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Full Length Research Paper

Effects of sunflower (*Helianthus annuus* L.) extracts on wheat (*Triticum aestivum* L.) and physicochemical characteristics of soil

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Pot-grown seedlings of wheat cultivars Margalla 99 and Chakwall 97 were given water mixed with extracts of sunflower leaves, stems, or roots 20 days after the seedlings had emerged. Data on growth and yield parameters, weed density, and hormone production were recorded. Physicochemical properties of soil were analysed before sowing sunflower and after harvesting wheat. Commonly observed effects of allelochemicals were (a) decreased production of indole-3-acetic acid and gibberellic acid and lower values of electrical conductivity, moisture content, weed density, and fresh and dry weight of weeds, and (b) increased production of abscisic acid and higher values of pH, Mn, Fe, Mg, Ca, K, 100-grain-weight, and fresh and dry weight of wheat plants.

Key words: Allelochemicals, atomic absorption, growth and yield parameters, HPLC, hormonal analysis, physico-chemical analysis of soil, wheat, weeds.

INTRODUCTION

Given the increasing emphasis on sustainable agriculture and concern about the adverse effects (contamination of the environment, greater resistance of seeds to herbicides, and high costs) of extensive use of synthetic chemicals, research attention is now focused on reducing the dependence upon synthetic herbicides and finding alternative strategies for weed management. Allelopathy promises to be one such strategy, which can be put to good use in several ways in agro-ecosystems. Sunflower (*Helianthus annuus* L.) in general, and its improved variety Hysun 38 in particular, are increasingly recognized as an important crop in several areas of Islamabad (Pakistan) given the suitability of the crop to agroclimatic conditions of the region, its importance as a source of edible oil and protein, resistance to drought, and its short duration, which makes it a suitable crop if sowing has to be delayed. However, yields of some crops when they follow 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 also be a factor although this remains unproven so far. Both sunflower and the crops that follow it receive routinely specified amounts of fertilizers, and there is no evidence of nutrient deficiency being the cause of lower yields. Sunflower is known to actively influence the growth of surrounding plants because of its high allelopathic potential. More than 200 natural allelopathic compounds have been isolated so far from different cultivars of sunflower. Most of the known allelochemicals affect seed germination (Wardle et al., 1991). Wheat (*Triticum aestivum* L.) is the staple food of the people of Pakistan, and wheat straw an integral part of the daily ration of livestock. Among various causes that lower the productivity of wheat, such as delayed sowing, inadequate doses of fertilizers, water shortage, non-availability of improved seed, diseases, and drought, weed infestation has emerged as a serious problem. Weeds not only compete with the crop for nutrients, water, space, light,

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Abbreviations: DW, Distilled water; **T_0 V_1**, control (DW); **T_1 V_1**, leaf extract (1 g + 10 ml DW); **T_2 V_1**, stem extract (1 g + 10 ml DW); **T_3 V_1**, root extract (1 g + 10 ml DW); **V_1**, Margalla 99; **T_0 V_2**, control (DW); **T_1 V_2**, Leaf extract (1 g + 10 ml DW); **T_2 V_2**, stem extract (1 g + 10 ml DW); **T_3 V_2**, root extract (1 g + 10 ml DW); **V_2**, Chakwall 97.

and carbon dioxide but also interfere with its normal growth by secreting biomolecules into the rhizosphere. The objective of the present study was therefore to assess the allelopathic effects of sunflower on wheat with reference to growth of wheat seedlings, production of indole-3-acetic acid, gibberellic acid, and abscisic acid by the seedlings, weed density and fresh and dry weight of weeds at 40 and 70 days after sowing, and fresh and dry weight of wheat and 100 seed weight and to analyse the soil after harvest of the sunflower and wheat plants.

MATERIALS AND METHODS

Sunflower plants (variety cv Hysun 38) were grown in pots in the Department of Plant Sciences, Quaid-i-Azam University, Islamabad. Three seeds were sown in each pot and were given a basal dose consisting of 2 g diammonium phosphate, 1 g urea, and 1 g potash. When the plants had reached the vegetative stage (40 days after sowing), they were uprooted and separated into their constituent parts (namely leaves, stems, and roots), washed thoroughly with distilled water, dried, pulverized in a mill, and stored in a cool place along with anhydrous $CaCl_2$ to keep the place dry. 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 sown in pots (3 seeds in each pot) and a basal dose consisting of 1 g urea and 1 g diammonium phosphate was applied to the 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 extract mixed with 9 ml water). Levels of the hormones, namely indole-3-acetic acid, gibberellic acid, and abscisic acid in wheat seedlings were measured 30 days after sowing and observations related to weeds were recorded 40 and 70 days after sowing. Wheat plants were harvested 145 days after sowing and relevant observations recorded. The experiment consisted of 3 replications.

Physicochemical properties of soil

Physical and chemical properties of soil were determined by following the standard method (Nelson and Sommers, 1982).

Macro- and micronutrients in soil

Soil samples, 5 g each, were collected from the experimental pots at a uniform depth of 5 cm, suspended in 50 ml of distilled water, stirred continuously for 20 min, and filtered. The filtrate was used for analysis.

Electrical conductivity

5 g of soil was mixed with 50 ml distilled water and stirred for 1 h. The suspension was left overnight to allow the soil to settle to the bottom. Electrical conductivity of the supernatant was determined using an EC meter.

Soil pH

Soil samples (25 g each) were placed in 100 ml beakers. To each beaker, 25 ml distilled water was added and the suspension stirred for 10 min before recording the pH with a pH meter (Recommended soil chemical test procedure, Whitney DA 1988).

Moisture content

Soil samples (20 g each) were taken from a uniform depth of 5 cm. Fresh weight of the samples was recorded. Dry weight was determined after oven-drying the soil for 72 h at 70°C to constant weight and moisture percentage calculated.

Plant height (cm)

At maturity, the wheat plants were harvested and their height was recorded.

Weed density

The number of weeds was recorded at 40 and 70 days after sowing.

Fresh weight and dry weight of weeds

For dry weight, the weeds were oven-dried at 70°C for 24 h.

Fresh weight and dry weight of wheat plants

Fresh weight of the plants was recorded upon harvest; dry weight was recorded after oven-drying the plants at 70°C for 24 h.

Determination of nitrogen, phosphorus, potassium, calcium, magnesium, iron, and manganese

Nitrate-nitrogen was determined by following the method described by Soltanpour and Schwab (1977). K, Mg, Mn, and Ca were extracted from the soil sample as described by Mehlich (1953 and 1984), and concentrations of Fe, Mg, Mn, and Zn were determined using an atomic absorption spectrophotometer (Shimadzu, AA-670). Solutions for the spectrophotometry were prepared as described by Whitney (1988).

Endogenous contents of phytohormones

Leaves (1 g each of samples) of wheat seedlings were ground in 80% methanol at 4°C with an antioxidant, namely butylated hydroxy toluene (BHT), and kept for 72 h with one change of the solvent. The extract was centrifuged and the supernatant reduced to its aqueous phase using a rotary film evaporator. The pH of the aqueous phase was adjusted to 2.5 – 3.0 and the phase was partitioned four times with half the volume of ethyl acetate each time. The ethyl acetate extract was fully dried using a rotary thin-film evaporator (RFE). The dried sample was re-dissolved in 1 ml methanol (100%) and analysed using HPLC with a UV detector and a C-18 column. For identifying hormones, samples filtered through 0.45-millipore filters were injected into the column. Pure IAA, GA, and ABA were used as standards for identification and quantification of the plant hormones. These growth hormones were identified on the basis of retention time and peak area of the standards. Methanol, acetic acid, and water (30:1:70) were used as the mobile phase. The wavelengths used for detection were 280 nm for IAA (Sarwar et al., 1992) and 254 nm for ABA and GA (Li et al., 1994).

Table 1. Physicochemical properties of soil after harvesting sunflower.

EC (dS/m)	pH	Mn (ppm)	Fe (ppm)	Mg (ppm)	Ca (ppm)	K (ppm)	Zn (ppm)	Pb (ppm)	Moisture (%)
130	6	1.01	1.30	13.27	210.91	30.26	0.15	5.66	16

Table 2. Physicochemical properties of soil after harvesting wheat variety Margalla 99.

Treatment	EC (dS/m)	pH	Mn (ppm)	Fe (ppm)	Mg (ppm)	Ca (ppm)	K (ppm)	Zn (ppm)	Pb (ppm)	Moisture (%)
T_0 V_1	120	6.5	1.60	2.01	15.10	149.50	40.10	0.19	5.10	20
T_1 V_1	115	6.7	1.99	2.80	14.07	130.49	55.60	0.30	7.19	17
T_2 V_1	117	6.4	1.70	2.40	13.01	140.10	45.01	0.23	6.10	19
T_3 V_1	116	6.6	1.95	2.55	14.00	131.10	53.10	0.25	6.90	18

Table 3. Physicochemical properties of soil after harvesting wheat variety Chakwall 97.

Treatment	EC (dS/m)	pH	Mn (ppm)	Fe (ppm)	Mg (ppm)	Ca (ppm)	K (ppm)	Zn (ppm)	Pb (ppm)	Moisture (%)
T_0 V_2	122	6.4	1.54	1.95	15.00	147.51	40.00	0.18	5.08	19
T_1 V_2	117	6.6	1.80	2.50	14.00	127.41	54.00	0.31	7.39	16
T_2 V_2	118	6.4	1.65	2.40	12.97	130.95	50.00	0.20	6.19	18
T_3 V_2	120	6.5	1.70	2.51	13.01	129.10	52.01	0.22	7.10	17

Table 4. Effect of sunflower leaf, stem, and root extracts on weed density in wheat 30 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	Weed density	Treatment	Weed density
T_0 V_1	6.00A	T_0 V_2	7.38A
T_1 V_1	3.01C	T_1 V_2	4.308D
T_2 V_1	5.00AB	T_2 V_2	5.735B
T_3 V_1	4.00BC	T_3 V_2	5.40C

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

Statistical analysis

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

RESULTS AND DISCUSSION

Soil analysis

Soil analysis was undertaken three times: before sowing sunflower, after harvesting sunflower, and after harvesting wheat. The following parameters were recorded in the second and the third analyses: electrical conductivity, pH, Zn, Pb, K, Ca, Mg, Fe, Mn, and moisture (Tables 1 – 3). In both the wheat cultivars, electrical conductivity (dS/m) decreased and pH increased in all the three treatments (extract of leaves, stems, or roots). These results are in agreement with those of Periturin (1913). Concentrations of all the nutrients except Ca (Mn, Mg, Fe, K, Zn, and Pb) also increased in all the three treatments.

Weed density

Table 4 shows that the number of weeds was maximum in the control pots and minimum in pots treated with leaf extract, which suggest that leaves contained the maximum amount of allelochemicals and stems contained the least, as was found also by Rice (1984), who proposed that allelopathy contributed to longevity of weed seeds through two mechanisms: (a) allelochemicals prevented the decay of weed seeds brought about by microorganisms and (b) allelochemicals kept the seed dormant and thus viable for a longer period.

Table 5. Effect of sunflower leaf, stem, and root extracts on fresh weight of weeds in wheat 40 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	Fresh weight of weeds (g)	Treatment	Fresh weight of weeds (g)
T_0 V_1	6.27A	T_0 V_2	8.49A
T_1 V_1	3.60D	T_1 V_2	5.00D
T_2 V_1	4.98B	T_2 V_2	6.49B
T_3 V_1	4.90C	T_3 V_2	5.90C

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

Table 6. Effect of sunflower leaf, stem, and root extracts on fresh weight of weeds in wheat 70 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	Fresh weight of weeds (g)	Treatment	Fresh weight of weeds (g)
T_0 V_1	8.20A	T_0 V_2	10.00A
T_1 V_1	5.62D	T_1 V_2	8.00C
T_2 V_1	6.10B	T_2 V_2	9.05B
T_3 V_1	5.97C	T_3 V_2	8.64BC

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

Table 7. Effect of sunflower leaf, stem, and root extracts on dry weight of weeds in wheat 40 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	Dry weight of weeds (g)	Treatment	Dry weight of weeds (g)
T_0 V_1	3.21A	T_0 V_2	5.31A
T_1 V_1	2.10D	T_1 V_2	2.64D
T_2 V_1	2.96B	T_2 V_2	3.09B
T_3 V_1	2.91C	T_3 V_2	3.07C

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

Table 8. Effect of sunflower leaf, stem, and root extracts on dry weight of weeds in wheat 70 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	Dry weight of weeds (g)	Treatment	Dry weight of weeds (g)
T_0 V_1	4.01A	T_0 V_2	6.04A
T_1 V_1	2.49D	T_1 V_2	3.89D
T_2 V_1	2.89B	T_2 V_2	4.08B
T_3 V_1	2.70C	T_3 V_2	3.97C

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

Fresh and dry weights of weeds at 40 and 70 days after sowing

In both the varieties, the maximum fresh and dry weights of weeds were recorded in the control pots, whether 40 days after sowing or 70 days after sowing (Tables 5 to 8).

Plant height

The tallest wheat plants of both the varieties were seen in pots that had been watered with extract of sunflower leaves, although in case of Chakwall 97, effects of root extract were on par with those of leaf extract (Table 9). The least effect was found in case of stem extract in two wheat varieties.

Weight of 100 grains

The parameter of 100-grain-weight confirmed the beneficial effects of allelochemicals: in both varieties, 100-grain-weight was maximum in wheat plants that had received the extract of sunflower leaves and minimum in those that had received no extract at all (Table 10).

Table 9. Effect of sunflower leaf, stem, and root extracts on height of wheat plants 145 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	Plant height (cm)	Treatment	Plant height (cm)
T_0 V_1	72.67C	T_0 V_2	85.44C
T_1 V_1	117.00A	T_1 V_2	120.00A
T_2 V_1	109.00B	T_2 V_2	112.30B
T_3 V_1	116.0A	T_3 V_2	120.00A

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

Table 10. Effect of sunflower leaf, stem, and root extracts on 100-grain-weight of wheat 145 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	100-grain-weight (g)	Treatment	100-grain-weight (g)
T_0 V_1	4.00D	T_0 V_2	3.98C
T_1 V_1	4.21A	T_1 V_2	4.141A
T_2 V_1	4.17C	T_2 V_2	4.13C
T_3 V_1	4.18B	T_3 V_2	4.140A

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

Table 11. Effect of sunflower leaf, stem, and root extracts on fresh weight of wheat plants 145 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	Fresh weight (g)	Treatment	Fresh weight (g)
T_0 V_1	4.00D	T_0 V_2	3.960D
T_1 V_1	4.90A	T_1 V_2	4.800A
T_2 V_1	4.450C	T_2 V_2	4.600C
T_3 V_1	4.710B	T_3 V_2	4.700B

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

Table 12. Effect of sunflower leaf, stem, and root extracts on dry weight of wheat plants 145 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	Dry weight (g)	Treatment	Dry weight (g)
T_0 V_1	0.70D	T_0 V_2	0.780D
T_1 V_1	0.89A	T_1 V_2	0.85A
T_2 V_1	0.77C	T_2 V_2	0.80C
T_3 V_1	0.87B	T_3 V_2	0.82B

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

Fresh and dry weights of wheat plants

Again, in both varieties, fresh and dry weights of whole plants were maximum in the plants that had received the extract of sunflower leaves and minimum in those that had received no extract at all (Tables 11 and 12).

Plant hormones: indole acetic acid, gibberellic acid, and abscisic acid

Broadly speaking, allelochemicals promoted the production of abscisic acid but depressed that of indole acetic acid and gibberellic acid (Tables 13 to 15).

Conclusions

Sunflower clearly exerted allelopathic effects on the crop (wheat) as well as on weeds. Commonly observed effects of allelochemicals were (a) decreased production of indole-3-acetic acid and gibberellic acid and lower values electrical conductivity, moisture content, weed density, and fresh and dry weight of weeds and (b) increased pro-

Table 13. Effect of sunflower leaf, stem, and root extracts on indole acetic acid content of wheat seedlings 30 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	IAA (µg/g)	Treatment	IAA (µg/g)
T_0 V_1	265.00A	T_0 V_2	195.00A
T_1 V_1	230.00C	T_1 V_2	186.70B
T_2 V_1	242.70B	T_2 V_2	194.00A
T_3 V_1	221.70C	T_3 V_2	192.00AB

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

Table 14. Effect of sunflower leaf, stem, and root extracts on gibberellic acid content of wheat seedlings 30 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	GA (µg/g)	Treatment	GA (µg/g)
T_0 V_1	510A	T_0 V_2	580A
T_1 V_1	500B	T_1 V_2	570B
T_2 V_1	508A	T_2 V_2	575AB
T_3 V_1	504AB	T_3 V_2	572AB

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

Table 15. Effect of sunflower leaf, stem, and root extracts on abscisic acid content of wheat seedlings 30 days after sowing (wheat varieties Margalla 99 and Chakwall 97).

Treatment	ABA (µg/g)	Treatment	ABA (µg/g)
T_0 V_1	110B	T_0 V_2	118B
T_1 V_1	119A	T_1 V_2	124A
T_2 V_1	116A	T_2 V_2	120B
T_3 V_1	117A	T_3 V_2	120B

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

duction of abscisic acid and higher values of pH, Mn, Fe, Mg, Ca, K, 100-grain-weight, and fresh and dry weight of wheat plants. Of the two varieties of wheat included in the experiment, Margalla 99 proved superior to Chakwall 97 in that it produced longer roots and shoots, greater biomass, and larger quantities of IAA and GA. Of the three plant parts tested, extract of sunflower leaves generally had the greatest allelopathic effect, followed by the extract of roots and of stems, in that order.

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