

## Phytotoxic effects of aqueous extract of eucalyptus, sunflower and sugar beet on seed germination, growth and photosynthesis of *Amaranthus retroflexus*

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### ABSTRACT

Laboratory and pot experiments were done to evaluate the allelopathic potential of shoot aqueous extracts of eucalyptus (*Eucalyptus camaldulensis* L.), sunflower (*Helianthus annuus* L.) and sugar beet (*Beta vulgaris* L.) on germination, growth and photosynthesis of redroot pigweed (*Amaranthus retroflexus* L.). Germination, growth and photosynthesis of *A. retroflexus* were inhibited by aqueous extract of the tested plant species. Sugar beet extract was more inhibitory to seed germination and seedling growth than sunflower and eucalyptus. Seed germination of *A. retroflexus* was significantly decreased by 57.4% and 92% at 10 and 20 gL<sup>-1</sup> extract concentration, respectively than control. While sunflower and eucalyptus extracts reduced the weed seed germination by 39% and 38%, respectively at 20gL<sup>-1</sup> concentration. High extract concentration (20gL<sup>-1</sup>) of sugar beet reduced the plant height, leaf area, shoot dry weight and leaf photosynthesis of *A. retroflexus* by 34.6%, 56.3%, 39.9% and 47.3%, respectively over the control, while, sunflower and eucalyptus extracts reduced the weed plant height by 23.1% and 20.6%, leaf area by 24.5% and 21%, shoot dry weight by 31.5% and 29.8% and leaf photosynthesis by 27% and 22.3%, respectively compared with control.

**Key words:** Allelopathy, germination, *Eucalyptus camaldulensis*, *Beta vulgaris*, *Helianthus annuus*, *Amaranthus retroflexus*, photosynthesis, weed.

### INTRODUCTION

Weeds compete with crops for growth factors and certain weeds interfere with crop plants through allelochemicals and thus inhibit crop growth and development (5). Yield losses caused by weeds may be upto as 24% than 16.4% and 11.2% for disease and insects, respectively (19). Existing weed control methods are expensive, hazardous and heavy doses of herbicides cause serious threat to environment and public health, this necessitated the search for alternative strategies.

Allelopathy is defined as the inhibitory/stimulatory effect(s) of one plant on other plants through the release of chemical compounds into the surrounding environment (31). Allelopathy interactions are primarily based on the ability of certain plant species to produce secondary chemical compounds that exert some biological effects on other organisms, many of which are unknown. Allelopathy is characterized by a reduction in

plant emergence or growth, reducing their performance in the association (18). It provides a relatively cheaper and eco-friendly weed control strategies (10).

Various *Eucalyptus* species possess allelopathic potential and suppresses the certain weed species (13, 26). Allelopathic effects of *Eucalyptus* are due to the presence of allelochemicals (phenolic and volatile compounds) in its foliage (16). Cao and Luo (12) reported that aqueous extract from bark and leaf, and volatiles from leaves of *Eucalyptus citriodora* showed inhibitory allelopathic effects on growth of 9- plant species (including the weeds; *Bidens pilosa*, *Digitaria pertenuis*, *Eragrostis cilianesi* and *Setaria geniculata*). The seed germination, seedling growth, chlorophyll content and respiration of some weed plants was drastically reduced by allelochemical extracted from *E. citriodora* (5). Dadkhah and Asaadi (13) also reported that foliage aqueous extract of *Eucalyptus camaldulensis* reduced the germination of *Acroptilon repens* and *Plantago lanceolata* but not that of *Portulaca oleracea*. Sunflower can actively influence the growth of surrounding plants due to its high allelopathic potential (4). Macias *et al.* (25) isolated 125 allelopathic compounds from different sunflower cultivars that were phytotoxic to many weed species. Bogatek *et al.* (9) reported that leaf aqueous extracts of sunflower at 10% concentrations decreased the seed germination and seedlings growth of *Sinapis alba*, while Kalburtji and Gagianas (24) reported poor performance of cotton plants following sugar beet.

Redroot pigweed is a problematic annual weed of cultivated lands in different part of the world (7). It is a strong competitor with different crops including vegetables and exerts allelopathic influences on different crops (29). Therefore, this study aimed to evaluate the effects of foliage aqueous extracts of *Eucalyptus camaldulensis*, sunflower (*Helianthus annuus*) and sugar beet (*Beta vulgaris*) on germination, growth and photosynthesis of *A. retroflexus*.

## MATERIALS AND METHODS

### I. Aqueous Extract Preparation

Aerial parts of sugar beet (*Beta vulgaris* L.) and sunflower (*Helianthus annuus* L.) were collected from Farm, Shrivani Agricultural College, North Khorasan Province, Iran and foliage of *Eucalyptus camaldulensis* L. were brought from north Iran (Golestan Province). Foliage parts were washed with distilled water and dried in shade at room temperature. Dried tissues were ground into a fine powder (using an electric mill). Twenty g ground tissue of each test species was placed in a 2 L Erlenmeyer flask and 1 L deionized water was added. Flasks were covered with aluminum foil to protect mixture from photodecomposition and placed on a rotary shaker (~250 revolutions per min.) for 24 h. The mixtures were then filtered through Whatman No.1 filter paper using a vacuum pump. The pH and electrical conductivity (EC) of extracts were determined using a digital pH meter and conductivity meters. The pH (from 6.9 to 6.1) and EC (from 2.03 to 3.1 mS/cm) ranges of aqueous extracts of tested plants were within the tolerance range of target weed. The filtrates were considered as full concentration stock solutions. A series of solutions including the stock solutions (extract of 20 g dry weight in 1.0 l water (S<sub>1</sub>), and concentration dilution of 10 gL<sup>-1</sup> (S<sub>2</sub>) (was prepared from the stock solution) and deionized

distilled water (control) were used for germination and seedling growth tests. The extra solutions were stored at  $-18^{\circ}\text{C}$  for later use.

## II. Petri Dish Experiment

A Petri dish assay based on a randomized complete block design with six replicates was done to screen the effects of different concentrations of aqueous extracts of *E. camaldulensis*, *B. vulgaris* (var. Dorotea) and *H. annuus* (var. Zaria) on germination and growth of *A. retroflexus*. Weed seeds were pre-sterilized with 2% sodium hypochlorite for 5 min and then washed with distilled water. Thirty seeds of weed (pre-tested seeds with more than 95% germination) were evenly distributed between two layers of Whatman No.1 filter papers in each of 9-cm plastic Petri dish. Five ml of dilute series was added to each Petri dish. Petri-dishes were placed in a germinator at 12h photoperiod and mean maximum and minimum temperatures of  $25\pm 1$  and  $16\pm 1^{\circ}\text{C}$  for 14 days. Germination (%) on root and shoot length of seedlings were recorded at 10 days after germination.

## III. Foliar Spray Experiment

Pot experiment was done in a completely randomized design with five replicates in glasshouse. Minimum and maximum temperature was  $20\pm 2^{\circ}\text{C}$  and  $35\pm 2^{\circ}\text{C}$ , respectively. Relative humidity was between 35-55%. Plastic pots of 15-cm diameter and 25-cm depth were filled with sandy-loam soil (pH: 7.65 and EC:  $2.6\text{ dS m}^{-1}$ ). Fifteen day-old seedlings of *A. retroflexus* (redroot pigweed) seedlings were carefully transplanted in pots (one seedling per pot). Aqueous extracts ( $20\text{gL}^{-1}$  and  $10\text{gL}^{-1}$ ) of foliage dry shoots of *B. vulgaris*, *H. annuus* and *E. camaldulensis* were prepared as described earlier. The extracts were sprayed on foliage parts of redroot pigweed plants (plants were at the 6-leaf stage) at 7 days after transplanting using hand sprayers. Plants were sprayed three times (at three days interval). The aqueous extracts were applied at 30 ml per pot. Plants in control treatment were sprayed with distilled water. Plants were harvested after 8 weeks from treatment and data on plant height, leaf area, and shoot dry weight were recorded.

Leaf photosynthesis of the intact young fully expanded leaves was measured using the Combined Infra Red Gas Analysis System (CIRAS-1 portable photosynthesis system) at 5 weeks after treatment. Measurements were made at a photon flux density of  $450\text{ }\mu\text{mol photon m}^{-2}\text{ s}^{-1}$ , at room temperature and ambient  $\text{CO}_2$  concentration. Chlorophyll content of *A. retroflexus* leaves was estimated according to Porra *et al.* (28). Phenolic content of aqueous extract of the tested plants was determined by Folin-Ciocalteu phenol as per method of Jindal and Singh (23).

## Statistical Analysis

Both experiments were laid out in a completely randomized design. Data were transformed using arcsine prior to statistical analysis. The data for all measured parameters were analysed using the analysis of variance procedure of Statistical Analysis System (SAS) software, version 6.12. Means were compared by Duncan's multiple range tests at the 0.01 probability level for all comparisons.

## RESULTS AND DISCUSSION

### Germination

The various concentrations of leaf aqueous extracts of sugar beet, sunflower and *E. camaldulensis* inhibited the germination (%) and seedling growth of *A. retroflexus*. The inhibitory effect was concentration-dependent. The sensitivity of *A. retroflexus* to aqueous extracts of different plants was variable. The low concentration ( $10 \text{ gL}^{-1}$ ) of aqueous extract of sugar beet, sunflower and eucalyptus caused inhibition of 57.4, 21.3 and 16.7%, respectively, in seed germination (%) of *A. retroflexus*. While the inhibition in seeds germination of *A. retroflexus* increased to 92, 39 and 38%, respectively, at their highest concentration ( $20 \text{ gL}^{-1}$ ) of sugar beet, sunflower and *E. camaldulensis* than control (Fig. 1).

Sugar beet extracts were more inhibitory than sunflower and eucalyptus (Fig. 1). The low concentration ( $10 \text{ gL}^{-1}$ ) of aqueous extract of sugar beet, sunflower and eucalyptus inhibited the seed germination of *A. retroflexus* by 74.2, 67.1 and 29%, respectively. However, the highest ( $20 \text{ gL}^{-1}$ ) extract concentration of sugar beet, sunflower and *E. camaldulensis* drastically inhibited the germination of *A. retroflexus* by 96.7, 76.5 and 79.6%, respectively, than control (Fig. 1).

### Seedling growth

The seedlings growth of *A. retroflexus* was severely inhibited at the higher concentration ( $20 \text{ gL}^{-1}$ ) of extracts. The low ( $10 \text{ gL}^{-1}$ ) and high ( $20 \text{ gL}^{-1}$ ) concentrations of sugar beet extract, decreased the root length of *A. retroflexus* seedlings by 42 and 95.5 %, respectively (Fig. 1). However, the root length of weed seedlings at low extract concentration ( $10 \text{ gL}^{-1}$ ) of sunflower and eucalyptus caused only 15.8 and 14.7% inhibition, while their high concentration caused inhibition of 46.2 and 35.9%, respectively, than control. Shoot length of *A. retroflexus* seedlings was also decreased by 24 and 82.6% at low ( $10 \text{ gL}^{-1}$ ) and high ( $20 \text{ gL}^{-1}$ ) concentrations of sugar beet extract over the control (Fig. 1). Shoot length of *A. retroflexus* seedlings at low extract concentration of sunflower and eucalyptus was slightly inhibited by 11.4 and 13% but inhibition at highest concentration was 17.5 and 14 %, respectively. Thus extracts caused less inhibition in shoot growth than in than roots. These results agree with Ahn and Chung (1) who found that length and dry weight of roots of *Echinochloa crusgalli* were more affected by hull extract than shoots. All phenolic compounds were more inhibitory to root than shoot length of *Vulpia myuros*(2). The strong inhibitory effects of extract on roots might be due to the direct contact with the extract and its chemicals.

Our findings agree with El-Rokiek and Eid (16) who reported the inhibitory effects of leaf extracts of eucalyptus on germination of some weeds and the magnitude of inhibition depended on the extract concentration. Inhibition in seedlings growth was more pronounced than in seed germination. Smith (32) and Ben-Hammouda *et al.* (6) found that aqueous leaf extracts of several species suppressed the seedling growth in target plants more than seed germination.

Leaf area, plant height, shoot dry weight and leaf chlorophyll content of *A. retroflexus* were all reduced at highest ( $20 \text{ gL}^{-1}$ ) aqueous extract concentration of

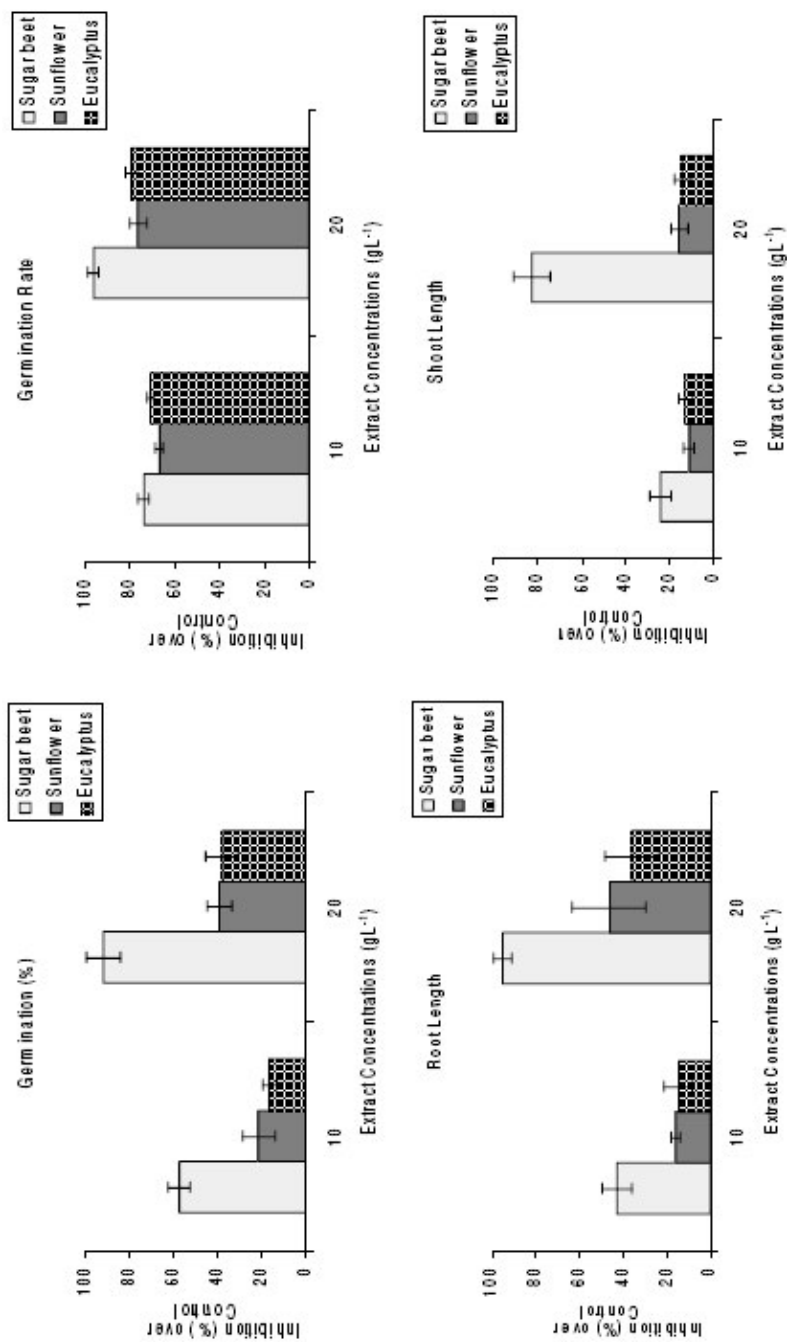


Figure 1. Inhibitory effects of aqueous extracts of Sugar beet, Sunflower and Eucalyptus on germination, germination rate, root length and shoot length of *A. retroflexus*. Each value is mean of 6-replications. Vertical lines are standard deviation of means.

Table 1. Effects of foliar application of aqueous extracts of test plants on leaf area, plant height, shoot dry weight, total chlorophyll content, photosynthesis and stomatal conductance. Each number is means  $\pm$  S.D of 4 measurements. Means followed by the same letter are not significantly ( $p \leq 0.05$ ) different by Duncan's multiple range test within rows.

Parameters	Control (0)		Eucalyptus		Sunflower		Sugar beet	
	10 g	20 g	10 g	20 g	10 g	20 g	10 g	20 g
Leaf area (cm <sup>2</sup> )	959 $\pm$ 35 <sup>a</sup>	810 $\pm$ 40 <sup>b</sup>	758 $\pm$ 22 <sup>b</sup>	724 $\pm$ 28 <sup>b</sup>	638 $\pm$ 20 <sup>c</sup>	540 $\pm$ 24 <sup>d</sup>		
Plant height (cm)	41.3 $\pm$ 3.5 <sup>a</sup>	36 $\pm$ 2 <sup>ab</sup>	33 $\pm$ 1 <sup>b</sup>	32 $\pm$ 3 <sup>b</sup>	30 $\pm$ 2 <sup>b</sup>	27 $\pm$ 3 <sup>b</sup>		
Shoot dry weight (g plant <sup>-1</sup> )	17.8 $\pm$ 2 <sup>a</sup>	15 $\pm$ 1 <sup>ab</sup>	12.5 $\pm$ 2 <sup>b</sup>	12.2 $\pm$ 1 <sup>b</sup>	13.1 $\pm$ 1 <sup>b</sup>	10.7 $\pm$ 1 <sup>c</sup>		
Total chlorophyll content	64.3 $\pm$ 5 <sup>a</sup>	57 $\pm$ 3 <sup>ab</sup>	50 $\pm$ 4 <sup>b</sup>	38 $\pm$ 2 <sup>c</sup>	55 $\pm$ 3 <sup>b</sup>	53 $\pm$ 4 <sup>b</sup>		
Photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	14.8 $\pm$ 1.5 <sup>a</sup>	12.3 $\pm$ 0.8 <sup>ab</sup>	11.5 $\pm$ 1.1 <sup>b</sup>	11.8 $\pm$ 0.8 <sup>b</sup>	10.8 $\pm$ 0.9 <sup>b</sup>	7.8 $\pm$ 1.2 <sup>c</sup>		
Stomatal conductance (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	173 $\pm$ 25 <sup>a</sup>	149 $\pm$ 15 <sup>ab</sup>	130 $\pm$ 13 <sup>b</sup>	123 $\pm$ 12 <sup>b</sup>	115 $\pm$ 16 <sup>b</sup>	93 $\pm$ 10		

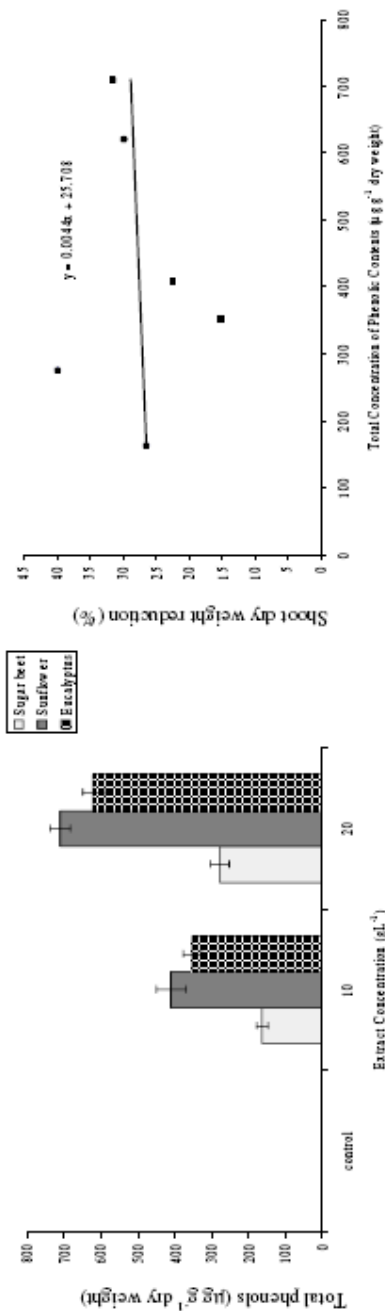


Figure 2. Total phenols contents in Sugar beet, Sunflower and eucalyptus aqueous extract concentrations. Each value is mean of 5-replications. Vertical lines are standard error of means.

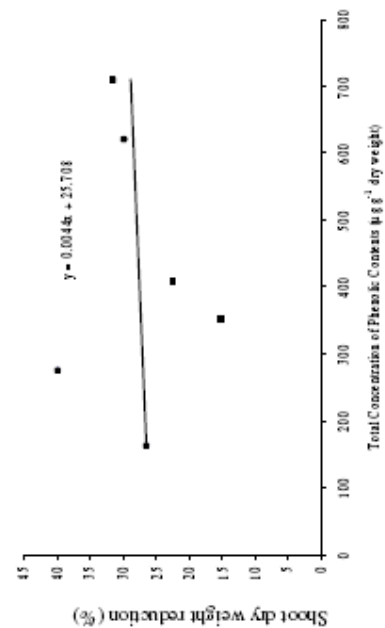


Figure 3. Relationship of reduction in shoot dry weight and total phenolics contents in aqueous extract of Sunflower, eucalyptus and Sugar beet.

eucalyptus, sunflower and sugar beet (Table 1). At the highest (20 gL<sup>-1</sup>) concentrations of eucalyptus, sunflower and sugar beet extract, the leaf area of *A. retroflexus* was decreased by 21, 24.5 and 56.3%, respectively than control. Likewise these extracts concentrations decreased the plant height of *A. retroflexus* by 20.6, 22.5 and 34.6%, respectively than control (Table 1). High (20 gL<sup>-1</sup>) extract concentrations of eucalyptus, sunflower and sugar beet decreased the shoot dry weight of *A. retroflexus* by 29.8, 31.5 and 39.9%, respectively, over the control (Table 1).

### Physiological processes

Leaf photosynthesis (*A*) was decreased with increasing extract concentrations of tested plants and this was similar to stomatal conductance (*gs*) (Table 1). Leaf photosynthesis and stomatal conductance of plants treated with high concentration (20 gL<sup>-1</sup>) of aqueous extract of sugar beet decreased by 47.3 and 46.2%, respectively, than control plants. While this high aqueous extract concentration of eucalyptus decreased *A* and *gs* by 22.3 and 24.9% and sunflower extracts decreased *A* and *gs* by 27 and 28.5%, respectively. Leaf chlorophyll content of *A. retroflexus* decreased with increasing aqueous extract concentration (Table 1). Sunflower extracts were more harmful to chlorophyll content than eucalyptus and sugar beet. High extract concentration of sunflower, eucalyptus and sugar beet decreased the leaf chlorophyll content of *A. retroflexus* by 40.9, 22.2 and 17.6%, respectively, than control.

The inhibitory effects of aqueous extracts of tested plants on seed germination and growth parameter of weeds may be due to the presence of allelochemicals (including phenolic and volatile compounds) in its leaves. Furthermore, the toxicity might be due to the interaction effects of allelochemicals rather than effects of single allelochemical. Phenolic acids are toxic to germination and plant growth (3,17,26). El-Rokiek and Eid (16) reported that the inhibitory effects of *E. citriodora* on weeds were correlated with accumulation of total phenols, compared to control. Accumulation of phenols often occurs under stress conditions (15,20). Some researchers reported that allelochemicals inhibits the physiological processes that leads to reduced growth (22). The effects of allelopathy on germination and growth of plants may occur through various mechanisms [reduced mitotic activity in roots and hypocotyls, suppressed hormone activity, reduced rate of nutrients uptake, inhibited photosynthesis and respiration, inhibition of protein formation, reduction in permeability of cell membranes and inhibition of enzyme action (31)]. Leaf area is reduced due to slower leaf production and of smaller leaves. Under stress conditions, leaf area decreases due to decrease in cells number and in cells size (14). A possible reason for reduction in dry matter in plant under allelochemical stress, could be owing to the drastic reduction in uptake and assimilation of mineral nutrients. Higher concentration of allelochemicals inhibits the amylase activity in wheat seedlings and application of allelochemicals at high concentrations decreases the protein content in wheat seedlings (19). The nature of inhibitory effects of allelochemicals on seed germination and plant growth could be attributed to the inhibition in water absorption (27).

Reduction in photosynthesis may be due to both stomatal limitation and non-stomatal factors such as reduction in light absorption by chlorophyll. In stomatal limitation, generally reduced stomatal conductance decreases the intercellular CO<sub>2</sub> concentration. Stomata function is influenced by many factors [water status, potassium concentration and ABA signals (30)]. One possibility is that phytotoxic chemicals

influenced the stomatal aperture indirectly by changing the water status, hormone balance and ions uptake. The phytotoxic chemicals reduce the chlorophyll accumulation in 3-ways (Inhibition of chlorophyll synthesis, stimulation of chlorophyll degradation or both) (34). Phytotoxic chemicals can potentially impair the performance of photosynthesis, stomatal control of CO<sub>2</sub> supply, the thylakoid electron transport (light reaction) and the carbon reduction cycle (dark reaction) (33). Dry matter accumulation may be decreased by reduction in photosynthetic area or assimilation rate per unit leaf area. A possible reason for higher reduction in shoot dry weight of plants treated with sugar beet extract could be due to greater reduction in leaf area and stomatal conductance, which reduces the net photosynthesis.

Although some researchers attributed more inhibitory effects of plant extract to higher concentration of phytotoxic chemicals such as phenolic compounds in extract, but our results did not confirm that because leaf aqueous extract of sugar beet had lower concentration of phenolics than eucalyptus and sunflower (Fig. 5) but sugar beet aqueous extract was more inhibitory. The correlation coefficient ( $R^2=0.0044$ ) of total concentration of phenolic contents of extracts with reduction (%) in shoot dry weight was very low (Fig. 6). This indicates that higher concentration of phenolic content may be more inhibitory to plant growth. Therefore, more inhibitory effects of *B. vulgaris* extract might be due to the presence of more active phenolic compounds in its aqueous extract. Hegab *et al.* (19) identified and quantified 8-phenolic compounds (shikimic acid, camphor, hydroxybenzoic, p-coumaric and vanillic acids, coumarin and protocatechuic acids) in water extract of *B. vulgaris* var. cical. These phenolic acids play main role in allelopathic interactions and their biological activities on growth of some crop plants and weeds. Chung *et al.* (11) demonstrated that p-hydroxybenzoic, p-coumaric acids were the most active compounds in rice hull extracts, which inhibited the growth of barnyardgrass seedlings. Allelopathic activity results from the interaction of mixtures of allelochemicals rather than to a single allelochemical that has synergetic inhibitory effect depending on their concentrations (8,9,21).

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