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Investigation of the Effects of Plant Extracts Containing Allelopathic Compounds as Natural Herbicides for Controlling yellow-thorn and wild safflower in Chickpea Crops

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Abstract

The use of plant extracts containing inhibitory compounds and the evaluation of their toxic effects on weeds can contribute to reducing the reliance on chemical herbicides. This study aimed to investigate the effects of plant extracts containing allelochemical compounds as natural herbicides for controlling yellow-thorn (*Picnomon acarna)* and wild safflower (*Carthamus oxyacantha*) in chickpea (*Cicer arietinum*) fields during the cropping seasons of 2021–2022 and 2022–2023. In both years, the experimental treatments included extracts of chamomile, artichoke, and peanut shells; pelargonic acid; the herbicide Challenge,; and a control (pure water). The results indicated that, among the applied treatments, chamomile extract presented the highest toxicity to both weed species. Compared with the control, the chamomile extract significantly reduced weed biomass by 55.53% and increased chickpea biomass by 73.81%. The combined data analysis revealed that the greatest inhibition of yellow-thorn and wild safflower growth was observed after treatment with chamomile extract in the first year, with inhibition rates of 70.47% and 82.83%, respectively. In the second year, yellow-thorn and wild safflower treated with chamomile extract presented a reduction in greenness of 55.36% and 53.44%, respectively, compared with the control in the same year, indicating the phytotoxic effect of the extract. Notably, the stomatal conductance of yellow-thorn and wild safflower treated with chamomile extract in the second year decreased by 37.40% and 37.85%, respectively, compared with that of the untreated control in the same year. The yellow-thorn plants in the pelargonic acid and control treatments in the first year presented the lowest chlorophyll fluorescence, which was 18.82% lower than that in the control in the second year. Additionally, chamomile extract treatment in the second year resulted in a 41.86% reduction in the chlorophyll fluorescence of wild safflower compared with that of the control in the first year. Gas chromatography–mass

spectrometry (GC–MS) analysis of chamomile essential oils revealed 21 compounds, with terpenoids being the primary active components. The compounds myrtenyl acetate, decanoic acid, spathulenol, and caryophyllene oxide were the most abundant constituents of the essential oil. The significant inhibitory and herbicidal activity observed in chamomile can be attributed to myrtenyl acetate. Overall, this study provides valuable insights into the substantial inhibitory effects of chamomile extract on yellow-thorn and wild safflower, which may be utilized for the development of environmentally friendly plant-based herbicides.

Keywords: Pelargonic acid, inhibition, chamomile extract, artichoke, myrtenyl acetate, greenness

Introduction

Chickpea (*Cicer arietinum* L.) is one of the three major pulse crops worldwide, ranking second in cultivated area (14.09 million hectares) and third in production (16.52 million tons) in 2023, following beans and peas [1]. Iran, with an annual production of approximately 176,100 tons, is the ninth largest chickpea producer worldwide [1]. The provinces of Kermanshah, Lorestan, and Kurdistan in western Iran account for the largest chickpea cultivation areas [2]. As an affordable and abundant source of protein with increasing demand, chickpea is highly important [3]. However, one of the primary constraints to successful chickpea cultivation is its limited ability to compete with weeds due to slow growth rates and restricted leaf development during the early growth and establishment stages [4]. Yield losses in chickpea can range from 24% to 63%, depending on the level of weed infestation [5]. If weeds are not effectively managed during the critical growth period of chickpea (35-60 days after emergence), yield reductions of up to 88% may occur [6].

The major weed species in chickpea fields in western Iran include pigweed (*Amaranthus retroflexus* L.), wild safflower (*Carthamus oxyacantha*), Syrian cephalaria (*Cephalaria syriaca* L.), chicory (*Cichorium intybus* L.), mustard (*Conringia orientalis* L.), common fumitory (*Fumaria officinalis* L.), yellow-thorn (*Picnomon acarna* L.), knotgrass (*Polygonum aviculare* L.), cleavers (*Galium aparine* L.), cow cockle (*Vaccaria hispanica* Mill.), hairy vetch (*Vicia villosa*), prickly lettuce (*Lactuca serriola* L.), field bindweed (*Convolvulus arvensis* L.), and round-leaved hare ear (*Bupleurum rotundifolium* L.), all of which cause significant damage to chickpea crops [7].

Among these, yellow-thorn is an annual weed that grows up to 50 cm in height, with yellow thorns (10–15 mm long) on its leaves [8]. Originating from the Mediterranean region, it is considered a problematic weed in rainfed agricultural systems across western Iran [9], Lebanon, and the Victoria Mallee and Wimmera regions of Australia [10]. This weed

reproduces via seeds and is widely distributed in various areas, including croplands, roadsides, fallow lands, and pastures. Once established, it competes with crops and complicates harvest operations [8,10]. Yellow-thorn is prevalent in major rainfed crops such as wheat (*Triticum aestivum* L.) and chickpea, where a density of 16 plants per square meter can reduce chickpea yield by 25% [8].

Wild safflower, an annual plant belonging to the Asteraceae family, is widespread in northwestern India, Turkey, Uzbekistan, Turkmenistan, Kazakhstan, Iran, and subtropical regions of western Iraq [11]. This weed feature nonwoody stems, yellow to orange flowers, alternate or opposite thorny leaves with deeply lobed and serrated margins, and can grow up to 1.5 m tall [12]. It thrives in various soils and is resilient to harsh environmental conditions and arid climates [13]. Its flowering period spans from March to June, making it a troublesome weed in spring crop fields [12]. It adversely affects the yield of crops such as chickpea and cereals, complicates harvesting, and causes economic losses in agriculture [14].

Weeds compete with chickpea for resources, absorbing nutrients and stored soil moisture, negatively affecting seed nutritional composition by reducing nitrogen and crude protein contents, and ultimately decreasing the nutritional value, total seed production, and seed quality of chickpea [5,15]. Consequently, effective weed management has become essential. While chemical herbicides have been highly successful in controlling most weed species, growing public concerns about environmental issues have highlighted the need for alternative weed management systems based on natural compounds [16]. Natural compounds (allelochemicals) represent a diverse range of natural herbicides derived from living organisms or their secondary metabolites [17]. These allelochemicals, owing to their structural organization, short half-life in soil, and synergistic effects, offer a promising approach for weed control when released into the environment [18,19]. Various chemical groups of allelochemicals, such as fatty acids, phenolics, alcohols, steroids, flavonoids, and terpenoids, have been identified and can be produced in different plant parts (leaves, flowers, aerial parts, fruits, seeds, and roots) [19,20]. By identifying and quantifying these natural allelochemicals, new natural herbicides can be developed [21]. These allelochemicals can affect plant physiological functions by disrupting respiration, nutrient uptake, enzymatic activities, cell division, and membrane permeability [22]. Unlike chemical herbicides, these natural compounds target the biochemical pathways of weeds naturally, making them a sustainable alternative to chemical herbicides [23].

Numerous studies have explored the herbicidal potential of various plant species against weeds in crop fields. Elghobashy et al. [21] reported that *Ononis vaginalis* extract reduced the germination and growth of *Rumex dentatus*

in faba bean (*Vicia faba*) fields in both pure and mixed cropping systems. Another study revealed that the methanolic extract of *Artemisia santolinifolia* presented the greatest herbicidal potential against major wheat weeds, including *Sinapis arvensis*, *Lolium multiflorum*, and *Parthenium hysterophorus*, causing permanent wilting and significant reductions in chlorophyll content, root length, hypocotyl length, and weed biomass [23]. Vashishth et al. [24] reported that aqueous leaf extracts of *Murraya koenigii* inhibited the growth of weeds such as *Anagallis arvensis*, *Poa annua*, *Lepidium didymum*, and *Vicia sativa* without affecting germination, growth, photosynthesis, the biochemical properties of chickpea and wheat, or soil physicochemical properties.

On the basis of findings from the past decade, plant extracts containing allelochemicals have shown considerable potential as natural herbicides for controlling weed populations in agriculture. However, there is limited evidence regarding the herbicidal potential of ethanolic plant extracts on weeds, particularly yellow-thorn and wild safflower, in crop fields. Therefore, this study aimed to evaluate the effects of ethanolic extracts of chamomile (*Anthemis dontostephana* Boiss.), peanut (*Arachis hypogaea* L.) shells, and artichoke (*Gundelia tournefortii* L.) containing allelochemical compounds as natural herbicides for controlling yellow-thorn and wild safflower in chickpea fields over two consecutive years.

2. Materials and Methods

2.1. Experimental site and climatic details

This study was conducted during the cropping seasons of 2021–2022 and 2022–2023 in an 80-hectare agricultural field belonging to the Jihad Self-Sufficiency Unit of the 35th Rapid Response Commando Brigade of the Islamic Republic of Iran Army, located in Jiranbolagh, Sarfiruzabad Dehestan, at 47°5'48" E longitude, 34°12'34" N latitude, and an elevation of 1728 m above sea level. The experimental site, which is situated 8 km south of Kermanshah, experiences a Mediterranean climate characterized by long, dry summers and cold winters with few and irregular snowy days. The long-term average annual precipitation in the region is 435 mm. The climate is classified as semiarid on the basis of the Selyaninov method.

Chickpea seeds (cv. Mansour) were obtained from Kaveh Mahidasht Seed Improvement Company. The field, previously cultivated with bread wheat in both years, was plowed to a depth of 25–30 cm after the first effective rainfall in early winter. The experimental plots consisted of six planting rows, each 3 m long with 0.5 m spacing, covering an area of 9 m^2 (3 × 3 m). The chickpea was sown mechanically via a seed drill at a density of 15 plants per

m² (45 kg/ha), following local techniques, when the soil moisture was adequate. Planting occurred on 16 March 2022 in the first year and 2 March 2023 in the second year.

During the first third of the growing season, weed density was assessed to aid in block design and plot selection across the field, following the methods of Minbashi et al. [25]. A total of 27 weed species were identified. In 2022, the total precipitation was 285.7 mm with an irregular distribution, peaking in May. In 2023, the precipitation reached 477.2 mm, which was also irregularly distributed, with the highest rainfall occurring in April (Figure 1). The spring temperatures (during the chickpea growing period) in 2023 were cooler than those in 2022.

2.2. Collection of plant materials

Aerial parts of artichoke and chamomile at the flowering stage were collected in early spring 2021 from elevations of 1300–1800 m in the Kuh-e-Sefid region of Kermanshah, which is part of the high Zagros range. Peanut pod shells were collected after the seeds were harvested from the experimental field of the Razi University Agricultural and Natural Resources Campus. Plants collected from the same region or taxonomic group may not produce identical quantities or qualities of secondary metabolites, leading to variations in allelochemical content [26].

2.3. Preparation of Plant Extracts

Following taxonomic confirmation by a botanist, plant extracts were prepared via the optimized method of Falleh et al. [27]. The collected plant samples were washed separately with distilled water and air-dried at room temperature in darkness for 7 days. The samples were then oven dried at 60°C for 2 hours and ground into powder. The powder was passed through a 35-mesh sieve for homogenization. Extracts were prepared using ethanol as a solvent at a 1:10 ratio of dry powder to solvent, and the mixtures were placed on a shaker at room temperature for 24 hours. Each extract was filtered twice—first through four layers of cheesecloth and then through Whatman No. 1 filter paper—to remove residues. The ethanol was evaporated via a rotary evaporator at 45°C, and the residues were redissolved in 1% Tween. The extract yield percentages were determined (Table 1). To preserve the allelochemical compounds and prevent degradation, the extracts were stored at 4°C in a refrigerator immediately after preparation and before use, as described by Aslani et al. [28].

2.4. Determination of total phenolic content

The total phenolic content was measured via the gravimetric method [29]. Five grams of powdered plant material was mixed with 50 mL of 10% acetic acid in ethanol. The suspension was incubated at 30°C on a shaker at 120 rpm. After 4 hours of settling, the mixture was filtered through Sartorius No. 389 filter paper. The filtrate volume was reduced to one-fourth of its original volume via a heater. A 30% ammonium hydroxide solution was added dropwise until phenolic compounds precipitated, and the mixture was filtered through preweighed filter paper. The filter paper was washed with 1% ammonium hydroxide, dried in an oven at 60°C for 30 minutes, and immediately transferred to a desiccator to prevent moisture absorption. The difference between the initial and final weights of the filter paper indicated the phenolic content, expressed as a percentage (Table 1).

2.5. DPPH free radical scavenging assay

The antioxidant capacity of the extracts was evaluated via the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method, which measures the ability of antioxidants to neutralize the lipid-soluble DPPH free radical that initiates lipid peroxidation and autoxidation chain reactions [30]. The reduction of DPPH by antioxidants results in the formation of stable H-DPPH molecules, decreasing the absorbance and producing yellow compounds with maximum absorption at 517 nm. To prepare a 4 mg/mL extract solution, the extract was dissolved in 2 mL of ethanol and diluted to 0.25 mg/mL. Two milliliters of DPPH solution (prepared by dissolving 2.4 mg of DPPH in 100 mL of ethanol) was added to 50 µL of various extract concentrations. After the samples were incubated in the dark for 30 minutes, their absorbance was measured at 517 nm via a spectrophotometer. Three replicates were prepared for each concentration. Butylated hydroxytoluene (BHT) at a concentration of 1 mg/mL was used as a synthetic antioxidant positive control [31]. The DPPH scavenging activity was calculated as a percentage via the following formula (Eq. 1) and is reported in Table 1:

$$\text{DPPH (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (1)$$

In this equation, $Abs_{control}$ represents the absorbance of the DPPH solution without the test sample (blank), whereas Abs_{sample} denotes the absorbance of the DPPH solution in the presence of the test sample. The difference between these absorbance values ($Abs_{control} - Abs_{sample}$) reflects the reduction in the DPPH radical concentration due to the antioxidant activity of the sample. This difference was then divided by the absorbance of the control to normalize the result, and the quotient was multiplied by 100 to express the scavenging activity as a percentage.

2.6. Experimental design and treatments

The experiment was conducted as a factorial design based on a randomized complete block design (RCBD) with three replications in both 2022 and 2023. The treatments included chamomile extract at a concentration of 50 g/L, artichoke extract at 100 g/L, peanut shell extract at 100 g/L, challenge herbicide at a rate of 2 per thousand, pelargonic acid at 5 per thousand, and a control (distilled water). Treatments were applied via foliar spraying combined with an adjuvant (provided by the Giah Company) to increase the penetration of active molecules through epicuticular waxes when the chickpea crop reached the 6–8 leaf stage and when most weeds were at the 2–6 leaf stage (critical period for weed control). In 2022, the critical weed control period occurred on 27 May, coinciding with the third growth stage (Set III, vegetative growth) of the five chickpea growth stages. Owing to late planting in mid-March and insufficient rainfall in April, the critical weed control period in 2022 was delayed until early June. In 2023, chickpea planting was carried out on 2 March, and treatments were applied on 9 May. Adequate rainfall in April 2023 resulted in an earlier critical weed control period than that in 2022.

2.7. Measured Traits

Forty-eight hours after treatment, the chlorophyll content was measured via a SPAD-502 device (Konica Minolta, Japan) [32]. To assess the quantum efficiency of photosystem II and the photosynthetic performance index (Pi), leaves were dark-adapted for 30 min via leaf clips two days after foliar application of the extracts and other treatments. The maximum photochemical efficiency of photosystem II, represented by the ratio of variable fluorescence to maximum fluorescence (Fv/Fm), was measured via a chlorophyll fluorescence device (Pocket PEA, UK). Stomatal conductance was recorded 48 hours posttreatment by placing the middle portion of the leaves inside the sensor of a photosynthesis meter (SD Portable, UK), with readings taken after 30 seconds. To minimize the effects of temperature and light variations on stomatal behavior, measurements were conducted between 7:00 and 9:00 a.m. [24]. For biomass estimation, chickpea plants and all weeds within a 2 m^2 area of each experimental plot were harvested in early July, oven-dried at 80°C for 48 hours until a constant weight was reached, and then weighed [32].

2.8 Weed Suppression and Population Indices

To evaluate the inhibitory effects of the various treatments on weed populations (St), the weed control efficacy of each treatment was compared with that of the control, following Motmainna et al. [33]. The weed suppression percentage was calculated via Eq. 2:

$$\mathrm{St} = \frac{\mathrm{W_u} - \mathrm{W_t}}{\mathrm{W_u}} \times 100 \quad (2)$$

where W_u represents the weed biomass (g/m²) measured at harvest under unrestricted conditions (control), and W_t represents the weed biomass recorded in each treated plot. This equation quantifies the reduction in weed biomass due to the applied treatments relative to the control, expressed as a percentage.

Weed population indices, including frequency, uniformity, density, mean density, and dominance index, were calculated via Eqs. 3 to 7, as described by Minbashi et al. [25].

$$F_k = \frac{\sum Y_i}{n} \times 100 \quad (3)$$

where F_k is the frequency of species *K*, Y_i indicates the presence (1) or absence (0) of species *K* in field *i*, and *n* is the number of fields surveyed. This formula expresses the proportion of fields where a specific weed species is present.

$$U_k = \frac{\sum_1^n \sum_1^m X_{ij}}{\sum_1^m m} \quad (4)$$

where U_k is the field uniformity for species *K*, *Xij* denotes the presence (1) or absence (0) of species *K* in quadrat *i* of field *j*, *n* is the number of fields surveyed, and m is the number of quadrats sampled. This equation assesses the evenness of species distributions across quadrats within fields.

$$D_{ki} = \frac{\sum_1^m Z_j}{m} \times 4 \quad (5)$$

where D_{ki} is the density (plants/m²) of species *K* in field *i*, *Zj* is the number of plants in a 0.25 m² quadrat, and m is the number of quadrats sampled. This formula converts quadrat counts to a per-square-meter density.

$$MFD_{ki} = \frac{\sum_1^n D_{ki}}{n} \quad (6)$$

where MFD_k is the mean density of species *K*, D_{ki} is the density of species *K* in field *i*, and *n* is the number of fields studied. This equation averages the density of a species across all fields.

$$AI_k = F_k + U_k + MFD_k \quad (7)$$

where AI_k is the dominance index of species *K*, combining its frequency, uniformity, and mean density to quantify its ecological prominence in the field [25].

The diversity within fields was assessed via the Shannon–Wiener index, which was calculated via Eq. 8:

$$H' = \sum_{p=1}^{s} [(P_i) \times ln(P_i)] \quad (8)$$

where s is the number of species recorded and P_i is the proportion of individuals belonging to species *i*. This index quantifies species diversity by accounting for both abundance and evenness.

2.9 GC–MS analysis

To separate and identify the constituents of chamomile essential oil, the oil was analyzed via gas chromatography–mass spectrometry (GC–MS) at the laboratory of the Research Institute of Forests and Rangelands, Iran. The GC–MS system was equipped with a capillary column (30 m length, 250 μm internal diameter, 0.25 μm film thickness). Helium was used as the carrier gas with a split ratio of 5:1 and an injection temperature of 230°C. The GC oven temperature was programmed from 60°C to 275°C, with a gradual increase of 5°C/min, reaching a final temperature of 265°C, which was maintained for 30 minutes. The helium carrier gas flow rate was 1 mL/min. The injector and detector temperatures were set at 300°C and 320°C, respectively. After injecting 1 μL of the essential oil, the resulting chromatogram revealed the presence of numerous compounds. Compound identification was performed by comparing their retention times (Rt) and mass spectra with those in the NIST/EPA/NIH Mass Spectral Library 2014, which is integrated with the GC–MS system [34].

2.10 Data analysis

The data were normalized, and the homogeneity of variance across the two years was tested. A combined analysis of variance (ANOVA) was performed via SAS software, with the year treated as a random factor. Mean comparisons were conducted via the least significant difference (LSD) test at the 5% probability level. Graphs were generated and interpreted via Microsoft Excel.

3. Results

In this study, the dominant weed species in the chickpea field were identified. The weed flora consisted of 27 species, ranked by density. Among these, yellow-thorn and wild safflower, with frequencies of 70% and 68%, were the fifth and sixth most dominant weeds, respectively. The average weed density in the field was 34.79 plants/m². The highest average density was recorded for cleavers (10.6 plants per m²) and chicory. The densities of chicory, creeping thistle

(*Cirsium arvense*), yellow-thorn, and wild safflower were approximately 5.0, 5.0, 3.8, and 3.2 plants per m², respectively, making them the dominant species in terms of density in the chickpea field. Additionally, cleavers, chicory, creeping thistle, yellow-thorn, and wild safflower had the highest relative density values of 30.47%, 14.37%, 14.37%, 10.92%, and 9.2%, respectively (Table 2). The Shannon–Wiener diversity index was calculated as 2.25.

3.1. Effects of the treatments on chickpea and weed biomass

Combined data analysis revealed that two weeks after treatment, chickpea biomass and weed biomass were significantly affected by the extracts at the 1% probability level but not by year. The interaction effect between year and extract was also significant for these traits (Table 3). The effect of the extracts on chickpea biomass was significant at the 1% probability level in 2022 but not in 2023, whereas the effect on weed biomass was significant in both years (Table 3). An evaluation of the effects of foliar application on chickpea biomass revealed that chamomile extract in 2022 was the most effective, with an average chickpea biomass of 353.8 g/m², followed by artichoke extract in 2023. In contrast, the lowest biomass was recorded in the control treatment in 2022, at 227.47 g/m². Specifically, foliar application of chamomile extract in 2022 had a greater inhibitory effect on weeds, resulting in a 55.53% increase in chickpea biomass compared with that of the control. Compared with the control, herbicide challenge also increased chickpea biomass by 10.62% in 2022 (Figure 2).

In both consecutive years, all the applied treatments had phytotoxic effects on weed biomass compared with the control. The chamomile extract in 2022 had the greatest inhibitory effect on weed biomass (72.77 g/m²). Although this treatment did not significantly differ from herbicide challenge (88.35 and 92.17 g/m² in 2022 and 2023, respectively) or pelargonic acid (87.92 and 91.94 g/m² in 2022 and 2023, respectively), its control effect was greater. In contrast, the control treatments in 2022 and 2023 presented the highest weed biomass, at 277.86 and 232.17 g/m², respectively. Compared with the control, the CM extract in 2022 reduced weed biomass by 73.81% and 68.66% in 2022 and 2023, respectively (Figure 2).

A comparison of the effects of the different treatments on chickpea and total weed biomass revealed an increase in chickpea biomass with the application of the plant extracts. This increase was particularly pronounced with chamomile extract in 2022, reaching approximately 83%. With this treatment, the total weed biomass was reduced to 17%, suggesting the high efficacy of chamomile extract for weed control and minimal damage to chickpea growth.

Additionally, in 2023, pelargonic acid was the most effective treatment, resulting in a 78% increase in chickpea biomass and a 22% reduction in weed biomass (Figure 2).

3.2. Inhibition

Analysis of variance revealed that treatment (extracts), year, and their interaction had varying effects on the inhibition of yellow-thorn and wild safflower growth. The interaction effect of the extracts in each year significantly influenced the inhibition of both weed species ($p < 0.01$) (Table 4). In both years, the application of the extracts increased the inhibition of both weed species. The chamomile extract from the first year (2022) was the most effective at suppressing weed growth, increasing the inhibition of yellow-thorn by approximately 70.47%. The artichoke extract had inhibition rates of 66.93% in the first year and 62.56% in the second year, indicating a stronger inhibitory effect than did the herbicide challenge (60.8% and 55.86% in 2022 and 2023, respectively) and pelargonic acid (54.36% and 49.56% in 2022 and 2023, respectively), although no significant differences were detected among them (Figure 3A).

Conversely, chamomile extract in the first year had a strong inhibitory effect on wild safflower growth (82.83%), with significantly greater efficacy than herbicide challenge. Following the chamomile extract, pelargonic acid had the highest inhibition rates of 82.63% and 78.6% in the first and second years, respectively (Figure 3B). In both years, no inhibition was observed in the control treatment, and the peanut shell extract had the least suppressive effect on the growth of both weed species.

3.3 Greenness

The greenness of yellow-thorn and wild safflower was significantly affected by the extract treatment and year ($p < 0.01$). Additionally, the greenness of both weed species was influenced by the year × extract treatment interaction. The interaction effect of treatments in the second year was significant for the greenness of both weed species ($p \leq 0.01$) (Table 4). The greenness of yellow-thorn ranged from 37.1--47.66% in the first year and from 25.67--57.50% in the second year. The chamomile extract in the second year presented the highest phytotoxicity and the lowest greenness (25.67) among all the treatments. In contrast, the greatest degree of greenness was recorded in the control treatment in the second year (57.50). Accordingly, chamomile extract application in the second year reduced yellow-thorn greenness by 55.36% compared with that of the control. Compared with herbicide challenge, chamomile extract reduced greenness by 41.70% in the first year and 48.79% in the second year (Figure 4A). In both years, the greenness

of wild safflower plants treated with plant extracts was lower than that of the untreated control plants. The lowest greenness (27.77) for wild safflower was recorded with chamomile extract in the second year, representing a 53.44% reduction compared with the control in the same year. In the second year, the herbicide challenge (35.27) performed similarly to the chamomile extract, reducing greenness by 40.87% compared with the control (Figure 4B).

3.4 Stomatal conductance

The combined data analysis revealed significant differences in the effects of treatment ($p < 0.01$) and the treatment × year interaction ($p < 0.05$) on the stomatal conductance of yellow-thorn and wild safflower. The year had a significant effect on the stomatal conductance of wild safflower leaves. The interaction effect of treatments (extracts) in the second year was significant for both weed species ($p < 0.01$) (Table 5). In the second year (2023), all concentrations of ethanolic extracts from chamomile, artichoke, and peanut shells significantly reduced the stomatal conductance of yellow-thorn and wild safflower compared with the control. The greatest reduction in stomatal conductance was observed with chamomile extract in the second year, with values of 26.55 mmol CO_2 m^{-2} s^{-1} for yellowthorn and 27.47 mmol CO_2 m^{-2} s^{-1} for wild safflower. This was followed by pelargonic acid, with values of 27.13 and 28.96 mmol CO_2 m^{-2} s^{-1}, respectively. Compared with the control, these treatments reduced the stomatal conductance of yellow-thorn by 37.40% and 32.03% and that of wild safflower by 37.85% and 23.85% in the second year. In contrast, the highest stomatal conductance for both weed species was observed in the control treatment in the second year, with values of 42.33 mmol CO_2 m^{-2} s^{-1} for yellow-thorn and 44.2 mmol CO_2 m^{-2} s^{-1} for wild safflower (Figure 5).

3.5 Chlorophyll fluorescence

The application of various plant extracts (treatments) and the treatment × year interaction significantly altered chlorophyll fluorescence in both yellow-thorn and wild safflower ($p < 0.01$). The interaction effect of treatments was significant in both years ($p < 0.01$) (Table 6). Chlorophyll fluorescence is a nondestructive and rapid technique for assessing photosynthetic responses. The pelargonic acid and control treatments in the first year presented the lowest chlorophyll fluorescence in yellow-thorn (0.69), which was 18.82% lower than that of the control in the second year. The next lowest values were recorded for pelargonic acid (0.72) and chamomile extract (0.73) in the second year and artichoke extract (0.74) in the first year. These five treatments showed no statistically significant differences. The highest chlorophyll fluorescence was observed in the control treatment in the second year (0.85) (Figure 6A). For wild

safflower, chamomile extract in the second year (0.50) and pelargonic acid in the first year (0.59) presented the lowest chlorophyll fluorescence among all the treatments. Compared with the control, these treatments reduced chlorophyll fluorescence by 41.86% and 32.18%, respectively, in the first year. Compared with those in the control, the chamomile and artichoke extracts in the first year and the pelargonic acid and artichoke extracts in the second year had similar effects on reducing chlorophyll fluorescence in wild safflower, with reductions of 29.90%, 27.60%, 26.44%, and 23%, respectively, in the first year (Figure 6B).

3.6 Identification of compounds in the essential oil of chamomile

The chamomile genus, comprising approximately 250 species, includes annual and perennial plants that are primarily distributed in temperate and cold regions. Most species are found in the Zagros region. Despite its ecological, agricultural, and medicinal importance, few studies have been conducted on chamomile. The field results revealed that chamomile extract had the greatest suppressive and herbicidal effects on the 17 studied weed species. Therefore, this study investigated the essential oil components and their allelopathic effects on weeds. The gas chromatogram from the analysis of chamomile essential oils is presented in Figure 7. GC–MS analysis revealed a diverse range of chemical compounds in chamomile essential oils, which appeared between 1 and 60 minutes. A total of 21 compounds were identified, accounting for 82.49% of the total essential oil. Among these, myrtenyl acetate (20.37%), decanoic acid (9.85%), spathulenol (7.99%), and caryophyllene oxide (5.12%) were the most abundant compounds in chamomile essential oils (Table 6).

4. Discussion

The results from field experiments conducted over two years demonstrated the significant herbicidal activity of ethanolic extracts from chamomile, artichoke, and peanut shells. However, this efficacy was selective, primarily affecting weed growth. Among all the treatments, the chamomile ethanolic extract had a pronounced negative effect on the growth of the tested weeds, suppressing yellow-thorn and wild safflower while enhancing the competitive ability of chickpea, leading to reduced weed biomass and increased chickpea biomass. The variations in plant sensitivity to allelochemicals may be explained by differences in absorption mechanisms through roots and surface cells, as well as diverse metabolic pathways and sites of action [19]. Pannacci et al. [35] reported similar findings, noting that mugwort (*Artemisia vulgaris* L.) extract inhibited seed germination, seedling emergence, and growth of

pigweed, a common weed associated with maize, without affecting maize (*Zea mays* L.) seed germination or seedling growth. The application of increasing doses of aqueous extract (12–21 L/ha) from mixtures of sorghum + sunflower and sorghum + sunflower + mulberry significantly reduced the total weed density and dry biomass of narrow-leaf weeds such as wild oat (*Avena fatua*) and littleseed canarygrass (Phalaris minor), as well as broadleaf weeds such as lamb quarters (*Chenopodium album*) and swinecress (*Coronopus didymus*). The application of a sorghum, sunflower, and mulberry aqueous extract mixture at 18 L/ha 40 and 55 days after wheat sowing resulted in an 87.14% reduction in total weed dry matter and a 19.5% increase in wheat grain yield [36].

In our study, the chamomile ethanolic extract significantly suppressed the growth of yellow-thorn and wild safflower seedlings in both experimental years (Figure 2). These inhibitory activities of various plant extracts have been documented in other studies. Dar et al. [37] demonstrated that *Anthemis cotula* extract enhanced the growth of wild oat, rice (*Oryza sativa*), wheat, and mung bean (*Vigna radiata*) plants. Similarly, German chamomile flower extract has shown potential for inhibiting the germination and growth of weeds associated with wheat crops, including *Lolium multiflorum* Lam., wild oat, and *Phalaris paradoxa* L. [16]. Reductions in weed growth and leaf chlorosis symptoms are attributed to the inhibition of chlorophyll synthesis, photosynthesis pathways, nitrogen metabolism, and porphyrin metabolism [38]. Many allelochemicals reportedly reduce the stimulatory effects of indole-3-acetic acid and gibberellin, leading to diminished plant organ growth [19]. Boukhalfa et al. [39] suggested that plant growth inhibition by essential oils may result from the production of reactive oxygen species (ROS), which damage proteins, DNA, lipids, cell membranes, and photosynthetic pigments, ultimately leading to plant mortality.

Chlorophyll is a critical parameter for studying the effects of allelochemicals on plant physiology [40]. Our findings revealed that, over the experimental years, weed leaf greenness decreased after exposure to chamomile extract, with plants wilting and yellowing. Aqueous stem extracts of *L. didymum* reduce the chlorophyll content in both Lens culinaris and Melilotus albus [41], which is consistent with our results. The reduction in weed chlorophyll levels may be attributed to excessive ROS production induced by bioactive compounds in the extracts [23]. Allelochemicals in plant extracts can reduce greenness by interfering with thylakoid membranes, decreasing chlorophyll synthesis, or inhibiting enzymes involved in chlorophyll biosynthesis [42]. Plant extracts have been shown to reduce photosynthesis and energy production by lowering chlorophyll content, resulting in visible symptoms such as chlorosis (leaf yellowing) [43].

Stomatal conductance measures the degree of stomatal opening in plants and is linked to the internal carbon dioxide content and leaf water potential in mesophyll cells, which are regulated by abscisic acid levels in chloroplasts [44]. These characteristics are influenced by multiple stress factors and indicate reduced photosynthetic efficiency [45]. Our results revealed that in both experimental years, the stomatal conductance of yellow-thorn and wild safflower was significantly reduced by foliar application of chamomile extract. These findings align with those of Bashar et al. [46], who reported that *P. hysterophorus* leaf extract at 100 g/L reduced photosynthesis, transpiration rate, stomatal conductance, carotenoid content, and chlorophyll content in bambara groundnut (*Vigna subterranea* L.), maize, and six weed species (*Digitaria sanguinalis*, *Eleusine indica, Ageratum conyzoides, Cyperus iria, Euphorbia hirta*, and *Cyperus difformis*). The most effective phytotoxic mechanism induced by allelochemicals involves photosynthesis inhibition, which is associated with reduced stomatal conductance and intracellular CO_2 concentration, suggesting that decreased photosynthetic efficiency is partly due to stomatal closure [47]. Stomatal conductance is closely linked to reduced transpiration rates. As stomata open, water is lost through evaporation and transpiration, while CO_2 is absorbed via photosynthesis [45]. Plant extracts can induce partial stomatal closure to prevent water loss, consequently reducing photosynthesis and transpiration, which slows plant growth [48].

Chlorophyll fluorescence is a powerful tool for studying the impact of stresses on photosynthetic processes, reflecting the maximum quantum yield of photosystem II (PSII) (Fv/Fm) [42]. The Fv/Fm test is designed to maximize the amount of light energy directed into the fluorescence pathway [24]. In this study, weeds treated with chamomile extract presented reduced chlorophyll fluorescence. Damage to thylakoid membranes and inhibition of energy transfer from antenna molecules to reaction centers can lead to photoinhibition and reduced Fv/Fm, potentially inhibiting initial photosynthetic reduction and hindering photosynthetic product formation [42]. Allelochemicals can significantly affect thylakoid electron transport during light reactions, stomatal regulation of CO_2, and carbon cycling in dark reactions [33]. Additionally, allelochemicals inhibit oxygen evolution and photosynthesis by interacting with photosystem II components [41]. According to Motmainna et al. [33], the Fv/Fm, photosynthesis rate, stomatal conductance, and transpiration of *Cyperus iria* are reduced by foliar application of *P. hysterophorus* extract.

In this study, chamomile essential oils were analyzed via gas chromatography–mass spectrometry (GC–MS) to identify active phytochemical compounds and their allelopathic potential on weeds. Twenty-one compounds were identified, with myrtenyl acetate (20.37%), decanoic acid (9.85%), spathulenol (7.99%), and caryophyllene oxide (5.12%) being the major constituents. These compounds belong to various chemical classes, including terpenes,

sesquiterpenes, and fatty acids. Among the identified compounds, myrtenyl acetate, caryophyllene oxide, and camphor have been reported as bioherbicides in various studies [49-51]. Essential oils comprise diverse organic groups of terpenoids, such as hydrocarbons, oxygenated monoterpenes, sesquiterpenes, and diterpenes. Terpenes are unsaturated hydrocarbons with distinct structural and chemical diversity based on simple isoprene molecules [52]. Terpenoids (primarily mono- and sesquiterpenes) are the main active components of essential oils, making them potential candidates for the development of new bioherbicides because of their strong phytotoxic activity against various weed species [39]. The phytotoxic potential of essential oils includes chlorosis, leaf burning, reduced plant growth, mitosis inhibition, membrane depolarization, reduced chlorophyll content, cellular respiration, and oxidative damage [42]. Thus, the significant inhibitory and herbicidal activity observed in the chamomile extract can be attributed to the high proportion of monoterpenes and sesquiterpenes, particularly myrtenyl acetate. Abd-ElGawad et al. [53] reported that sesquiterpenes, along with monoterpenes, especially oxygenated components of *Pergularia tomentosa*, exhibited herbicidal activity against *Dactyloctenium aegyptium* and Bidens pilosa. The application of essential oils from *Satureja alpina*, *Thymus satureioides*, and Myrtus communis significantly reduced the stem and root length and chlorophyll content of pigweed. This herbicidal property was attributed to pulegone (81.15%) in *S. alpina*, carvacrol (33.05%) in *T. satureioides*, and myrtenyl acetate (43.5%) in M. communis [54].

Conclusion

The results demonstrated that among all the treatments, the ethanolic extract of chamomile enhanced the competitive ability and growth of chickpea by suppressing weed growth. Chamomile extract significantly reduced the greenness, stomatal conductance, and chlorophyll fluorescence of both weed species, yellow-thorn and wild safflower, in both years of the experiment. Furthermore, compared with the challenge herbicide, chamomile extract inhibited weed growth and exhibited superior herbicidal potential. Phytochemical analysis identified 21 compounds in chamomile essential oils. These results provide evidence that Myrtenyl acetate, which is isolated from chamomile essential oils, has herbicidal potential. These findings suggest that chamomile extract can be used as a novel plant-based herbicide for weed management in chickpea crops without adverse environmental impacts. However, further studies are needed to investigate its mechanism of action on weeds and to develop a natural herbicide.

Data availability

The raw data of this article will be made available by corresponding author according to the personal requests.

Declarations

Ethics approval and consent to participate

All methods performed in this study including the collection of plant materials were in compliance with the relevant institutional, national, and international guidelines and legislation.

Experimental research and field studies on plants

The growing plants sampled comply with relevant institutional, national, and international guidelines and domestic legislation of Iran.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests

Funding Declaration

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Authors' Contributions

S.J., A.G., M.R.A., and A.B. conceived and designed the experiments. S.J. and A.B. conducted the field experiments and collected the data. M.R.A. performed the GC-MS analysis and interpreted the chemical data. S.J. and A.G. analyzed the data and conducted the statistical analysis. S.J. wrote the initial draft of the manuscript. A.G. and M.R.A. contributed to revising and editing the manuscript. All authors reviewed and approved the final manuscript.

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Figures

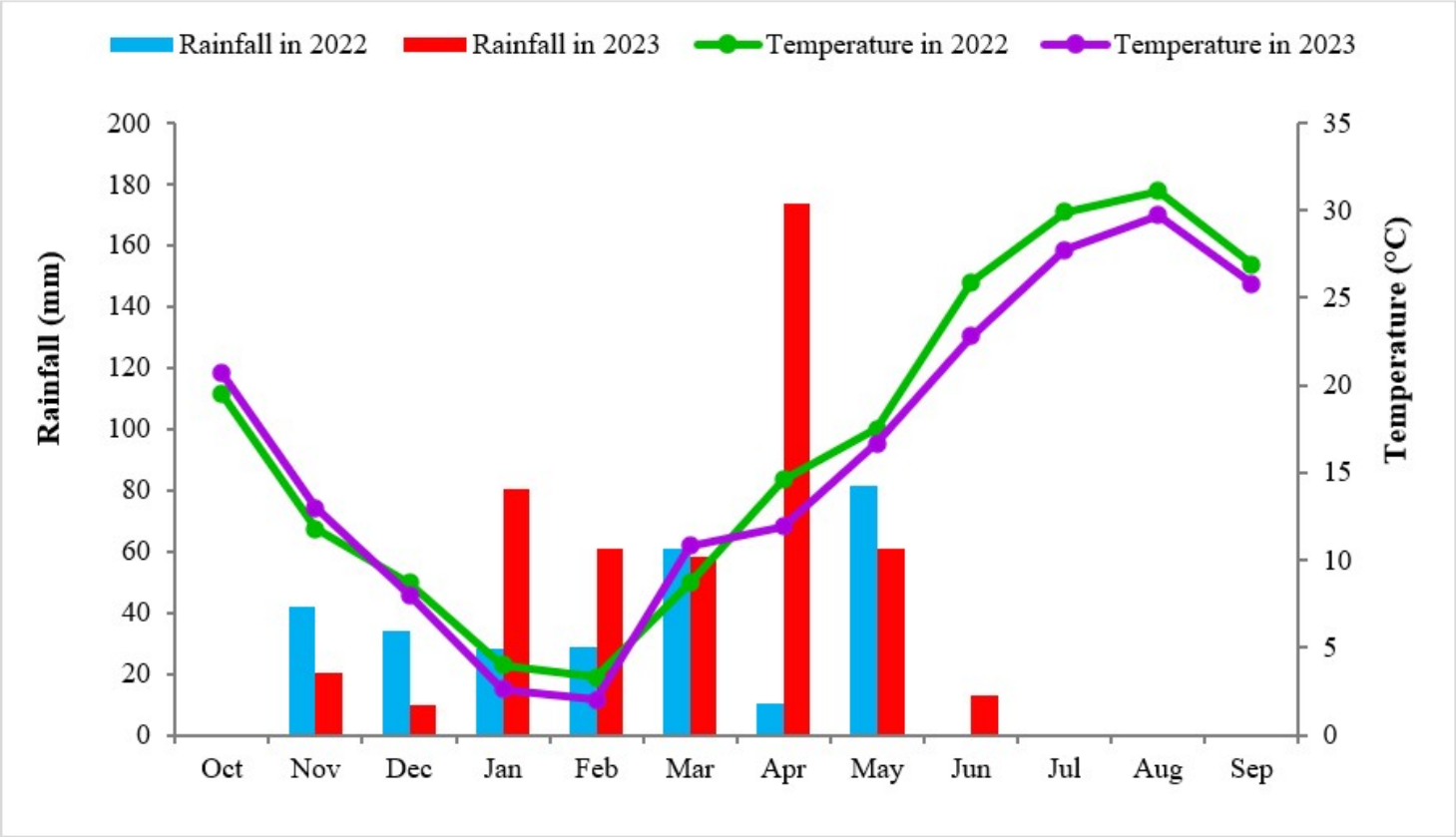


Figure 1

Climatic variations during the experimental months in 2022 and 2023

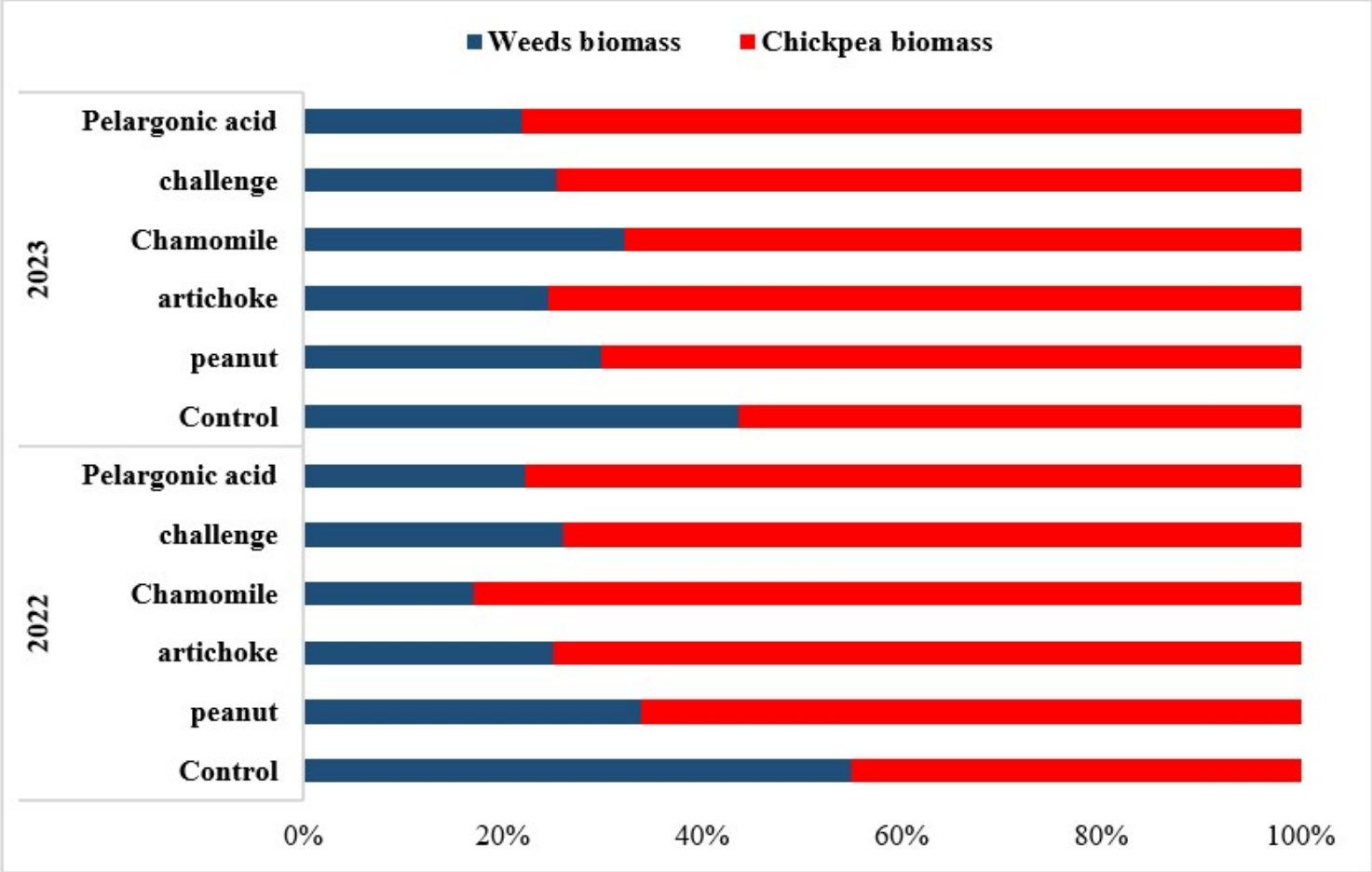


Figure 2

Effect of treatments on the percentage of total weed and chickpea biomass in plots

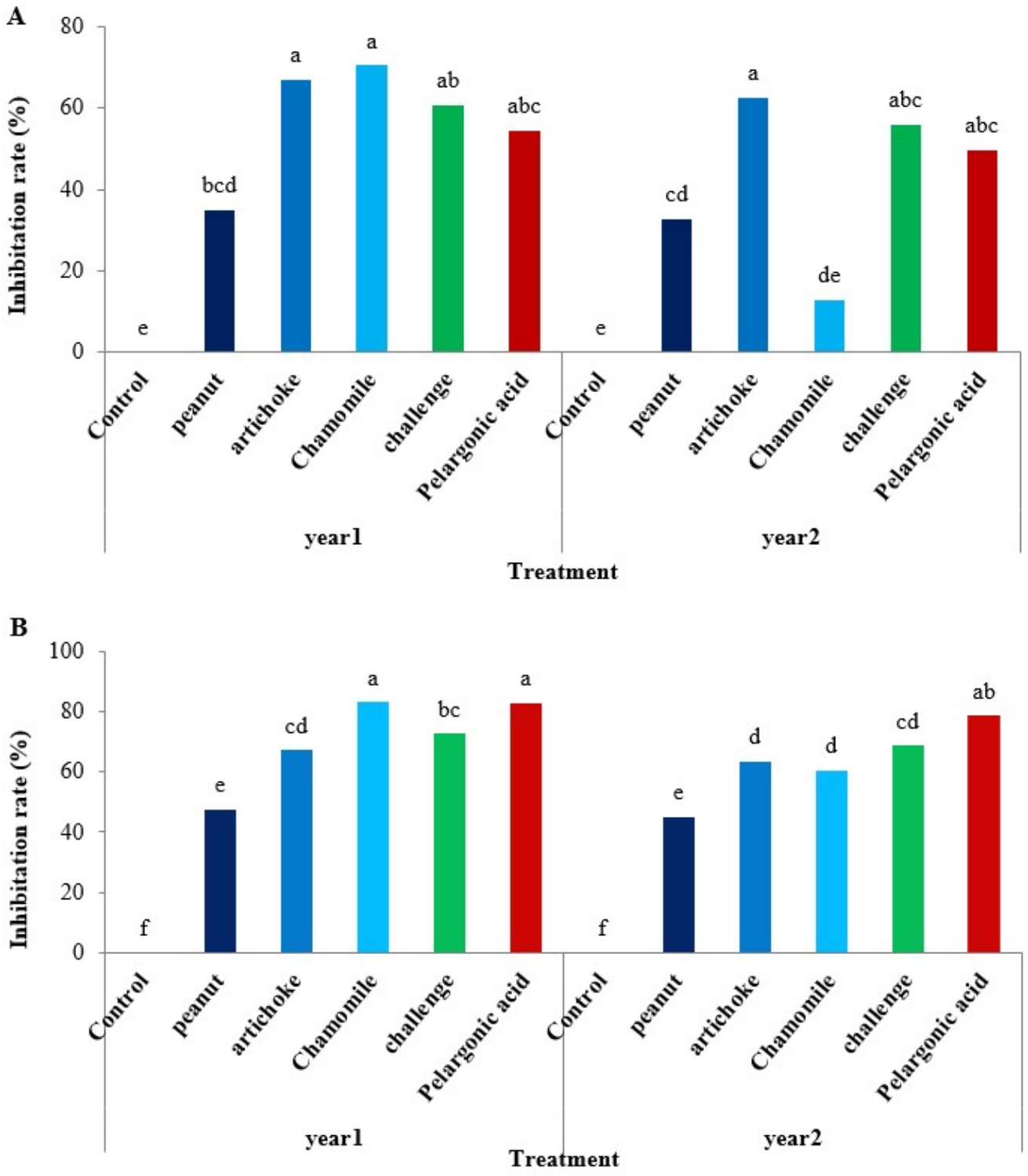


Figure 3

Comparison of the mean effect of treatments on the inhibition of weed growth for yellow-thorn (A) and wild safflower (B). Means sharing the same letter in each column are not significantly different based on the Least Significant Difference (LSD) test at a 5% probability level.

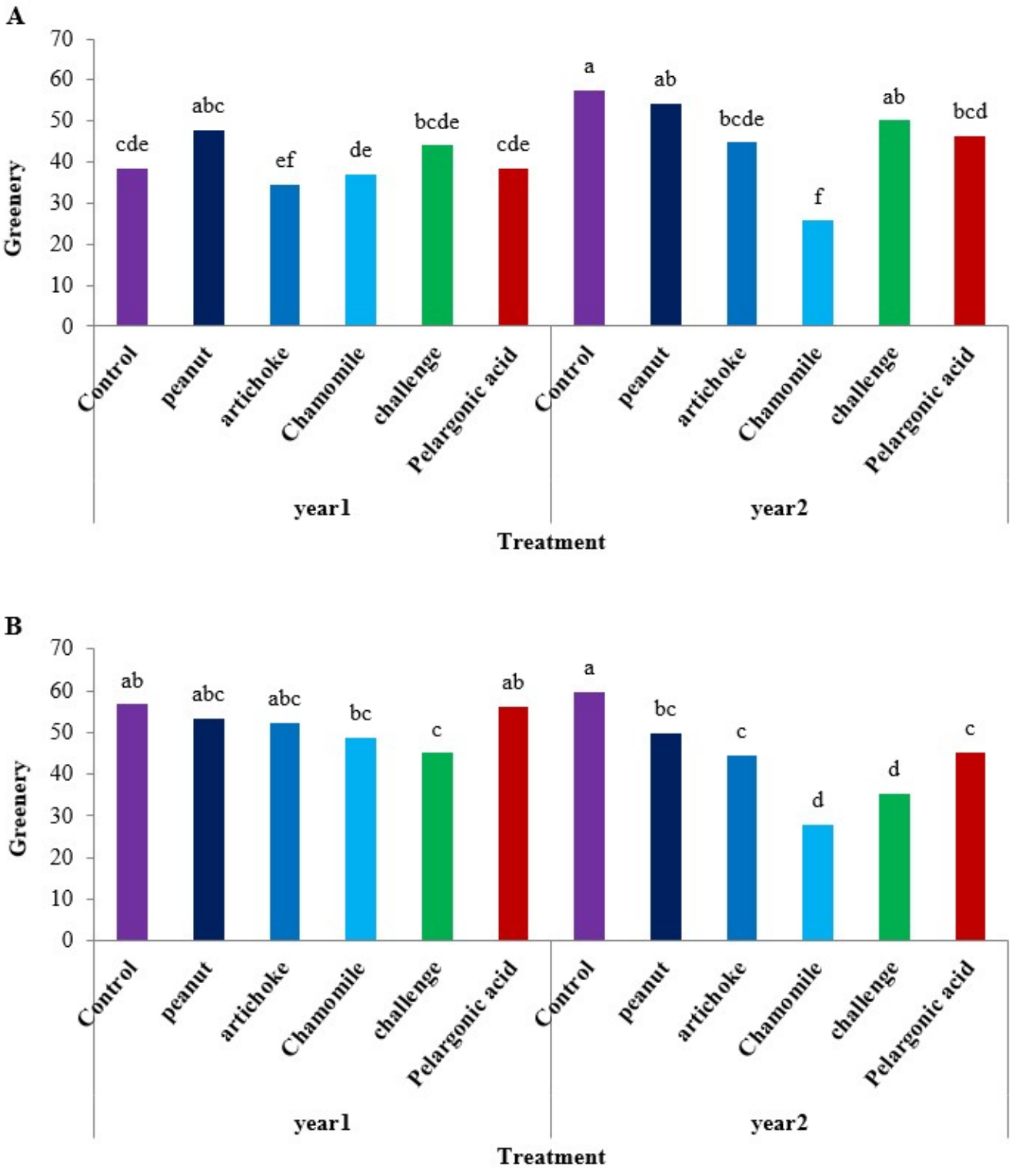


Figure 4

Comparison of the mean effect of treatments on the greenness of weeds yellow-thorn (A) and wild safflower (B). Means sharing the same letter in each column are not significantly different based on the Least Significant Difference (LSD) test at a 5% probability level.

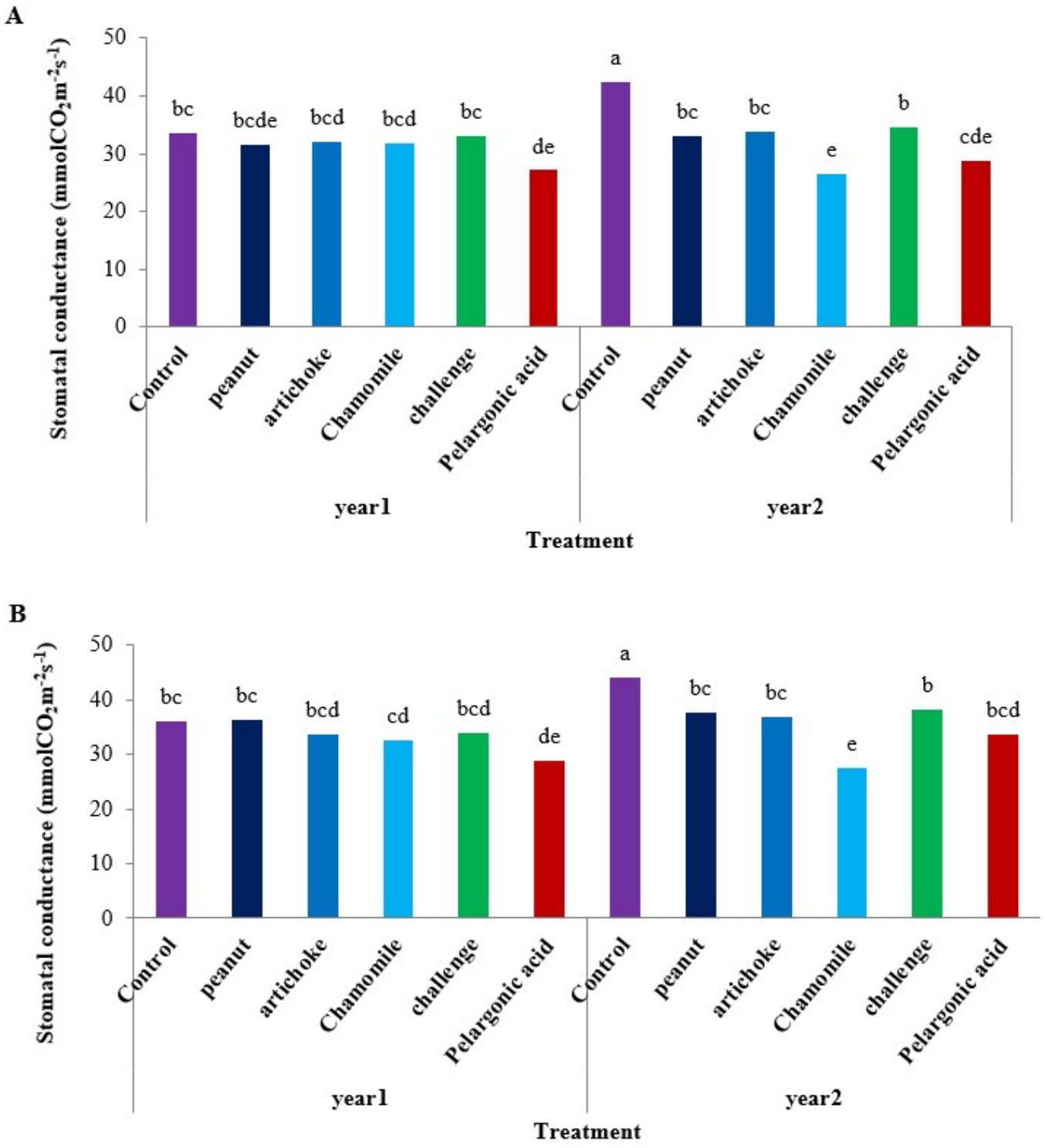


Figure 5

Comparison of the mean effect of treatments on the stomatal conductance of weeds yellow-thorn (A) and wild safflower (B). Means sharing the same letter in each column are not significantly different based on the Least Significant Difference (LSD) test at a 5% probability level.

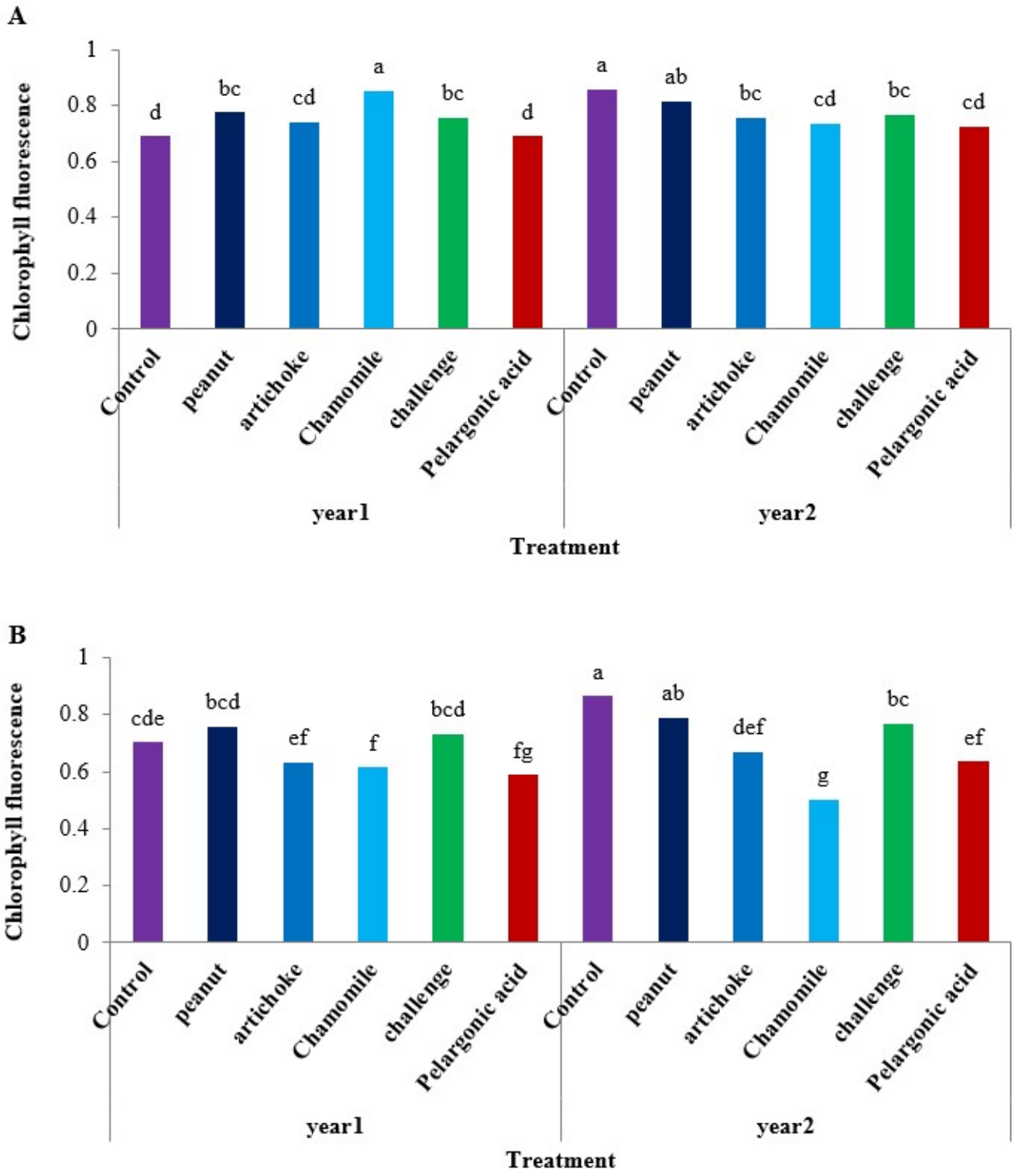


Figure 6

Comparison of the mean effect of treatments on chlorophyll fluorescence of weeds yellow-thorn (A) and wild safflower (B). Means sharing the same letter in each column are not significantly different based on the Least Significant Difference (LSD) test at a 5% probability level.

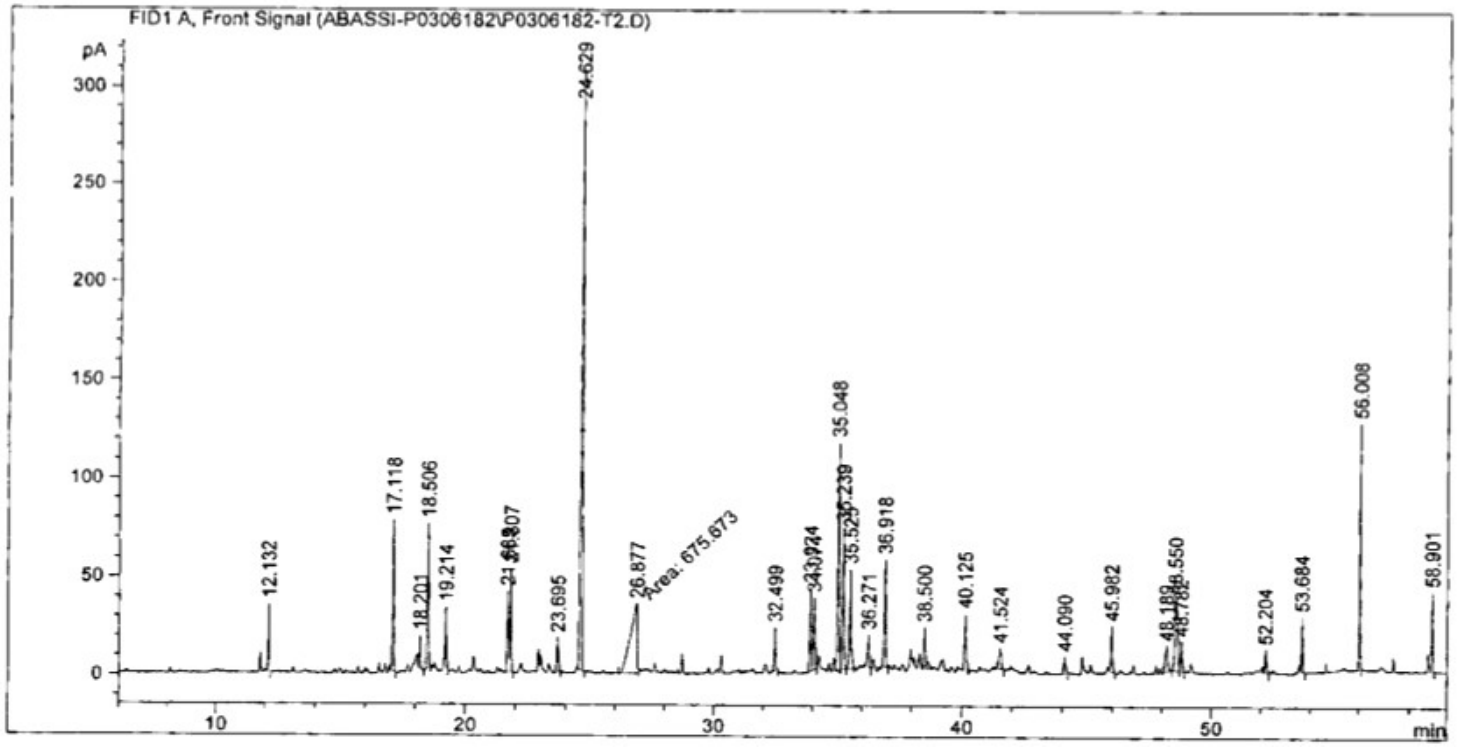


Figure 7

Gas chromatogram obtained from GC-MS analysis of chamomile essential oil

Supplementary Files

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- Table.docx