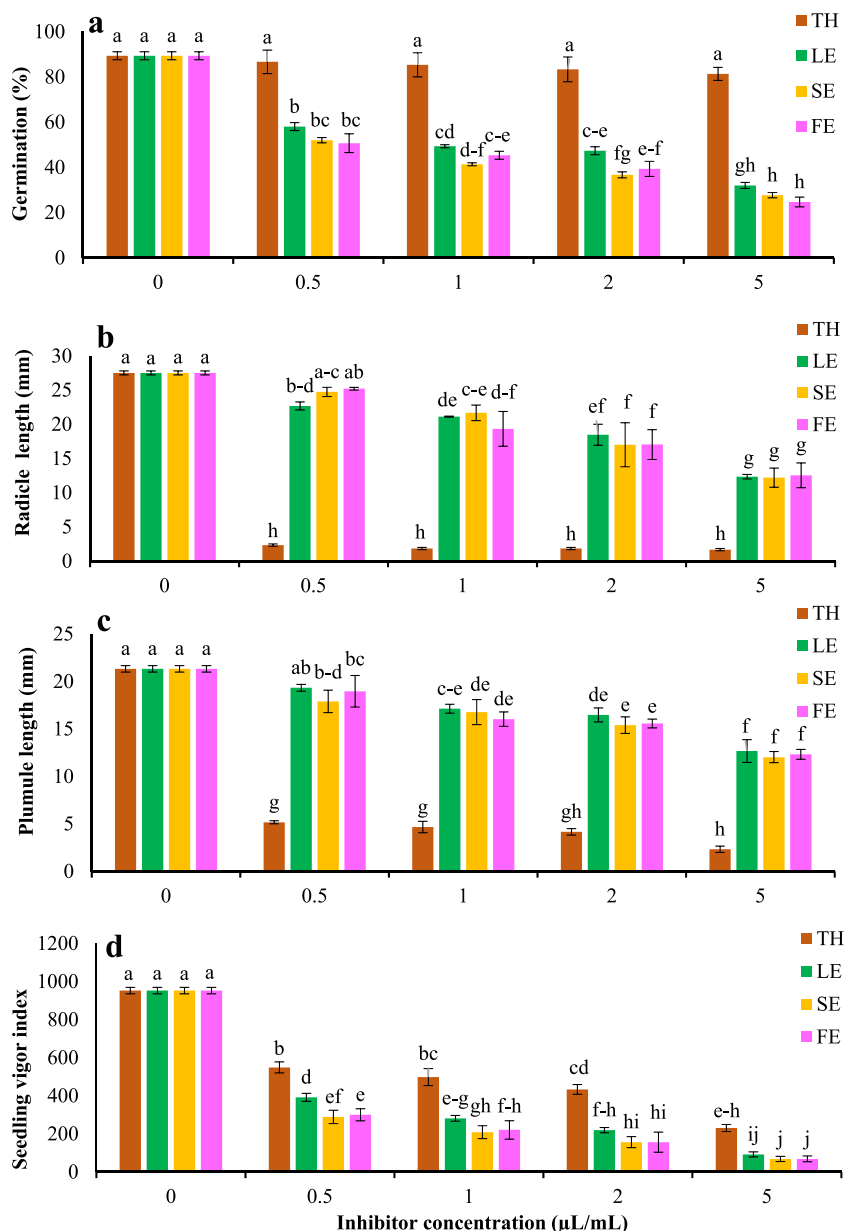


**Fig. 2.** The interaction of inhibitor type and growth inhibitor concentrations on germination percentage (a), radicle length (b), plumule length (c) and seedling vigor index (d) in *Chenopodium album*. Means with different letters have statistically significant differences based on the LSD test ($P < 0.05$). TH, LE, SE, and FE are Treflan herbicide and essential oils of leaf, stem, and flower of *Echinophora cinerea*, respectively. Bars represent ± SE.

significant ($P < 0.01$; Table S2). The measurement of malondialdehyde concentration serves as an indicator of membrane lipid peroxidation, as shown in Fig. 1b. A dose-dependent linearity was observed for membrane lipid peroxidation (malondialdehyde). The observed difference between the control and the group treated with 0.5 µL/mL was not statistically significant ($P > 0.05$). However, a significant difference was observed compared to the control ($P < 0.05$; Fig. 1c) when the concentration of the treatment reached $\geq 1$ µL/mL. The concentration of malondialdehyde at 5 µL/mL exhibited a 96 % increase compared to the control (Fig. 1c).

### 3.3. Electrolyte leakage

As indicated in Table S2, the concentration of inhibitor substances had a significant impact on the amount of electrolyte leakage, which is a measure of membrane permeability ($P < 0.01$). However, the observed feature was not affected by the type of inhibitor or the interaction between the type and concentration ($P > 0.05$; Table S2). The electrolyte leakage at a concentration of 0.5 µL/mL did not show a significant difference compared to the control ($P > 0.05$). However, at concentrations of 1, 2, and 5 µL/mL, the electrolyte leakage rate increased by 9 %, 20 %, and 28 % respectively, in comparison with the control ($P < 0.05$; Fig. 1d).

### 3.4. Cell viability

The impact of inhibitory substances on the cellular viability of weed was found to be statistically significant ($P < 0.01$; Table S2). According to the data presented in Fig. 1e, there was a negative linear relationship between cell viability and the concentration of inhibitory substances. At a concentration range of 1–5 µL/mL, the viability of cells exhibited a reduction of 11–28 % in comparison with the control ($P < 0.05$; Fig. 1e).

### 3.5. Proline content

Table S2 presents the ANOVA results for proline content. Compared to the control, inhibitor substances at concentration of $\geq 1$ µL/mL significantly increased the proline content of *C. album* by 28–76 % (Fig. 1f).

### 3.6. Germination percentage

The analysis of variance (ANOVA) results indicated a significant impact of the type and concentration of inhibitor substances, as
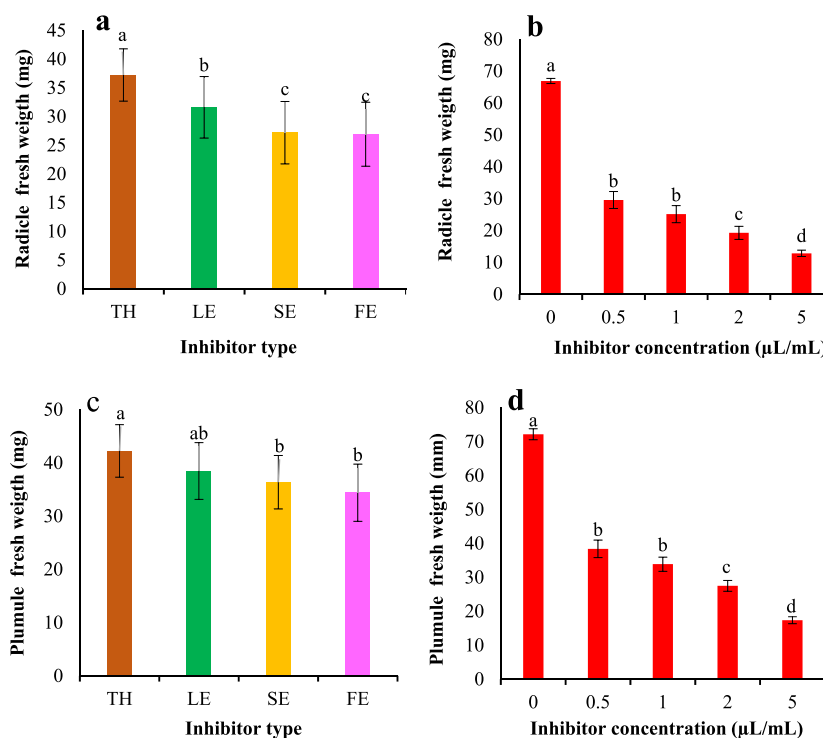
**Fig. 3.** The effect of different concentrations of growth inhibitory substances on radicle fresh weight (a, b) and plumule fresh weight (c, d) in *Chenopodium album*. Means with different letters have statistically significant differences based on the LSD test ($P < 0.05$). TH, LE, SE, and FE are Treflan herbicide and essential oils of leaf, stem, and flower of *Echinophora cinerea*, respectively. Bars represent ± SE.

well as their interaction, on the germination percentage ($P < 0.01$; Table S3). Different concentrations of Treflan herbicide did not affect the weed germination ($P > 0.05$; Fig. 2a). However, the EO of different parts of Fedaleh reduced the weed germination percentage compared to the control ($P < 0.05$; Fig. 2a). Compared to the control, exposure to the concentrations of 0.5–5 µL/mL of leaf, stem and flower essential oil resulted in reduction in percentage of weed germination by 35–65, 42–69 and 43–73 % (Fig. 2a).

### 3.7. Radicle length

The length of the radicles was affected by inhibitory substances, as well as the interaction between the type and concentration of inhibitor ($P < 0.01$; Table S3). Compared to the control, various concentrations of Treflan significantly reduced radicle growth ($P < 0.05$). However, there was no significant difference observed among the different herbicide concentrations ($P > 0.05$). The EO of Fedaleh different parts at $\geq 1$ µL/mL and the concentration of 0.5 µL/mL of leaf essential significantly reduced the length of the radicle compared to the control (Fig. 2b). Compared to the control, the inhibition of radicle growth exposed to a concentration of 5 µL/mL of Fedaleh EO was approximately 55 %. Treflan herbicide prevented radicle growth after a certain amount of radicle emergence (Fig. 2b).

### 3.8. Plumule length

The ANOVA indicates that the effects of the inhibitory type, concentration, type × concentration interaction, on the plumule length were significant ($P < 0.01$; Table S3).

Compared to the control, the plumule length was reduced when exposed to different concentrations of Treflan ($P < 0.05$; Fig. 2c). Compared to the control, the concentrations of 0.5–5 µL/mL of Treflan reduced the plumule length by 76–89 %. Similarly, the concentrations of 0.5–5 µL/mL of leaf, stem, and flower EO reduced the plumule length by 9–41 %, 16–44 %, and 11–42 %, respectively. These reductions were significant, except for 0.5 µL/mL of leaf EO (Fig. 2c). Additionally, The EO extracted from the stem and flower had a greater impact on reducing the length of the plumule compared to the EO extracted from the leaf (Fig. 2c).

### 3.9. Radicle fresh weight

The amount of radicle fresh weight was affected by inhibitory substances (Table S3). The radicle fresh weight exposure to the EO of leaf, stem, and flower was 15, 26, and 27 % lower than that of the Treflan treatment, respectively ($P < 0.05$; Fig. 3a). Compared to the control, concentrations of 0.5–5 µL/mL of the inhibitor significantly reduced the radicle fresh weight. The reduction in weight for concentrations of 0.5, 1, 2, and 5 µL/mL was 56, 62, 71, and 81 %, respectively ($P < 0.05$; Fig. 3b).

### 3.10. Plumule fresh weight

As shown in the ANONA results (Table S2), the fresh weight of the plumule was influenced by the type and concentration of inhibitory substances. However, the interaction between type and concentration was not found to be significant. There were no significant differences in the weight of the plumule exposed to the essential oil from different parts of Fedaleh ($P > 0.05$). Compared to Treflan, the essential oil extracted from the stem and flower significantly decreased the fresh weight of the plumule ($P < 0.05$; Fig. 3c).

A dose-dependent negative linear relationship was observed for the plumule fresh weight. Compared to the control, the reduction in plumule fresh weight was 47, 47, 62, and 76 % at concentrations of 0.5, 1, 2, and 5 µL/mL of inhibitory substances, respectively (Fig. 3d).

### 3.11. Seedling vigor index

The seedling vigor index was affected by the inhibitory substances, as well as their interaction ($P < 0.01$; Table S3). In Fig. 2d, it can be observed that various concentrations of Treflan herbicide and Fedaleh EO significantly decreased the seedling vigor index compared to the control. However, the reduction in this index was greater for the seedlings exposed to the essential oils of different Fedaleh parts (leaf, stem, and flower) compared to Treflan herbicide. Concentrations $\geq 0.5$ µL/mL of Treflan reduced the seed germination index by 42–76 %. However, the reduction for the essential oil of the leaf, stem, and flower of Fedaleh was 59–90, 70–93, and 68–93 %, respectively (Fig. 2d).

## 4. Discussion

Our results indicated an increase in the concentration of $H_2O_2$ by increasing the concentration of the essential oils. The accumulation of $H_2O_2$ in *C. album* seedling has been attributed to the presence of allelochemical compounds in Fedaleh EO, such as α-pinene, α-phellandrene, γ-terpinene, linalool, β-myrcene, neric acid, β-phellandrene, carvacrol, thymol, and spathulenol [36]. Some studies have demonstrated that the presence of monoterpenes in the essential oil (EO) of certain allelopathic plants can lead to the accumulation of $H_2O_2$ in certain plants [37,38]. In the investigation conducted by Singh et al. [10], it was demonstrated that α-pinene has the ability to enhance the production of ROS in five plant species, including *Amaranthus viridis*, *Pisum sativum*, *Cassia occidentalis*, *Triticum aestivum*, and *Cicer arietinum*. Among the ROS produced in the plant cell in response to stress, $H_2O_2$ acts as a molecular signal. In low concentrations, it defends the cell against stress, but at high concentrations, it induces cell damage in plants [39]. Hydrogen peroxide inhibits the SH-group and enzymes, reduces the activity of photosynthesis and chloroplasts, and limits growth [40]. The

results showed that the concentration of H$_2$O$_2$ increased by 33–57 % at $\geq$1 μL/mL, and this amount of ROS can cause oxidative stress and disrupt the metabolic activities of cells [10].

Malondialdehyde is created by the peroxidation of unsaturated fatty acids in membranes, which indicates cell membrane damage of plant species [41] by ROS accumulation [42]. Monoterpenes present in the essential oil have been found to induce membrane damage [43] and elevate malondialdehyde levels. Essential oils of plants such as *Schinus areira* and *Tagetes minuta*; and monoterpenes, including ocimenone, ocimene, α-pinene, thymol, 1,8-cineole, geraniol, camphor, and menthol inhibited corn growth by increasing MDA [44]. In addition, the EO has the potential to enhance the permeability of the cell membrane in weeds. This phenomenon is caused by the penetration of terpenes through the cell wall and membrane, the leakage of cellular potassium, and inhibition of glucose-dependent respiration [20]. Monoterpene compounds are lipophilic, and may stimulate cell membrane expansion, destroy membrane structure, increase fluidity, and inhibit membrane enzymes [37,45].

The significant increase of malondialdehyde in weed seedlings exposed to concentrations of inhibitory substances $\geq$1 μL/mL may be caused by the high concentration of H$_2$O$_2$ in these treatments (Fig. 1a). Conversely, the non-significance of the interaction also indicates a similar trend between the essential oil and Treflan herbicide. Therefore, the production of ROS and the disruption of membrane lipid peroxidation are similar between Fedaleh EO and Treflan herbicide. Accumulation of H$_2$O$_2$ is closely associated with electrolyte leakage and malondialdehyde levels. Excessive H$_2$O$_2$ can react with phospholipids in the cell membrane, leading to oxidative stress and lipid peroxidation [38]. Chloroplasts and mitochondria are locations where significant electron transport processes occur, leading to the creation of substantial electro-chemical imbalances across membranes involved in energy conversion. These circumstances create favorable conditions for the formation of H$_2$O$_2$ through the dismutation of O$_2$ [46], therefore, its production and lack of control damages the cellular composition.

Previous studies have demonstrated that there is a significant presence of compounds, such as α-pinene, α-phellandrene, γ-terpinene, linalool, β-myrcene, β-phellandrene, carvacrol, and thymol, in Fedaleh EO [36]. Additionally, it has been observed that the excessive production of H$_2$O$_2$ (Fig. 1a) in the presence of these monoterpenes leads to oxidative stress and high damage to the cell membrane. Consequently, this condition results in an increase in the amount of electrolyte leakage in the cell. Our findings align with previous studies conducted by Kaur et al. [16] and Singh et al. [47], who have also demonstrated a direct correlation between membrane integrity and permeability disorder, electrolyte leakage with increasing concentrations of monoterpenes. The accumulation of H$_2$O$_2$ leads to an increase in lipid peroxidation and oxidative stress, disrupting the metabolic activities of weed cells and altering cell membrane permeability [38]. The change in membrane permeability affects the biochemical and physiological functions related to the cell membrane [48]. For instance, the breakdown of the cell membrane results in the release of lipids into the cytoplasm of the cell [44]. Furthermore, the elevated levels of electrolyte leakage, H$_2$O$_2$, and MDA caused by high concentrations of *Artemisia fragrans* EO inhibit the growth of *Convolvulus arvensis* [49].

Monoterpenes have been found to induce cellular damage, as demonstrated by Singh et al. [10]. Therefore, the decrease in cell viability of *C. album* may be related to the existence of terpenes in Fedaleh EO. These monoterpenes include hydrocarbon monoterpenes such as α-thujene, camphene, α-pinene, sabinene, β-myrcene, β-pinene, α-phellandrene, γ-terpinene, α-terpinene, β-phellandrene, and terpinolene. Additionally, alcohol monoterpenes like linalool, terpin-4-ol, and α-terpineol, ketone monoterpenes such as camphor and sis-jasmone, monoterpenes aldoide like citronellal and geranial, phenol monoterpenes like thymol and carvacrol, and phenylpropene monoterpenes such as methyl eugenol may also contribute to this decrease in cell viability. Several studies have demonstrated that monoterpenes, owing to their lipophilic nature, exert an effect on oxidative phosphorylation, leading to an imbalance in cellular energy [37]. Cell viability, indirectly representing cellular respiration [50], decreases upon exposure to Fedaleh EO, indicating interference with energy metabolism related to macromolecule synthesis and therefore, growth impairment. This observation is consistent with previous findings by Singh et al. [47].

In addition, increased ROS concentrations cause lipid peroxidation within cell membranes. This process is further accelerated by the presence of inhibitors, leading to the release of lipid substances within the membrane and ultimately to cell death [51]. Our findings indicated that when the concentration of Fedaleh EO was equal to or greater than 1 μL/mL, it led to an excessive ROS in the cellular membrane. This, in turn, results in the disruption and destruction of cell viability in *C. album*. Yan et al. [52] demonstrated that the viability of *Lactuca sativa* radicle tip cells declined as the concentration of artemisinin increased. This decline was attributed to the excessive production of ROS, as well as membrane lipid peroxidation and disruptions in physiological processes.

Proline content is effective in osmotic regulation, ROS detoxification, plant recovery after stresses, and preservation of membrane integrity [53]. The increase in proline levels in response to oxidative stress is associated with the generation of ROS during periods of environmental stress [52]. Concentrations equal to or greater than 1 μL/mL have been found to significantly elevate the levels of H$_2$O$_2$, MDA, and electrolyte leakage. These findings suggest the presence of severe oxidative stress and toxicity caused by inhibitory substances, as shown in Fig. 1a–d. Accumulation of proline in these treatments serves as a mechanism to mitigate the toxicity caused by inhibitory substances. However, it is important to note that the toxicity of these substances surpasses the capacity of proline to maintain cell membrane stability and prevent cell death, as presented in Fig. 1e. Several studies have indicated that the proline content in plants is known to increase when exposed to α-pinene, primarily due to the generation of ROS [54]. In certain instances, proline may be ineffective in scavenging ROS, resulting in cytotoxicity and subsequent cell death [55].

The findings of this study indicate that the inhibitory effect of flower EO on germination was more pronounced compared to the essential oil derived from stems and leaves. Additionally, it was observed that the herbicide Treflan did not have a significant impact on the percentage of weed germination ($P > 0.05$). The strong inhibition of flower essential oil can be attributed to the high levels of ingredients such as β-myrcene, α-phellandrene, β-phellandrene, sabinol, verbenol, (−)-spathulenol, and patchoulane, as indicated in Table S1. Other studies have demonstrated that essential oils and monoterpenes with high oxygen content exhibit significant inhibitory effect on weed germination [43,56]. This inhibitory agent either activates membrane lipid peroxidation or inhibits the activity of

certain antioxidant enzymes, particularly superoxide dismutase. Therefore, the phytotoxicity of essential oils can be attributed to the existence of synergistic or antagonistic compounds [38]. The present study showed that the administration of Fedaleh EO induced oxidative stress. This was evident by increased levels of ROS, especially $H_2O_2$, and subsequent accumulation and damage to cellular membrane. Additionally, the study revealed that Fedaleh EO increased membrane lipid peroxidation. Furthermore, the EO was found to modulate the activity of antioxidant enzymes by activating or deactivating them, thereby inhibiting the seed germination of *C. album*. Seed germination plays a crucial role in the overall growth, and development of plants. Changes in physiological, biochemical, and morphological processes during germination have a significant impact on the survival of seedlings. In this regard, plant biological herbicides can inhibit weed germination by blocking initial cell division and hydrolysis [57]. The inhibition of the seed germination by some plant extracts includes the osmotic effect on the amount of absorption, which ultimately prevents germination and especially cell elongation [58]. The EO derived from *Nepeta meyeri* has been found to inhibit the germination of several plant species, including *Bromus danthoniae*, *Amaranthus retroflexus*, *Lactuca serriola*, *Bromus intermedius*, *Portulaca oleracea*, and *Chenopodium album*. This inhibitory effect can be attributed to the EO's ability to induce oxidative stress, leading to an increase in ROS such as $H_2O_2$. The presence of these ROS causes membrane damage and enhances the level of lipid peroxidation in the membranes. Additionally, the EO deactivates antioxidant enzymes, further exacerbating the oxidative stress [38]. The study conducted by Pouresmaeil et al. [49] found that the EO of *Artemisia fragrans* contains α-thujone, camphor, β-thujone, and 1,8-cineole compounds. The presence of these compounds resulted in an increase in the amounts of hydrogen peroxidation, malondialdehyde, and membrane electrolyte leakage in *Convolvulus arvensis*.

Fedaleh EO contains compounds such as α-pinene, α-phellandrene, γ-terpinene, linalool, β-myrcene, neric acid, β-phellandrene, carvacrol, thymol, and spathulenol, which were observed to inhibit the growth of seedlings [36]. Compared to the radicle, the radicle is exposed to inhibitory substances sooner, and the permeability of its tissue is higher than other plant organs [59]. In addition, phytotoxic substances prevent the growth of radicle tissue by affecting the genes responsible for their cellular characteristics [60]. Hazrati et al. [61] reported that α-pinene, 1,8-cineole, verbenone, camphor, camphene, and borneol are the main components of *Rosmarinus officinalis* (L.) that prevent the growth of *Amaranthus retroflexus*. Additionally, the presence of ketone monoterpene compounds such as thymol, piperitenone oxide, and piperitone oxide in the EO of *Mentha longifolia* reduced the radicle length of *Cyperus rotundus* and *Echinochloa crus-galli* [62]. Some monoterpenes present in the ginger plant, including camphene, limonene, α-pinene, β-myrcene, and α-phellandrene, have been found to cause a decrease in seedling length [63]. The carvacrol, which has a high herbicidal effect, caused a 64–96 % reduction in the growth of radicles and plumules of *Cheniopudium album* and *Amaranthus retroflexus* [64,65]. It has also been reported that the compound eugenol has a stronger inhibitory effect on the growth of *Avena fatua* radicles compared to its effect on aerial parts. This is attributed to the absence of a cuticle on the radicle, which allows for greater penetration of monoterpenes [21]. The presence of α-phellandrene, linalool, carvacrol, thymol, and eugenol in Fedaleh essential oil slowed down the growth of the weed's radicle. This decrease may be caused by a reduction in the speed of cell division and cell length, which is a result of the activity of allelochemicals and a decline in the mitotic index [66].

The phytotoxic potential of the EO is mainly attributed to specific compounds called monoterpenes [67]. Therefore, the difference in plumule length can be attributed to compounds such as p-cymene, α-pinene, α-phellandrene, linalool, carvacrol, and β-myrcene, which are more prevalent in the essential oils of flowers compared to leaves. Kordali et al. [68] demonstrated that thymol and carvacrol inhibit the germination of *A. retroflexus*, *R. crispus*, and *C. album*. The compounds, such as α-thujone, camphor, eucalyptol, and β-thujone, found in the essential oil of *Seriphidium terrae-albae*, were found to cause toxicity and reduce the plumule length of *Poa annua* and *Amaranthus retroflexus* [69]. Plumule length is one of the major growth components for plant emergence. In general, plumule growth is less sensitive to phytotoxicity compared to root growth [59]. The reduction of root and shoot length in weed species can be caused by changes in DNA synthesis in the tips of meristems or mitochondrial metabolism [70], cell division, or a combination of both [71]. The lack of an increase in plumule length may be the result of direct interference of phytochemicals with cell division and a change in the balance of growth hormones [72]. It has been reported that the presence of composites such as tannins, terpenoids, and alkaloids in the leaf extract of *Mimosa pigra* slows down the plumule length of *Lactuca sativa* [73].

The stronger inhibitory effect of Fedaleh EO on radicle fresh weight compared to plumule was consistent with findings from other studies [60]. Similarly, other researchers have reported a relationship between the inhibition of radicle growth and oxidative stress, disruption of cell division, and induction of cell death in *Chenopodium murale* [74]. It appears that the inhibitors in Fedaleh EO [36] have had a greater impact on inhibiting the growth of weed roots compared to Treflan. This is achieved by disrupting the balance of gibberellin, ethylene, cell division, and cell metabolism [75].

Seedling weight may be affected by changes in plant growth rate and relative water content when exposed to natural essential oil or allelochemicals [65]. Furthermore, essential oils and monoterpene compounds can induce physiological and anatomical alterations in seedlings. These changes lead to the accumulation of lipid globules in the cytoplasm, induction of membrane permeability and respiration, and inhibition of DNA and RNA synthesis [76]. In the present study, the decrease in plumule fresh weight can be related to the concentration of essential oil, as well as physiological and anatomical changes. In another study, the length of the plumule and radicle in *C. album* exposed to the EO of *Satureja hortensis* (L.) showed a declining pattern in a dose-dependent manner [77].

The decrease in seedling vigor index of weeds exposed to Treflan was primarily attributed to a decrease in seedling growth (Fig. 2b and c). However, for weeds exposed to Fedaleh EO, the decrease in seedling vigor index was mainly due to a decrease in germination percentage (Fig. 2a). Therefore, the mechanism of action of the EO includes preventing and disrupting the cell division of the embryo, as well as inhibiting the growth of the seedling. As the seedling continues to grow, the intensity of the inhibitory effects decreases. In this study, the significant reduction in the seedling vigor index was consistent with the findings of Han et al. [18], who used the EO of *Ambrosia artemisiifolia* (L.) on *Poa annua*, *Amaranthus retroflexus*, and *Setaria viridis*. In addition, some studies have shown that β-pinene, limonene, and caryophyllene [78–80], which are the main components of the EO in *Ambrosia artemisiifolia* (L.), have phytotoxic effects

on weeds. Conversely, other researchers have suggested that plant chemicals might directly impede the activity of antioxidant enzymes within the cell, leading to an accumulation of ROS and oxidative stress, ultimately inhibiting plant growth [81]. Phytotoxic compounds can disrupt plants by inducing abnormal cell division, chromosome breakage, interference with various stages of mitosis, and alteration of chromosome structure [82].

## 5. Conclusion

The Fedaleh EO had significant potential as a growth disruptor and oxidative stress inducer for weed control. The significant decrease in weed germination exposed to Fedaleh EO indicated that the essential oil interferes with the stages of cell division and is highly effective in preventing weed growth, surpassing the efficiency of Treflan herbicide. Furthermore, exposure of seedlings to essential oils, similar to the herbicide Treflan, altered the biochemical substances in the plant, including ROS accumulation, lipid peroxidation, and membrane electrolyte leakage. These changes led to acute oxidative stress, and proline production was ineffective in protecting weed seedling. Consequently, the growth of the weed seedlings was significantly reduced. The high efficiency of the Fedaleh EO in reducing the seed germination index of weeds indicates that this essential oil is a viable alternative to the chemical herbicide Treflan. Therefore, the utilization of this natural essential oil in agricultural ecosystems can be effective in mitigating pollution caused by chemical herbicides.

*Additional information*

Supplementary content related to this article has been provided as appendix.

## Data availability statement

Data will be made available on request.

## CRediT authorship contribution statement

**Ali Nasiri:** Writing – original draft, Methodology, Investigation, Formal analysis. **Sina Fallah:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Amir Sadeghpour:** Writing – review & editing, Methodology, Conceptualization. **Hossein Barani-Beiranvand:** Writing – review & editing, Methodology.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36085.

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