ALLELOPATHIC EFFECTS OF SUNFLOWER (*HELIANTHUS ANNUUS*) ON GERMINATION AND GROWTH OF WILD BARLEY (*HORDEUM SPONTANEUM*)

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Abstract

Sunflower [*Helianthus annuus* (L.) Koch.] contains watersoluble allelochemicals that inhibit the ermination and growth of other species. This characteristic could be used in weed management programmes. Greenhouse and laboratory experiments were conducted to determine the effects on wild barley (Hordeum spontaneum Koch.) germination and seedling growth of(i) preceding crops, (ii) fresh sunflower residue incorporation, and (iii) sunflower leaf, stem, flower and root water extract concentrations. Growth of wild barley, as indicated by plant height and weight, was significantly reduced when grown in soil previously cropped to sunflower compared with that cropped to wild barley. Soil incorporation off fresh sunflower roots and both roots and shoots reduced wild barley germination, plant height and weight when compared with a no-residue control. In bioassays, sunflower extracts reduced wild barley hypocotyl length, hypocotyl weight, radicle weight, seed germination, and radicle length by as much as 44, 578, 61, 686 and 79 %, respectively, when compared with a water control. Increasing the water extract concentrations from 4 to 20 g per 100 ml of water of all sunflower parts significantly increased the inhibition of wild barley germination, seedling length and weight. Based on 8-day-old wild barley radicle length, averaged across all extract concentrations, the degree oftoxicity ofdifferent sunflower plant parts can be ranked in the following order of inhibition: leaves > flowers > mixture of all plant parts > stems > roots.

Key words: allelopathy , sunflower — Helianthus annus (L.) Koch. , wild barley ,Hordeum spontaneum Koch., , water extracts, inhibition germination and growth

Introduction

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the release of chemical compounds into the environment (Rice 1984). Several phytotoxic substances causing germination and/or growth inhibitions have been isolated from plant tissues and soils. These substances, collectively known as allelochemicals, are usually secondary plant products or waste products of main metabolic pathways of plants (Whittaker and Feeny 1977, Hall and Henderlong 1989,

Chon and Kim 2002). Sunflower is well known for its allelopathic compounds. Several phenols and terpenes have been reported in various cultivars of sunflower (Spring *et al*. 1992; Macias *et al.* 2002). They are often watersoluble substances that are released into the environment through root exudation, leaching and decomposition of plant residues. Several Asteraceae species have been reported as having allelopathic effects on other plant species, reducing seed germination and emergence of subsequent small-grain crops when grown in rotation (Bialy et al. 1990, Muehlchen et al. 1990). Several putative allelochemicals have been isolated from Asteraceae and their allelopathic potential demonstrated in bioassays. For example, allyl-isothiocyanate (ITC) isolated from sunflower residues inhibited the germination and growth of various grass species (Vaughn and Boydston 1997). Benzyl-ITC, a breakdown product of white mustard (Brassica hirta L.), is phytotoxic to velvetleaf (Abutilon theophrastis Medic.), sicklepod (Senna obtusifolia L.) and sorghum [Sorghum bicolor (L.) Moench] (Josefsson 1968, Tollsten and Bergstrom 1988). Other breakdown products of glucosinolate like ionic thiocyanate (SCN)) inhibit the root or shoot growth of several species (Brown et al. 1991, Brown and Morra 1993). However, studies with other species have reported that the response to allelochemicals may be concentrationdependent. Allelochemicals that inhibit the growth of some species at certain concentrations might in fact stimulate the growth of the same or different species at different concentrations (Narwal 1994). It is thus essential to identify concentrations at which each specific response occurs if allelopathic interactions are to be used in weed management programmes. In addition, various plant parts may vary in their allelopathic potential (Chon and Kim 2002, Economou et al. 2002). Information about the allelopathic potential of the flora of Mediterranean regions remains scarce.

The present study was conducted to determine the allelopathic potential of sunflower towards wild barley, a problematic weed in Mediterranean regions. The objectives were to determine the effects of(i) preceding crops on germination and seedling growth of wild barley, (ii) fresh sunflower residue incorporation on early growth of wild barley, and (iii) the effects of water extract concentration of various sunflower parts on wild barley seed germination and seedling growth.

Materials and Methods

Greenhouse experiments

Effects of preceding crops

The effects of preceding crops were studied by growing sunflower and wild barley in soils from fields in north Iran(Mazanderan state) cropped in the previous season with either species, to assess the existence of long-term allelopathicity of sunflower. Ten wild barley seeds were planted in pots (150 mm wide and 150 mm high) each containing soil (loam) from adjacent fields previously cropped either to wild barley (wild barley soil) or sunflower (sunflower soil). Each treatment, wild barley grown in wild barley soil and wild barley grown in sunflower soil, was replicated eight times and arranged in a completely randomized design. A similar experiment was conducted with barley, planting five seeds per replicate pot. Plants were grown at constant temperature (26 ºC) with a 16-h light 8-h dark cycle for 35 days. At the end of the growth period, germination percentage, plant height and fresh weight were recorded.

Effects of fresh residue incorporation

The effects of incorporating fresh sunflower or wild barley whole plants or roots only on wild barley were studied to test for the existence of short-term sunflower allelopathicity. Treatments were arranged in a 2 × 3 factorial assigned to a randomized complete block design with four replications. Treatment combinations included source of residues (sunflower or wild barley) and type of residues incorporated [whole plants, roots only or no residue (control)]. Ten sunflower or wild barley plants were grown for 30 days in pots (170 × 165 mm) kept in a greenhouse. At the end of this period, whole plants or roots only were mixed into the soil in situ. Control treatments contained only soil. Four days after incorporation, 10 wild barley seeds were planted in each pot, including control pots. Germination, plant height and dry weight were recorded 30 days after planting.

Laboratory experiments

Preparation of extracts

Sunflower plants were collected from fields in north Iran (Mazanderan state) during the 2004–05 growing season. Fresh Sun flower plants were separated into leaves, stems, roots and flowers. Tissues from each plant part were soaked in distilled water for 24 h at 25 ºC in a lighted room to give concentrations of 4, 8, 12, 16, and 20 g of tissue per 100 ml of water.

After soaking, solutions were filtered through four layers of cheesecloth and the filtrate was then centrifuged (1500 g) for 4 h. The supernatant was filtered again using a 0.2 mm Filter ware unit to give the final water extract. Ten-millilitre aliquots from each plant part extract were mixed together to constitute whole-plant extracts.

Seed bioassays

Hundred wild barley seeds were surface sterilized with water : bleach solution (10 : 1) and were placed evenly on filter paper in sterilized 9 cm Petri dishes. Ten millilitres of extract solution from each plant part was added to Petri dishes and distilled water was used as a control. All Petri dishes were placed in a lighted room at 25 ºC. Treatments (extracts from the various plant parts and the distilled water control) were arranged in a completely randomized design with four replications. After 7 days, germination was determined by counting the number of germinated seeds and expressed as total percentage. Radicle and hypocotyl lengths were determined after 78 days by measuring 24 representative seedlings. After measuring the radicle and hypocotyl lengths, the seedlings were separated into hypocotyl and radicle parts. The plants were then dried and their respective dry weights recorded.

Water uptake by seeds

One-gram samples of wild barley seeds were soaked for 4, 8, 12 and 16 h in sunflower leaf water extracts at concentrations of 4, 8, 12, 16 and 20 g per 100 ml of water. Distilled water was used as the control. Treatments were arranged in a completely randomized design with four replications. After soaking, seeds were taken from the solution, blotted for 2 h and weighed. Water uptake was calculated by subtracting the original seed weight from the final seed weight and expressed in milliliters.

Statistical analyses

All experiments were repeated twice and pooled mean values were separated using least significant differences (LSD) at the 0.05 probability level following an analysis

ofvariance; except for the experiment investigating the effects of preceding crops, for which t-tests were used.
Statistical analyses were made with the MSTAT statistical program (Michigan State University, East Lansing, MI).

Results and Discussions

Greenhouse experiments

Effects of preceding crops

Growth of sunflower, as indicated by plant height and fresh weight per plant 35 days after planting, was significantly reduced in soil previously cropped to sunflower compared with that cropped to wild barley (Table 1). However, the preceding crop did not affect sunflower germination. In the case of wild barley, differences in germination percentage, plant height and fresh weight per plant caused by preceding crops were all significant. All variables were significantly lower when the preceding crop was sunflower than when it was wild barley. These results suggest that sunflower has a long-term potential to reduce the growth of plants from other (i.e. allelopathicity) or the same species (i.e. autotoxicity). Other species, e.g. alfalfa (Medicago sativa L.), have both allelopathic and autotoxic potentials (Chung and Miller 1995, Chon and Kim 2002).

Effects of residue incorporation

Wild barley germination percentage, plant height and dry weight per plant 35 days after planting were all significantly lower with fresh sunflower or wild barley residue incorporation than the controls, suggesting the presence of short-term allelopathic and autotoxic effects (Table 2). However, germination and growth inhibition of wild barley were 16–28 % greater with sunflower than with wild barley incorporation. Allelopathicity and autotoxicity were also greater when whole plants were incorporated than when roots only were incorporated. This response could be attributable to a greater contribution of allelochemicals from leaves or simply to the greater amount of residues incorporated with whole plants.

Laboratory experiment

Germination

Extracts from fresh sunflower leaves, stems, flowers, roots and their mixture greatly inhibited wild barley seed germination at all concentrations when compared with a water control (Table 3).

Germination reductions ranged between 12 and 67 %. The degree of inhibition increased for all

tissues with increase in extracts concentration from 4 to 20 g per 100 ml of water. Plant parts varied in their allelopathicity to wild barley germination.

Leafextracts had the greatest allelopathic potential at all concentrations and stems the lowest. Leaf extract reduced germination by 34, 48, 53, 59 and 64 % at concentrations of 4, 8, 12, 16 and 20 g per 100 ml of water, respectively. These results are in accordance with other studies that reported that allelopathicity may vary among plant parts (e.g. Chon and Kim 2002, Economou et al. 2002) and in accordance with data of Turk and Tawaha (2002), who reported that sunflower leaves had the greatest inhibitory effect on lentil (Lens culinaris Medik.).

Seedling length

All extracts, except that from stems, significantly reduced hypocotyl length at all concentrations when compared with the water control (Table 4). Reductions ranged between 7 and 46 %. Hypocotyl length was not affected by stem extracts at any concentrations. For all other extracts, allelopathicity increased with increases in concentrations. At all concentrations, reduction was greatest with leaf extracts compared with extracts from other parts.

Radicle length appeared more sensitive to allelochemicals than was hypocotyl length. These results are in agreement with the finding that water extracts of allelopathic plants generally have more pronounced effects on radicle, rather than hypocotyl, growth (Chung and Miller 1995,Turk and Tawaha 2002). This may be attributable to the fact that radicles are the first to come in contact with allelochemicals. Extracts from all plant parts caused a marked reduction in radicle length of wild barley seedlings, ranging between 11 and 55 % when compared with the water control. Again, allelopathicity increased with an increase in extract concentration of all plant parts and was greatest with leafextracts. Radicle length inhibition was lowest with root extracts. Besides the inhibition of radicle elongation, many of the extracts also altered radicle morphology, appearing distorted and twisted when compared with the control seedlings. Allelochemicals also affect root morphology in the alfalfa autotoxic response (Jennings and Nelson 2002).

Seedling weight

All sunflower extracts caused a marked reduction in wild barley hypocotyl dry weight at all concentrations when compared with the water control, ranging between 30 and 77 % (Table 5). For all tissues, hypocotyl dry weight also decreased as the extract concentration increased. Leaf extracts were again the most inhibitory at all concentrations compared with the water control, and reduced hypocotyl dry weight by 58, 64, 68, 72 and 76 % at concentrations of 4, 8, 12, 16 and 20 g 100 ml per water, respectively. The response of wild barley radicles was similar to that of hypocotyls, although inhibition was somewhat lower, sunflower extracts causing weight reductions ranging between 5 and 58 %.

Water uptake by seeds

Increasing the concentration of water extracts from leaves significantly inhibited water uptake by wild barley seeds (Table 6). For all soaking times, the greatest inhibition in water uptake when compared with the water control occurred at the 20 g per 100 ml ofwater concentration, averaging 57 %. These results suggest that allelopathicity of sunflower may be mediated in part through a regulation of water uptake and inhibition of seeds. This could be due to a reduction of seed protease activity, which plays a key role in protein hydrolysis during germination, and which is to a large extent related to water imbibition and water uptake of seeds (Rice 1984).

Conclusions

In these studies, sunflower demonstrated short- and long-term harmful allelopathic effects on wild barley, including reduced seed germination and reduced seedling growth. Overall, the allelopathic potential of sunflower on wild barley germination and seedling growth increased with

increased concentration and varied among tissues ranking from the most allelopathic to the least in the following order: leaves, flowers, mixture of all tissues, stems and

roots, although this order varied slightly depending on the growth variable under consideration. The inhibitory substances present in sunflower plants causing this allellopathicity could be used as a potential natural herbicide resource, but they must first be identified and their mode ofaction studied.

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TABLES

Table 1: Germination and growth of wild barley and sunflower 35 days after planting in soils previously grown with sunflower or wild barley

	sunflower			wild barley		
Soil	Germination (%)	Plant height (cm)	Fresh weight per plant (g)	Germination (%)	Plant height (cm)	Fresh weight per plant (g)
Sunflower	68.0	6.1	0.5	81.3	22.0	0.77
wild barley	64.1	7.3	0.12	94.0	29.6	1.24
t-test	ns	*	*	*	*	*

ns, not significantly different ($P > 0.05$). *Significantly different at $P < 0.01$.

Table 2: Wild barley seed germination, plant height and weight 35 days after planting as affected by species and tissues incorporated into soil

Tissue incorporated	Species incorporated		
	sunflower	Wild barley	LSD (0.05)
Germination (%)			
None (control)	91.0	96.5	4.8
Roots only	63.1	71.8	5.6
Whole plant	44.7	66.0	4.3
LSD (0.05)	5.6	4.7	
Plant height (cm)			
None control)	41.1	38.7	ns
Roots only	22.3	24.3	2.4
Whole plant	14.0	17.6	3.0
LSD (0.05)	4.6	3.8	
Plant dry weight (g)			
None (control)	1.42	1.35	ns
Roots only	0.77	1.2	0.17
Whole plant	0.62	0.87	0.22
LSD (0.05)	0.21	0.17	

LSD, least significant differences; ns, not significantly different ($P > 0.05$).

Table 3: Effect of the concentrations of water extracts made from various sunflower plant parts on the germination of wild barley seeds

Tissues extracted	Concentration (g per 100 ml of water)					LSD (0.05)
	4	8	12	16	20	
Germination (%)						
Leaves	55	48	40	35	31	3.0
Stems	88	80	80	72	68	2.8
Flowers	65	56	51	50	45	3.9
Roots	77	70	66	67	67	2.0
Mixture	70	67	60	65	54	3.1
LSD (0.05)	4.0	4.4	3.2	4.0	4.8	

LSD, least significant differences. Water control = 98. The mixture consisted in mixing equal parts ofleaf , stem, flower and root extracts

Table 4: Effects ofthe concentration ofwate r extracts made from various sunflower plant parts on the hypocotyl and radicle length of 7-day- old wild barley seedlings

	Concentration (g per 100 ml of wat er)					LSD
Tissues extracted	4	8	12	16	20	(0.05)
Hypocotyl length (cm)						
Leaves	3.6	3.5	3.2	3.0	2.6	0.3
Stems	5.1	4.8	4.7	4.5	4.3	ns
Flowers	4.1	3.9	3.6	3.3	2.9	0.3
Roots	4.8	4.5	4.2	3.7	3.3	0.4
Mixture	4.6	4.1	3.6	3.3	3.0	0.2
LSD (0.05)	0.2	0.3	0.3	0.2	0.2	
Radicle length (cm)						
Leaves	3.6	3.1	2.8	2.6	2.5	0.3
Stems	5.1	4.8	4.5	4.1	3.8	0.4
Flowers	4.2	3.8	3.6	3.3	3.0	0.3
Roots	5.6	5.2	4.8	4.5	4.3	0.2
Mixture	4.5	4.2	3.8	3.5	3.1	0.3
LSD (0.05)	0.2	0.2	0.3	0.1	0.3	

LSD, least significant differences; ns, not significant.
Water control hypocotyl = 4.6. Water control radicle = 5.7. The mixture consisted in
mixing equal parts of leaf , stem, flower and root extracts.

Table 5: Effects of the concentration of water extracts made from various sunflower and plant parts on the hypocotyl and radicle dry weight of 7-day- old wild barley seedlings

	Concentration (g per 100 ml of water)					LSD
Tissues extracted	4	8	12	16	20	(0.05)
Hypocotyl weight (mg)						
Leaves	0.63	0.58	0.55	0.50	0.45	0.05
Stems	1.40	1.33	1.30	1.27	1.23	0.06
Flowers	1.10	1.00	0.97	0.94	0.91	0.04
Roots	1.20	1.03	0.99	0.95	0.93	0.03
Mixture	0.90	0.86	0.84	0.81	0.78	0.04
LSD (0.05)	0.04	0.05	0.04	0.03	0.04	
Radicle weight (mg)						
Leaves	0.51	0.47	0.45	0.41	0.38	0.03
Stems	0.73	0.70	0.67	0.64	0.61	0.05
Flowers	0.64	0.61	0.58	0.55	0.54	0.05
Roots	0.86	0.82	0.79	0.75	0.73	0.04
Mixture	0.77	0.74	0.70	0.67	0.65	0.03
LSD (0.05)	0.03	0.03	0.04	0.06	0.03	

LSD, least significant differences.
Water control hypocotyl = 1.90. Water control radicle = 0.95. The mixture consisted in
mixing equal parts of leaf , stem, flower and root extracts.

Table 6: Water uptake by wild barley seeds soaked in sunflower leafwater extract at different concentrations

Soaking time (h)	Concentration (g per 100 ml of water)						LSD (0.05)
	0	4	8	12	16	20	
4	1.38	0.91	0.80	0.71	0.59	0.50	0.02
8	1.24	0.88	0.82	0.74	0.68	0.52	0.04
12	1.33	0.94	0.89	0.81	0.65	0.61	0.03
16	1.54	0.95	0.88	0.84	0.62	0.63	0.05
LSD (0.05)	0.08	0.6	0.08	0.03	0.02	0.04	

LSD, least significant differences.