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Full Length Research Paper

Allelopathic potential of sunflower (*Helianthus annus* L.) on soil metals and its leaves extracts on physiology of wheat (*Triticum aestivum* L.) seedlings.

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The effect of a sunflower (*Helianthus annuus* L.) variety, namely Hysun 38, on metals and of aqueous extracts of its leaves on germination in two varieties of wheat, namely Margalla 99 and Chakwall 97, were studied under laboratory conditions. In particular, the effect of leaf extract on hormones produced by wheat seedlings and on electrical conductivity, pH, Mn, Ca, K, P, and soil moisture content was examined. The leaf extract significantly inhibited the rate of germination and growth of seedlings.

Key words: Allelopathy, atomic absorption, HPLC, hormones analysis (IAA, GA, ABA), sunflower, soil analysis, wheat seedlings.

INTRODUCTION

Given the increasing emphasis on sustainable agriculture and concern about the adverse effects (e.g. 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 agroecosystems. 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). Germination consists of several phases, including a catabolic phase, and ends with the anabolic phase that results in protrusion of the radicle. Therefore, radical emergence is considered to mark the completion of the process of germination and the beginning of seedling growth.

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, 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 effect of sunflower on wheat – especially on seedling growth, production of indole-3-acetic acid, gibberellic acid, and abscisic acid by

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the seedlings, and yield – and to analyse the soil before and after a crop of sunflower.

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 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.

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 9 cm Petri dishes (15 seeds in each dish) on a layer of filter paper moistened with either distilled water (control) or with different concentrations of the extracts of sunflower leaves and incubated at 26°C for 24 h (9 h of light and 15 h of darkness) before recording the rate of germination. These conditioned were maintained for 30 days in order to study the parameters like fresh weight, dry weight, root length, shoot length and hormonal analysis of wheat seedlings. The experiment consisted of 3 replications.

Physicochemical analysis 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 the 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 each filled with 25 ml of distilled water, and stirred for 10 min before recording the pH (Recommended soil chemical test procedure, 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 drying the soil in oven for 72 h at 70°C to constant weight and moisture percentage calculated.

Fresh weight and dry weight

Fresh weight of the seedlings was recorded upon harvest. Dry weight was recorded after drying the seedlings in an oven 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

The plant leaves (samples of 1 g each) 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 partitioned four times with ½ volume of ethyl acetate. 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), 254 nm for ABA and GA (Li et al., 1994).

Statistical analysis

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

RESULTS AND DISCUSSION

Soil analysis

The soil was sampled twice, once before sowing and once after the crop had been harvested. Electrical conductivity, pH, Zn, Pb, K, Ca, Mg, Fe, Mn, and moisture content were determined (Table 1). After harvesting the crop of sunflower, values of the following parameters had decreased: Ec (from 130 dS/m before the harvest to 110 dS/m after the harvest), Ca (from 210.91 to 120.02 ppm), P (5.66 to 4.10 ppm), and moisture (20 to 16%). A decrease in moisture content was also reported by Aldrich (1984), who maintains that allelopathy manifests itself as stress: stressed source plants often release a

Table 1. Physico-chemical analysis of soil before sowing and after harvesting the crop of sunflower.

EC (dS/m)	EC (dS/m)	pH	Mn (ppm)	Fe (ppm)	Mg (ppm)	Ca (ppm)	K (ppm)	Zn (ppm)	P (ppm)	Moisture content (%)
Before sowing	130	6	1.01	1.30	13.27	210.91	30.26	0.15	5.66	20
After harvesting	110	7	2.20	2.10	20.29	120.02	50.20	0.21	4.10	16

Table 2. Effect of sunflower extract on germination of wheat varieties Margalla 99 and Chakwall 97.

Treatment	Germination (%)	
	Margalla 99	Chakwall 97
T1	14.67 A	13.00 A
T2	10.00 D	09.00 C
T3	11.33 CD	10.00 B
T4	12.00 BC	11.67AB
T5	13.33 AB	12.67A

T1 = Control (distilled water; DW); T2 = Undiluted extract (9 g extract + 10 ml DW); T3 = 75% extract + 25% DW (7.5 g + 10 ml); T4 = 50% extract + 50% DW (5 g +10 ml); T5 = 25% extract + 75% DW (2.5 g + 10 ml).

greater array and higher concentrations of allelochemicals, and stressed target plants are likely to be more susceptible to the allelochemicals. Measuring the effects of allelochemicals along stressor gradients should help in elucidating the relationship between allelopathy and stress. The decrease of phosphorus after harvesting of sunflower crop is also due to decrease of moisture contents is mainly due to allelopathy; this phenomena was also reported by Rice (1984). In the present experiment, values of the following parameters had increased after the harvest: Mn (from 1.01 to 2.20 ppm), Fe (1.30 to 2.10 ppm), Mg (13.27 to 20.29 ppm), K (30.26 to 50.20 ppm), Zn (0.15 to 0.21 ppm), and pH (6 to 7). The increase in pH was also reported by Periturin (1913).

Rate of germination

The data on the rate of germination (Table 2) reveal that germination was maximum in the control, which was on par with that in T5; the percentages in T4 and T3 were also on par with each other. Germination was maximum in T2 in Margalla 99 and in T1 in Chakwall 97, followed, in that order in Chakwall 97, by T5, T4, T3, and T2. Overall, Margalla 99 was better than Chakwall 97 in terms of germination percentage.

Indole acetic acid (IAA)

The data indicate that in Margalla 99, IAA content of leaves (Table 3) was maximum in T3 and T2, followed by that in T4 and T5. The least quantity of IAA was found in T1. In the case of roots of Margalla 99 (Table 3), T1 recorded the maximum level of IAA, followed by T2, T3, T4, and T5. In Chakwall 97, the maximum quantity of IAA in leaves was found in T4, followed by T1, T3, T2, and T1 (Table 4) whereas in its roots, T2 contained the highest quantity of IAA, followed by T3, T1, T4, and T5 (Table 4). The quantities differed between the two varieties as well as among the treatments comprising varying concentrations of extracts of sunflower leaves.

Gibberellic acid (GA)

The quantity of GA in leaves of Margalla 99 was maximum in T1 followed by T2 and T3 and the least in T4 and T5 (Table 3); in the roots of that variety (Table 3), the maximum quantity was in T2, followed by T3. The least quantity was found in T5. In Chakwall 97, GA in leaves (Table 4) was maximum in T1, followed by T5, T4, T3, and T2 whereas that in roots (Table 4) was maximum in T2, which was on par with that in T1, T3, and T4. The least quantity was observed in T5.

Abscisic acid (ABA)

In leaves of Margalla 99, abscisic acid was maximum in T3 (Table 3) and minimum in T1. In roots of the same variety (Table 3), the maximum value was observed in T1, followed by T2, T3, and T4; the lowest value was recorded in T5. In leaves of Chakwall 97 (Table 4), the maximum quantity of ABA was observed in T4, which was on par with that in T3 and T1. The least quantity was observed in T5 and T2. In roots of Chakwall 97 (Table 4), the maximum values were observed in T2 and T3 and the minimum in T5.

Shoot length

Data on shoot length reveal that in both Margalla 99 and Chakwall 97, the maximum values were observed in T1 and the minimum in T2 (Table 5).

Root length

In Margalla 99, root length was maximum in T1 and minimum in T2. In Chakwall 97, the maximum value was observed in T1 followed by T5, T4, T3, and T2.

Table 3. Effect of leaf extract on IAA, GA, and ABA content (µg/g) of leaves and roots of wheat variety Margalla 99.

Treatment	Leaves			Roots		
	IAA	GA	ABA	IAA	GA	ABA
T1	90.000 D	500.00 A	68.00 D	190.00 A	415.00 AB	167.00 A
T2	163.00A	430.00 B	160.00 B	140.00 B	422.00 A	137.00 B
T3	175.00A	400.00 C	180.00 A	130.00 C	418.00 AB	120.00 C
T4	115.00 C	390.00 D	130.00 C	125.00 C	415.00 AB	116.00 C
T5	120.00 C	385.00 D	135.00 C	110.00 D	410.00 B	102.00 D

Table 4. Effect of leaf extract on IAA, GA, and ABA content (µg/g) of leaves and roots in wheat variety Chakwall 97.

Treatment	Leaves			Roots		
	IAA	GA	ABA	IAA	GA	ABA
T1	120.00 B	510.00 A	101.00 BC	105.00 C	425.00 B	92.00 C
T2	105.00 C	407.00 D	90.33 C	130.00 A	445.00 A	120.00 A
T3	115.00 B	430.00 C	113.00 AB	120.00 B	450.00 A	118.00 A
T4	130.00 A	460.00 B	120.00 A	105.00 C	450.00 A	102.00 B
T5	102.00 C	465.00 B	95.00C	91.00 D	410.00 C	81.00 D

Table 5. Effect of leaf extract of sunflower on shoot length, root length, fresh weight, and dry weight of seedlings of wheat varieties Margalla 99 and Chakwall 97.

Treatment	Margalla 99				Chakwall 97			
	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
T1	14.00 A	18.30 A	0.91A	0.87A	13.00 A	17.50 A	0.89 A	0.86 A
T2	9.50 C	9.00 D	0.20E	0.17 E	8.70 D	8.60 E	0.81 E	0.16 E
T3	10.20 C	11.40C	0.30 D	0.27 D	9.80 C	10.60 D	0.28 D	0.24 D
T4	12.00 B	14.00B	0.60 C	0.57 C	11.20 B	13.60 C	0.50 C	0.47 C
T5	13.00 AB	15.00 B	0.81 B	0.77 B	12.60 A	14.70 B	0.89 A	0.68 B

Fresh weight

Data on fresh weight indicate that in both Margalla 99 and Chakwall 97, T1 showed the maximum fresh weight, followed by T5, T4, T3, and T1.

Dry weight

Data on dry weight indicate that in both Margalla 99 and Chakwall 97, T1 showed the maximum dry weight and T2 the minimum.

Conclusions

Allelochemicals secreted by sunflower inhibited germination and lowered the levels of hormones GA and IAA, fresh weight, dry weight, root length, and shoot length but increased the levels of ABA in wheat seedlings. The variety Margalla 99 was better than Chakwall 97 in terms of germination percentage, root length, shoot length, fresh weight, dry weight, and the contents of IAA and GA. Extract of sunflower leaves diluted to 25% with water had the greatest effect on the seedlings, followed by that diluted to 50 and 75%. The extract increased the pH and levels of Mn, Fe, Mg, K, and Zn but decreased electrical conductivity, Ca, P, and moisture content of soil in the rhizosphere.

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REFERENCES

Aldrich JD (1984). Weed-crop ecology: principles and practices. Breton Publishers, pp. 215-241.

Bogatek R, Gniazdowska A, Zakrzewska W, Oracz K, Gawroński SW (2005). Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. Biol. Plant. In Press.

Li JC, Shi J, Zhao XL, Wang GYU, Ren YJ (1994). Separation and determination of three kinds of plant hormone by high performance liquid chromatography. Fenxi-Huaxue. 22: 801-804.

Mehlich A (1953) determination of P, Ca, Mg, K, Na and NH_4. North Carolina Soil test Division (Mimeo).

Mehlich A (1984). Mehlich 3 soil test extractant. A modification of the Mehlich 2 extractant. 15: 1409-1416.

Nelson DW, Sommers LE (1982). Total carbon, organic carbon and organic matter. In: Page AL, Miller RH, Keeny DR (eds,), Methods of Soil Analysis. Part 2: Chemical and Microbiological properties, 2nd ed. American Society of Agronomy, Madison, WI, pp. 538-580

Periturin FT (1913). Izv.Mosk. S-kh.Inst.kn.4. Recommended soil chemical test procedure for North region (1988) North Regional Publication No. 221 (NDSU Bull. No. 499. WP5. Estlt ests 04/11/91.

Rice EL (1984). Allelopathy 2nd ed. New York Acadmic Press.

Sarwar M, Arshad M, Martens DA, Frankenberger WT Jr (1992). Tryptophan-dependent biosynthesis of auxins in soil. Plant Soil 147: 207-215.

Soltanpour PN, Schwab AP (1977). A new soil test for simultaneous extraction of macro and micro nutrients in alkaline soils. Commun. Soil Soc. Plant Anal. 8: 195-207.

Wardle DA, Ahmad M, Nicholson KS (1991). Allelopathic influence of nodding thistle (*Cardus nutans* L.). seed on germination and radicle growth of pasture plants. N. Z. J. Agric. Res. 34: 185-191.

Whitney DA (1988). Micronutrients soil tests for zinc, iron, manganese and copper. pp. 20-22. In recommended chemical soil test procedure for the North Chemical region. Ed. WC Dahnke. pp. 117-130. North Dakota agric. Exp. Stn Bull. No. 499.