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Full Length Research Paper

Impact of allelopathy of sunflower (*Helianthus annuus* L.) roots extract on physiology of wheat (*Triticum aestivum* L.)

Javed Kamal

Department of Plant Sciences, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan. E-mail: javed1743@yahoo.com. Tel: +923046886850.

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Petri-dishes grown seedlings of wheat cvs. Margalla 99 and Chakwall 97 were treated with distilled water and with different extracts of sunflower roots. Data on physiology of wheat seedlings including germination rate, protein, proline, sugars, DNA, peroxidase, superoxide dismutase and chlorophyll contents were recorded; all increased with allelochemicals treatments when compared with the controls.

Key words: Allelopathy, chlorophyll contents, DNA, peroxidase, protein, proline, sunflower, sugar, superoxide dismutase, wheat.

INTRODUCTION

There is increasing emphasis on sustainable agriculture and concerns about the adverse effects of extensive use of synthetic chemicals, such as contamination of the environment, greater plant resistance to herbicides and high costs. Consequently, research attention is now focused on reducing the dependence upon synthetic herbicides and finding alternative strategies for weed management. Allelopathy is one promising strategy, which can be put to good use in several ways in agro-ecosystems. Sunflower (*Helianthus annuus* L.) in general and cv. Hysun 38 in particular is increasingly recognized as an important crop in several areas of Islamabad, (Pakistan) given the suitability of the crop to local agro-climatic conditions, its importance as source of edible oil and protein, resistance to drought and its short duration, which makes it a suitable crop if sowing is delayed. However, yields of some crops following sunflower are lower than normal, possibly because of inadequate nutrition and chemical inhibition. Sunflower is often grown when rainfall is marginal and depletion of soil moisture by sunflower may be a factor, although, this remains unproven. Both sunflower and the crops that follow it receive routinely specified amounts of fertilizers and there is no evidence that nutrient deficiency is the cause of lower yields. Sunflower is known to actively influence the growth of surrounding plants because of its high allelopathic potential and > 200 natural allelopathic compounds have been isolated from sunflower cultivars. Most of these known allelochemicals affect seed germination (kamal and Bano, 2008). Wheat (*Triticum aestivum* L.) is the staple food of Pakistan. Various factors lower the productivity of wheat, such as delayed sowing, inadequate doses of fertilizers, water shortage, non-availability of improved seed, diseases and drought and now weed infestation has emerged as a serious problem. Weeds compete with the crop for nutrients, water, space, light and carbon dioxide and also interfere with its normal growth by secreting biomolecules into the rhizosphere. Thus, the objective of the present study was to assess the allelopathic effects of sunflower root extracts on germination , contents of chlorophyll, protein, proline, deoxyribonucleic acid, sugar, superoxidase dismutase (SOD) and peroxidase (POD) in wheat.

MATERIALS AND METHODS

Sunflower plants (cv. Hysun 38) were grown in pots in the Department of plant sciences, Quaid-i-Azam University, Islamabad. 3 seeds were sown in each pot and were given a basal fertilizer dose of 2 g diammonium phosphate, 1 g urea, and 1 g potash. When the plants reached the vegetative stage (40 d after sowing), they were uprooted and separated into leaves, stems and roots. They were then washed thoroughly with distilled water, dried,

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pulverized in a mill and stored in a cool place along with anhydrous $CaCl_2$ to maintain dryness. Allelopathic extracts of sunflower at different concentrations were prepared as described by Bogatek et al. (2005). The solution was centrifuged at low speed (3000 rpm) for 15 min and the supernatant filtered through one layer of Whatman No. 42 filter paper. The extracts were stored at < 5°C until use. The extracts were determined to be free of fungal contamination. Seeds of 2 wheat varieties cvs. Margalla 99 and Chakwall 97 were sown in pots (3 seeds per pot) and a basal fertilizer dose of 1 g urea and 1 g diammonium phosphate applied to soil in each pot at the time of sowing through either plain water (control) or water mixed with extracts of sunflower leaves, stems or roots (1 g of extract mixed with 9 ml of water). Contents of chlorophyll, protein, proline, sugar, SOD and POD were determined. The experiment had 3 replications.

Plant material and growth conditions

Allelopathic extracts of sunflower at different concentrations were prepared as described by Bogatek et al. (2005). The solution was centrifuged at low speed (3000 rpm) for 15 min and the supernatant filtered through one layer of Whatman No. 42 filter paper. The extracts were stored below 5°C until use. It was ascertained that the extracts were free of fungal contamination. Seeds of two wheat varieties, namely Margalla 99 and Chakwall 97 were germinated in pots. The experiment consisted of 3 replications.

Leaf protein contents

Protein contents of leaves were determined following the methods of Lowry et al. (1951) using BSA as the standard. Phosphate buffer (Stock solution), Monobasic sodium phosphate (27.6 g) was dissolved in 1000 ml of distilled water.

Chlorophyll content of leaves

The fresh leaves of wheat were collected at 50% flowering for extraction of chlorophyll. The chlorophyll estimation of leaves followed the method of Arnon (1949) and Kirch (1968). The crude preparation (1 ml) was mixed with 4 ml of 80% (v/v) acetone and allowed to stand in the dark at room temperature for 10 min. It was centrifuged at 2000 rpm for 5 min to clear the suspension. The supernatant, which contained soluble pigment, was used to determine chlorophyll. Absorbance of the solution was read at 645 nm for chlorophyll a and at 663 nm for chlorophyll b on a spectrophotometer against 80% (v/v) acetone blank. Total chlorophyll was determined with the equation of Arnon (1949):

Total chlorophyll (mg/L) = (20.2 × a 645.A645) + (8.02 × b 663.A663)

Sugar content of leaves

Sugar content of wheat leaves at flowering stage was estimated by the method of Dubo et al. (1956) as modified by Johnson et al. (1966). Fresh plant material (0.5 g) was homogenized with 10 ml of distilled water in a clean mortar. It was centrifuged at 3000 rpm for 5 min. Then to 0.1 ml of supernatant, 1 ml of 5% (v/v) phenol was added. After 1 h incubation at room temperature, 5 ml of concentrated H_2SO_4 was added. The absorbance of each sample was recorded at 420 nm. The concentration of unknown samples was calculated with reference to a standard curve made using glucose.

Proline content of leaves

Proline content of leaves was estimated at flowering stage by using the following method (Bates et al., 1973). Fresh plant material (0.1 g) was homogenized with 5 ml of 3.0% sulfosalicylic acid in a mortar. Samples were centrifuged at room temperature at 3000 rpm for 5 min. Supernatant was adjusted to 5 ml with distilled water, to which 5 ml of glacial acetic acid and 5 ml of ninhydrin (1.25 g of ninhydrin dissolved in a solvent prepared by mixing 30 ml of glacial acetic acid, 8 ml of orthophosphoric acid and 12 ml distilled water). The reaction mixture was shaken and the contents placed in tubes and heated in a boiling water bath for 1 h. Then tubes were cooled and the mixture extracted with 10 ml of toluene in a separating funnel. The absorbance of the toluene layer was recorded at 520 nm. A standard curve was constructed from a dilution series of 10 to 100 g of proline at increments of 10 g. The concentration of the unknown sample was calculated with reference to the standard curve.

Peroxidase of leaves

To 3 ml of assay buffer (20 mM mixed phosphate buffer, Ph 7.0 to 7.5 at 30°C) was added 100 µl of 100 mM guaiacol and 300 µl of partially purified enzyme preparation. The mixture was incubated for 1 min at 30°C and the reaction initiated by addition of 1 µmol H_2O_2 (30% v/v). Activity was measured colorimetrically at 436 nm and calculated using an extinction coefficient of 6.39 mM/cm for the guaiacol dehydrogenation product (Putter, 1974).

SOD of leaves

SOD (EC 1. 15. 1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971) taking into account the notes by Beyer and Fridovich (1987). Leaf samples were homogenized in 4 volumes (w/v) of an ice-cold buffer containing 0.1 M Tris-HCL (pH 7.8), 0.1 mM EDTA and 0.05% Triton X- 100. The homogenates were filtered through 4 layers of cheesecloth and centrifuged at 4°C for 30 min at 15,000 rpm. The crude extracts were dialyzed for 24 h against half-strength extraction buffer without Triton X-100, centrifuged at 4°C for 30 min at 15,000 rpm and the supernatants were used for SOD assay. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.053 Mm NBT, 10 mM methionine, 0.0053 mM riboflavin and an appropriate aliquot of enzyme extract. The reaction was started by switching on the light and allowed to run for 7 min. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction as monitored at 560 nm.

DNA analysis

Total DNA was extracted according to Gumin and estimated using an UV spectrophotometer (Spectrophotometer 601) as adopted by Ogur and Rosen genomic DNA was extracted from 60 mg of frozen tissue (young leaves ground in liquid nitrogen) using Genomicprep cells and tissue DNA Isolation Kit (Amersham Pharmacia Biotech Inc.) according to the instruction manual. The DNA concentration in samples was measured by UV-absorption spectroscopy at 260 nm at which DNA gives a maximum absorption at a concentration of approximately 50 µg/g of double-stranded DNA. DNA was treated with 4 restriction enzymes (Bam HI, EcoRI, HinfI and SmaI at 5 U per reaction) according to manufacturer's instructions. The reaction mixture was incubated for 1 h at 37°C except SmaI (25°C). The restriction fragments were size-fractioned using 2% agarose gel

Table 1. Effect of sunflower root extract on germination (%) of wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	13.67^{A}	12.00^{A}	14.00^{A}	12.33^{A}
T_2	10.33^{C}	9.00^{C}	9.333^{C}	6.667^{C}
T_3	11.67^{BC}	9.667^{C}	10.67^{BC}	7.667^{C}
T_4	12.00^{ABC}	10.67^{B}	11.00^{B}	9.333^{B}
T_5	12.67^{AB}	11.33^{A}	11.67^{B}	9.667^{AB}

Values followed by the same letter within a column are not significantly different.

Table 2. Effect of root extract of sunflower on root length (cm) of seedlings of wheat varieties Margalla 99 Chakwall 97.

Treatment	1st year		2nd year	
	V1	V2	V1	V2
T_1	13.00^{A}	17.50^{A}	12.57^{A}	17.00^{A}
T_2	9.633^{C}	13.20^{B}	8.283^{C}	11.88^{C}
T_3	10.50^{BC}	13.67^{B}	9.950^{B}	13.68^{B}
T_4	10.90^{B}	14.20^{B}	11.65^{A}	12.01^{C}
T_5	12.40^{A}	16.96^{A}	12.30^{A}	13.98^{B}

Values followed by the same letter within a column are not significantly different.

Table 3. Effect of root extract of sunflower on shoot length (cm) of seedlings of wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	18.30^{A}	17.50^{A}	18.43^{A}	17.00^{A}
T_2	11.86^{D}	12.50^{C}	11.64^{D}	12.92^{B}
T_3	12.66^{CD}	14.20^{B}	13.03^{CD}	13.17^{B}
T_4	13.19^{C}	12.13^{C}	13.40^{C}	13.70^{B}
T_5	14.63^{B}	14.70^{B}	15.35^{B}	16.32^{A}

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5 g + 10 ml); T_4 = 50% extract + 50% DW (5 g +10 ml); T_5 = 25% DW (2.5 g + 10 ml).

electrophoresis. The agarose gels were stained with ethidium bromide and photographed under UV light. The results were documented by Image analyzer gel Doc 2000 (Bio Rad).

Statistical analysis

The data were analysed statistically using MStatC. A completely randomized design was followed.

RESULTS

Effect of sunflower root extract on germination (%) of wheat varieties Margalla 99 and Chakwall 97

The data regarding germination rate under the effect of sunflower roots extract are presented in Table 1. A perusal of the data revealed that in case of both wheat varieties, Margalla 99 and Chakwall 97, the TI (control) showed maximum germination, followed by T5 (25% extract), whereas minimum germination was shown by T2 (undiluted extract). Similar pattern was followed in the 2nd year).

Effect of root extract on root length and shoot length (cm) of seedlings of wheat varieties Margalla 99 and Chakwall 97

Root length and shoot length indicates the productive efficiency of a crop. The higher the root and shoot length, the greater the efficiency and vice versa. The data given in Tables 2 and 3 revealed that in case of both wheat

Table 4. Effect of root extract of sunflower on fresh weight (g) of seedlings of wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st Year		2nd year	
	V1	V2	V1	V2
T^1	0.8900^{A}	0.8900^{A}	0.9100^{A}	0.8300^{A}
T^2	0.2333^{E}	0.7500^{B}	0.1900^{E}	0.7100^{B}
T^3	0.3200^{D}	0.8300^{A}	0.2900^{D}	0.7900^{A}
T^4	0.6333^{C}	0.8667^{A}	0.6000^{C}	0.8233^{A}
T_5	0.8300^{B}	0.8733^{A}	0.7500^{B}	0.8400^{A}

Values followed by the same letter within a column are not significantly different.

Table 5. Effect of root extract of sunflower on dry weight (g) of seedlings of wheat varieties Margalla 99 Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	0.8600^{A}	0.8600^{A}	0.8933^{A}	0.8333^{A}
T_2	0.1833^{E}	0.5433^{C}	0.1633^{E}	0.5167^{D}
T_3	0.2833^{D}	0.6300^{B}	0.2633^{D}	0.6133^{C}
T_4	0.5833^{C}	0.6700^{B}	0.5567^{C}	0.6467^{BC}
T_5	0.7833^{B}	0.6833^{B}	0.7533^{B}	0.6700^{B}

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5 g + 10 ml); T_4 = 50% extract + 50% DW (5 g +10ml); T_5 = 25% DW (2.5 g + 10 ml).

Table 6. Effect of root extract of sunflower on GA ($\mu g\ g^{-1}$) content of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	500.0^{A}	490.00^{A}	503.0^{A}	492.00^{A}
T_2	404.00^{D}	351.00^{C}	402.00^{D}	348.7^{C}
T_3	418.7^{C}	388.3^{BC}	416.7^{C}	386.0^{BC}
T_4	527.0^{C}	394.3^{BC}	424.7^{C}	391.7^{BC}
T_5	480.0^{B}	437.0^{AB}	477.0^{B}	434.0^{AB}

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5 g + 10 ml); T_4 = 50% extract + 50% DW (5 g +10 ml); T_5 = 25% DW (2.5 g + 10 ml).

varieties Margalla 99 and Chakwall 97, Tl (control) showed the maximum root and shoot length, followed by T5 (25% extract) < T4 (50% extract) < T3 (75% extract), and T2 (undiluted extract), in the second year, the results were the same.

Effect of sunflower stem extract on fresh weight and dry weight (g) of seedlings of wheat varieties Margalla 99 and Chakwall 97

The data in Tables 4 and 5 revealed that in the case of both first and second year, wheat variety Margalla 99 showed better response. In the two wheat varieties, Tl (control) showed maximum response, followed by T5 (25% extract), whereas T2 (undiluted extract) showed minimum response. In the case of Margalla 99, T4 was at par with T3 (75% extract), while in the case of Chakwall 97, T4 (50% extract) was at par with T2 (undiluted extract).

Effect of root extract of sunflower on gibberellic acid ($\mu g\ g^{-1}$) contents of leaves and roots in wheat varieties Margalla 99 and Chakwall 97

The data on the GA content in leaves are given in Tables 6 and 7. The maximum GA contents in case of wheat

Table 7. Effect of root extract of sunflower on GA (µg g^{-1}) content of roots in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	505.3^A	422.3^A	407.0^A	419.7^A
T_2	392.3^B	318.0^C	301.0^E	313.0^E
T_3	399.0^B	360.0^B	349.7^D	349.0^D
T_4	411.7^B	364.7^B	364.3^C	362.0^C
T_5	482.3^A	417.0^A	417.0^A	412.3^B

Values followed by the same letter within a column are not significantly different.

Table 8. Effect of root extract of sunflower on IAA (µg g^{-1}) content of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V1	V2	V1	V2
T_1	172.7^A	131.0^A	190.0^A	132.0^A
T_2	100.0^E	102.0^E	113.0^E	96.00^D
T_3	127.0^D	107.0^D	127.0^D	106.0^C
T_4	139.3^C	114.0^E	139.0^C	107.0^C
T_5	160.0^B	122.0^B	149.0^B	121.0^B

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = Undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5 g + 10 ml); T_4 = 50% extract + 50% DW (5 g +10 ml); T_5 = 25% DW (2.5 g + 10 ml).

Table 9. Effect of root extract of sunflower on IAA (µg g^{-1}) content of roots in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V1	V2	V1	V2
T_1	193.0^A	164.0^A	164.0^A	134.0^A
T_2	117.0^D	94.00^E	96.00^E	93.30^D
T_3	129.0^C	120.0^D	125.7^D	109.0^C
T_4	135.0^C	139.0^C	140.3C	113.0^C
T_5	155.7^B	153.0^B	155.0^B	123.0^B

Values followed by the same letter within a column are not significantly different.

variety Margalla 99 were given by T5 (25% extract) which was at par with T1 (control), the minimum amount of GA was found in T2 (undiluted extract), while in the second year TI (control) showed the maximum amount of GA and T2 (undiluted extract) showed the minimum amount of GA. In the wheat variety Chakwall 97, in both years, TI (control) showed the maximum and T2 (undiluted extract) showed the minimum quantity of GA in wheat leaves.

Effect of root extract of sunflower on indole-3-acetic acid content of leaves and roots in wheat varieties Margalla 99 and Chakwall 97

The data regarding IAA contents in leaves are presented in Tables 8 and 9. A perusal of the tables revealed that in the case of both wheat varieties Margalla 99 and Chakwall 97, the maximum IAA contents in leaves samples were calculated in TI (control) and the least amount were found in T2 in the first year as well as second year.

Effect of root extract of sunflower on abscisic acid (µg g^{-l}) content of leaves and root in wheat varieties Margalla 99 and Chakwall 97

The data regarding ABA contents in leaves presented in Tables 10 and 11 revealed that in both the wheat varieties Margall 99 and Chakwall 97, maximum amount of ABA was recorded in T2 (undiluted extract) and the minimum in TI (control). Similar result was found in the

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Table 10. Effect of root extract of sunflower on ABA (μg g^{-1}) contents of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V1	V2	V1	V2
T_1	68.00^{E}	90.30^{E}	66.33^{E}	91.61^{E}
T_2	177.3^{A}	177.3^{A}	180.3^{A}	180.0^{A}
T_3	156.0^{B}	159.0^{B}	159.0^{B}	162.3^{B}
T_4	133.7^{C}	132.7^{C}	136.3^{C}	135.0^{C}
T_5	98.00^{D}	102.0^{D}	100.7^{D}	104.0^{D}

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5 g + 10 ml); T_4 = 50% extract + 50% DW (5 g +10 ml); T_5 = 25% DW (2.5 g + 10 ml).

Table 11. Effect of root extract of sunflower on ABA (μg g^{-1}) content of roots in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	100.0^{C}	81.33^{D}	98.67^{C}	82.33^{D}
T_2	151.0^{A}	113.7^{A}	153.0^{A}	116.3^{A}
T_3	130.0^{B}	106.0^{B}	132.0^{B}	108.0^{B}
T_4	105.7^{C}	96.67^{C}	107.7^{C}	98.67^{C}
T_5	100.3^{C}	84.00^{D}	102.7^{C}	86.00^{D}

Values followed by the same letter within a column are not significantly different.

Table 12. Effect of root extract of sunflower on DNA (mg /100 g F. wt) contents of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	512.9^{A}	485.3^{A}	515.0^{A}	487.0^{A}
T_2	150.4^{D}	101.3^{E}	148.7^{D}	99.33^{E}
T_3	239.0^{C}	206.7^{D}	237.7^{D}	204.7^{D}
T_4	272.3^{C}	269.3^{C}	269.0^{C}	266.7^{C}
T_5	398.3^{B}	396.7^{B}	395.3^{B}	393.7^{B}

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5g + 10ml); T_4 = 50% extract + 50% DW (5 g +10 ml); T_5 = 25% DW (2.5 g + 10 ml).

second year.

Effect of root extracts of sunflower on deoxyribonucleic acid content of leaves in wheat varieties Margalla 99 and Chakwall 97

The data regarding DNA is presented in Table 12. The data pertaining to the DNA contents in leaves of wheat varieties were also affected significantly by leaf, stem and root extract of sunflower. In both wheat varieties Margalla 99 and Chakwall 97, the maximum DNA was observed in TI (control) and the minimum in T2 (undiluted extract) in the first as well as second year of experiment under the effect of leaves, stem and root extract of sunflower.

Effect of root extracts of sunflower on chlorophyll content (mg/g) of leaves in wheat varieties Margalla 99 and Chakawall 97

The data regarding effect of leaf stem and root extract on chlorophyll contents are presented in Table 13. From the table, it is revealed that the maximum chlorophyll contents under the effect of leaf, stem and root extract of sunflower were recorded in TI (control) and the minimum

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Table 13. Effect of root extract of sunflower on chlorophyll (mg/ 100 g F. wt) content of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V1	V2	V1	V2
T_1	109.7^{A}	107.0^{A}	111.9^{A}	108.0^{A}
T_2	87.34^{D}	84.00^{D}	86.66^{D}	83.00^{D}
T_3	94.34^{CD}	93.00^{C}	93.00^{AB}	92.00^{C}
T_4	100.00^{BC}	98.67^{BC}	99.00^{AB}	97.67^{BC}
T_5	105.3^{AB}	103.3^{AB}	104.3^{A}	102.3^{AB}

Values followed by the same letter within a column are not significantly different.

Table 14. Effect of root extract of sunflower on proline (mg/ 100 g F. wt) contents of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	82.70^{E}	65.06^{D}	81.00^{E}	64.63^{D}
T_2	130.0^{A}	119.7^{A}	131.3^{A}	121.0^{A}
T_3	121.0^{B}	112.4^{A}	123.0^{B}	113.4^{A}
T_4	114.7^{C}	101.3^{B}	116.7^{C}	102.3^{B}
T_5	96.00^{D}	91.67^{C}	97.00^{D}	92.67^{C}

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5 g + 10 ml); T_4 = 50% extract + 50% DW (5 g +10 ml); T_5 = 25% DW (2.5 g + 10 ml).

Table 15. Effect of root extract of sunflower on sugar (mg/ 100 g F. wt) content of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V1	V2	V1	V2
T_1	175.0^{C}	180.0^{CD}	174.0^{C}	169.0^{C}
T_2	235.0^{A}	226.0^{A}	222.0^{A}	212.3^{A}
T_3	215.0^{B}	210.0^{B}	202.7^{B}	197.0^{B}
T_4	190.0^{C}	184.3^{C}	178.0^{C}	175.3^{C}
T_5	182.0^{E}	177.7^{D}	176.0^{C}	172.0^{C}

Values followed by the same letter within a column are not significantly different.

chlorophyll content was found in T2 (undiluted extract) in both wheat varieties Margalla 99 and Chakwall 97. In the second year, results were found similar.

Effect of root extract of sunflower on proline content (mg/g) of leaves in wheat varieties Margalla 99 and Chakwall 97 Data presented in Table 14 revealed that proline contents of leaves under the effect of leaf, stem and root extract were affected significantly by sunflower extract.

A perusal of the tables revealed that in both the wheat varieties, T2 (undiluted extract) showed the maximum proline contents under the effect of leaf, stem and root extract and TI (control) showed the minimum proline contents. Results were found similar in the second year of experiment.

Effect of root extract of sunflower on sugar content (mg/g) of leaves of wheat varieties Margalla 99 and Chakwall 97

Effect of root extract of sunflower on sugar content of leaves in wheat varieties is given in Table 15. A perusal of the table showed that in the case of wheat variety Margalla 99, T2 (undiluted extract) showed the maximum while T5 (25% extract) showed the minimum amount of

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Table 16. Effect of root extract of sunflower on protein (mg/ 100 g F. wt) activity of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	1685^{D}	1766^{B}	1753^{D}	1675^{C}
T_2	1723^{A}	1785^{A}	1783^{A}	1725^{A}
T_3	1720^{A}	1775^{AB}	1775^{B}	1760^{B}
T_4	1712^{B}	1772^{B}	$1768B^{C}$	1755^{A}
T_5	1695^{C}	1765^{B}	1761^{A}	1692^{C}

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5 g + 10 ml); T_4 = 50% extract + 50% DW (5 g +10 ml); T_5 = 25% DW (2.5 g + 10 ml).

Table 17. Effect of root extract of sunflower on superoxidase (mg/ 100 g F. wt) of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V_1	V_2	V_1	V_2
T_1	2.670^{C}	3.000^{B}	3.000^{B}	3.000^{BC}
T_2	6.000^{A}	5.000^{A}	5.330^{A}	5.000^{A}
T_3	5.000^{AB}	4.000^{AB}	5.000^{A}	4.330^{AB}
T_4	3.000^{C}	2.670^{B}	4.670^{A}	3.670^{ABC}
T_5	3.330^{BC}	2.330^{B}	3.000^{B}	2.330^{C}

Values followed by the same letter within a column are not significantly different.

sugar contents; this is in par with TI (control), and TI (control) showed the minimum values in the Chakwall 97. Similar result was found in the second year of experiment.

Effect of root extract of sunflower on protein content (mg/g) in leaves of wheat varieties Margalla 99 and Chakwall 97

Effect of leaf and root extract of sunflower on protein content in leaves of wheat varieties is shown in Table 16. A perusal of the table revealed that in the case of both wheat varieties Margalla 99 and Chakwall 97, T2 (undiluted extract) showed the maximum contents of protein under the effect of leaf and root extract, which was followed by T3 (75% extract), and TI (control) showed minimum amount of protein.

Effect of root extract of sunflower on superoxide dismutase (mg/100 g fresh weight) of leaves in wheat varieties Margalla 99 and Chakwall 97

From Table 17, it is revealed that in the case of both wheat varieties Margalla 99 and Chakwal1 97, T2 (undiluted extract) showed the maximum superoxide dismutase (SOD) content under the effect of sunflower leaf and stem extract, followed by T3 (75% extract), and T4 (50% extract) whereas TI (control) showed minimum amount of SOD. Similar results were found in the second.

Effect of root extract of sunflower on peroxidase (POD) content (mg/100 g fresh weight) of leaves in wheat varieties Margalla 99 and Chakwall 97

From Table 18, it is revealed that in the case of both wheat varieties Margalla 99 and Chakewall 97, T2 (undiluted extract) showed the maximum POD content under the effect of leaf and stem extract of sunflower, which was followed by T3 (75% extract) and T4 (50% extract), whereas TI (control) showed minimum amount of POD. Similar result was shown in the second year.

DISCUSSION

The allelopathic phenomenon has received much attention, as shown by numerous reports on the subject (Anaya, 1999; khan et al., 2005; Narwal et al., 1998; Reigosa et al., 2006; Singh et al., 2001; Weston and Duke, 2003; Kamal and Bano, 2009; Kamal, 2011). The present study has shown that sunflower (*H. annus* L.)

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Table 18. Effect of root extract of sunflower on peroxidase (mg/ 100 g F. wt) activity of leaves in wheat varieties Margalla 99 and Chakwall 97.

Treatment	1st year		2nd year	
	V1	V2	V1	V2
T_1	8.000^{C}	7.000^{B}	7.670^{C}	6.00^{B}
T_2	14.67^{A}	13.00^{A}	15.33^{A}	14.00
T_3	13.67^{AB}	11.00^{A}	14.67^{A}	11.67^{A}
T_4	12.33^{AB}	10.67^{A}	13.33^{AB}	11.33^{A}
T_5	10.67^{BC}	10.33^{A}	11.00^{B}	8.00^{C}

Values followed by the same letter within a column are not significantly different. T_1 = Control (distilled water; DW); T_2 = undiluted extract (9 g extract + 10 ml DW); T_3 = 75% extract + 25% DW (7.5 g + 10 ml); T_4 = 50% extract + 50% DW (5 g +10 ml); T_5 = 25% DW (2.5 g + 10 ml).

extracts (leaves, stems, and roots) affect seed germination; so, aqueous extracts of sunflower parts, like leaves, stems, and roots, significantly affected germination. This indicates that reduction in these parameters (fresh weight, dry weight, root length, and shoot length) might have been the result of water-soluble allelochemicals in the extracts and their inhibitory effect or phytotoxicity on the measured parameters. Results show that sunflower plant parts varied in their allelopathic activity against wheat seedlings extracts of leaves, followed by roots which showed a higher allelopathic potential on the test plants as compared to stems. These results are in accordance with previous studies reporting that allelopathy may vary among plant parts (Turk and Tawaha, 2002; Peng et al., 2004). For all extracts, allelopathic activity increased with increases in extract concentrations. This is in agreement with the finding that under certain conditions, the rate of an elementary reaction is positively related to the reactant concentration. Previous studies have shown that the phytotoxicity of extracts was significantly increased as their concentration increased (Rice, 1984; Putnam, 1994; Sinkkonen, 2001; Peng et al., 2004; kamal and Bano, 2009). Wheat seed germination was reduced by all extracts of sunflower as compared with the untreated control. The observed inhibition of the two wheat varieties, namely Margalla 99 and Chakwall 97, for germination of seeds could be attributable to a contribution of allelochemicals released from the sunflower extracts of leaves, stems, and roots, as the allelochemicals are water-soluble and can accumulate upon release in seeds in direct contact with bioactive concentrations. There are several reports from literature showing that addition or incorporation of plant residues into the growth environment of another plant can result in growth inhibition (Patterson, 1981; Qasem, 1994; Chung and Miller, 1995; Al-Khatib et al., 1997). This study observed seed chlorosis on wheat seed at high rates of sunflower extracts; this may be due to a sunflower phenolic allelochemical effect on Mg-chelatase activity and chlorosis of seeds. The effect of higher concentration of extracts of sunflower suggests that reduction in measured parameters may have the result of either or both the osmotic potential of the extracts or the presence of allelochemicals in the extracts. Beside possible allelochemicals, higher chemical concentrations in the extracts might possibly cause an osmotic stress during seed germination and seedling growth. The inhibition of seed germination and seedling growth was concentration-dependent; the present results are similar to those obtained by other workers, who observed inhibition of seed germination and seedling growth on other crops and weeds by sunflower extracts (Batish et al., 2002; Bogatek et al., 2005; Anjum et al., 2005; kamal and Bano, 2008). The allelochemicals inhibit germination and seedling growth, probably by affecting the cell division and elongation process that are very important at this stage or by interfering with enzymes involved in the mobilization of nutrients necessary for germination. Levizou et al. (2002) found that mitosis in root apex of lettuce retarded with leaf extracts. Growth hormones, like GA and IAA, are very important in agriculture. Growth-inhibiting compounds of agricultural importance have recently received considerable research attention (Siquira et al., 1991; Inderjit, 1996). These compounds which affect phytohormones are phenols with allelopathic characteristics (Schenk et al., 1999; Callaway and Aschhoug, 2000). Allelopathy is the plant harm to other plants in their vicinity or is an adverse influence of one plant on another (Rice, 1984). In plants exposed to sunflower extracts, changes in ABA were also recorded. In the leaves, the ABA content increased. In the case of roots, levels of ABA are very low in control plants and in treated plants they were high. This was probably due to the fact that in roots, as being very affected by allelechemicals early, cell death would occur. In roots with gradually dying cells, most of the already synthesized ABA is probably transported to the shoot, as a stress signal messenger. The increase of ABA in leaves correlates well with changes described earlier in the plant water status; that is, loss of turgor, lowered osmotic potentials and RWC, and the increase in leaf diffusive resistance. The involvement of ABA in plants coping with

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abiotic and biotic stresses, especially those dealing with cell water deficit is well documented in the literature (Davies and Jones., 1991). This study clearly shows that ABA also plays an important role in the defense processes against allelopathic stress. An increase of ABA levels in seedlings of wheat exposed to allelopathy stress was observed in another study (Bernat et al., 2003). However, in this study, conversely to this work, the increase was recorded not only in leaves but also in roots. Different patterns of ABA accumulation in these two growth stages by wheat plants due to the same stress suggest that there are differences in mechanisms of coping with allelopathy stress or in sensitivity to this stress. Results presented in this work demonstrated that plants of sunflower cv. Hysun 38 are the donors of allelopathic compounds which affect acceptor plants via disorder in many physiological processes and that these changes, being mostly statistically significant showed a dose- and time-dependent manner. Recently, some progress has been made in the study of molecular processes involved in morphological and physiological adaptation of plants exposed to allelopathic chemicals of other plants. The present research was carried out to study the allelopathic effects of leaf stem and root on morphological, biochemical, and molecular criteria of wheat leaves. These extracts can be used in biological control as natural herbicides to reduce the risk of manufactured herbicides. With respect to the total soluble protein and the nucleic acids contents, there was a highly significant increase in the level of DNA in all treated samples of wheat seedlings. Wheat seedlings treated with sunflower extracts showed a highly significant increase in the level of DNA. These results were confirmed partially by (Duhan et al., 1995) who revealed a drastic increase in the level of nucleic acids and decrease in the level of soluble proteins in legume crops in response to plant extracts. They attributed this effect to the enzymes of synthetic pathways. In accordance with these results, Baziramakenga et al. (1997) reported that many phenolic acids reduced the incorporation of phosphorus into DNA in soybean. The present results are in controversy with those obtained by (Padhy et al., 2000*),* who reported that chlorophyll synthesis in leaves, as well as protein, carbohydrate, and nucleic acid (DNA and RNA) contents, in both shoots and roots of seedling was also decreased with increases in extracts concentrations. It was found that the activities of SOD and POD of many plants were affected by (allelochemicals) drought. Numerous studies have shown that the degree of injury caused by allelochemicals was negatively correlated to the increase of activities of SOD and POD (Dhindsa and Matow, 1981; Chowdhury and Choudhuri, 1985). But some results were the same as this; for example, Sun et al. (2010a) found that SOD and POD activities increased under allelochemical water stress (Wang et al., 2002); water stress and allelochemicals are correlated with each other because more allelochemicals are produced during stress conditions. Some others got the similar results (Iturbe-ormatexe et al., 1998). In this study, it was found that SOD and POD activities in roots and leaves increased with allelochemicals. These differences may result in different treatment methods. In this study, the allelochemicals were applied during the whole growth period. Allelochemicals probably acted directly on roots, whereas leaves could reduce water transpiration by curling and stoma regulation. Furthermore, the degree of leaf injury caused by allelochemicals could be lessened through morphological adaptation and regulation of stem and sheath water content, stem diameter, or plant height. This showed that the ability of crops to resist allelochemicals was connected to the activities of protective enzymes and their defensive function. These mechanisms may be the main physiological action and defense from injury of crops under allelochemicals. There were significant differences in endogenous concentrations of GA and IAA in both leaves and roots of two wheat varieties during their growth when different concentrations of extracts of sunflower were applied. The GA and IAA concentration in leaves as well as roots decreased when applied different concentrations of extracts of sunflower were applied, while a higher reduction in both IAA and GA concentration was found when more concentrations of extracts of sunflower were applied in 2 wheat varieties. This decrease in leaves' GA and IAA may be attributed to slow transport of growth-promoting hormones from vegetative organs. Phytohormones may also enhance root development under growing conditions. Kuroha et al. (2002) reported that plant hormones stimulate adventitious root formation. Similar findings have been reported previously (Oda et al., 2003). The ability of the plants to absorb nutrients efficiently can be attributed to a higher number of adventitious root formations, and GA promotes this (Wahyuni et al., 2003; Kono, 1995; Watanabe, 1997). Root initiation and early development of root are also stimulated by auxin (Bellamine et al., 1998; Pan and Tian, 1999). There were significant decreases in endogenous concentrations of GA and IAA in the T1 and T5 treatments in both varieties (Margalla 99 and Chakwall 97). In controlling stomatal resistance, the most important signal is considered to be the concentration of plant hormones in xylem sap (Borel et al., 1997). Root-produced organic compounds in the xylem sap, such as hormones and amino acids, are considered to be vital for plant development (Oda et al., 2003). Extracts of sunflower (leaves, stems, and roots) decreased IAA and GA and increased ABA concentration in both leaves and roots compared to the control. Plant responses to allelopathy (stress) can also be determined by the variations in IAA, GA, and ABA concentration (Naqvi, 1999). A rapid decrease in leaf ABA concentration is observed in low concentrations of allelochemicals and in control plant. The GA concentration was decreased in plants under water stress conditions (Aharaoni, 1979; Guinn-Brummet,

1988; Yang et al., 2001; Xie et al., 2003). The increase in ABA may possibly be attributed to an induction in ABA synthesis. Abscisic acid contents in seedlings of leaves treated with extracts of sunflower were greater than those of control (Yang et al., 2001, 2004). Allelochemicals increased in the concentrations of ABA in leaves and in roots. This may be due to a higher concentration of growth-promoting hormones. During the present investigation, it was found that extracts of sunflower significantly increased the accumulation of soluble sugars in both wheat varieties. In these experiments, extracts of leaves, followed by root extracts and stem extracts, significantly increased the soluble sugar contents of wheat varieties Margalla 99 and Chakwall 97. Allelochemical treatment (T1) markedly increased sugar content in leaves. This increase may be due to the positive effect of ABA on assimilate translocation. Assimilate translocation to the developing seeds is reported to be under the control of ABA ('Brenner and Cheikh, 1995; Yang et al., 1999, 2004). Allelochemicals decreased the accumulation of sugars markedly in leaves. The allelochemical treatments also showed an increase in sugar contents both in leaves. Ahmadi and Bakicer (1999) reported that ABA is involved in osmolyte regulation under moisture stress conditions. Mahajan and Tuteja (2005) reported that under severe drought, growth was inhibited by high concentrations of ABA and sugar, whereas low concentrations promote growth. Increased rates of photosynthesis and higher chlorophyll content might cause accumulation of sugars due to ABA or allelochemical treatments (Dong et al., 1995; Ndung et al., 1997). Drought tolerance in plants is enhanced by ABA caused by allelochemical treatment, possibly due to the accumulation of osmolytes, such as sugars. The present investigation indicated that application extracts of sunflower parts, like leaves, stems, and roots, caused accumulation of protein in both wheat varieties, Margalla 99 and Chakwall 97. Nevertheless, the intensity of increase was higher in Margalla 99 and Chakwall 97. The application of extracts of sunflower significantly increased the protein accumulation in both wheat varieties Margalla 99 and Chakwall 97. In these experiments, the extracts of leaves have more of an effect, followed by root extracts, and the lowest effect was observed in the case of stem extract. It was observed that allelochemicals increased protein content in leaves. This may be due to a positive role of ABA on protein accumulation. Guerrero and Mullet (1986) and Schmitz et al. (2000) reported that protein synthesis in developing seeds is induced by ABA. Zhang et al. (2001) reported that protein phosphorylation is enhanced under water stress due to increased concentration of ABA. Bartels and Sunkar (2005) and Ingram and Bartels (1996) investigated late embryo-genesis-abundant (LEA) proteins induced in vegetative tissues of plants in response to osmotic stress that may interact with carbohydrates to prevent cellular damage during dehydration. The recovery of leaf protein levels in drought-stressed plants is more rapid at panicle initiation. In the present study, it is shown that allelopathy of sunflower caused accumulation of proline in both wheat varieties Margalla 99 and Chakwall 97. The intensity of increase was higher in Margalla 99. The application of extracts of sunflower significantly increased the proline accumulation in both wheat varieties. However, a significantly higher increase was recorded in Margalla 99. The efficiency of sunflower leaves, followed by root extracts, to increase proline contents was higher than root and stem extracts. Applications of allelochemical treatments markedly increased proline content in leaves of two wheat varieties, Margalla 99 and Chakwall 97. This magnitude of the increase was similar to that under drought stress. Under drought stress, in addition to its role as an osmoregulator, proline like other soluble organic compounds, may also act as osmo-protectants (Kamelie and Lose, 1995). ABA was considered to be involved in the accumulation of proline (Hose et al., 2000; Trotel Aziz et al., 2000; Nayyar and Walia, 2003), carbohydrates (Ahmadi and Baker, 2001), and other osmolytes in plants (Popova et al., 2000). Allelochemical-caused stress increased the accumulation of proline significantly in leaves. ABA enhanced the accumulation of proline contents in leaves. This increase may be due to the role of ABA, which may stimulate proline accumulation under water deficit conditions. Allelochemicals in T2, followed by T3, T4, and T5, caused a significant increase in proline levels compared to control (Bajji et al., 2001). Root length, shoot length, fresh weight, and dry weight of wheat seedling were significantly differernt in both the varieties with respect to different concentrations of extracts of sunflower; leaf extract showed more of an effect, followed by root extract, and the lowest effect was found in the case of stem extracts of sunflower.

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Conclusions

From the experiments, these were concluded: allelochemicals secreted by sunflower inhibited germination and lowered the level of hormones, GA, IAA, shoot length, root length, fresh weight, dry weight, chlorophyll contents, and DNA, while they increased the values of ABA, protein, proline, sugar, SOD, and POD contents in wheat seedlings.

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