Journal of Horticultural Science & Ornamental Plants 2 (1): 32-37, 2010
ISSN 2079-2158

Allelopathic Effect of *Helianthus annuus* (Sunflower) on *Solanum nigrum* (Black Nightshade) Seed Germination and Growth in Laboratory Condition

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Abstract: A greenhouse and laboratory experiments were conducted to determine the effects on Black Nightshade (*Solanum nigrum*) germination and seedling growth of preceding crops, fresh Sunflower residue incorporation and Sunflower leaf, stem and root water extract concentrations. Sunflower [*Helianthus annuus* (L.) Koch.] contains watersoluble allelochemicals that inhibits the germination and growth of other species. This characteristic could be used in weed management programmes. Growth of *S. nigrum* , as indicated by plant height and weight, was significantly reduced when grown in soil previously cropped to Sunflower compared with that cropped to *S. nigrum.* Soil incorporation off fresh Sunflower roots and both roots and shoots reduced *S. nigrum* germination, plant height and weight when compared with a no-residue control. In bioassays, Sunflower extracts reduced *S. nigrum* hypocotyl length, hypocotyl weight, radicle weight, seed germination and radicle length by as much as 56, 64, 61, 77 and 81%, respectively, when compared with a water control. Increasing the water extract concentrations from 5 to 25 g per 100 ml of water of all Sunflower parts significantly increased the inhibition of *S. nigrum* germination, seedling length and weight. Based on 8-day-old *S. nigrum* radicle length, averaged across all extract concentrations, the degree of toxicity of different Sunflower plant parts can be ranked in the following order of inhibition: leaves > flowers > mixture of all plant parts > stems > roots.

Key words: Phytotoxicity effect · Sunflower - *Helianthus annuus* (L.) · Black Nightshade · *Solanum nigrum* · Germination inhibition

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 [1-4]. Several phytotoxic substances causing germination and/or growth inhibitions have been isolated from plant tissues and soils [5, 6]. These substances, collectively known as allelochemicals. They are usually secondary plant products or waste products of main metabolic pathways of plants [1, 4, 5, 7, 8, 9]. Sunflower is well known for its allelopathic compounds. Several phenols and terpenes have been reported in various cultivars of Sunflower [1, 10- 13]. Ben-Hammouda *et al.* [7] reported that this same cultivar of Sunflower was autotoxic to other cultivars of Sunflower, though not to it [7, 14]. Leaves were the most important source of allelopathic substances. This same cultivar of Sunflower was also found to be phytotoxic to durum wheat (*Triticum durum*) and bread wheat (*T. aestivum*). Seedling growth bioassays demonstrated that the two wheat species responded differently to the allelopathic potential of Sunflower with a greater sensitivity shown by the bread wheats [15- 17]. For both wheat species, radicle growth was more depressed than coleoptile growth, though stimulation of seedling growth was observed for durum wheat. Leaves and roots were the most phytotoxic Sunflower plant parts for durum and bread wheats, respectively. Results suggested that the response by durum wheat and bread wheat varied depending on the source of allelochemicals (i.e. plant part) and the growth stage of the Sunflower plant. Consequently, Sunflower should be considered a depressive prior crop for both durum wheat and bread wheat in a field cropping sequence. However, studies with other species have reported that the response to allelochemicals may be concentration dependent. 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 [1, 18- 21]. It is thus essential

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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 [15, 22-25]. 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 *S. nigrum*, a problematic weed in Mideast regions. The objectives were to determine the effects of preceding crops on germination and seedling growth of *S. nigrum*, fresh Sunflower residue incorporation on early growth of *S. nigrum* and the effects of water extract concentration of various Sunflower parts on *S. nigrum* 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 *S. nigrum* in soils from fields in center Iran (Eslamshahr, Tehran state) cropped in the previous season with either species, to assess the existence of long-term allelopathicity of Sunflower. Ten *S. nigrum* seeds were planted in pots (150 mm wide and 150 mm high) each containing soil (loam) from adjacent fields previously cropped either to *S. nigrum* (S. nigrumsoil) or Sunflower (Sunflower soil). Each treatment, *L. holosteoide* sgrown in *S. nigrum* soil and *S. nigrum* grown in Sunflower soil, was replicated eight times and arranged in a completely randomized design. A similar experiment was performed with Sunflower, planting five seeds per replicate pot. Plants were grown at constant temperature (26 °C) 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 *S. nigrum* whole plants or roots only on *S. nigrum* were studied to test for the existence of short-term Sunflower allelopathicity. Treatments were designed in a 2 × 3 factorial assigned to a randomized complete block design with four replications. Treatment combinations included source of residues (Sunflower or *S. nigrum*) and type of residues incorporated [whole plants, roots only or no residue (control)]. Ten Sunflower or *S. nigrum* plants were grown for 30 days in pots (170 × 165 mm) kept under greenhouse condition. 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 *S. nigrum* 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 center Iran (Tehran state) during the 2007-2008 growing season. Fresh Sunflower plants were separated into leaves, stems, roots and flowers. Tissues from each plant part were soaked in distilled water for 24 h at 25 °C 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 *S. nigrum* 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 °C. 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 *S. nigrum* 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.

Table 1: Germination and growth of *Solanum nigrum* and Sunflower 35 days after planting in soils previously grown with Sunflower or *S. nigrum*

	Sunflower			*S. nigrum*		
Soil	Germination(%)	Plantheight (cm)	Fresh weightper plant (g)	Germination(%)	Plantheight (cm)	Fresh weightper plant (g)
Sunflower	68.0	6.1	0.5	81.3	22.0	0.77
wild oat	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.

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 of variance; 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: Growth of Sunflower, as indicated by plant height and fresh weight of 35 days grown plant, was significantly reduced in soil previously cropped to Sunflower compared with that cropped to *S. nigrum* (Table 1). However, the preceding crop did not affect Sunflower germination. In case of *S. nigrum*, 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 *S. nigrum*. 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 [14, 15].

Effects of Residue Incorporation: *Solanum nigrum* germination percentage, plant height and dry weight of 35 days grown plant were all significantly lower with fresh Sunflower or *S. nigrum* residue incorporation than the controls, suggesting the presence of short-term allelopathic and autotoxic effects (Table 2). However, germination and growth inhibition of *S. nigrum* were 16-28 % greater with Sunflower than with *S. nigrum* 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.

Table 2: *Solanum. nigrum* seed germination, plant height and weight 35 days after planting as affected by species and tissues incorporated into soil

	Species incorporated		
Tissue incorporated	Sunflower	*S. nigrum*	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 *S. nigrum* seeds

	Concentration (g per 100 ml of water)					
Tissues extracted	4	8	12	16	20	LSD(0.05)
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 of leaf, stem, flower and root extracts

Laboratory Experiment, Germination: Extracts from fresh Sunflower leaves, stems, flowers, roots and their mixture greatly inhibited *S. nigrum* seed germination at all concentrations compared to 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

Table 4: Effects of the concentration of water extracts made from various Sunflower plant parts on the hypocotyl and radicle length of 7-day- old *S. nigrum* seedlings

	Concentration (g per 100 ml of water)					
Tissues extracted	4	8	12	16	20	LSD(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.

S. nigrum germination. Leaf extracts had the greatest allelopathic potential at all concentrations and stems had the lowest. Leaf extracts 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 which reported the allelopathicity may vary among plant parts [14, 23] and in accordance with data of Turk and Tawaha [26], who reported that Sunflower leaves had the greatest inhibitory effect on lentil.

Seedling Length: All extracts, except that of stems, significantly reduced hypocotyl length at all concentrations compared to 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 to 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 [15, 26]. 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 *S. nigrum* seedlings, ranging between 11 and 55 % compared to water control. Again, allelopathicity increased with an increase in extract concentration of all plant parts and was greatest with leaf extracts. 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 compared to control seedlings. Allelochemicals also affect root morphology in the alfalfa autotoxic response [27].

Seedling Weight: All Sunflower extracts caused a marked reduction in *S. nigrum* hypocotyl dry weight at all concentrations compared to 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

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 *S. nigrum* seedlings

	Concentration (g per 100 ml of water)					
Tissues extracted	4	8	12	16	20	LSD(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 *S. nigrum* seeds soaked in Sunflower leaf water extract at different concentrations

	Concentration (g per 100 ml of water)						
Soaking time (h)	0	4	8	12	16	20	LSD(0.05)
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.

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 *S. nigrum* 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 leaf extracts significantly inhibited water uptake by *S. nigrum* 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 of water 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 imbibitions and water uptake of seeds.

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