

Effect of sunflower (*Helianthus annus* L. cv. Azargol) extracts on seedling growth, photosynthesis and enzyme activities of *Sorghum halepense* and *Sinapis arvensis*

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Abstract: The present study was carried out to study the allelopathic effect of sunflower (*Helianthus annus* L. cv. Azargol) on seedling growth, photosynthesis and enzyme activities of johnson grass (*Sorghum halepense*) and wild mustard (*Sinapis arvensis*). Physiological parameters of the selected weeds such as photosynthesis, lipid peroxidation, and sucrose synthase activity were reduced by applying higher concentrations of sunflower extracts. Sunflower extracts negatively affected the shoot dry weight and seedling height of the weeds. The highest concentration of malondialdehyde (MDA) in johnson grass and wild mustard were 0.065 and 0.069 $\mu\text{mol g}^{-1}$ FW respectively when the highest concentration of extracts was used. The photosynthetic rate was 6.1 $\mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1}$ in the former and 2.1 $\mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1}$ in the later weed. The sunflower extracts inhibited the wild mustard and johnson grass seedling growth and increased lipid peroxidation causing cell wall damage. The results suggest that sunflower extracts can be considered as weed biological control agents due to their inhibitory and toxic effects on plant cellular structures, physiological mechanisms, and biochemical reactions.

Key words: Wild mustard; Johnson grass; Malondialdehyde; Sucrose synthase

1. Introduction

Allelopathy is defined as the effect of one plant on another plant through the release of allelochemical compounds into the environment (Rice, 1984). The production of allelochemicals in crop plants and their releases into the soil via root exudation, volatilization, leaching, and residue decomposition (Lorenzo et al., 2011) can negatively influence the germination and growth of plant species (Farooq et al., 2011; Rice 1984). Allelopathic crops offer strong potential for the development of cultivars that are more highly weed-suppressive. Allelochemical compounds decreased photosynthesis, leaf area and dry mass of target plants (Lorenzo et al., 2011; Farhoudi and Lee, 2012). Increases in evidences also suggest that allelopathic mechanisms can raise oxidative stresses which are underlying components of most abiotic stresses (Oracz et al., 2007). The production of Reactive Oxygen Species (ROS) in cells increases during abiotic and biotic stresses including salt, drought, heat and allelopathic stresses, result in increasing the level of ROS-induced damage. Elevated production of ROS can induce serious disorderliness in cellular metabolisms through oxidative damage to lipids, proteins, and nucleic acids. Also, it was previously reported that the membrane injury induced by allelochemicals in target plant species is related to an enhanced production of ROS (Oracz et al., 2007; Farhoudi and Lee, 2012). To resist against the ROS, plants possess

antioxidant enzymes such as catalase and peroxidase to scavenge the cells. Antioxidant capacity of plants is directly related to their stress tolerances (Oracz et al., 2007).

The sunflower (*Helianthus annus*) allelochemicals have a potential as possible alternative for achieving sustainable weed management (Azania et al., 2003; Oracz et al., 2007; Nikneshan et al., 2011). Allelochemicals such as phenolic (Alsaadawi et al., 2010) and terpenoids (Macias et al., 2002) compounds have been found in the most suppressive potential sunflower genotypes which have high impacts in other plant growth. Sunflower extracts decrease wild mustard seed germinations and seedling growth via increase in accumulations of ROS resulting in cell membrane damages (Oracz et al., 2007). Several researchers have documented that the sunflower extract increased lipid peroxidation, oxidative stress, and cell membranes damage in wild mustard (*Sinapis arvensis*) seedlings (Bogatek et al., 2006; Kupidłowska et al., 2006; Oracz et al., 2007). On the other hand, it can induce α -amylase activity and germination in wild mustard (Farhoudi and Lee, 2012). Moreover, sunflower extracts negatively effects on wheat by reducing the seedling growth (Ghafar et al., 2000). Allelochemicals associated in sunflower extracts also influences on biochemical and developmental processes such as reduction of sucrose synthase activity and seedling growth in wild oat (Farhoudi et al., 2012). The phytotoxicity of sunflower extracts makes them good candidates as a source of a natural herbicide against weed species (Oracz et al., 2007).

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johnson grass (*Sorghum halepense*) is a robust, aggressive, and large, warm-season, C4 perennial, herbaceous graminoid (Ruyle and Young, 1997) and overwinters as rhizomes (primary rhizome) or seeds (Warwick and Black, 1983). It has been identified as one of 10 important weeds in the world (Nouri et al., 2012). Wild mustard is one of the most common annual weeds in cultivated fields in (Modhej et al., 2013). It is a difficult weed to control in oilseed and cereal crops such as canola (*Brassica napus* L.), soybeans (*Glycine max* L. Merr.), wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.), causing considerable damages (Mulligan et al., 1975; Friesen et al., 1990).

The purpose of the present study was to evaluate the effect of sunflower aqueous extracts on seed germinations, seedling growth, lipid peroxidations, antioxidant enzyme activities, and sucrose synthase activity of johnson grass and wild mustard.

2. Material and methods

2.1. Solution preparations

The experimental design was arranged on the basis of a complete randomized design (CRD), with five replications. Sunflower (*Helianthus annuus* L. cv. Azargol) was harvested at the beginning of the flowering stage. The entire aerial parts of harvested plants were dried at 50°C for 72 hours, powdered and sieved afterward. To prepare the stock solution of sunflower aqueous extract, the amount of 100g of powdered sample was dissolved in 1 liter distilled water and kept for 24 hours at 24°C. The solution was filtrated and designated as a stock solution of 100% (w/v) strength. Different sunflower aqueous extract concentrations were prepared at 10, 20, and 30% (w/v) according to volumetric method; and distilled water (0%) was used as a control treatment. Five germination boxes containing a seed of the weed species were provided separately for each individual weed, johnson grass or wild mustard. Germinated seedlings have been allowed to grow for 40 days at alternating temperature of 24/15 °C and fluctuation of 16 hours light and 8 hours darkness. Treatments of sunflower aqueous extracts were performed 27 and 30 days after the emergence through watering. The plants were harvested 10 days after the last application of the sunflower aqueous extracts and dried at 70 °C for 48 hours.

2.2. MDA assay

The amount of malondialdehyde (MDA) formation was measured using the thiobarbituric acid method described by Heath and Packer (1968), Valentovic (2006) as an indicator of lipid peroxidation. The amount of 1.5 ml of 10% trichloroacetic acid (TCA) was mixed with 0.1 g of wild mustard seedling tissues. The mixture was centrifuged at 10,000×g for 15 min and supernatant (350µl) was mixed with 350µl of 0.6% (w/v)

thiobarbituric acid (TBA). The final solution was heated at 95 °C for 30 min and quickly cooled on ice for 5min afterward. The centrifugation at 10,000×g for 10 min at 4 °C was applied to the prepared solution and the absorbance of the reaction mixture was measured at 450, 532 and 600 nm. The concentration of MDA (µM L⁻¹) was calculated according to the formula below (Heath and Packer, 1968):

$$[\text{MDA}] = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

Where 6.45 and 0.56 are constant coefficients; A₅₃₂, A₆₀₀, and A₄₅₀ represent the absorbance of the mixture at 450, 532, and 600 nm, respectively.

2.3. CAT, POX and Sucrose synthase activity assay

To measure the variations of catalase (CAT) and peroxidase (POX) enzyme activities as indicators of oxidative damages in plant cells, CAT (EC:1.11.6) and POX (EC:1.11.1.13) were extracted by homogenizing frozen fresh seedling material in ice-cold solution containing 100 mMTris (pH 7.0), 10 mMd-isoascorbic acid, 20 g L⁻¹ PVP- 10, 1.5 g insoluble PVP, 0.1mM EDTA and 2mL L⁻¹ Triton X-100. CAT activity was determined following Chanes and Maehly (1995) by monitoring the disappearance of H₂O₂ and measuring the reduction in absorbance at 240 nm of a reaction mixture containing 1.9mL H₂O, 1.0mL of 5.9mM H₂O₂ in potassium phosphate buffer (pH 7.0), and 1.0mL of the extract.

POX activity was determined following the protocol of Chanes and Maehly (1995) using guaiacol as a reactant and measured by monitoring the H₂O₂-dependent oxidation of reduced 2, 3, 6-trichloroindophenol at 675 nm using a UV-vis spectrophotometer (Model U-2001, Hitachi, Tokyo, Japan). Sucrose synthase activity was assessed by Counce and Gravois (2006) methods. An infrared, open gas exchange system LI-6400 was used to measure Photosynthesis on the same leaf in all plants (Martins et al., 1992) and Chlorophyll a and b were assayed by Gunes et al. (2007), methods.

3. Data analysis

The data analysis was performed using MSTAT-C statistical program and mean comparisons were performed using Duncan test (P<0.01). Before data analysis, normality and homogeneity of variance was tested.

4. Results and discussion

4.1. The effects of sunflower foliar extracts on Johnson grass

The analysis variance and mean comparison of data related to the effects of sunflower foliar extracts on johnson grass showed different extract concentrations significantly affected on growth indicators of the weed such as plant height and dry

weight as well as physiological parameters including photosynthetic rate (PR) and chlorophyll contents

(Table 1) The negative effects of the extract applications were also observed in enzymes activity

Table 1: Means comparison of allelopathic effect of sunflower extracts on photosynthesis, chlorophyll contents and seedling growth in johnson grass

Sunflower extracts concentrations (%)	Photosynthesis ($\mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1}$)	Chlorophyll a ($\text{mg g}^{-1} \text{ Fw}$)	Chlorophyll b ($\text{mg g}^{-1} \text{ Fw}$)	Shoot dry weight (g)	Root dry weight (g)	Shoot height (cm)
0	14.80 a	0.98 a	0.78 a	1.21 a	0.11 a	22.1 a
10	12.21 a	0.74 b	0.57 b	1.12 a	0.12 a	17.3 b
20	9.61 b	0.68 b	0.44 c	0.85 b	0.11 a	14.1 c
30	6.10 c	0.37 c	0.41 c	0.52 c	0.10 a	12.6 d
MS	176.3**	4.7**	5.11**	0.075 ^{ns}	1.86**	102.1**

Means followed by the same letter(s) are not significantly different at $P = 0.01$ according to Duncan test MS: Mean square ** and *: Significant

such as catalase, peroxidase, and sucrose synthase (Table 2).

at the 0.01 and 0.05 level of probability according to Duncan test, respectively. Ns: Not significant

Table 2: Means comparison of allelopathic effect of sunflower extracts on enzymes activity, MDA concentration and sucrose synthetase activity in johnson grass

Sunflower extracts concentrations (%)	Catalase activity ($\text{unit mg}^{-1} \text{ pro}$)	Peroxidase activity ($\text{unit mg}^{-1} \text{ pro}$)	MDA concentration ($\text{nmol g}^{-1} \text{ FW}$)	Sucrose synthase activity ($\text{nmol mg pro}^{-1} \text{ min}^{-1}$)
0	5.50 a	12.10 a	0.0011 d	6.13 a
10	5.46 a	13.30 a	0.037 c	4.18 b
20	3.12 b	7.45 b	0.048 b	4.11 b
30	2.16 c	4.17 c	0.065 a	2.13 c
MS	43.0**	18.1**	0.12**	11.4**

Means followed by the same letter(s) are not significantly different at $P = 0.01$ according to Duncan test MS: Mean square ** and *: Significant at the 0.01 and 0.05 level of probability according to Duncan test, respectively. Ns: Not significant

The difference in the PR was significant among different treatments of sunflower extracts. As the solution concentration increased the PR was decreased. The lowest PR in johnson grass was at the highest concentration of sunflower foliar extract (30%) by $6.1 \mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1}$ (Fig1). Studies showed sunflower allelopathic extracts reduced seedling photosynthesis of target plants (Nikeshana et al., 2011). Allelopathic compounds are thought to negatively interfere with physiological processes including photosynthesis (Lorenzo et al., 2011). Our findings also indicate increase in extract concentration reduced the amount of chlorophyll a and b in Johnson grass leaf while the highest chlorophyll a and b contents obtained in the control treatment. Decrease in chlorophyll content (Fig 2) resulted in low photosynthetic rate (Table 1).

In addition to the negative effects of sunflower extracts on physiological variables these extracts decreased growth indicators such as seedling length, root and shoot weight of johnson grass (Table 1 and 2). The most reduction was obtained in 30% (w/v) of sunflower foliar extracts by 9.5 cm followed by 20% and 10% (w/v) sitting at 7 and 4.7 cm, respectively (Table 1). Moreover, it was previously reported that the wheat and mustard seedlings growth were significantly reduced by allelopathic

effects of sunflower aqueous extracts, which can prove our results (Kamal 2011; Oracz et al., 2007). Furthermore, enzyme activities of johnson grass including lipid peroxidation; and Malondialdehyde (MDA) concentrations increase under application of sunflower extract treatments (Table 2) indicating the occurrence of ROS in the cells.

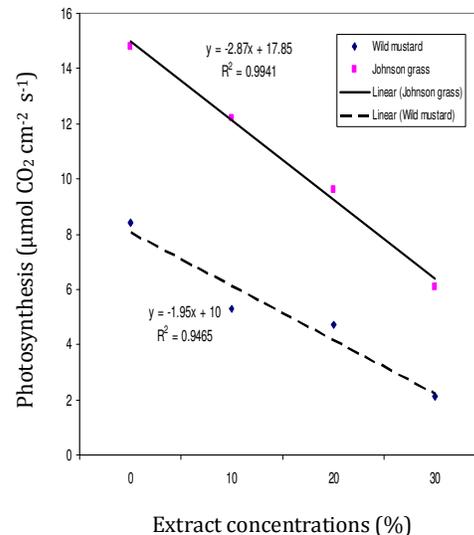


Fig. 1: Trend of regression changes of wild mustard and Johnson grass photosynthesis ($\mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1}$) at different sunflower extract concentrations

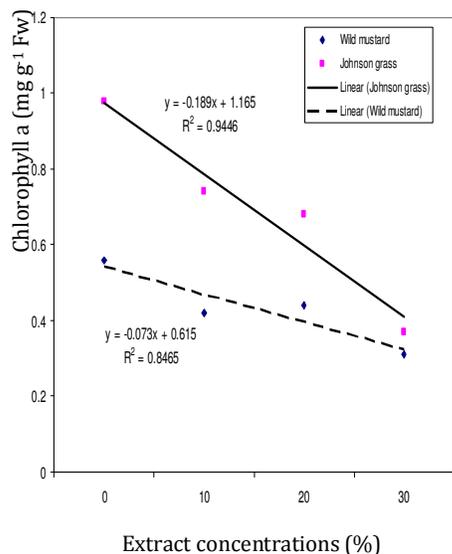


Fig. 2: Trend of regression changes of wild mustard and johnson grass chlorophyll a at different sunflower extract concentrations

Results showed increase sunflower extract concentrations, decreased catalase and peroxidase activity in johnson grass seedling compared to the control. The lowest antioxidant enzymes activities obtained at 30% sunflower extract foliar concentration (Table 2). The possible roles of oxidative stress in phytotoxicity has been known, the changes in the main antioxidant enzymes and MDA concentrations have been investigated during seed treatment on sunflower tissue extract (Farhoudi and Lee, 2012). The results of the present research are supported by the other researches expressing that exposure of cucumber roots (Yu et al., 2003) and wild mustard (Oracz et al., 2007) to phytotoxic compounds significantly increased oxidative stress. One of the best known targets of oxidative stress is lipid peroxidation which is a free-radical chain process leading to the deterioration of polyunsaturated fatty acids. Lipid peroxidation is likely to increased MDA present in membranes, leading to severe damage to membranes (Oracz et al., 2007).

The sucrose synthase activity was inhibited by applying the sunflower foliar extracts in johnson grass and as the sunflower extract concentrations were gradually increased the inhibitory effects increased (Fig 3). The lowest sucrose synthase

activity obtained in 30% sunflower extract by 2.13 nmol mg pro⁻¹min⁻¹ (Table 2). The most significant compound in conversion of sucrose to starch is sucrose synthase; thus, the activity of sucrose synthase is a valuable predictor of sink strength, plant growth and phytosentesis (Counce et al., 2006). Previously, Farhoudi et al. (2012), reported wheat aqueous extract can decrease sucrose synthase and α-amylase activity of wild oat because of oxidative stresses and cell membrane damage caused by allelopathic compounds of wheat destructing enzyme activities (Farhoudi et al., 2012); the results support our findings.

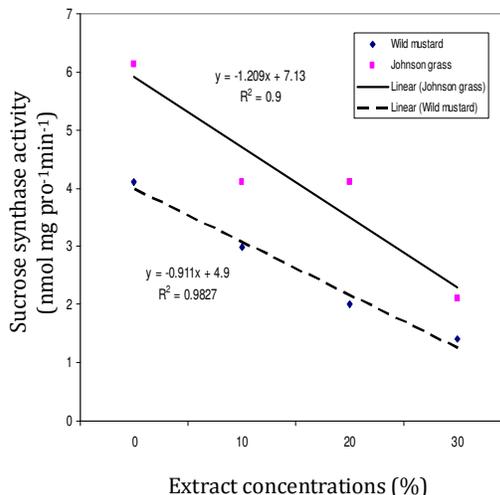


Fig. 3: Trend of regression changes of wild mustard and johnson grass sucrose synthase activity at different sunflower extract concentrations

4.2. The effects of sunflower foliar extracts on wild mustard

The wild mustard seedling growth was significantly affected by different sunflower foliar extract concentrations (Table 3). The mean comparisons among different treatments were significantly different for measured physiological, morphological and growth parameters. Growth indicators such as shoot height and shoot/root dry weight were influentially and negatively affected by sunflower foliar extracts (Table 3).

Table 3: Means comparison of allelopathic effect of sunflower extracts on photosynthesis, chlorophyll contents and seedling growth in wild mustard

Sunflower extracts concentrations (%)	Photosynthesis (μmol CO ₂ cm ⁻² s ⁻¹)	Chlorophyll a (mg g ⁻¹ Fw)	Chlorophyll b (mg g ⁻¹ Fw)	Shoot dry weight (g)	Root dry weight (g)	Shoot height (cm)
0	8.40 a	0.56 a	0.39 a	0.98 a	0.092 a	15.2 a
10	5.31 b	0.42 b	0.26 b	0.73 b	0.091 a	12.1 b
20	4.70 b	0.44 b	0.24 c	0.54 c	0.083 a	10.0 c
30	2.10 c	0.31 c	0.16 c	0.41 d	0.064 b	7.12 d
MS	22.6**	1.6**	2.91**	1.35 ^{ns}	6.71**	13.3**

Means followed by the same letter(s) are not significantly different at $P = 0.01$ according to Duncan test MS: Mean square ** and *: Significant at the 0.01 and 0.05 level of probability according to Duncan test, respectively. Ns: Not significant

Applications of sunflower aqueous extracts caused serious reductions in shoot dry weight (SDW) of wild mustard in comparison with the control. The lowest SDW (0.52 g) was obtained after applying the highest extract concentration (30% w/v) compared to the control with 0.98 g. The mean comparisons for these parameters among different treatments were significantly different. Previous study confirmed that the effect of aqueous sunflower extracts on mustard germination is dose-dependent (Bogatek et al., 2006). The maximum shoot height of wild mustard observed in the control treatment by 15.2 cm while 30% extract treatment remarkably reduced the plant height by 8.08 cm in average (Table 3). A reason of less SDW in treated wild mustard can be the reduction of height causing decrease in the

competitive ability of the weed for light with the desired plants dominating the canopy since this capability is directly correlated with height (Zobel 1992). Consequently, it can indirectly limit the negative effects of the weeds. Nikneshana et al. (2011) indicated that the allelopathic properties of some sunflower cultivars can affect seedling growth of some weed species such as *Lolium rigidum* and *Hordeum spontaneum*. The same results were reported in wild mustard seedling growth and seed germinations negatively affected by safflower and canola allelopathic extracts (Modhej et al., 2013). Moreover, sunflower extracts decreased seed germination and seedling growth of wheat (Ghafar et al. 2000).

Our results showed sunflower extract decreased wild mustard PR compared to the control (Fig 1). The lowest wild mustard PR was detected at 30% of sunflower extract and we did not observe any significant differences between 0 and 10% of sunflower extracts (Table 4).

Table 4: Means comparison of allelopathic effect of sunflower extracts on enzymes activity, MDA concentration and sucrose synthetase activity in wild mustard

Sunflower extracts concentrations (%)	Catalase activity (unit mg^{-1} pro)	Peroxidase activity (unit mg^{-1} pro)	MDA concentration (nmol g^{-1} FW)	Sucrose synthase activity ($\text{nmol mg pro}^{-1}\text{min}^{-1}$)
0	4.12 a	12.10 a	0.0014 d	4.11 a
10	5.00 a	8.03 b	0.027 c	2.98 b
20	2.96 b	8.09 b	0.039 b	2.01 c
30	2.02 c	4.18 c	0.069 a	1.46 d
MS	37.2**	61.1**	0.052**	10.3**

Means followed by the same letter(s) are not significantly different at $P = 0.01$ according to Duncan test MS: Mean square ** and *: Significant at the 0.01 and 0.05 level of probability according to Duncan test, respectively. Ns: Not significant

Photosynthetic rate reduction can occur due to different mechanisms. One of the crucial mechanisms affected by allelochemicals is stomatal movements influencing the PR by changing in CO_2 concentration reported in *Vicia faba* (Rai et al., 2003). Chlorophyll damage has been also reported as a substantial factor decreasing PR under allelopathic stresses (Lorenzo et al., 2011). On basis of our results, chlorophyll a and b contents of wild mustard were reduced by applications of sunflower foliar extracts. The lowest amount on chlorophyll a and b were 0.31 and 0.16 compared to the control 0.56 and 0.39 respectively (Table 3). Our results are supported by Farhoudi and Lee (2012) findings reporting safflower extract decreased photosynthetic rate and chlorophyll a content of *Lolium* spp. as well as *Avena ludoviciana* seedlings (Farhoudi and Lee, 2012).

Increasing in concentration of sunflower foliar extracts caused increase in the MDA content of wild mustard. The MDA content was 0.033, 0.052 and 0.063 nmol g^{-1} FW from the lowest to the highest extract concentrations respectively (Table 4). Oxidative stresses were associated with increase in MDA concentration during the growth of plants.

Enzymes activities were also reduced by sunflower extracts; at 30% (w/v) extract concentration, catalase with 2.02 mg^{-1} pro and peroxidase 4.18 mg^{-1} pro were in the lowest amount compared to the control treatment with 4.12 and 12.10 mg^{-1} pro, respectively (Table 4). Oracz et al. (2007) stated catalase and superoxide dismutase activities decrease under sunflower extract treatments. As allelochemicals secreting from a plant can cause environmental stresses in neighboring plants, they can induce oxidative stresses occurring in plants subjected to the these conditions and producing ROS (Yu et al., 2003; Oracz et al., 2007). One of the consequences of oxidative stress is increasing in lipid peroxidation which is a free-radical chain process leading to the deterioration of polyunsaturated fatty acids. Lipid peroxidation is likely to elevate MDA present in membranes, result in severe damage to membranes (Oracz et al., 2007). Allelochemicals have significant effects on enzyme function, signal transduction as well as gene expression (Rice, 1984). Moreover, Farhoudi and Lee (2012) indicated safflower extract decreased α -amylase activity of wild mustard seedling (Farhoudi and Lee, 2012). Our findings also showed sunflower foliar extracts reduced the sucrose synthase activity of wild mustard (Table 4). The lowest sucrose synthase activity was 1.43 $\text{nmol mg pro}^{-1}\text{min}^{-1}$ obtained in 30% (w/v) extract concentration. Sucrose synthase and α -amylase activity were also

reduced in wild oat by applying wheat aqueous extracts containing allelopathic agents (Farhoudi and Lee, 2012).

5. Conclusion

The results of the present research showed that the MDA concentration increase under sunflower extract foliar applications due to the oxidative stresses causing lipid peroxidation. Furthermore, the extracts can substantially reduce the sucrose synthase activity result in increasing cell membrane damage. The results exhibited that sunflower allelopathic compounds damage cell membranes stability and antioxidant enzyme structures (Bogatek et al., 2006). The same results were reported in safflower (Farhoudi and Lee, 2012). The data demonstrates that the deleterious effect of sunflower phytotoxins on plant growth may occur through the imposition of an oxidative stresses (Yu et al., 2003). Thus, higher concentration of sunflower extracts increase lipid peroxidation (MDA production) while decreased antioxidants enzymes and sucrose synthase activity activates. The decrease in wild mustard and johnson grass germination was well related to increased membrane damage defined by increased malondialdehyde contents. Additionally, plant growth, photosynthesis, and chlorophyll contents were negatively affected. The effect of aqueous sunflower extracts on both target weeds was dose-dependent and the reduction of photosynthesis rate (Fig 1), chlorophyll content (Fig 2), and sucrose synthase activity (Fig 3) in the growing seedlings was more affected in johnson grass compared with wild mustard that may relate to morphological and physiological differences (Ashrafi Zoheir et al., 2010).

The low growth rate in the target plants can cause losses of their abilities to compete with desired plants without any special treatments including herbicides applications that are usually hazardous to the environment and costly. The former can have negative effects on the sustainable agriculture and the later can raise the market price of the products.

Further investigations require determining which tissues of selected plants are more susceptible to these toxic substrates of sunflower extracts. The results of the present study can help us to have better understanding of plant physiology and biology under environmental stresses; and may eventually lead researches to find a biological integrative control method to deal with selected weeds. Consequently, an extreme reduction can occur in herbicide consumptions since the allelopathic substances which potentially safer than chemical herbicides can be used as biological control agents.

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