https://doi.org/10.26651/allelo.j/2019-46-1-1198 0971-4693/94 Euro 20.00
Allelopathy Journal 46 (1): 55-72 (January, 2019) *International Allelopathy Foundation* 2019
Table: 2, Figs : 6

Allelopathic effects of redroot pigweed (*Amaranthus retroflexus* L.) aqueous extract on cucumber and wheat

H. Bakhshayeshan-Agdam, S.Y. Salehi-Lisar* and R. Motafakkerazad
Department of Plant Sciences, Faculty of Natural Sciences,
University of Tabriz, Tabriz-5166616471, Iran
E. Mail: y_salehi@tabrizu.ac.ir

(Received in revised form: February 12, 2018)

ABSTRACT

We conducted an hydroponic experiment to evaluate the physiological aspects of redroot pigweed's allelopathic effects on cucumber and wheat plants. Plants were cultivated hydroponically in greenhouse on perlite and treated with 0 and 0.25% aqueous extracts of redroot pigweed's. Aqueous extracts reduced the stomata opening and photosynthetic pigments contents, disrupted the membrane integrity, induction of oxidative stress and consequently reduced the growth and biomass production in treated plants. Therefore, allelopathic effects of redroot pigweed reduced the test plants growth due to the negative effects of its allelochemicals. Wheat proved more resistant to allelopathic effects of redroot pigweed than cucumber. In GC-MS analysis, twenty compounds were identified from amaranth aqueous extract. In aqueous extracts, the Docosane, Triacontane and Silane, ethoxytrimethyl- were found the most important allelopathic compounds.

Key words: Allelochemicals, allelopathy, *Amaranthus retroflexus*, cucumber, compounds, GCMS, growth, hydroponics, physiology, redroot pigweed, resistance, wheat.

INTRODUCTION

Plants produce numerous organic compounds; some of these have allelopathic potential. These compounds named allelochemicals are derived from the secondary metabolism of plants and are specie and tissue specific (17,25,28,45,52). Allelopathic organic compounds are: phenolic compounds, terpenoids, alkaloids, non-protein amino acids, saponins and benzoxazinones (25,27,28,35,39,46). These compounds are released from the plants into the environment as leachates, volatiles, root exudates and from biomass decomposition (28,30,42,45). Their action mechanism affecting the receiver plants also differs. Some of these compounds such as volatile compounds, directly impact the receiver plant, while other compounds needs microorganism's intermediation (45,49,53).

Allelopathy denotes interactions between the plants in natural ecosystems and agricultural fields. Weeds reduce the crops yield in fields due to their allelopathic effects. Allelopathic interactions in receiver plants occur at molecular, biochemical, physiological and morphological levels (13,20,23,32,47,50,51). The weeds in arable systems produce allelopathic compounds (5,41,42,49). Redroot pigweed (*Amaranthus retroflexus* L.) is major invasive and allelopathic weed worldwide. Many crops are vulnerable to its invasion (2,5,8,11,41). Moreover, it has become resistant to herbicides, hence, very difficult to control (9,36). It inhibits the growth of various plants viz., corn (5), maize (15), soybean (5), barley (41), alfalfa (4), garden cress (31), common bean (4), and so on. Its plants contains the allelopathic compounds viz., aldehydes, alkaloids, apocarotenoids, flavonoids, steroids, xyloids, chlorogenic acid and saponins (40,41).

*Correspondence author

The majority of studies had focused on germination and seedlings growth of receiver plants and physiological aspects but the allelopathic interactions were not studied. Hence, this study aimed to evaluate the allelopathic effects of redroot pigweed on growth and physiology of cucumber and wheat plants.

MATERIALS AND METHODS

Redroot pigweed fresh material (Root, stem, leaf and flower) was collected from crop fields of Khosrowshah, East Azerbaijan, Iran: [37°56′59″N 46°03′10″E, altitude 1307 m, mean maximum and minimum temperature is 24.3 and -1 °C respectively and annual precipitation: 322 mm)] The plant material was oven dried under lab conditions (in 25 °C temperature for two weeks) and powdered using a mill. Plant species were identified by Agricultural and Natural Resources Research Centre, East Azerbaijan, Iran.

Aqueous extract preparation: Ten g powdered material were suspended in 100 ml sterile double distilled water and mixed for 24 h using shaker (120 rpm) to produce uniform suspension. It was filtered by two layers of sterile cheesecloth and filtrate was considered as aqueous extracts of 10 % concentrations (31,40,41). Initially, an experiment was conducted to determine appropriate and effective aqueous extracts concentration on test species (results not shown). Accordingly, 0.25% concentration of amaranth aqueous extract was selected for physiological assays. Aqueous extracts 0.25% was prepared by diluting the 10 % aqueous extract using double distilled water.

Seeds of cucumber (*Cucumis sativus* L. "Basmenj") and wheat (*Triticum aestivum* L. "Pishgam") were obtained from Agricultural and Natural Resources Research Centre, Tabriz and stored at 4 °C until use. The seeds were disinfected using 1 % (v/v) sodium-hypochlorite solution for 5 min and thereafter, sufficiently washed with sterile distilled water.

Pot culture: Experiment was conducted in May/July of 2014 in pots (2 L pot size, 17 and 12 cms top and bottom dia, respectively, depth 11 cm) filled with perlite (Medium size and F.C: 165 ml per 100 gr), using completely randomized design (CRD) with three replications. Twenty and 10 seeds of cucumber and wheat, respectively, were sown by forceps at 2 cm depth and 4 cm distance on 26 May 2014. To accelerate seed germination (seeds with negative phytoblasty), dark treatment was applied for 2-days. Thereafter all pots were transferred to growth chamber with controlled conditions (25-30 °C, 16/8 (light/dark) photoperiod and relative humidity of 60 %). After 27-days on 21 June 2014, plants were harvested.

Hydroponics: Twenty and 10 seeds of cucumber and wheat, respectively, were sown at 2 cm depth in pots (2 L pot size, 17 and 12 cm top and bottom dia, respectively, depth 11 cm) filled with sterile perlite and irrigated with sterile distilled water for 7 days. The 7 days old seedlings were transferred to Hoagland medium (Table 1) and grown for 7 days. Then 14 days old seedlings were treated with 0.25% aqueous extract of redroot pigweed. In control plants only Hoagland solution was used. After two days, when allelopathic symptoms (leaf chlorosis, wilting, etiolation and leaf necrosis) were observed, plants were harvested and used for growth analysis and physiological assays.

Table 1. Chemical composition of Hoagland solution (24).

Macro elements			Micro elements		
Compounds name	Elements	Concentration (mM)	Compounds name	Elements	Concentration (μM)
KNO_3	N	16	KCl	Cl	50
	K	6	Fe-EDTA	Fe	20
$Ca(NO_3)_2$	Ca	4	H_3BO_3	B	25
$NH_4H_2PO_4$	P	2	$CuSO_4$	Cu	0.5
$MgSO_4$	Mg	2	$MnSO_4$	Mn	2
	S	2	H_2MoO_4	Mo	0.5
			$ZnSO_4$	Zn	2

Plant harvest: Biochemical and physiological assays were done using the fresh samples before plants harvest (on 21 June). After measuring the shoot height, root length and leaf area, the harvested plants were partitioned into the roots and shoots. Samples were washed with water to remove excess nutrients solution from root surface and dust on leaves surface, immediately dried with towel paper weighed and kept in oven at 70 °C for 72 h for dry weight. Thereafter, dry weight of samples was recorded.

Photosynthetic gas exchange parameters: Photosynthetic gas exchange parameters were measured using a portable leaf chamber analyzer (ADC, LCA4, UK). During the measurements, the leaf chamber temperature was 25±1 °C, CO_2 concentration varied between 350-400 ppm and photosynthetic photon flux density (PPFD) was 300 µmol m^{-2} s^{-1}. Water use efficiency (WUE) was calculated by using net photosynthesis (A) and transpiration (E) (26) as under:

$$WUE= A/E$$

Photosynthetic pigments content: Photosynthetic pigments content (chlorophyll a, b, total chlorophyll, and total carotenoids) was measured as per Dere (1998). Briefly, 0.5 g fresh leaf sample was homogenized with 10 ml of >99% acetone using a mortar and pestle on ice bath. Homogenates were filtered using a Whatman No. 42 filter paper and the absorbance of extracts was recorded at 662, 645 and 470 nm. The pigments contents were determined as under:

$$\text{Chlorophyll a } (\mu g g^{-1}) = C_a = 11.75A_{662} - 2.350A_{645}$$
$$\text{Chlorophyll b } (\mu g g^{-1}) = C_b = 18.61A_{645} - 3.960A_{662}$$
$$\text{Carotenoids } (\mu g g^{-1}) = C_{x+c} = 1000A_{470} + 2.270C_a - 81.4C_b /227$$

Membrane stability index (MSI): Leaf samples (0.1 g) were sliced into discs with uniform size and transferred into test tubes containing 10 ml of double distilled water. For each assay, two sets of samples were provided. One set was kept at 40 °C for 30 min (C_1) and another at 100 °C in boiling water for 15 min (C_2). Then, electrical conductivity's of C_1 and C_2 samples were measured by electric conductivity meter (HANNA 9812). Finally, the MSI of samples were calculated using following formula (37).

$$MSI= [1-(C_1/C_2)] \times 100$$

Relative water content (RWC): Relative leaf water content (RWC) was determined by method of Sairam (2002). Leaf samples (0.5 g) were kept in 100 ml distilled water for 4h. The turgid weights of leaf samples were recorded and dried on towel paper, and transferred to 70 °C for 48 h. Finally, the dry weight of samples was measured and RWC was calculated as under:

$$RWC= [(FW-DW)/ (TW-DW)] \times 100$$

Where, RWC : Relative water content, FW : Fresh weight, DW : Dry weight and TW : Turgid weight.

Total soluble protein content and antioxidant enzymes activity: The samples were homogenized in 10 ml of 0.1 M potassium phosphate (Na_2HPO_4/NaH_2PO_4, pH=7) buffer solution on the ice bath using mortar and pestle. Homogenates were centrifuged at 10000 g for 10 min at 4 °C and supernatants used to estimate the soluble protein and enzymes content. Soluble protein content was determined as per method of Bradford (1976).

Catalase: The activity of catalase (CAT, EC1.11.16) was measured at 240 nm after the decomposition with H_2O_2 for 2 min. The reaction mixture contained 50 mM of potassium phosphate buffer (pH=7), 10 mM of H_2O_2 and 50 µM of enzyme extract. CAT specific activity was calculated using the extinction coefficient of H_2O_2 (0.041 $mM^{-1}cm^{-1}$) and one unit of CAT activity was considered as the amount of enzyme necessary to reduce 1 µMH_2O_2 per minute (10).

Peroxidase: To evaluate the peroxidase activity (POD, EC1.11.1.7) the appropriate amount of supernatants was added to the reaction mixture containing 10 mM of potassium phosphate buffer (pH=7), 5 mM of H_2O_2 and 4 mM of guaiacol. The increase in absorbance at 470 nm during the polymerization of guaiacol totetraguaiacol was recorded for 3 min. Finally, POD specific activity was calculated by using the extinction coefficient of guaiacol (25.5 $mM^{-1}cm^{-1}$) and one unit of POD activity was considered as the enzyme amount capable to oxidize 1 µM guaiacol to tetraguaiacol per minute (10).

Malondialdehyde content (MDA): Lipid peroxidation was evaluated by measuring the malondialdehyde (MDA) content in plant material as per Boominathan and Doran (2002). The samples were homogenized with 0.1 % (W/V) trichloroacetic acid (TCA) and centrifuged at 10000 g for 5 min. Then, 0.5 ml of supernatants were mixed with 2 ml of 20 % TCA containing 0.5 % of 2-thiobarbituric acid and heated for 30 min in hot water at 95 °C. The mixtures were immediately transferred to ice bath and then centrifuged at 10000 g for 15 min. Finally, the absorbance of supernatants recorded in 532 nm and MDA concentration was calculated according to standard curve prepared using 3,1,1,3-tetraethoxy propane (0-100 nM) and expressed as µg g^{-1}FW.

GC-MS analysis: The required quantity of whole plant powder of amaranth was transferred to a flask, treated with double distilled water until the powder was fully immersed. Then, the suspension was shaken overnight and filtered through a Whatman No. 42 filter paper. The filtrate was dried by freeze drying, dissolved in absolute alcohol and sodium sulfate was added to remove the sediments and traces of water (18,33,34). GC-MS analysis of the aqueous extract of redroot pigweed was performed using a Agilent 6890 GC system and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a HP-5MS (5 % diphenyl/95 % dimethyl poly siloxane) fused a capillary column (30 cm × 0.25 mm ID × 0.25 µm df). An electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999 %) was used as a carrier gas at a constant flow rate of 1 ml min^{-1}. The injector temperature was maintained at 150 °C, the oven temperature was programmed from 50 °C (isothermal for 3 min), with an increase of 10 °C min^{-1} to 120 °C, then 10 °C min^{-1} to 150 °C, ending with a 5 min isothermal at 240 °C. The mass-detector used in this analysis was HP 5989A, and the software adopted to handle mass spectra and chromatograms was a chemstation.

Identification of Components: The interpretation of GC-MS spectrum was done using the database of National Institute Standard and Technology (NIST) with more than 62,000 patterns. The spectrum of the unknown compounds was compared with the spectrum of known compounds stored in the NIST library. The name, molecular weight, and structure of the test materials compound's were determined (18).

Statistical analysis: Analysis of variance was done with t-test using SPSS software (ver. 16) and reading was considered significant when $p \leq 0.01$. Microsoft excel 2007 software was used for the preparation of figures.

RESULTS AND DISCUSSION

Growth parameters: The redroot pigweed aqueous extract significantly affected the plants growth and the effects varied with studied species ($p \leq 0.01$). The plant height and shoot fresh and dry weight of cucumber plants were increased (8.83, 17.83 and 10.32 %, respectively), but these were decreased in wheat plants (8.37, 26.5 and 13.76 %, respectively). Root fresh and dry weight of cucumber plants were decreased significantly (60.15 and 36.72%, respectively) ($p \leq 0.01$), while root fresh weight of wheat plants was only decreased 21.51% than control. Redroot pigweed aqueous extract significantly reduced the root length of both tested species ($p \leq 0.01$) and the reduction was 27.7 and 50.7 % in wheat and cucumber plants, respectively. Likewise, the leaf area was decreased in both species than control ($p \leq 0.01$) (Fig. 1,2).

The effects of aqueous extract of pigweed on cucumber growth parameters were higher than on wheat. Negative effects of redroot pigweed aqueous extract on growth of barley (41), maize (15), common bean (4), and garden cress (31) had been reported. There was drastic reduction in biomass (fresh and dry weight) of wheat shoot than its root biomass. Amaranth pigweed aqueous extract had stimulatory effects on shoot and root of cucumber plant. Although, it led to significant decline in root biomass, but caused increase in shoot biomass.

Photosynthetic gas exchange parameters: Redroot pigweed aqueous extract increased the stomatal conductance (16.15 %) in cucumber plants. However, significant decrease in stomatal resistance (68.16 %) and water use efficiency (9.44 %) was observed ($p \leq 0.01$). In wheat plants, significant decline in net assimilation rate (48.71 %), transpiration rate (60.28 %) and stomatal conductance (79.63 %) were observed, but stomatal resistance and water use efficiency increased (524.2 and 49.58 % respectively) than control ($p \leq 0.01$) (Fig. 3).

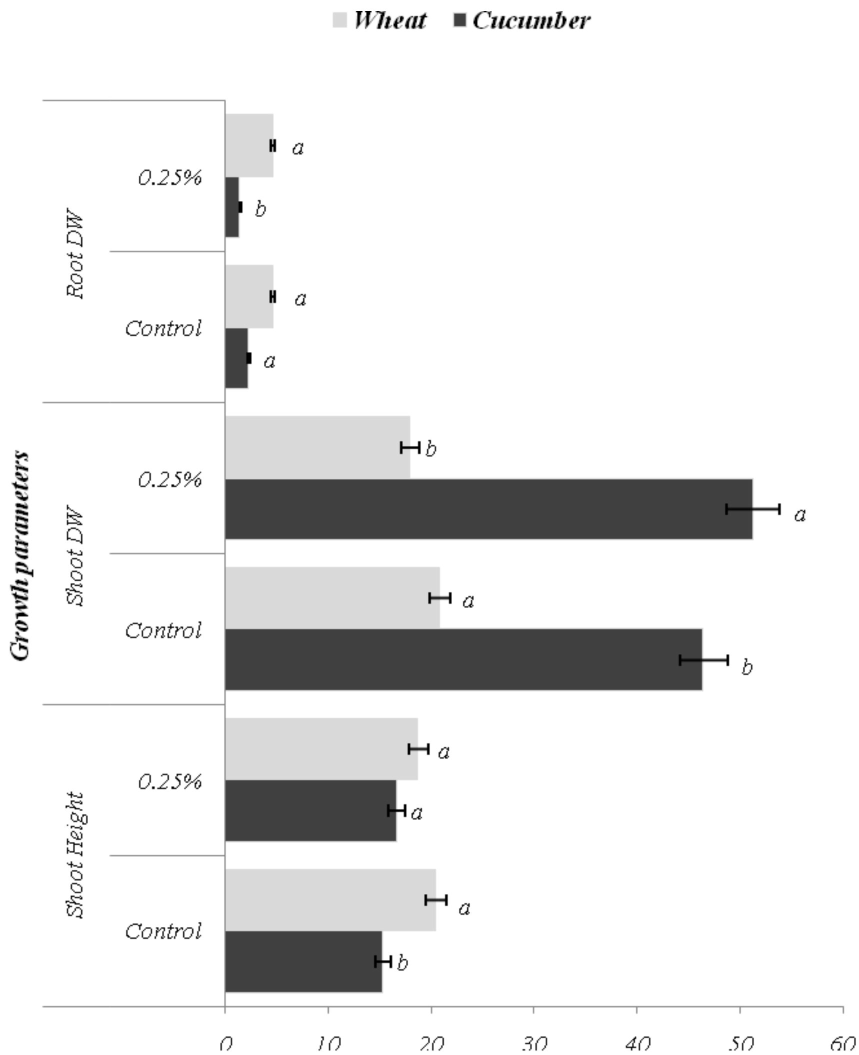


Figure 1.Effects of redroot pigweed aqueous extract on shoot height (cm) and shoot and root dry weight (mg) of cucumber and wheat plants. The data represent the mean of tree replications ± SD and different letters in each parameter for each species indicate significantly different values at *p* ≤0.01
DW: Dry Weight.

In treated wheat plants, photosynthesis and transpiration rate were decreased due to increasing stomatal resistance and decline in stomatal opening, which can be part of resistance procedure to increase management of water use efficiency. Water use efficiency increased due to stomatal closure. Results for relative water content of wheat plants confirm this subject and had significant positive correlation with WUE ($r^2 = 0.917$) ($p \leq 0.01$). Significant decline in net assimilation rate of treated wheat plant were observed, but this parameter was not decreased in cucumber plant. Therefore, reduction in wheat shoot biomass by amaranth aqueous extract can be due to reduced net assimilation rate.

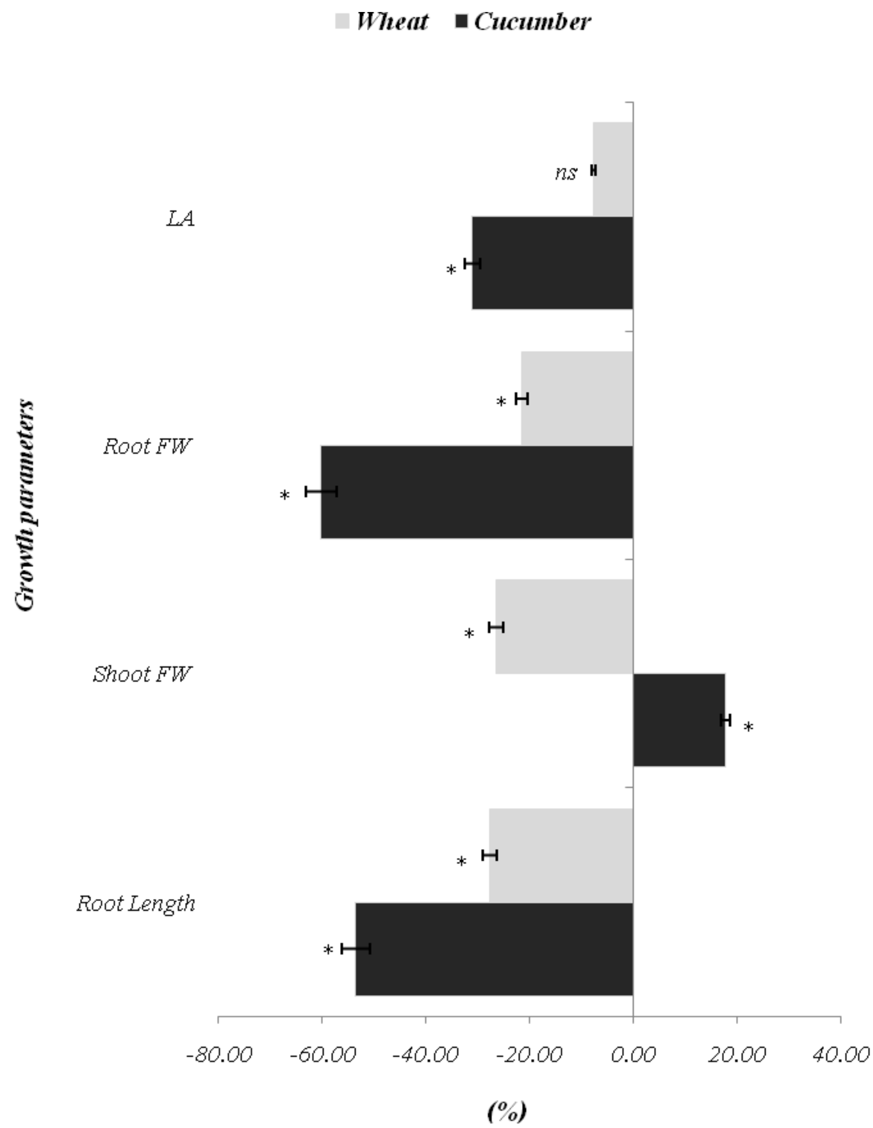


Figure 2. Effects of redroot pigweed aqueous extract on root length (cm) shoot and root fresh weight (mg), and leaf area (mm^2) of cucumber and wheat plants. The data represent the mean of tree replications ± SD and * and ns indicate significant at $p \leq 0.01$ and non significant respectively
FW: Fresh Weight, LA: Leaf Area.

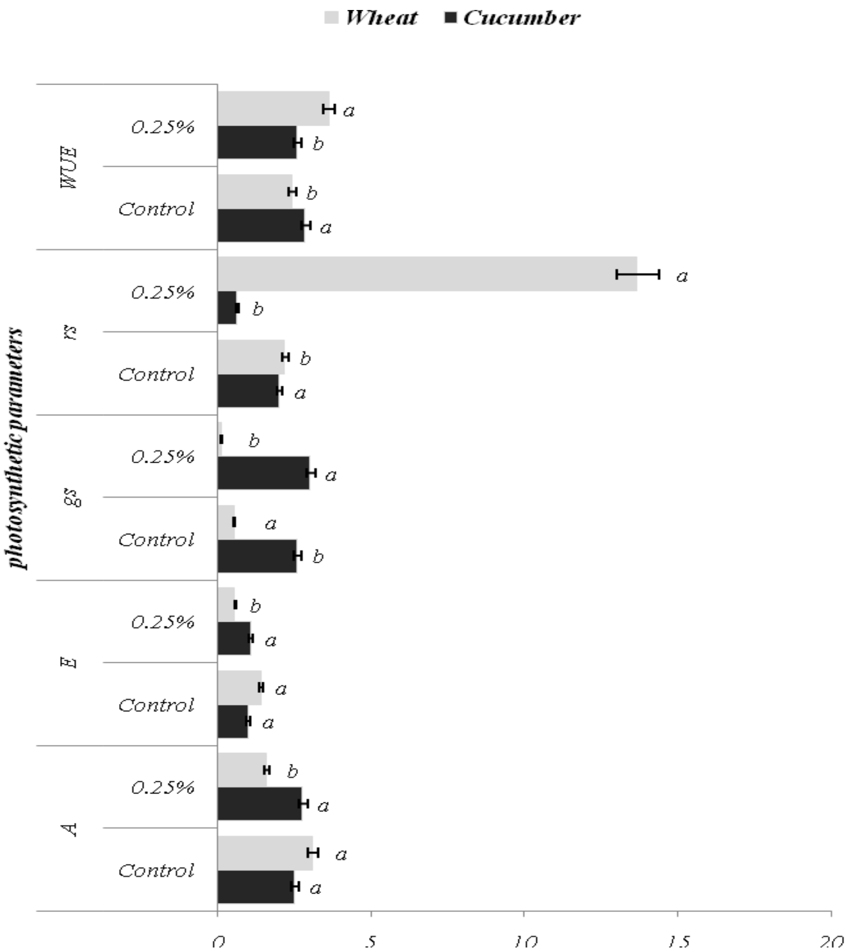


Figure 3. Effects of redroot pigweed aqueous extract on photosynthetic gas exchange parameters of cucumber and wheat plants. The data represent the mean of tree replications ± SD and different letters in each parameter for each species indicate significantly different values at $p \leq 0.01$
A: Net assimilation rate (µmol $m^{-2} s^{-1}$), E: Transpiration rate (µmol $m^{-2} s^{-1}$), g_s: Stomatal conductance (mol $m^{-2} s^{-1}$), r_s: Stomatal resistance (mol $m^{-2} s^{-1}$), WUE: Water Use Efficiency (µmol mol^{-1}).

However, there was no decline in wheat root biomass that led to higher root to shoot ratio. Probably higher allocation of photosynthetic products to the roots in treated plants is the cause of this observation. Such a change in root to shoot ratio is the well-known plants response to some other soil stress such as water deficiency (38) and phosphorus deficiency (29). In cucumber plants, the stability in net assimilation rate of treated plants increased the shoot biomass. However, this reason cannot explain the sharp reduction in root biomass in this plant. This may be done to the lower allocation of photosynthetic products to the roots. Consequently, it seems that in wheat plants (as tolerant species) despite the decline in photosynthesis, main parts of photosynthetic products participated to the roots for maintaining its normal role as first plant resistance barrier against allelochemicals and keep its fundamental roles in resistance condition to maintain plant growth. Obtained results for root length in the studied species confirm this explanation.

Photosynthetic pigments content: Despite changes in pigments content of treated cucumber plants than control, the difference was statistically significant only in carotenoids content ($p \leq 0.01$), which showed an increase (85.93 %). In wheat plants chlorophyll a concentration dramatically decreased (13.47 %) by aqueous extract treatment that was statistically significant ($p \leq 0.01$) as well. The concentration of carotenoids in wheat plants was not affected by 0.25 % redroot pigweed aqueous extract (Fig.4).

In addition to influence of stomatal factor in reduction of photosynthesis, decreasing chlorophyll content was the other factors, which decreased the photosynthesis in wheat plants. Decline in leaf chlorophyll content, especially chlorophyll *a* in wheat plants have been reason of leaf chlorosis, a phenomenon that reported by various researches (13,43). The opposite point of all items those described for wheat plants is mentionable for treated cucumber plants. Treated cucumber plants had significant increase in carotenoid pigments content, which can be interpreted as a mechanism of resistance in this species. Carotenoides are not only photosynthetic pigments, but also are low molecular weight antioxidant compounds and there are various reports about increasing in concentration of these compounds including carotenoides and anthocyanins under allelopathic stress (1,13,43).

Membrane stability index: Redroot pigweed aqueous extract injured membrane stability, but the effect was not the same in studied species. Membrane stability index decreased by 61.33 % in wheat plants, but in cucumber the changes was not statistically significant in comparison with the control ($p \leq 0.01$) (Fig.5).

Relative water content (RWC): Amaranth aqueous extract distinctly influenced water content of cucumber and wheat (Fig. 5). A significant reduction in RWC of cucumber was happened (7.77 %), while significantly increase in treated wheat plants was observed (21.85 %) ($p \leq 0.01$).

Total soluble protein content and antioxidant enzymes activity: Amaranth aqueous extract showed variable effects on catalase activities of cucumber and wheat plant (Fig.6). While, activities of this enzyme significantly decreased in cucumber plants (32.87 %), a significant increase (14.88 %) was observed in catalase activity in wheat plant ($p \leq 0.01$). Peroxidase activity stimulated by redroot pigweed aqueous extract in cucumber and wheat (307.41 and 12.73 %) (Fig. 6), but it was only significant in cucumber plant ($p \leq 0.01$). Total soluble protein content affected from amaranth aqueous extract in both studied species (Fig. 6). In cucumber, significant reduction (26.95 %) was observed ($p \leq 0.01$). In wheat plant an increase in total soluble protein (0.83 %) was detected, but it was not significant in comparison to the control ($p \leq 0.01$).

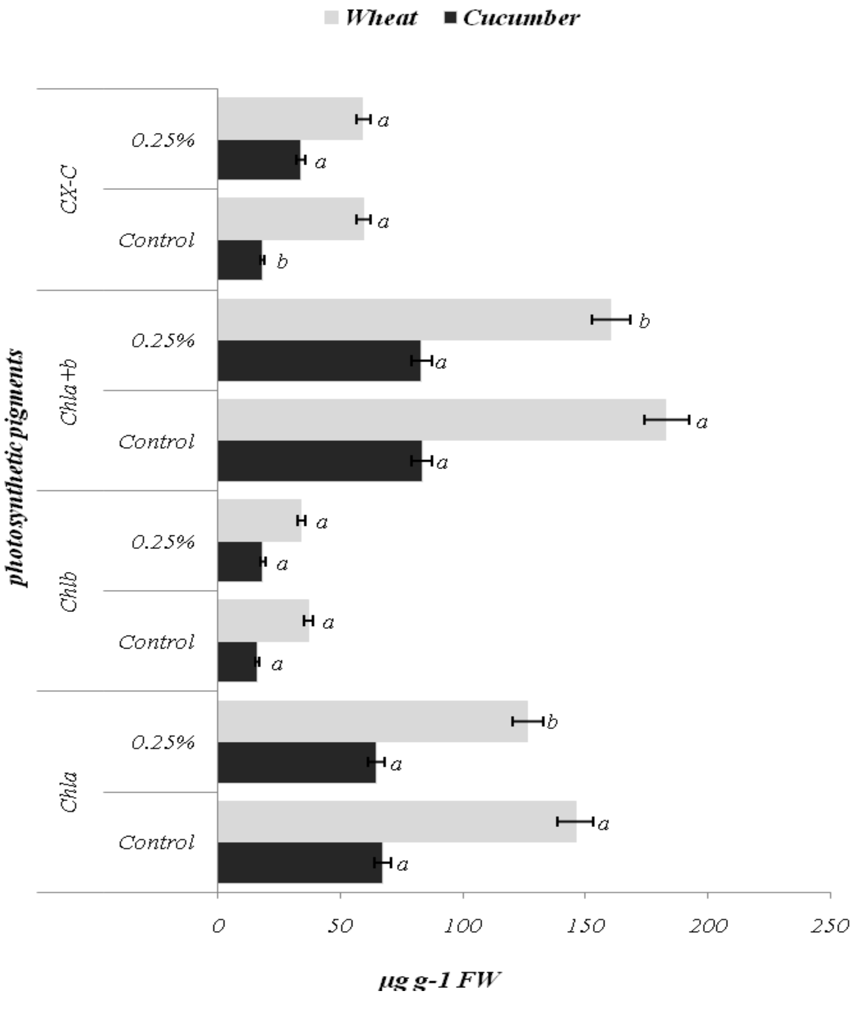


Figure 4. Effects of redroot pigweed aqueous extract on photosynthetic pigments content (µg g^{-1} FW) of cucumber and wheat plants. The data represent the mean of tree replications ±S D and different letters in each parameter for each species indicate significantly different values at $p \leq 0.01$
Chl_a: Chlorophyll a, Chl_b: Chlorophyll b, Chl_{a+b}: Total Chlorophyll), $C_{X\text{-}C}$: Carotenoid.

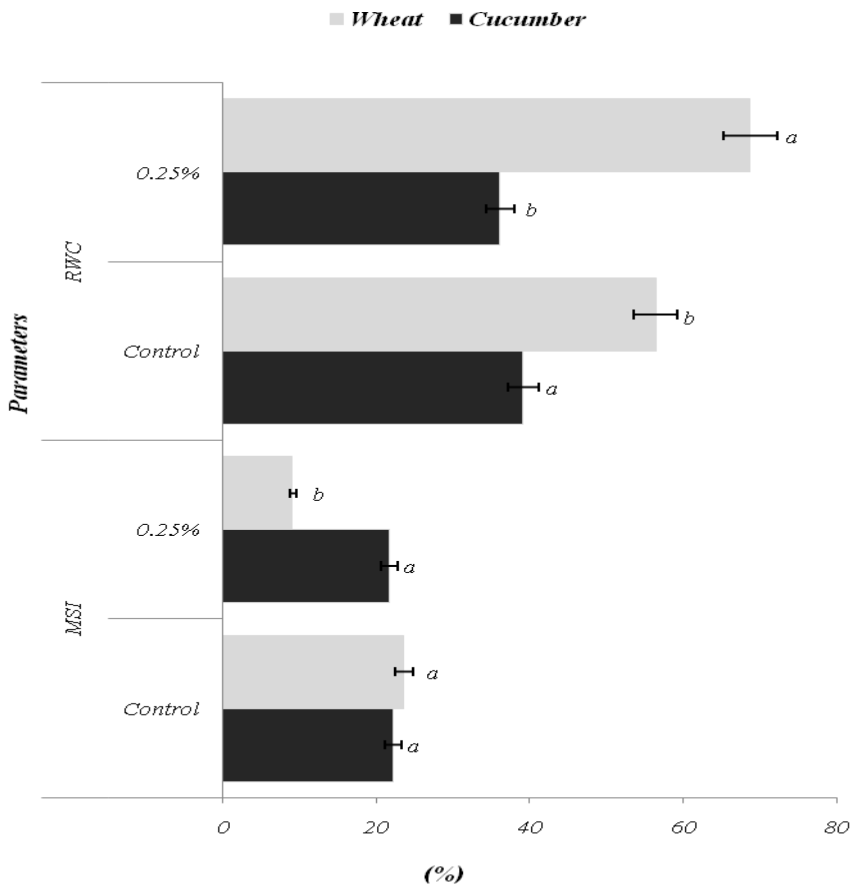


Figure 5. The effects of redroot pigweed aqueous extract on membrane stability index and relative water content (%) of cucumber and wheat plants. The data represent the mean of tree replications and error bars indicate SD. Different letters in each species show significantly different values at $p \leq 0.01$.
MSI: Membrane Stability Index, RWC: Relative Water Content.

Malondialdehyde content (MDA): The redroot pigweed aqueous extract variably affected the Malondialdehyde content in cucumber and wheat (Fig. 6). While, MDA content in cucumber decreased significantly (62.5 %), but was slightly increased in wheat (1.46 %) ($p \leq 0.01$).

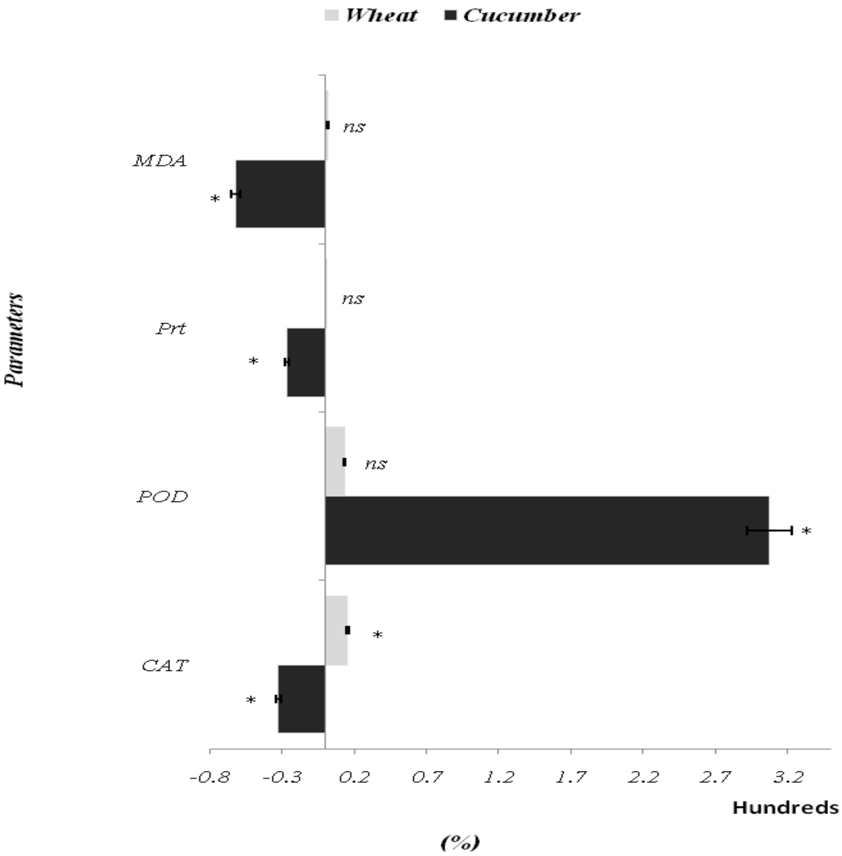
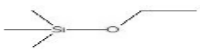
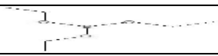
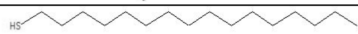
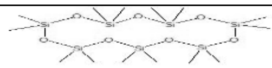
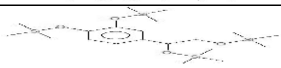
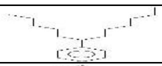
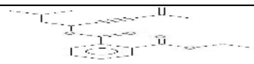
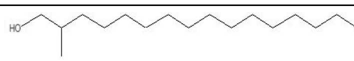
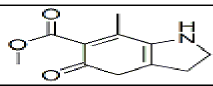


Figure 6. Effects of redroot pigweed aqueous extract on catalase and peroxidase (Unit mg $^{-1}$ protein) specific activity and total soluble protein (mg g^{-1} FW) and malondialdehyde content (µg g^{-1} FW) of cucumber and wheat plants. The data represent the mean of tree replications ± SD and * and ns indicate significant at $p \leq 0.01$ and non significant respectively
CAT: Catalase, POD: Peroxidase, Pr_t: Total soluble protein, MAD: Malondialdehyde.

Activities of catalase and peroxidase as well-known antioxidant enzymes in both studied plants have shown notable changes. Increasing in the activity of antioxidant enzymes such as catalase (1), peroxidase (43), polyphenol oxidase (22) and superoxide dismutase (50) by allelochemicals were previously reported in different plant species. In cucumber plants, amaranth aqueous extract led to a reduction in catalase activity, but peroxidase activity increased considerably. In wheat plants catalase activity increased and peroxidase activity remained constant in comparison with the control. While, the content of MDA in treated cucumber plant significantly declined, in wheat plant MDA content showed non-significant increase. MDA content is the biological marker for lipid

Table 2. Chemical compounds identified in the aqueous extractof redroot pigweed by GC-MS.

S. No.	RT	Name of the compound	Molecular Formula	Mol. Wt.	Peak area%	Structure
1.	2.152	Docosane	$C_{22}H_{46}$	310	68.35	
2.	3.028	Undecanal dimethyl acetal	$C_{13}H_{28}O_2$	216	0.72	
3.	3.761	Silane, ethoxytrimethyl-	$C_5H_{14}OSi$	118	3.17	
4.	3.927	Triethyl borate	$C_6H_{15}BO_3$	146	2.09	
5.	12.316	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444	0.16	
6.	14.580	1-Hexadecanethiol	$C_{16}H_{34}S$	258	0.08	
7.	17.021	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	0.75	
8.	20.883	Silane, [[4-[1,2 bis[(trimethylsilyl)oxy]ethyl]-1	$C_{20}H_{42}O_4Si_4$	458	0.82	
9.	20.916	Benzeneacetic acid, .alpha.,3,4-tris [(trimethylsilyl)oxy] -	$C_{18}H_{34}O_5Si_3$	414	0.11	
10.	22.126	Benzene, (1-pentylheptyl)-	$C_{18}H_{30}$	246	0.20	
11.	22.203	Benzene, (1-butyloctyl)-	$C_{18}H_{30}$	246	0.29	
12.	23.479	Dotriacontane, 1,32-dibromo-	$C_{32}H_{64}Br_2$	606	0.05	
13.	24.123	Hexadecane, 1-chloro-	$C_{16}H_{33}Cl$	260	0.19	
14.	25.188	Oxirane, 2-ethyl-2-methyl-	$C_5H_{10}O$	86	0.06	
15.	26.109	Phthalic acid, 2,7-dimethyloct-7-en-5-yn-4-yl iso	$C_{20}H_{24}O_4$	328	0.13	
16.	28.040	1-Bromo-11-iodoundecane	$C_{11}H_{22}BrI$	360	0.09	
17.	33.300	Triacontane	$C_{30}H_{62}$	422	20.23	
18.	33.866	Octacosane	$C_{28}H_{58}$	394	0.01	
19.	34.321	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	0.57	
20.	34.487	1H-Indole-6-carboxylic acid, 2,3,4,5-tetrahydro-7-	$C_{10}H_{11}NO_3$	194	0.03	

peroxidation due to ROS accumulation (21), and it is usually used for the illustration of plants sensitivity to oxidative stress (12). Probably lower MDA content is the result of lower peroxidation of membrane lipid possibly due to better activity of antioxidant systems. Results for MDA content in this study, perfectly matched with peroxidase activity in treated plants. A significant negative correlation between MDA content and POD activity was observed in studied plants (r^2 = 0.962 & 0.918 for cucumber and wheat, respectively) ($p \leq 0.01$). Low peroxidation of membrane lipids can lead to better membrane integrity (32). Results for membrane stability index from this study confirm this subject as well. Disorder in membrane integrity can be considered as reason of photosynthesis decline in treated wheat plants.

In addition to growth and physiological effects, some morphological symptoms were seen in treated plants including: leaf margin chlorosis and necrosis, ethiolation, decline in root proliferation, browning roots apex and adventitious root formation in cucumber plants collar, despite the existence of main root.

GC-MS analysis: GC-MS analysis identified 20 compounds (benzene compounds, alkanes, cyclic silicone compounds) of different groups in plant materials (Table 2). The major compounds in amaranth aqueous extract were Docosane (68.35%), Triacontane (20.23%) and Silane, ethoxytrimethyl- (3.17%).

Based on GC-MS analysis results, the 20-compounds were characterized and identified from the peaks of aqueous extract of redroot pigweed in comparison to the mass spectra of the compounds with the NIST library. Some of these compounds and their derivatives were characterized in several plants such as damas tree (34), thistles (19), and heaven tree (3). It was reported that some detected compounds such as Docosane, Triacontane, and Octacosane have allelopathic activities on different organisms (19,33,34,). According to the present result, Docosane, Triacontane, and Silane, ethoxytrimethyl- were found major allelopathic compounds in amaranth aqueous extract. However, other compounds including aldehydes, alkaloids, apocarotenoids, flavonoids, steroids, xyloids, chlorogenic acid and saponins were reported by other researchers as active allelochmicals in redroot pigweed (40,41). In addition, because researchers selected different methods for extraction and analysis of redroot pigweed therefore, different compounds have been reported as active allelochemical in this plant. So that, the selection of suitable extraction and analysis methods for the accurate evaluation of redroot pigweed allelochemicals is necessary.

CONCLUSIONS

Redroot pigweed aqueous extract inhibited the growth, development, physiological and morphological aspects of cucumber and wheat plants. The decline in growth of wheat plants was higher than cucumber. Aqueous extract of redroot pigweed affects different aspects of plant physiological traits viz., photosynthesis, membrane integrity, antioxidant system etc., but the effect was not similar in different species. Among the 20-compounds detected in aqueous extract of redroot pigweed, some are well-known allelochemical but the role of other compounds needs further investigation in future.

ACKNOWLEDGEMENTS

The authors are grateful to the Research Affairs of the University of Tabriz for financial and other support. We are also thankful to Dr. Nader Farsad for assistance in statistical analysis of results and Mr.Amirhosein Talebpour for collection of plant samples.

REFERENCES

1. Ahrabi, F., Enteshari, S. and Moradshahi, A. (2011). Allelopathic potential of para-hydroxybenzoic acid and coumarin on canola: talaieh cultivar. *Journal of Medicinal Plant Research* **5**: 5104-5109.
2. Akubugwo, I.E., Obasi, N.A., Chinyere G.C. andUgbogu, A.E. (2007). Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo, Nigeria. *African Journal of Biotechnology* **6**: 2833-2839.
3. Albouchi, F., Hassen, I., Casabianca, H. and Hosni, K. (2013). Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of *Ailanthus altissima* (Mill.) Swingle leaves. *South African Journal of Botany* **87**: 164-174.
4. Bakhshayeshan-Agdam, H., Salehi-Lisar, S.Y., Motafakkerazad, R., Talebpour, A.H. andFarsad, N. (2015). Allelopathic effects of redroot pigweed (*Amaranthus retroflexus* L.) on germination and growth of cucumber, alfalfa, common bean and bread wheat.*ActaA griculturae Slovenica* **2**: 193-202.
5. Bhowmik, P.C. and Doll, J.D. (1982). Corn and soybean response to allelopathic effects of weed and crop residues. *Agronomy Journal* **74**: 601-606.
6. Boominathan, R. and Doran, P.M. (2002). Ni induced oxidative stress in the roots of Ni hyperaccumulator, *Alyssum bertoloni*L. *New Phytologist* **156**: 202-205.
7. Bradford, M.M. (1976). A rapid and sensitive method to quantify the microgram quantities of protein using the principle of protein- dye binding. *Analytical Biochemistry* **72**: 248-254.
8. Bradow, J.M. andConnick J.W.J. (1987). Allelochemicals from palmer amaranth, *Amaranthus palmeri* S. Wats. *Journal of Chemical Ecology* **13**: 185-202.
9. Costea, M., Weaver, S. and Tardif, F. (2004). The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. (Update). *Canadian Journal of Plant Science* **84**: 631-668.
10. Chance, B. and Maechly, A.C. (1955). Assay of catalases and proxidases. *Methods in Enzymology* **2**: 764-775.
11. Chou, C.H. (1999). Roles of allelopathy in plant biodiversity and sustainable agriculture.*Critical Reviews in Plant Sciences* **18**: 609-636.
12. Debiane, D., Garcon, G., Verdin, A., Fontaine, J., Durand, R., Grandmougin-Ferjani, A., Shirali, P. and Sahraoui, L.H.A. (2008). *In-vitro* evaluation of the oxidative stress and genotoxic potentials of anthracene on mycorrhizal chicory roots. *Environmental and Experimental Botany* **64**: 120-127.
13. Dehghani, F., Yahyaabadi, S. andRanjbar, M. (2014). Allelopathic potential of petal, leaf and seed extracts of different ecotypes of sunflower on *Zea mays*. *International Journal of Biosciences* **5**: 136-144.
14. Dere, S.H., Gunes, T. andSivaci, R. (1998). Spectrophotometric determination of chlorophyll-a, b and total carotenoid contents of some algae species using different solvents. *Turkish Journal of Botany* **22**: 13-17.
15. Dogaru, G.V., Budoi, S.G. andSandoiu, D.D.I. (2012). Determination of the *Amaranthus retroflexus* damage threshold in maize crop. *Advances in Agriculture and Botanic* **4**: 1-5.
16. Duke, S.O., Scheffler, B.E. and Dayan, F.E. (2001). Strategies for using transgenes to produce allelopathic crops. *Weed Technology* **15**: 826-834.
17. Einhellig, F.A. (1996). Intractions involving allelopathy in cropping system. *Agronomy Journal* **69**: 13-23.
18. Ezhilanand, B.P. and Neelamegam, R. (2012). GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. *Pharmacognosy Research* **4**: 11-14.
19. Formisano, C., Rigano, D., Senatore, F., De Feo, V., Bruno, M. andRosselli, S. (2007). Composition and allelopathic effects of essential oils of two thistles: *Cirsium creticum* (Lam.) D.'Urv. ssp. *triumfetti* (Lacaita) Werner and *Carduusnutans* L. *Journal of Plant Interactions* **2**: 115-120.
20. Gniazdowska, A., Krasuska, U., Andrzejczak, O. and Soltys, D. (2015). Allelopathic compounds as oxidative stress agents: yes or no. In: *Reactive Oxygen and Nitrogen species Signaling and Communication in Plants* (Eds., K.J. Gupta and A.U.Igamberdiev) Vol. **23**: 155-176. Springer, New York, USA.
21. Gunes, A., Pilbeam, D. andInal, A. (2009). Effects of arsenic-phosphorus interaction on arsenic induced oxidative stress in chickpea plants. *Plant and Soil* **314**: 211-220.

22. Haddadchi, G.R. andGerivani, Z. (2009). Effects of phenolic extracts of canola (*Brassica napuse*L.) on germination and physiological responses of soybean (*Glycin max* L.) seedlings. *International Journal of Plant Production* **3**: 63-74.
23. Haddadchi, G.R. andMassoodi, K.F. (2006) Allelopathic effects of aqueous extracts of *Sinapis arvensis* on growth and related physiological and biochemical responses of *Brassica napus*. *Journal of Science* University of Tehran **32**: 23-28.
24. Hoagland, D.R. andArnon, D.I. (1950). The water-culture method for growing plants without soil. *California Agricultural Experiment Station* **347**: 1-32.
25. Inderjit, Dakshinamurthy, M. andEinhellig, F.A. (1993). Allelopathy: organism, processes and applications. *Journal of the American Chemical Society* **123**: 7518-7533.
26. Jiang, Q.D., Roche, T.A. Monaco and Durham, S. (2006). Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14-barley genetic lines in response to salinity. *Field Crop Research* **96**: 269-278.
27. Khan, A.L., Hussain, J., Hamayun, M., Kang, S.M., Kim, H.Y., Watanabe, K.N. and Lee, I.N. (2010). Allelochemical, eudesmane-type sesquiterpenoids from *Inulafalconeri*. *Molecules* **15**: 1554-1561.
28. Kohli, R.K., Singh, H.P. andBatish, D.R. (2001). *Allelopathy in Agroecosystems*. Food Products Press. New York. USA.
29. Li, J., Xie, Y., Dai, A., Liu, L. and Li, Z. (2009). Root and shoot traits responses to phosphorus deficiency and QTL analysis at seedling stage using introgression lines of rice. *Journal of Genetics and Genomics* **36**: 173-183.
30. Li, Y.P., Feng, Y.L., Chen, Y.J. and Tian, Y.H. (2015). Soil microbes alleviate allelopathy of invasive plants. *Science Bulletin* **60**: 1083-1091.
31. Mlakar, S.G., Jakop, M., Bavec, M. andBavec, F. (2012). Allelopathic effects of *Amaranthus retroflexus* and *Amaranthus cruentus* extracts on germination of garden cress. *African Journal of Agricultural Research* **42**: 325-31.
32. Monem, R., Mirsharifi, S.M. andMirtaheri, S.M. (2012). Evaluation of allelopathic effects of barley shoot aqueous extracts on germination, seedling growth, cell membrance permeability and malondialdehyde content of corn. *Advances in Environmental Biology* **6**: 2490-2495.
33. Phatak, R.S. (2015). GC-MS analysis of bioactive compounds in the methanolic extract of *Kalanchoe pinnata* fresh leaves. *Journal of Chemical and Pharmaceutical Research* **7**: 34-37.
34. Raman, V.B., La, S., Saradhi, P.M., Rao N.B., Krishna, A.N.V., Sudhakar, M. andRadhakrishnan, T.M. (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian Journal of Pharmaceutical and Clinical Research* **5**: 99-106.
35. Razavi, S.M. (2011). Plant coumarins as allelopathy agents. *International Journal of Biological Chemistry* **5**: 86-90.
36. Rosskopf, E., Charudattan, R., DeValerio, J. and Stall, W. (2000). Field evaluation of *Phomopsis amaranthicola*, a biological control agent of *Amaranthus* spp. *Plant Disease Journal* **84**:1225-1230.
37. Sairam, R.K., VeerabhadraRao, K. andSrivastava, G.C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science* **163**: 1037-1046.
38. Salehi-lisar, S.Y. and Bakhshayeshan-agdam, H. (2016). Drought stress in plants: Causes, consequences, and tolerance. In: *Drought Stress Tolerance in Plants* (Eds., M.A. Hossain*et al*.) Vol. **1**: 1-16. Springer Press. New York. USA.
39. Schulz, M., Marocco, A., Tabaglio, V., Macias, F.A. and Molinillo, J.M. (2013). Benzoxazinoids in rye allelopathy from discovery to application in sustainable weed control and organic farming. *Journal of Chemical Ecology* **39**: 154-174.
40. Shahrokhi, S., Darvishzadeh, M., Mehrpooyan, M. andFarboodi M. (2012). Comparison of allelopathic effects of *Amaranthus retroflexus* L. different organs extracts on germination and initial growth of Alvand and Zarrin wheat cultivars. *International Journal of Agronomy and Plant Production* **3**: 489-494.
41. Shahrokhi, S., Hejazi, S.N., Khodabandeh, H., FarboodiM. andFaramarzi, A. (2011). Allelopathic effects of aqueous extracts of pigweed, *Amaranthus retroflexus* L. organs on germination and growth of five barley cultivars. *International Conference on Chemical, Biological and Environmental Engineering,* Singapore **20**: 80-84.
42. Singh, H.P., Daizy, R., Batish, D.R. andKohli, R.K. (2001). Allelopathy in agroecosystems an overview. *Journal of Production Agriculture* **4**: 121-161.
43. Singh, N.B. andSunaina (2014). Allelopathic stress produced by bitter gourd (*Momordica charantia* L.). *Journal of Stress Physiology and Biochemistry* **10**: 5-14.

44. Sodaeizadeh, H. and Hosseini, Z. (2012). Allelopathy an environmentally friendly method for weed control. *International Conference on Applied Life Sciences* **18**: 387-392.
45. Soltys, D., Krasuska, U., Bogatek, R. and Gniazdowska, A. (2013). Allelochemicals as bioherbicides-Present and Perspectives. In: *Herbicides-Current Research and Case Studies in Use* (Eds., A.J. Price and J.A. Kelton), pp. 517-542. Licensee In Tech Press. New York, USA.
46. Wang, C.M., Chen, H.T., Li, T.C., Weng, J.H., Jhan, Y.L. and Lin, S.X. (2014). The role of pentacyclic triterpenoids in the allelopathic effects of *Alstonia scholaris*. *Journal of Chemical Ecology* **40**: 90-98.
47. Weir, T.L., Park, S.W. and Vivanco, J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology* **7**: 472–479.
48. Willis, R.J. (2007). *The History of Allelopathy*.Springer Press. New York. USA.
49. Wortman, S.E., Drijber, R.A., Francis, C.A. and Lindquist, J.L. (2013). Arable weeds, cover crops and tillage drive soil microbial community composition in organic cropping systems. *Applied Soil Ecology* **72**: 232-241.
50. Xiao-Jun, Y., Hui-Xing, S., Guang-Li, L. and Qi-Bing, C. (2013). Allelopathic effects of *Paeonia decomposita* on seed germination and protective enzymes activities of wheat. *Journal of Medicinal Plant Research* **7**: 1057-1062.
51. Ye, C., Zhang, M. and Yang, Y. (2013). Photosynthetic inhibition in the microalga *Phaeodactylum tricornutum* by the dried macroalga *Gracilaria tenuistipitata*. In: *Energy and Environment Materials* (Eds., Tang, X.F., Wu, Y., Yao, Y., and Zhang, Z.Z), pp. 725-731. China Academic Journal Electronic Publishing House, Beijing.
52. Zeng, R.S. (2014). Allelopathy-the solution is indirect. *Journal of Chemical Ecology* **40**: 515-516.
53. Zuo, S.P., Li, X.W., Ma, Y.Q. and Yang, S.Y. (2014). Soil microbes are linked to the allelopathic potential of different wheat genotypes. *Plant and Soil* **378**: 49-58.