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# Allelopathic potential of sunflower (*Helianthus annus*) water extracts to reduce the pendimethalin herbicide dose to control *Chenopodium album* in corn (*Zea mays*)

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## ABSTRACT

In field studies, we evaluated the combinations of sunflower (*Helianthus annus*) extract and reduced doses of herbicide pendimethalin to control *Chenopodium album* in corn field. The experimental treatments were: control, pendimethalin full dose (600 mL $ha^{-1}$), pendimethalin half dose (300 mL $ha^{-1}$), sunflower extracts (10 and 20%), pendimethalin half dose + 10% sunflower extract and pendimethalin half dose + 20% sunflower extract. Combinations of sunflower extract (20%) with pendimethalin half dose, drastically decreased the *C. album* density and seedling weight (81.3% and 82.8 % inhibition over control) than other treatments except pendimethalin full dose. The minimum LAI of *C. album* was with pendimethalin full dose (75.5 %) and pendimethalin half dose + 20% sunflower extract (71 %). Pendimetalin full dose and pendimethalin half dose + 20% sunflower extract decreased the photosynthesis to 59% and 55% of control, respectively. All treatments increased the ABA contents in *C. album* seedling, but the highest ABA content was with pendimethalin full dose (74.7%) and pendimethalin half dose + 20% sunflower extract treatments (75.4 % over the control). We concluded that the phytotoxic effects of sunflower extract tank mix with pendimethalin could reduce the herbicide doses for *C. album* control.

**Key word**: Abscisic acid, allelopathy, antioxidant enzyme, lipid peroxidation, pendimethalin,

## INTRODUCTION

In the World, corn is third major crop after wheat and rice. It is used as food, livestock and poultry feed and raw materials for many industries (11). Weeds compete with crops for growth resources (like nutrients, moisture, sunlight and space) and reduce their yields (15). Today agriculture is heavily reliant on herbicides for weed control (3), it has resulted in contamination of ground water resources and development of herbicide resistance in weed biotypes, hence, till now 290 weeds biotypes have become resistant to herbicides (1). In post-emergence weed control, use of herbicides at reduced dose is main approach to reduce the herbicide dose in integrated weed management system (2).

The production of some allelochemicals in plants and their release into the environment decreased the germination, seedling growth, photosynthesis, cell membrane stability, leaf area and seedling weight of target plants (5,6,9,10,14). The sunflower

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allelopathic chemicals (sesquiterpene, lactones and terpenes) from the crude leaf extract have herbicidal potential for weed management (18). Sunflower extract decreases the wild mustard seed germination and seedling growth (20) due to increased lipid peroxidation and cell membranes damage to wild mustard seedling. Sunflower extracts also decreases the weeds seedling growth in wheat and safflower (18). Iqbal and Cheema (12) reported effective control of purple nutsedge using the natural plant extracts from various allelopathic crops (sorghum, sunflower and brassica) in cotton field.

*Chenopodium album* (lambsquarters) is major weed in corn (*Zea mays* L.) fields and has competitive advantage due to early emergence before the crop. It produces large numbers of seeds that remains viable in soil for many years and contribute to future populations. Farmers use MCPA, 2, 4-D and pendimethalin to control this weed in corn field. The study aimed to find, if reduced rates of pendimethalin combined with sunflower water extracts could control the *C. album* in corn fields.

## MATERIALS AND METHODS

### I. Field experiment

Field experiments were conducted in 2012 at Islamic Azad University, Shoushtar, Iran [150 m above sea level (25° 29' N and 35° 22' E), mean annual precipitation : 355 mm] and GC/MS analysis was done in Dankook University, Korea. The study site at shoushtar, had moderate winter and dry and hot summer. Field soil was clay-loam, pH 6.4, EC 1.9 ds $m^{-2}$ and 0.94 % organic matter. Based on the soil test, 120 kg N $ha^{-1}$ and 50 kg $P_2O_5$ $ha^{-1}$ as urea and triple super phosphate were applied before sowing. Each plot was 3m × 3m. Corn was sown in row (75 cms apart) in dry soil on 1$^{th}$ July, 2012 on both sides of ridges and field irrigated after seed sowing,. Corn density was thinned to 10 seedling $m^2$ two weeks after emergence. All agronomical trials were done in fields previously heavily infested with *C. album*.

There were 6- treatments: (i). control, (ii). pendimethalin full dose (600 mL $ha^{-1}$), (iii). Pendimethalin half dose (300 mL $ha^{-1}$), (iv). sunflower extracts (10 and 20%), (v). pendimethalin half dose + 10% sunflower extract and (vi). pendimethalin half dose + 20% sunflower extract. The treatments were replicated 4- times in complete block design. Field grown sunflower (*Helianthus annus* L. cv. Azargol) was collected at maturity from the field, its residues (shoot + leaf) were oven-dried at 70°C for 48 h and chopped into 3-4 cm long pieces. This chopped material was soaked in water in ratios of 1:10 and 2:10 (w/v) for 48 h and water extract was collected by passing it through the sieves. Pendimethalin was used at 1200 mL $ha^{-1}$ (full dose) and 600 mL $ha^{-1}$ (half dose) as post emergence herbicide. Pendimethalin and sunflower water extract were tank mixed and sprayed by hand sprayer with flat fan nozzle (13) between the corn rows in morning at 30 days after sowing.

### II. *C. album* seedlings growth and physiological parameters assay

The *C. album* density and dry weight was recorded at 14 days after spraying in three randomly selected quadrates (50 × 50 cm) from each experimental plot and *C. album* dry weight was recorded after oven drying at 70°C for 48 h. *C. album* LAI was determined by LAI portable meter (modle Co-B: 1218) 7 days after spraying.

**III. Physiological Parameters**

**(i). Photosynthesis:** *C. album* seedlings were harvested 72 h after spraying to determine the physiological characteristics. An infrared, open gas exchange system LI-6400 was used 5 days after spraying to measure the photosynthesis in leaf of all plants. Gas exchange rates were measured on intact leaves using a LICOR Infra Red Gas Analyser IRGA, LI-6400 XT (LICOR Inc., Lincoln, NE, USA), fitted with a leaf chamber mounted with light. The fully expanded leaves were used for photosynthesis measurements (15). Chlorophyll a and b were assayed 5 days after spraying according to Gunes *et al.* (11).

**(ii). Lipid peroxidation:** The extent of seedling lipid peroxidation was determined by measuring the amount of Malondialdehyde (MDA) formed with Triocloro Acetic Acid method as per Valentovic *et al.* (23). 0.2g of *C. album* fresh tissue was thoroughly mixed with 3 ml of 10% Triocloro Acetic Acid (TCA), centrifuged at 10,000×g for 15 min; supernatant (350 µl) was then poured off, mixed with 350 µl of 0.6% (w/v) Thiobarbituric acid in a new microtube. The resulting mixture was heated at 95°C for 30min and then quickly cooled on ice for 5min. After centrifugation at 10,000×g for 10 min at 4°C, the absorbance of reaction mixture was measured at wavelengths of 450, 532 and 600 nm. MDA concentration was calculated based on the following formula:

$$[MDA] = 6.45 \times (A532 - A600) - 0.56 \times A450$$

Where, A532, A600 and A450 represent the absorbance of the mixture at 450, 532, and 600 nm, respectively.

**(iii). Enzymes activities:** Catalase (CAT: EC:1.11.6), Glutathione Reductase (GR: EC 1.6.4.2) and Guaiacol peroxidase (POD: EC:1.11.1.13) are key antioxidant enzymes scavenging the plant cells. CAT and POD were extracted by homogenizing the frozen fresh leaf material in ice-cold solution containing 100 mM Tris (pH 7.0), 10 mM-isoascorbic acid, 20 g $L^{-1}$ PVP+, 1.5 g insoluble PVP, 0.1 mM EDTA and 2mL $L^{-1}$ Triton $X^{-100}$ (7). CAT activity was determined by monitoring the disappearance of $H_2O_2$ by measuring the decrease in absorbance at 240 nm of a reaction mixture [1.9 mL $H_2O$, 1.0 mL of 5.9 mM $H_2O_2$ in potassium phosphate buffer (pH 7.0)] and 1.0 mL extract (7). POD activity was determined following the protocol of Chanes and Maehly (7) using guaicol as a reactant. POD activity was measured by monitoring the $H_2O_2$-dependent oxidation of reduced 2, 3, 6-trichloroindophenol at 675 nm using a UV-vis spectrophoto-meter (Model U-2001, Hitachi, Tokyo, Japan). GR activity was determined in 800 µl of 0.1M potassium phosphate buffer (pH 7.8) containing 0.5mM β-nicotinamide adenine dinucleotide 2′-phosphate, reduced form (2′-NADPH), 10 mM glutathione, oxidized form 3 mM $MgCl_2$ and 50 µl enzymatic extract. The NADPH oxidation-dependent absorbance was declined at 340 nm every 30 sec for 6-8 min using spectrophotometer (Pharma Spec UV-1700; Shimadzu) (20).

**(iv). ABA, GA and IAA**: For Gibberellins (GA), Indole Acetic Acid (IAA) and Abscisic Acid (ABA) contents assays, the weed seedling were ground in 80% methanol at 4 °C with antioxidant (butylated hydroxy toluene: BHT) and kept for 72 h with change of

solvent every 24 h. The extract was centrifuged and the supernatant was reduced to its aqueous phase using a rotary thin film evaporator. The pH of aqueous phase was adjusted to 2.5 -3.0 and partitioned 4x with 1/3rd volume of ethyl acetate. The ethyl acetate extract was fully dried using the rotary evaporator. The dried sample was re-dissolved in 1ml methanol (100%) and analysed using HPLC (model Agilent 1100, USA). Pure ABA, GA and IAA were used as standards to identify and quantify plant hormones (14).

**IV. GC/MS analysis**

To identify the sunflower extract components, Agilent gas chromatography model 6890 N, equipped with MSD model 5973 N and fused silica capillary column (HP-5MS, 30m- 0.25mm) was used. The GC oven temperature was held at 50 °C for 5 min, then programmed from 50 °C to 240 °C at a rate of 3 °C min-1 and from 240 °C to 290 °C at a rate of 5 °C min-1, held for 2 min at 290 °C, using He gas as the carrier (1.0 ml $min^{-1}$). The temperatures of injector and detector were 240 °C and 280 °C. The percentage composition of extracts was computed from GC peak areas without using any correction factors. Qualitative analysis was based on comparison of retention times and indices on both columns and mass spectra using computer mass spectra libraries model Agilent Technologies 5973 Network and corresponding data available in literature. Total phenolic and flavonoid content assay according Yrdon et al. (27) method.

**Statistical Analysis:** One way ANOVA was carried out using MSTATC software. Post hoc tests (Duncan) were performed only if F-test was significant at $p \leq 0.01$.

## RESULTS AND DISCUSSION

**Sunflower extract compounds**

The 1,8-cineole, sabinene, β-pinene, camphor and α-pinene were major compounds in sunflower extract (Table 1). Monoterpenoids (1,8-cineole, β-pinene, camphor and α–pinene) potentially acts as allelochemicals and inhibits the cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings (19). The α-pinene, one of the common monoterpenoids emitted from several aromatic plants including forest trees, is known for its growth-inhibitory activity. It inhibits the early root growth of *Cassia occidentalis*, *Amaranthus viridis*, *Triticum aestivum*, *Pisum sativum* and *Cicer arietinum* and causes oxidative damage in root tissues through enhanced generation of ROS, as shown by increased lipid peroxidation, disruption of membrane integrity and elevated antioxidant enzyme levels (22). Rai *et al.* (21) found that volatile monoterpenes from *Prinsepia utilis* L. leaves inhibits the stomatal openings in *Vicia faba* seedlings. Total phenolic and Flavonoids content of sunflower were 4.19±0.15mg GA/100 g DW and 1.53 ±0.03, mg CE/100 g DW) respectively (Table 2).

***C. album* density, growth and LAI:** Fourteen days after spraying, full dose pendimethalin dratically decreased the density of *C. album* (91% inhibition over control) (Figure 1.a). While 20% sunflower extract foliar application decreased the *C. album* density (42.5 %

Table 1. Chemical composition and percentage composition of the sunflower extract

| Compound | Compound (%) | RI* | Compound | Compound (%) | RI* |
|---|---|---|---|---|---|
| α-Thujene | 0.51 | 920.9 | ά –Humulene | 0.74 | 1443.1 |
| 1,8-Cineole | 18.3 | 924.2 | ά –Muurolene | 0.22 | 1471.6 |
| Tricyclene | 0.21 | 934.8 | Germacrene D | 0.68 | 1482.4 |
| Thuja-2,4(10)-diene | 0.21 | 955.9 | β –Bisabolene | 0.29 | 1505.1 |
| β-pinene | 4.0 | 971.6 | cubebol | 0.36 | 1548.4 |
| Sabinene | 20.1 | 977.1 | Elemol | 0.17 | 1559.6 |
| α –Phellandrene | 0.51 | 1003.6 | Spathulenol | 0.22 | 1581.6 |
| ά—Terpinene | 0.90 | 1012.5 | Germacrene-D | 0.46 | 1591.5 |
| Camphor | 3.21 | 1020.2 | Salvial-4(14)-en-1-one | 0.16 | 1600.7 |
| β -Phellandrene | 0.78 | 1027.4 | Humulene epoxide II | 0.12 | 1607.4 |
| α –Pinene | 5.1 | 1038.1 | Cedryl acetate | 0.15 | 1657.8 |
| Cis- β -ocimene | 0.29 | 1045.7 | Chamazulene | 2.1 | 1673.9 |
| Trans- β -ocimene | 0.83 | 1058.0 | (E,E)-Farnesyl acetate | 0.21 | 1698.2 |
| Cis-sabinene hydrate | 0.76 | 1067.5 | manoyl oxide | 0.61 | 1702.1 |
| Linalool | 2.13 | 1087.4 | ά –Cadinol | 0.23 | 1711.4 |
| Cis-p-menth-2-en-1-ol | 0.34 | 1117.3 | ά –bisabolol | 10.6 | 1725.1 |
| α –Terpineol | 0.64 | 1184.9 | γ-Eudesmol acetate | 0.34 | 1759.0 |
| Cis-dihydrocarvone | 0.11 | 1195.6 | | | |
| Trans-dihydrocarvone | 0.23 | 1202.7 | | | |
| Piperitone | 0.98 | 1241.7 | | | |
| Nerylformate | 0.39 | 1275.3 | | | |
| Carvacrol | 1.74 | 1314.6 | | | |
| Eugenol | 0.44 | 1325.1 | | | |
| Trans-β-caryophyllene | 0.15 | 1402.7 | | | |
| Camphor | 0.86 | 1419.5 | | | |
| Borneol | 1.73 | 1421.1 | | | |
| Terpinen-4-ol | 8.6 | 1425.0 | | | |

*: Retention indices

Table 2. Total flavonoids and phenolic contents in sunflower extracts

| **Compounds** | **Content (mg GA/100 g DW)** |
|---|---|
| Total Phenolics | 4.19±0.15 |
| Total Flavonoids | 1.53 ±0.03 |
| **Phenolic acid content** | **Content (%)** |
| Catechin | 32.5±2.20 |
| Gallic acid | 18.0±0.12 |
| 3,4-Dihydroxybenzoic | 10.1±0.84 |
| Benzoic acid | 7.8±0.85 |
| Luteolin | 3.46±0.003 |
| Salicylic acid | 2.9±0.09 |
| Vanillin | 2.22±0.01 |

over the control) and 10 % sunflower extract. Combinations of sunflower extract (20%) with pendimethalin half dose, significantly decreased the *C. album* population (81.3% inhibition over control) than other treatments, except pendimethalin full dose (Figure 1.a).

The Pendimethalin, sunflower extract and combination of sunflower extract and pendimethalin reduced the biomass of *C. album*. The sunflower extract of 20% and 10% decreased the *C. album* dry weight compared to control (Figure1.b). The lowest *C. album* dry weight was in pendimethalin full dose (90 % inhibition over control) and pendimethalin half dose + 20% sunflower extract (82.8 % inhibition over control). The use of sunflower extracts and pendimethalin decreased the LAI (Leaf Area Index) in *C. album* (Figure 1.c). The minimum LAI of *C. album* was in pendimethalin full dose (75.5 % inhibition) and pendimethalin half dose + 20% sunflower extract (71 % inhibition). The 10% sunflower extract did not influence the LAI of *C. album* than control. Pendimetalin full dose and pendimethalin half dose + 20% sunflower extract decreased the LAI of *C. album* up to 70% than control. Application of sunflower water extracts combined with reduced herbicide dose significantly inhibited the *C. album* growth. These decreases in weed density and dry weights might be owing to the phytotoxic effects of allelochemicals present in crop water extracts like sunflower (10,18). The use allelopathy for weed control is to use the extracts of allelopathic plants as herbicides, because these biosynthesized herbicides are easily biodegradable and are much safer than synthesized herbicides. Iqbal *et al*. (13) found that *Purple nutsedge* density and dry weight were decreased when sorghum and sunflower water extracts were used in combination with reduced dose of glyphosate. Awan *et al*. (2) reported that combination of sorghum and sunflower extract with pendimethalin decreased the weed density and weed growth in sunflower crop.

**Photosynthesis and chlorophylls contents**: The application of sunflower extract and the pendimethalin decreased the photosynthesis and chlorophylls contents in *C. album* leaves (Figure 2.a-c). Pendimetalin full dose and pendimethalin half dose + 20% sunflower extract decreased the photosynthesis to 59% and 55% of control, respectively (Figure 2.a). These treatments also decreased the chlorophyll a and b content in *C. album* leaves (Figure 2.b and figure 2.c). The allelochemicals have inhibitory effects on physiological processes that leads to growth inhibition (5,9,16). The effects of allelopathy on plants growth may occur through various mechanisms [reduced mitotic activity in roots and shoots, suppressed hormone activity, inhibited photosynthesis and respiration, protein formation, decreased permeability of cell membranes and/or inhibition of enzyme action (5,6,9)]. Allelopathic compounds apparently decreases the physiological processes like photosynthesis (15). The presence of monoterpenes in leaves of *Prinsepia utilis* L. inhibits the stomatal opening in *Vicia faba* (21).

**Lipid peroxidation and antioxidant enzymes activates:** The applied sunflower extracts and pendimethalin increased the lipid peroxidation in *C. album* seedlings (Figure 3.a). The full dose of pendimethalin and pendimethalin half dose + 20% sunflower extract increased the MDA contents by 97.5% and 96%, respectively, over control. Allelopathy induces the membrane injury in different plant species is related to enhanced production of oxidative stress (5,6,9,18). Allelochemicals can damage the cell membranes through direct interaction with constituent of membrane, or can impair some metabolic processes necessary for maintenance of membrane functions (25,26). Likewise the applied extract of *Callicarpa acuminate* and *Sicyos deppei* increased the lipid peroxidation in tomato, bean and corn roots (7). Farhoudi *et al*. (10) found that sunflower extract increased the lipid

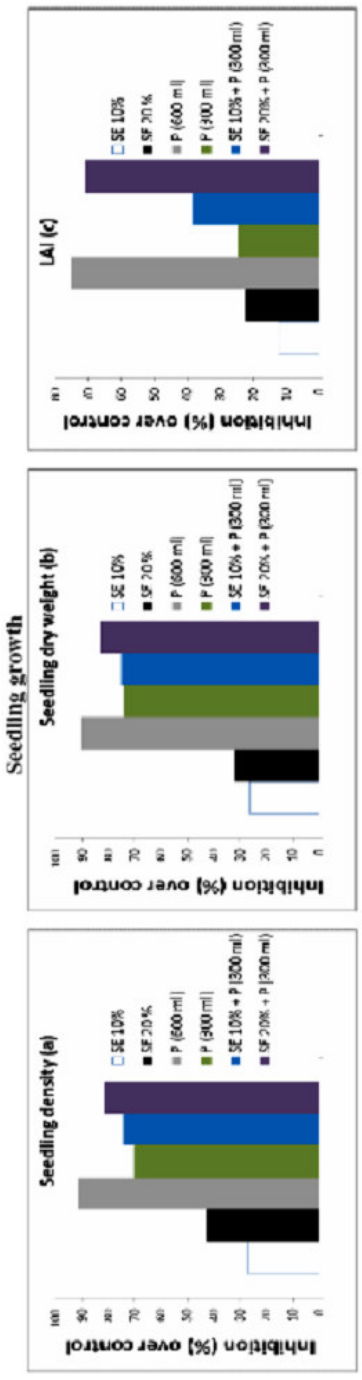


Figure 1. Effects of sunflower extract and Pendimethaline herbicide dose on inhibition (%) over control of *C. album* seedling density (a), seedling dry weight (b) and leaf area index (c).SE: sunflower extract; P: Pendimethaline.

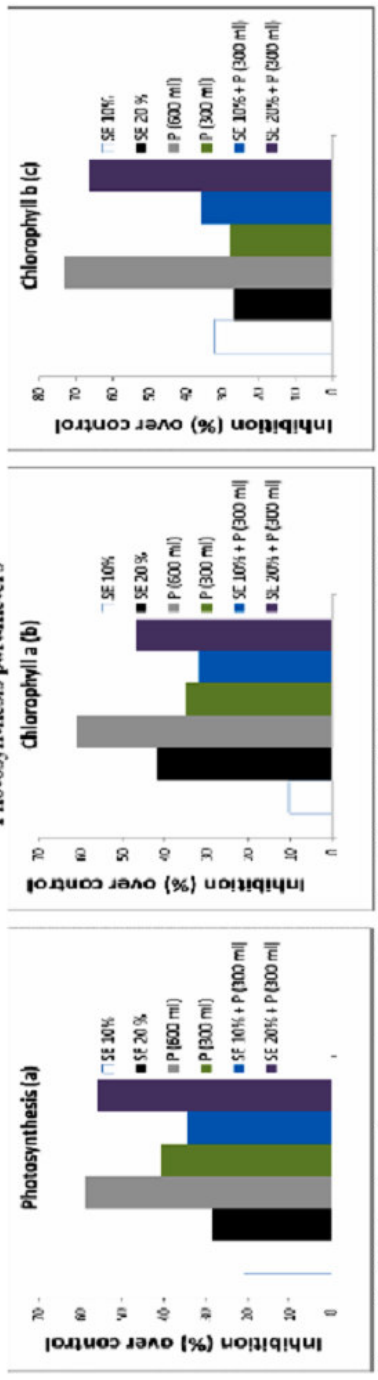


Figure 2. Effects of sunflower extract and Pendimethaline herbicide dose on inhibition (%) over control of *C. album* Photosynthesis (a), Chlorophyll a (b) and Chlorophyll b (c). SE: sunflower extract; P: Pendimethaline.

peroxidation in johnson grass (*Sorghum halepnse*) and wild mustard (*Sinapis arvensis*) seedlings. Orcaz *et al.* (20) reported that sunflower water extract decreased the wild mustard (*Sinapis arvensis*) seed germination and seedling growth, because the sunflower allelopathic compounds increased the cell membrane damage and caused the oxidative stress in mustard seedling. Lipid peroxidation may increase the MDA content in membranes resulting in severe damage to cells (9).

**Enzyme activities:** The sunflower extracts, pendimethalin and combination of sunflower extract and pendimethalin increased CAT, POD and GR activity in *C. album* seedling (Figure 3). The lowest GR activity was found in pendimethalin half dose + 20% sunflower extract (14.1%) followed by pendimethalin full dose (28%) treatment. The highest change in CAT activity was found in sunflower extracts (10 %), sunflower extracts (20 %), pendimethalin half dose (600 mL ha-1) and pendimethalin half dose + 10% sunflower extract treatment (45%, 51%, 45% and 48% over control), respectively. These results confirmed the findings of earlier studies, that degree of injury caused by allelochemicals was negatively correlated to the increased activities of antioxidant enzymes (9,10,18). The evidences suggests that allelopathy induces the oxidative stress which is key component of most abiotic stresses. The production of Reactive Oxygen Species (ROS) in cells increases during the abiotic and biotic stresses [salt, drought, heat and allelopathic stress], as does the level of ROS-induced damage (3). Higher production of ROS seriously disrupts the cellular homeostasis and normal metabolisms through oxidative damage to lipids, protein, Phytohormones and nucleic acid (23). Yu *et al.* (26) showed that exposure of cucumber roots to phytotoxic compounds significantly increased the oxidative stress and injury in cucumber seedlings. Many environmental stresses disrupts the cellular homeostasis, enhances the production of ROS and there is correlation between the allelochemicals and increase in ROS production (3,5,6,9,18,19). The herbicide and sunflower extract produced the oxidative stress in *C. album* seedlings because these changed the antioxidant activities in this weed. Plants have antioxidant enzymes in *C. album* and antioxidant compounds to scavenge these ROS. The antioxidant capacity of plants is directly related to their stress tolerance but high concentrations of phytotoxic compounds decreased the antioxidants activities because these compounds damaged the protein structure in cells (20). Farhoudi and Lee (9) found that high concentration of sunflower extract decreased the seedling growth and antioxidants activities in wild barley (*Hordeum spontoneum)* and wild oat (*Avena ludoviciana)* seedling.

**Phytohormones content:** The sunflower extract foliar application decreased the GA and IAA contents in *C. album.* The highest GA concentration in *C. album* was with 10% and 20% sunflower extract, pendimethalin half dose +10% sunflower extract and pendimethalin half dose + 20% sunflower extract (27%, 33%, 24% and 34% respectively). While the lowest IAA content in *C. album* was with 20% sunflower extract (51% over control) and pendimethalin half dose + 20% sunflower extract (49% over control) (Figure 4. a and 4.b). All treatments increased the ABA contents in *C. album* seedling, but the highest change was with pendimethalin full dose (74.7% over the control) and pendimethalin half dose + 20% sunflower extract treatments (75.4 % over the control) (Figure 4.c). The sunflower extract at 10% and 20% increased the ABA content of

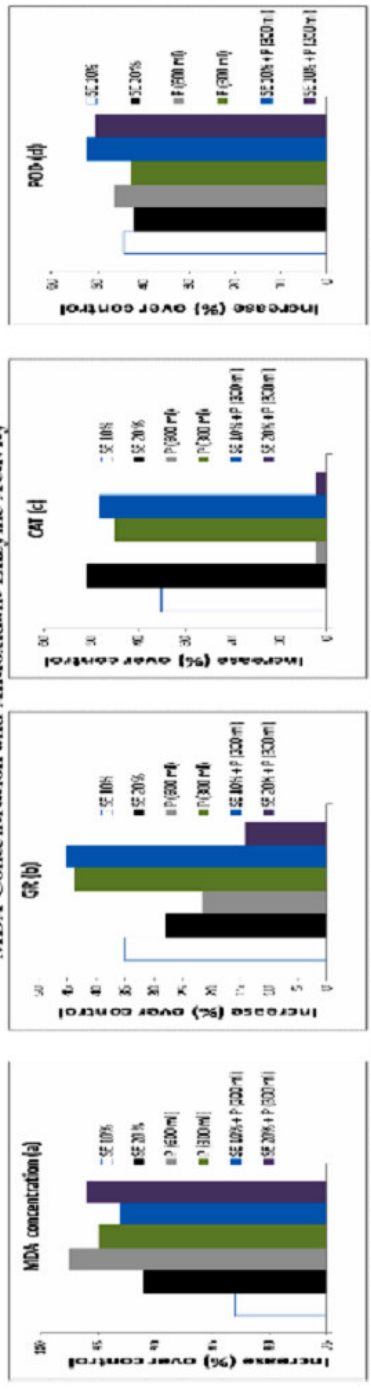


Figure 3. Effects of sunflower extract and Pendimethaline herbicide dose on increase (%) over control of *C. album* MDA concentration (Figure 3.a), GR (Figure 3. b), CAT (Figure 3.c) and POD (Figure 3.d) activity. SE: sunflower extract; P: Pendimethaline.

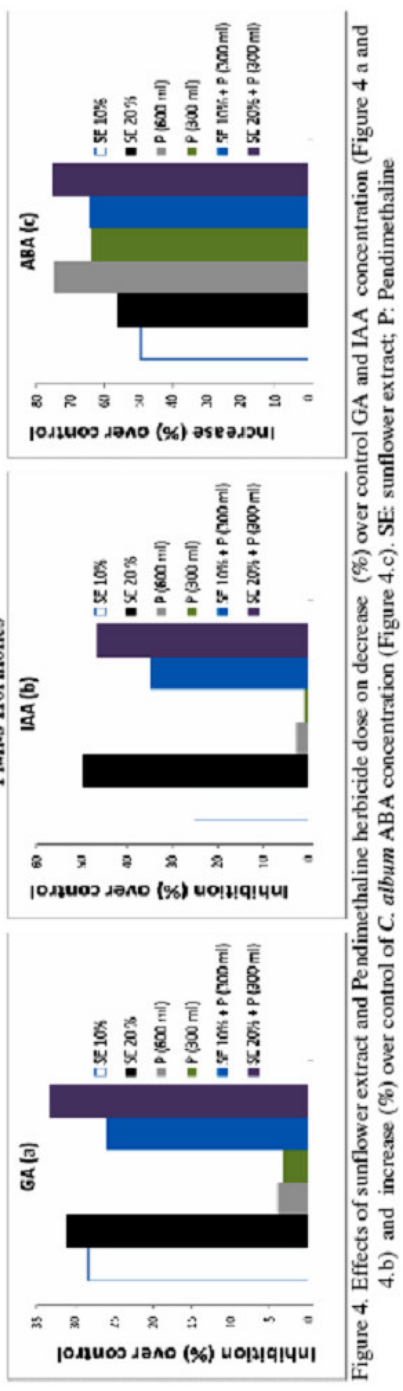


Figure 4. Effects of sunflower extract and Pendimethaline herbicide dose on decrease (%) over control GA and IAA concentration (Figure 4 a and 4.b) and increase (%) over control of *C. album* ABA concentration (Figure 4.c). SE: sunflower extract; P: Pendimethaline

*C. album* seedling to 49 % and 56% than control, respectively. These results indicated that sunflower extracts caused stress in *C. album* seedlings, because ABA is well-known stress hormone (13). Plant growth regulators play major role in physiological processes in plants and are influenced by allelopathic stress (5). Some e physiological processes stimulated by GA and IAA are: stem elongation, cell division and seed germination (5,14) but allelochemical compounds decreased the IAA and GA contents in plants (16,22). Bernart *et al.* (4) found that sunflower extract increased the ABA level in roots of mustard and wheat seedlings but decreased the IAA and GA contents. They reported that ABA decreased the seedling growth of mustard and wheat under allelopathic stress conditions. Yang *et al.* (25) reported that some allelochemical compounds of *Ageratina adenophora* increased the ABA and decreased the IAA content in roots of rice seedlings. Sunflower extracts decreases the IAA and GA content but increases the ABA content in both leaves and roots of wheat seedling than control (14).

## CONCLUSIONS

Sunflower water extract is rich in volatile monoterpenes compounds (1, 8-Cineole, β-pinene, camphor and α-pinene), which inhibited the seedling growth of *C. album.* Sunflower water extract increased the lipid peroxidation and ABA contents in *C. album* seedling but decreased the IAA and GA contents, thereby, suppressed the weed seedling growth. The 20% sunflower water extract combined with pendimethalin half dose reduced the *C. album* density, LAI and seedling dry weight up to 81%, 76% and 71%, respectively, and also reduced the herbicide dose to half compared to pendimethalin full dose. This information may help in the development of biosynthesized herbicides and other biological methods to control *C. album.*

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