



REGULAR ARTICLE

RESPONSE OF GERMINATION AND GROWTH OF THORN-APPLE (*DATURA STRAMONIUM* L.) SEEDLINGS TO ALLELOPATHIC EFFECTS OF WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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SUMMARY

Greenhouse and laboratory experiments were conducted to determine the allelopathic effects of Winter wheat (*Triticum aestivum* L.) on Thorn-apple (*Datura stramonium* L.) germination and seedling growth. Growth of *D. stramonium*, as indicated by plant height and weight, was significantly reduced when grown in soil previously cropped to Winter wheat compared with that cropped to *D. stramonium*. Soil incorporation of fresh Winter wheat roots and both roots and shoots reduced *D. stramonium* germination, plant height and weight when compared with a no-residue control. In bioassays, Winter wheat extracts reduced *D. stramonium* hypocotyl length, hypocotyl weight, radicle weight, seed germination, and radicle length. Increasing the water extract concentrations from 4 to 20 g per 100 ml of water of all Winter wheat parts significantly increased the inhibition of *D. stramonium* germination, seedling length and weight. Based on 6-day-old *D. stramonium* radicle length, averaged across all extract concentrations, the degree of toxicity of different Winter wheat plant parts can be ranked in the following order of inhibition: leaves > flowers > mixture of all plant parts > stems > roots.

Keywords: allelopathy, Winter wheat (*Triticum aestivum* L.), Thorn-apple (*Datura stramonium* L.), seed, germination and growth.

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1. 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, 1995). 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 (Rice 1995, Whittaker and Feeny 1977, Hall and Henderlong 1989, Chon and Kim 2002; Ashrafi et

al. 2007). Winter wheat is well known for its allelopathic compounds. Several phenols and terpenes have been reported in various cultivars of Winter wheat (Spring et al. 1992; Ben-Hammouda et al. 2001) Ashrafi et al. 2008 reported that this same cultivar of Winter wheat was autotoxic to other cultivars of Winter wheat, though not to itself. Leaves were the most important source of allelopathic substances. This same cultivar of Winter wheat was also found to be phytotoxic to durum wheat (*Triticum durum*) and bread wheat (*T. aestivum*).

Leaves and roots were the most phytotoxic Winter wheat plant parts for durum and bread wheat, respectively. 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; Ashrafi et al. 2007). Information about the allelopathic potential of the flora of Mediterranean regions remains scarce.

The present study was conducted to determine the allelopathic potential of Winter wheat towards *D. stramonium*, a problematic weed in Mediterranean regions. The objectives of the present study were to determine the effects of (1) preceding crops on germination and seedling growth of *D. stramonium*, (2) fresh Winter wheat residue incorporation on early growth of *D. stramonium*, and (3) the effects of water extract concentration of various Winter wheat parts on *D. stramonium* seed germination and seedling growth.

2. Materials and Methods

Greenhouse experiments on the Effects of preceding crops

The effects of preceding crops were studied by growing Winter wheat and *D. stramonium* in soils from fields in Central Iran (Tehean state) cropped in the previous season with either species, to assess the existence of long-term allelopathicity of Winter wheat. Ten *D. stramonium* seeds were planted in pots (150 mm wide and 150 mm high) each containing soil (loam) from adjacent fields previously cropped either to *D. stramonium* (Thorn-apple soil) or Winter wheat (Winter wheat soil). Each treatment, *D. stramonium* grown in *D. stramonium* soil and *D. stramonium* grown in Winter wheat soil, was replicated eight times and arranged in a completely randomized design. A similar experiment was performed with Winter wheat, 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 Winter wheat or *D. stramonium* whole plants or roots only on *D. stramonium* were studied to test for the existence of short-term Winter wheat 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 (Winter wheat or *D. stramonium*) and type of residues incorporated [whole plants, roots only or no residue (control)]. Ten Winter wheat or *D. stramonium* 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 *D. stramonium* seeds were planted in each pot, including control pots. Germination, plant height and dry weight were recorded 30 days after planting.

Winter wheat plants were collected from fields in central Iran (Tehran state) during the 2006–07 growing season. Fresh Winter wheat 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 *D. stramonium* 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 *D. stramonium* seeds were soaked for 4, 8, 12 and 16 h in Winter wheat 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 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).

3. Results and Discussions

Greenhouse experiments

Growth of Winter wheat, as indicated by plant height and fresh weight of 35 days grown plant, was significantly reduced in soil previously cropped to Winter wheat compared with that cropped to *D. stramonium* (Table 1). However, the preceding crop did not affect Winter wheat germination. In case of *D. stramonium*, 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 Winter wheat than when it was *D. stramonium*. These results suggest that Winter wheat has a long-term potential to reduce the growth of plants from other (i.e. allelopathicity) or the same species (i.e. autotoxicity).

Table 1: Germination and growth of *D. stramonium* and Winter wheat 35 days after planting in soils previously grown with Winter wheat or *D. stramonium*

Soil	Winter wheat			<i>D. stramonium</i>		
	Germination (%)	Plant height (cm)	Fresh weight per plant (g)	Germination (%)	Plant height (cm)	Fresh weight per plant (g)
Winter wheat	68.0	6.1	0.5	81.3	22.0	0.77
<i>D. stramonium</i>	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.

Effects of residue incorporation

D. stramonium germination percentage, plant height and dry weight of 35 days grown plant were all significantly lower with fresh Winter wheat or *D. stramonium* residue incorporation than the controls, suggesting the presence of short-term allelopathic and autotoxic effects (Table 2). However, germination and growth inhibition of *D. stramonium* were 16–28 % greater with Winter wheat than with *D. stramonium* 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 Winter wheat leaves, stems, flowers, roots and their mixture greatly inhibited *D. stramonium* seed germination at all concentrations compared to water control (Table 3). Table 3: Effect of the concentrations of water extracts made from various Winter wheat plant parts on the germination of *D. stramonium* 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 of leaf, stem, flower and root extracts

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 *D. stramonium* germination. Leaf extracts had the greatest allelopathic potential at all concentrations and stems had 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 which reported the allelopathicity may vary among plant parts (e.g. Chon and Kim 2002, Economou et al. 2002). Table 2: *D. stramonium* seed germination, plant height and weight 35 days after planting as affected by species and tissues incorporated into soil

Tissue incorporated	Species incorporated		LSD (0.05)
	Winter wheat	<i>D. stramonium</i>	
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).

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 (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 *D. stramonium* 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. 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 (Jennings and Nelson 2002).

Table 4: Effects of the concentration of water extracts made from various Winter wheat plant parts on the hypocotyl and radicle length of 7-day-old *D. stramonium* seedlings

Tissues extracted	Concentration (g per 100 ml of water)					LSD (0.05)
	4	8	12	16	20	
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.

Seedling weight

All Winter wheat extracts caused a marked reduction in *D. stramonium* 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 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 *D. stramonium* radicles was similar to that of hypocotyls, although inhibition was somewhat lower; Winter wheat extracts causing weight reductions ranging between 5 and 58 %.

Table 5: Effects of the concentration of water extracts made from various Winter wheat and plant parts on the hypocotyl and radicle dry weight of 7-day-old *D. stramonium* seedlings

Tissues extracted	Concentration (g per 100 ml of water)					LSD (0.05)
	4	8	12	16	20	
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.

Water uptake by seeds

Increasing the concentration of water leaf extracts significantly inhibited water uptake by *D. stramonium* seeds (Table 6).

Table 6: Water uptake by *D. stramonium* seeds soaked in Winter wheat leaf water 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.

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 Winter wheat 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).

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