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Differential allelopathic potential of sunflower (*Helianthus annuus* L.) genotypes on weeds and wheat (*Triticum aestivum* L.) crop

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Studies were conducted to screen eight sunflower (*Helianthus annuus* L.) genotypes for their allelopathic potential against weeds and wheat crop, which customarily follows sunflower in Iraq. All sunflower genotypes significantly inhibited the total number and biomass of companion weeds and the magnitude of inhibition was genotype dependent. Among the eight genotypes tested, Sin-Altheeb and Coupon were the most weed-suppressing cultivars, and Euroflor and Shumoos were the least. A subsequent field experiment indicated that sunflower residues incorporated into the field soil significantly inhibited the total number and biomass of weeds growing in the wheat field. Sunflower genotypes Sin-Altheeb and Coupon appeared to inhibit total weed number and biomass more and significantly increased wheat yield compared with the least-suppressive genotypes (Euroflor and Shumoos). Chromatographic analyses by HPLC revealed the presence of 13 secondary metabolites in residues of the tested sunflower genotypes. All the isolated compounds appeared to be phenolic, with the exception of terpinol, which is a terpenoid derivative. The total concentration of Phytotoxins (phenolic compounds) was found to be higher in the most-suppressive potential genotypes compared with the least-suppressive genotypes.

Keywords: allelopathy; sunflower genotypes; weed control; wheat crop; phenolic acids

Introduction

Allelopathy plays a major role in natural ecosystems by determining vegetation pattern, plant dominance, succession and biodiversity, preventing seed decay and causing seed dormancy (Rice 1984). Also, allelopathy has a potential role in agricultural ecosystems (Singh et al. 2001). Work on different aspects of allelopathy over the past four decades has led scientists to put much effort into using this phenomenon to reduce the reliance on chemical herbicides for weed control (Weston and Duke 2003). Several crops, including sunflower, have been reported to have allelopathic potential against weeds and considerable variations among cultivars of the test crop have been reported (Putnam and Duke 1974; Leather 1983, 1987; Alsaadawi et al. 1986).

Several genotypes of sunflower were introduced into Iraq during the recent past for cultivation beside local genotypes. Preliminary field observations revealed that the growth and population of companion weeds were variable among stands of

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selected genotypes. Also, differential growth and population variation were observed in weeds grown in the field after sunflower harvest. This suggests that allelopathy might be the mechanism responsible for the variation in weed growth and population, and the differences among stands could be attributed to the variable allelopathic potential of the cultivars. The aim of this study was to: (1) screen some sunflower genotypes for their ability to control the growth and population of companion weeds, (2) determine the supressive potential of residues of high and low allelopathic potential genotypes on growth of wheat crop and on weeds in wheat field, and (3) isolate and identify phytotoxic compounds found in the residues of high and low allelopathic potential genotypes.

Materials and methods

Plant materials

Five local sunflower genotypes (Zahrat Al-Iraq, Sin-Altheeb, Akmar, Sabah, Shumoose) and three introduced genotypes (Euroflor, Coupon and Flammy) commonly used by farmers in Iraq were used in the experiments. Local wheat (*Triticum aestivum* L. cv. Abu-Ghraib) was used as a test cultivar.

Effects of sunflower genotypes on companion weeds

The experiment was conducted in a field located 180 km south of Baghdad. The site was located at 32°34′N, 45°50′E. The field is characterized by calcareous soil and loamy sand of pH 7.2 and electrical conductivity 3.5 dS m^{-1}; average annual rainfall was ~50 mm and day/night temperatures during the growing season were 20–40/10–20°C. Field plots (1.5 × 1.5 m) were randomly made on 25 February 2008 in a field heavily infested with weeds. The plots were plowed by spade to a depth of 30 cm and received urea (46% N) at 240 kg ha^{-1} (50% before planting and 50% after two weeks from planting) and triple superphosphate (46% P_2O_5) at 240 kg ha^{-1} at planting time as a source of P.

Seeds of eight test sunflower genotypes were sown in their respective plots in rows, with a distance of 25 cm between seeds and 50 cm between rows. Plots without crops were used as a control. No herbicides were used during the course of the experiment. The experiment was conducted in a randomized complete block design with three replications for each treatment. At the end of crop maturity (15 July 2008), plants of each sunflower genotype were removed and the total number of weeds was recorded. Weeds were clipped at the ground surface, oven-dried for three days at 75°C and weighed for biomass determination using a digital balance.

Effect of sunflower residues on the wheat crop and its companion weeds

Based on the results of a previous field experiment, Coupon and Sin-Altheeb proved to be highly suppressive genotypes, whereas Euroflor and Shumoos were the least suppressive to weeds. Therefore, these genotypes were used in the following experiment to test their effects on weeds in a wheat crop and on wheat yield.

Field plots of 1.5 × 1.5 m were randomly selected in a field adjacent to that of the first experiment and which had been previously cultivated with broad bean. Plant parts (stems and leaves only) of the highly suppressive (Coupon and Sin-Altheeb) and least suppressive genotypes (Shumoose and Euroflor) were collected at the end

of growing season from the field of the first experiment. The plant parts were chopped into pieces (2–3 cm), air-dried under the sun during July and kept until use. Residues of each genotype were added to field plots at a rate of 600 and 1400 g m^{-2}. All plots were plowed by spade to depth of 30 cm and prepared for wheat cultivation. Grains of wheat cv. Abu Ghraib were sown in lines on 25 November 2008 in the plots. Control plots were made in the same manner, except that sunflower residues were not added (control 1). Other control plots (control 2) without residues and without wheat were also used to demonstrate the effect of wheat plants on weeds. The experiment was conducted in a randomized complete block design in split-plot arrangement with three replications, keeping sunflower cultivars in the subplot and sunflower residues in the main plot. Nitrogen as urea (46% N) at 200 kg ha^{-1} and phosphorus as triple superphosphate (46% P_2O_5) at 200 kg ha^{-1} were applied to the plots. All the phosphorus and half of the nitrogen was applied at planting, with the remaining nitrogen applied at the tillering stage. The number of weeds and oven-dried above-ground weed biomass were recorded three months after the beginning of the experiment. At the end of experiment (15 June 2009), wheat plant height, straw air-dried biomass, number of spikes per plant, number of tillers per plant, number of grains per spike and weight of 100 grains were recorded. The data were analyzed using analysis of variance (ANOVA). The least significant differences test was used to compare the means of treatments (Steel and Torrie 1980).

Separation, identification and quantification of secondary metabolites

Water extract of the residues of the highest suppressive and the least suppressive cultivars were prepared according to Singh et al. (1989) with some modification. One gram of residues of test genotypes was soaked in 100 ml hot distilled water (70–80°C) acidified with 1 ml of acetic acid. The mixture was heated gently, mixed thoroughly using ultrasonic apparatus to exclude air bubbles from the residues and allowed to stand for 4 h. The mixture of each sample was filtered by filter paper under vacuum and kept in a refrigerator until use. For identification, 50 μl of the extract of each sample was injected into a HPLC Shimadzu-C-6A using the procedure outlined by Hartley and Buchan (1979). The separation conditions are listed in Table 1. The peaks were detected using a UV detector. Standards of suspected phytotoxins were run similarly for identification and quantification. The data of total phenolic compounds were analyzed by ANOVA. The least significant differences test was used to compare the means of treatments (Steel and Torrie 1980).

Table 1. HPLC conditions for the separation of phytotoxins from sunflower residues.

Parameter	Characteristic
Diatomite	Supleco wax 10
Column dimensions	50 × 2.6 mm
Attenuation	0.01 mg l^{-1}
Rate of recorder	10 mm/minutes
Detector	UV spectrophotometer at 254 nm
Volume injection sample	50 μl
Type of Column	NS-C18
Mobile face	1% acetic acid in buffer phosphate 0.01 M: acetyl nitrite 3:2 v/v
Temperature	25°C

Results

Allelopathic potential of sunflower genotypes against companion weeds

The results indicated that the numbers of weed plants recorded differed among the tested sunflower genotypes (Table 2). Zahrat Al- Iraq. Akmar, Shabah, Sin-Altheeb, Coupon and Flammy reduced the total number of weeds growing in the field by 67.0, 46.1, 50.5, 47.2, 86.8 and 63.7% of control, respectively, while Euroflor and Shumoos were the least supressive genotypes with reductions of 21.59 and 5, respectively.

Total above-ground biomass for weeds was significantly reduced by all sunflower test genotypes (Table 2). The magnitude of the reduction was genotype dependent. Coupon and Sin-Altheeb were the most inhibitive with a reduction of 80.79 and 74.23% of control, respectively, whereas the reduction ranged from 33.67 of control in Shumoos to 60.81% of control in Shabah.

Effect of selected sunflower genotype residues on wheat field weeds

Plots without sunflower residues and without wheat recorded the maximum total number and total biomass of weeds compared with all treatments containing sunflower residues (Table 3). Residues of all selected sunflower genotypes significantly reduced total weed biomass compared with control treatments, and the reduction was affected by both the genotype of the sunflower residues and the amount of incorporated residues. In most cases, the reduction increased with increasing residue concentrations.

Coupon and Sin-Altheeb recorded the highest inhibition of above-ground weed biomass with a reduction of 53.96 and 64.52% at the low residue rate and 62.22 and 66.75% at the high residues rate, respectively. The total number of weeds was significantly inhibited by the residues of all selected genotypes of sunflower compared with controls (Table 3). Inhibition increased markedly with increasing residue rate for all test genotypes. Genotypes Coupon and Sin-Altheeb showed the highest reduction in total number of weeds, followed by Shumoos and Euroflor.

Table 2. Allelopathic potential of sunflower genotypes on companion weeds under field conditions.

Sunflower genotypes	Total weeds number per plot	Reduction (% of control)	Total weeds dry weight (g plot^{-1})	Reduction (% of control)
Euroflor	47.62	21.5	93.90	42.28
Zahrat Al- Iraq	20.00	67.0	77.20	52.54
Shumoos	54.85	9.5	107.90	33.67
Akmar	32.67	47.2	91.63	43.67
Shabah	30.00	50.5	63.76	60.81
Sin-Altheeb	32.00	46.1	41.93	74.23
Coupon	8.00	86.8	31.26	80.79
Flammy	22.00	63.7	90.34	44.46
Control (- sunflower residue)	60.67	–	162.66	–
LSD = 0.05	6.75	–	32.37	–

Note: Results are an average of three replicates.

Effect of residues of selected sunflower genotypes on wheat crop:

Application of all sunflower residues except Shumoos resulted in reduced plant height (Table 4). However, the increase in residue rate did not influence plant height significantly. The number of tillers per plant was increased by residue treatments for all test genotypes. However, the increase was not statistically different from control. Plant dry weight increased significantly with residue rates for all sunflower genotypes tested, except for the low residues rate in Euroflor and Shumoose, where the increase was not statistically significant. The magnitude of the increase was in the following order: Sin-Altheeb > Coupon > Euroflor > Shumoos at high residue concentrations and Sin-Altheeb > Coupon > Shumoos > Euroflor at low residue concentrations.

Results (Table 5) revealed no significant difference in spike number of wheat due to the application of residues of different sunflower genotypes. Nonetheless, the

Table 3. Effect of residues of selected sunflower genotypes on the growth of weeds in a wheat field[a].

Sunflower		Weed			
Genotypes	Residue rate ($g\ m^{-2}$)	Weed dry weight ($g\ m^{-2}$)	Reduction (% of control)	Total weed number per m^2	Reduction (% of control)
Euroflor	600	31.53	19.33	21.33	39.62
	1400	18.43	52.86	18.00	49.05
Shumoos	600	34.30	12.27	26.67	24.51
	1400	23.33	40.33	18.67	49.09
Sin-Altheeb	600	13.00	64.52	13.00	47.15
	1400	13.00	66.75	9.00	74.52
Coupon	600	18.00	53.96	15.67	55.62
	1400	14.77	62.22	11.67	66.96
Control (1)[b]	–	39.10	0.00	35.33	0.00
Control (2)[c]	–	41.33	–[d]	36.67	–[d]
LSD = 0.05		6.43		4.66	

Notes: [a]Each values is an average of three replicates. [b]Plots cultivated without sunflower, weed biomass was increased by 5.7 and 3.79% over control. [c]Plots without wheat or sunflower residues. [d]Weeds numbers.

Table 4. Effect of residues of selected sunflower genotypes on some agronomic traits of wheat.

Sunflower		Wheat		
Genotypes	Residue rate ($g\ m^{-2}$)	Plant height (cm)	Number of tillers per plant	Plant dry weight (g)
Euroflor	600	74.72	4.20	387.3
	1400	74.28	4.74	408.2
Shumoos	600	84.52	4.11	369.4
	1400	80.24	4.14	399.5
Sin-Altheeb	600	75.72	4.44	490.4
	1400	73.00	4.51	544.1
Coupon	600	74.30	4.14	416.5
	1400	73.21	4.66	471.9
Control	0	83.05	3.98	364.0
LSD = 0.05	–	4.05	NS	30.87

Note: Each value is an average of three replicates.

Table 5. Effects of residues of test sunflower genotypes on yield and yield components of wheat.

Sunflower		Wheat			
Genotypes	Residue rate (g m^{-2})	Number of ear per plant	Number of grains per ear	Weight of 100 grains (g)	Plant yield (g)
Euroflor	600	2.67	19.33	3.58	1.91
	1400	3.00	31.33	4.00	3.68
Shumoos	600	2.67	18.67	3.33	1.64
	1400	2.67	26.00	3.58	2.45
Sin-Altheeb	600	3.67	32.00	4.25	4.93
	1400	4.00	40.67	4.48	6.77
Coupon	600	3.33	30.00	4.08	4.00
	1400	3.67	40.33	3.46	5.06
Control	0	2.67	15.33	3.41	1.56
LSD = 0.05		NS	4.69	0.81	1.70

Note: Each value is an average of three replicates. NS, not significant.

highest ear number per plant was achieved by residues of Coupon and Sin-Altheeb and by a high concentration of Euroflor.

The number of grains per ear was significantly increased by the residues of all test genotypes and with increasing rate of residues for all genotypes. The highest number of grains per ear was recorded for Coupon and Sin-Altheeb followed by Euroflor and Shumoos. The weight of 100 grains was increased by residues of the test genotypes. Increasing residue rates of Coupon and Sin-Altheeb only caused significant increase in weight of 100 grains. Wheat plant yield was significantly increased by residues of the test genotypes. In most cases, plant yield increased significantly with increasing residue rates. Coupon and Sin-Altheeb gave the highest plant yield, and Euroflor and Shumoos residues the lowest.

Separation, identification and quantification of secondary metabolites

HPLC analyses indicated the presence of 13 secondary metabolites in the residues of the tested sunflower genotypes (Table 6). All the isolated compounds appeared to have different retention times and were identified as phenolic compounds, with the exception of terpinol, which is a terpenoid derivative. The profile for each compound differed among the test genotypes. The concentration of the isolated compounds was in the order: protocatecheuic acid < isochlorogenic acid < terpinol < catechol < hydroxybenzoic acid < caffeic acid < chlorogenic acid < gallic acid < ferulic acid < *p*-coumaric acid < sinapic acid < syringic acid < vanillic acid in Coupon and hydroxybenzoic acid < caffeic acid < catochol < ferulic acid < protocatecheuic acid < gallic acid < chlorogenic acid < isochlorogenic acid < *p*- coumaric acid < syringic acid < vanillic acid < sinapic acid in Euroflor.

The total concentration of secondary metabolites appeared to be much higher in Coupon and Sin-Altheeb than in Euroflor and Shumoos.

Discussion

The differential inhibition of weeds among the sunflower genotypes could be mainly attributed to differences in the allelopathic potential of the genotypes through root

Table 6. Identification and quantification of phytotoxins (phenolic compounds) from residues of test sunflower genotypes.

	Phytotoxin concentration (mg l^{-1})*				
	Genotype				Retention
Phytotoxins	Euroflor	Shumoos	Coupon	Sin-Altheeb	time (min)
Chlorogenic acid	127.4	111.2	123.7	138.0	11.05
Isochlorogenic acid	147.9	60.0	83.7	55.1	1.85
Caffeic acid	64.7	90.5	75.0	127.4	2.87
Gallic acid	111.0	122.5	221.2	152.3	3.49
Protocatecheic acid	95.8	39.2	88.0	43.5	4.38
Syringic acid	184.8	116.7	2589.7	292.5	5.14
Hydroxybenzoic acid	21.7	53.1	126.2	86.0	6.16
p-Coumaric acid	157.8	175.7	142.3	269.1	7.01
Ferulic acid	90.9	54.1	122.9	171.0	7.69
Vanillic acid	224.2	118.4	85.0	320.4	8.68
Catochol	79.8	63.0	132.0	61.6	9.75
Sinapic acid	264.8	341.5	107.7	292.0	10.93
Terpinol	0.0	12.7	65.5	57.7	11.69
Total**	1571.2 c	1359.2 c	3963.4 a	2067.3 b	—

Notes: *Average of two replicates. **Numbers within a row followed by the same letter are not significantly different at 0.05, according to Duncan's Multiple Range Test.

exudation. Differences in the sunflower genotypes' competitive ability for light, moisture or nutrients was probably not the main reason for the observed differences in weed suppression because the most suppressive genotypes had lower biomass than the least-suppressive ones (data not shown). Differential allelopathic potential among crops, including sunflower, has been reported and well documented by several investigators (Leather 1983; Alsaadawi et al. 2007). The suppressive ability of sunflower residues on weeds growing in a wheat field could be attributed to both allelopathy and an effect involving nutrient cycling. Differences in the allelopathic potential of several allelopathic crops other than sunflower have been documented previously (Dilday et al. 1998; Reberg-Horton et al. 2005; Alsaadawi et al. 2007).

Our studies clearly demonstrated that residues of sunflower genotypes showed an inhibitory effect against weed density and biomass in a wheat field, and in most cases the inhibition was affected by the residues incorporated into the soil. Differences in weed dry weight further confirmed the allelopathic potential of the tested sunflower genotypes. In most cases, the reduction in total weed dry weight was parallel to the reduction in weed number. Sunflower genotype Sin-Altheeb, which caused the highest reduction in weed number also exhibited the maximum reduction in total weed biomass. This suggests that the sunflower residues contain secondary metabolites, which were released into the soil by the action of microorganisms and affected the roots of the receiver plant species (Chou and Lin 1976; Rice 1984; Blum 2006). The strong effects of Sin-Altheeb and Coupon residues, positive on wheat yield and negative on weeds, could also be due to an indirect effect of residues on nutrient availability in the soil, which would favor wheat rather than weeds. However, this hypothesis is very unlikely because: (1) the same amount of residue was incorporated for the same cultivars; and (2) in this experiment, wheat seemed to compete with weeds, because weeds biomass was almost the same in plots with and without wheat (compare controls 1 and 2 in Table 3).

It is noteworthy that the genotypes with high allelopathic potential through root exudates (Coupon and Sin-Altheeb) had the highest allelopathic effects through their residues. No attempt was made to study the biological activity of root exudates of sunflower genotypes. However, previous studies on other sunflower genotypes revealed that root exudates significantly inhibited the growth of the test weeds (Wilson and Rice 1968; Balasem 2001). Also, the allelopathic effects of sunflower are not restricted to companion weeds only, but were also observed on weeds, which appeared in the wheat crop field. This result is very striking from a management point of view because it provides a background to using residues and root exudates in a rotational sequence to control weeds in cropping systems. Several investigators have indicated that residues and root exudates of allelopathic crops have great potential in improving crop productivity, genetic diversity, disease management, weed control and nutrient conservation (Barker and Bhowmik 2001; Batish et al. 2001; Singh et al. 2001).

Residues of sunflower genotypes, particularly the highly suppressive ones (Coupon and Sin-Altheeb), favorably affected some agronomic traits and yield components of wheat. The increase in the growth and yield of wheat might be attributed to suppression of weeds by sunflower residues and elimination of competition with the wheat crop. Allelopathic crops, including sunflower, can be used as a potential means to control weeds and enhance crop production using different strategies such as using plant extract, plant residues as a cover and mulch, crop rotation, crop mixture and intercropping practices (Einhellig and Leather 1988; Putnam 1990; Cheema et al. 2000; Dahiya and Narwal 2003; Anjum and Bajwa 2005; Alsaadawi and Dayan 2009).

Chemical analyses using HPLC indicated the presence of several phenolic acids in a water extract of the residues from sunflower genotypes. These phenolic acids are known to inhibit ion uptake (Olmsted and Rice 1970), chlorophyll biosynthesis (Weir et al. 2004), cell membrane stability (Keck and Hodges 1973), protein and hormone biosynthesis (Rice 1984; Holappa and Blum 1991) and cell division, and change the ultrastructural components of cells (Sánchez-Moreiras et al. 2004). Thus, the results of chemical analysis showed additional evidence that sunflower residues contain allelopathic agents. Highly suppressive genotypes (Coupon and Sin-Altheeb) in terms of weed suppression were confirmed by the high concentration of total phenolic compounds in these genotypes compared with the others. A possible suppressive effect of secondary metabolites other than phenolics could not be excluded. Several researchers have indicated that terpenoids and flavanoids isolated from sunflower have considerable suppressive ability against plants (Macias et al. 1998, 1999; Anjum and Bajwa 2007; Dayan and Duke 2009).

The differential allelopathic potential of sunflower genotypes suggested the possible use of highly allelopathic cultivars for managing weeds through root exudation and/or residue incorporation, thereby enhancing crop production.

Conclusion

Differential ability to suppress weeds was observed among the eight tested cultivars of sunflower genotypes. The highly suppressive cultivars not only inhibit companion weeds, but also suppress the population density and biomass of weeds when their residues are incorporated into soil. The concentration of isolated phytotoxins appeared to be higher in residues of the most suppressive cultivars than in residues of

the least suppressive cultviars. Apparently, the residues favorably affected some agronomic traits and yield and yield components of wheat by suppressing weeds and eliminating competition with the wheat crop. This method may provide a possible alternative for achieving sustainable weed management.

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