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Inhibitory Effects of Aqueous Extracts from Black Mustard (*Brassica nigra* L.) on Germination and Growth of Wheat

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Abstract: Aqueous extracts of *Brassica nigra* leaf, stem, flower and root plant part were made to determine their effects on germination, dry weights of hypocotyl and total length of 5 days old wheat seedlings over a range of concentrations. Increasing the aqueous extract concentrations of separated *Brassica nigra* plant parts significantly inhibited the wheat germination, seedling length and weight. Radicle length was more sensitive to extract source than seed germination or hypocotyl length. Based on 5-d-old wheat plant radicle length growth, averaged across all extract concentrations, the degree of toxicity of different *Brassica nigra* plant parts can be classified in order of decreasing inhibition as follows: leaf, flower, mixture of all plant parts, root and stem.

Key words: Black mustard, *Brassica nigra* L., wheat, germination, seedling length, allelopathy

Introduction

Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment (Brown *et al.*, 1991). Many of the phytotoxic substances, suspected of causing germination and growth inhibition have been identified from plant tissues and soils. These substances are termed as allelochemics (Whittaker and Feeny, 1971) or, more commonly, allelochemicals. These are the waste products of main metabolic pathways in plants. These may be water-soluble substances that are released into the environment through leaching, root exudation, volatilization and decomposition of plant residues. Most research on allelopathy has focussed on the effect of interactions among weed species (Narwal, 1994) weeds and crops (Rice, 1984) and crop species (Hedge and Miller, 1990). *Brassica* species is a wild plant, which naturally grows on the plains and hilly areas of North Jordan and neighbouring countries. In North America and Europe, *Brassica* species are important oil seed crops, and have potential for use as green manure (Grodzinsky, 1992). Members of Brassicaceae have frequently been cited as allelopathic crops (Bell and Muller, 1973). Some *Brassica* species have harmful effects on crops including reduced seed germination and emergence of subsequent small-grain crops when grown in rotation (Bialy *et al.*, 1990; Muehlchn *et al.*, 1990). Benzyl-ITC, a breakdown product of white mustard (Josefsson, 1968; Tollsten and Bergstrom, 1988) was phytotoxic to velvet leaf, sicklepod (*Senna obtusifolia* L. formerly *Cassia obtusifolia* L.), and sorghum [*Sorghum bicolor* (L.) Moench]. Other breakdown products of glucosinolate like ionic thiocyanate (SCN-) inhibited the root or shoot growth of many crop species (Brown *et al.*, 1991). Volatile isoprenoid and benzenoid compounds released from *Brassica* tissue degradation may suppress weed growth (Tollsten and Bergstrom, 1988). It was also found that allelochemicals, which inhibited the growth of some species at certain concentrations, might stimulate the growth of same or different species at lower concentration (Narwal, 1994). The stimulatory (negative) allelopathic compounds of any plant on the other plant can be used to develop ecofriendly, cheap and effective, 'Green growth Promoter's' (Oudhia *et al.*, 1988). Allelopathy is a relatively new branch of science and not much work has been done on allelopathic potential of Jordanian flora. The present research was conducted to evaluate the effects of aqueous extracts from various plant parts of black mustard (*Brassica nigra* L.) on wheat seed germination and seedling growth.

Materials and Methods

General procedure: Black mustard was collected from the plains and hilly areas of North Jordan during 2000/ 2001 growing season. The experiment was carried out at the Crop Production Department, Faculty of Agriculture, Jordan University of Science and Technology (JUST), Irbid, Jordan, from March to July 2001.

Plant sampling and preparation of extracts: Fresh plants were separated into leaves, stems, roots and flowers for vegetative stage. Fresh tissue from each plant part was soaked in distilled water for 24 h at 24 °C in a lighted room to give concentrations of 4, 8, 12 and 16 g kg^{-1} of tissue per 100mL of water. These solutions were filtered through four layers of cheesecloth and centrifuged at 3000-revolution min^{-1} for 4 h. the supernatant was filtered again using a 0.2 mm filterware unit. Ten milliliter aliquot from each plant part extract were mixed together to give whole-plant extracts.

Seed bioassay: Germination tests were conducted for each plant part extract as follows: 100 wheat seeds (c.v. Hourani) were surface- sterilized with 10:1 water/ bleach (5.25% w/v NaOCl) solution and were evenly placed on filter paper in sterilized 9-cm Petri dishes. Ten milliliters of extract from each plant part were added to each Petri dish and distilled water was used as a control. All petri dishes were placed in a lighted room at 24 °C. Germination was determined by counting the germinated seeds at 24-h intervals over a 4 days period and expressed as total percent germination. Radicle and hypocotyl lengths were determined 5-d after seeding by measuring 24 representative seedlings. After measuring the radicle and hypocotyle lengths, the seedlings were separated into hypocotyle and radicle parts for measuring dry weight. The duration of the experiment was four months.

Experimental design and statistical analysis: Germination and seedling growth bioassays were conducted in a complete randomized design (CRD) with four replications. The experiments were repeated twice and the pooled mean values were separated on the basis of least significant difference (LSD) at 0.05 probability level.

Results and Discussion

Germination percentage: Extracts from fresh black mustard leaves, stems, flowers, roots and their mixture solutions showed inhibitory effects on wheat seed germination (Table I). The degree of inhibition increased with the increased extract concentration. At the highest concentration (16 g kg^{-1}), all aqueous extracts significantly reduced seed germination compared with the distilled water control (Table I). This finding is supported by Chung and Miller (1995), who found that, degree of inhibition is increased with the increase in extract concentration. Mixture extracts of all plant parts were the most inhibitory at all concentrations, and the extract of stem was the least inhibitory. The results found in this study are congruency with data of Bell and Muller (1973), but inconsistent with Ballester *et al.* (1979), who reported the most inhibitory effect of allelopathic plants, by flower extracts. The

Table 1: Influence of various concentrations of different aqueous extracts made from *Brassica nigra* plant parts on proportion of germinated wheat seeds after 5 days of inhibition at 24 °C

Plant parts	% germination seeds, by extract conc., g kg^{-1}				LSD (0.05)
	4	8	12	16	
Leaf	68.3	60.5	57.3	54.3	3.1
Stem	92.5	88.0	84.3	80.1	3.7
Flower	63.5	59.3	55.5	52.4	2.9
Root	88.5	83.0	80.1	77.1	2.8
Mixture	60.5	58.5	54.3	50.1	3.1
Control= 98.5%					
LSD (0.05)	3.0	3.7	3.1	3.0	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing of equal parts from leaf, stem, flower, and root extracts prepared the mixture.

Table 2: Influence of various concentrations of different aqueous extracts made from *Brassica nigra* plant parts on the hypocotyl length (cm) of 5-d old wheat seedlings

Plant parts	Hypocotyl length by extract conc., g kg^{-1}				LSD (0.05)
	4	8	12	16	
Leaf	3.4	3.2	3.0	2.8	0.1
Stem	4.0	4.0	3.5	3.2	0.2
Flower	3.3	3.2	2.9	2.7	0.2
Root	3.6	3.5	3.3	3.2	0.2
Mixture	3.4	3.2	3.1	3.0	0.1
Control= 4.1 cm					
LSD(0.05)	0.2	0.2	0.1	0.1	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing of equal parts from leaf, stem, flower, and root extracts prepared the mixture.

Table 3: Influence of various concentrations of different aqueous extracts made from *Brassica nigra* plant parts on the radicle length (cm) of 5-d old wheat seedlings

Plant parts	Radicle length by extract conc., g kg^{-1}				LSD (0.05)
	4	8	12	16	
Leaf	2.5	2.4	2.1	1.8	0.1
Stem	2.9	2.8	2.6	2.5	0.1
Flower	2.6	2.4	2.2	1.9	0.2
Root	2.8	2.8	2.6	2.4	0.1
Mixture	2.6	2.6	2.4	2.2	0.2
Control = 3.2 cm					
LSD(0.05)	0.1	0.2	0.2	0.1	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing of equal parts from leaf, stem, flower, and root extracts prepared the mixture.

Table 4: Influence of various concentrations of different aqueous extracts made from *Brassica nigra* plant parts on the total length (cm) of 5-d old wheat seedlings

Plant parts	Total length by extract conc., g kg^{-1}				LSD (0.05)
	4	8	12	16	
Leaf	5.8	5.1	4.5	3.4	0.6
Stem	7.1	6.5	5.3	4.0	0.5
Flower	5.9	5.4	4.4	3.3	0.5
Root	6.5	6.2	6.0	4.2	0.4
Mixture	6.1	5.3	4.7	4.0	0.6
Control = 9.8 cm					
LSD(0.05)	0.4	0.4	0.3	0.3	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing of equal parts from leaf, stem, flower, and root extracts prepared the mixture.

degree of reduction increased as the extract concentration progressively increased from 4 to 16 g kg^{-1} (Table I). The effect of leaf extracts was statistically similar to those of flower extracts at 8, 12 and 16 g kg^{-1} concentrations. Mixture extract reduced the proportion of germinated seeds by 38.0, 40.0, 44.2, and 48.4 % as compared with control, at 4, 8, 12, and 16 g kg^{-1} concentrations respectively (Table I).

Table 5: Influence of various concentrations of different aqueous extracts made from *Brassica nigra* plant parts on the dry weight (g) of the hypocotyl 5-d old wheat seedlings

Plant parts	Hypocotyl dry wt., by extract conc., g kg^{-1}				LSD (0.05)
	4	8	12	16	
Leaf	0.85	0.81	0.73	0.71	0.03
Stem	0.96	0.95	0.85	0.78	0.04
Flower	0.83	0.78	0.70	0.68	0.05
Root	0.89	0.88	0.82	0.79	0.04
Mixture	0.83	0.81	0.74	0.78	0.03
Control = 1.01g					
LSD(0.05)	0.03	0.06	0.04	0.04	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing of equal parts from leaf, stem, flower, and root extracts prepared the mixture.

Table 6: Influence of various concentrations of different aqueous extracts made from *Brassica nigra* plant parts on the total dry weight (g) of 5-d old wheat seedlings

Plant parts	Total dry wt., by extract conc., g kg^{-1}				LSD (0.05)
	4	8	12	16	
Leaf	1.45	1.30	1.13	0.85	0.08
Stem	1.80	1.65	1.31	0.99	0.07
Flower	1.50	1.37	1.10	0.83	0.08
Root	1.63	1.57	1.50	1.06	0.06
Mixture	1.53	1.36	1.18	1.03	0.09
Control = 1.94 g					
LSD(0.05)	0.06	0.05	0.04	0.07	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing of equal parts from leaf, stem, flower, and root extracts prepared the mixture.

Hypocotyl length: Hypocotyl length was not affected by stem extracts at 4 and 8 g kg^{-1} concentrations and slightly decreased by 14.6 and 21.9 % at 12 and 16g kg^{-1} concentration, respectively (Table 2). Extract from different allelopathic plant parts showed significant differences in phytotoxicity to wheat seedling (Ben-Hammouda *et al.,* 1995). At 16 g kg^{-1} concentration, the flower and leaf extracts caused the greatest reduction in hypocotyl length (31.7 and 34.1 % respectively) when compared with other plant part extracts (Table 2). The mixture of all extracts significantly reduced hypocotyl length at all concentrations when compared with control (Table 2). The results of this study add support to previous studies by Chung and Miller (1995), who found that mixture of all the extracts significantly reduced the hypocotyl length at all concentrations when compared with control.

Radicle length: Radicle length was relatively more sensitive to autotoxic allelochemicals than was hypocotyl length (Table 3). These results are in agreement with earlier studies, reporting that effects of water extracts of allelopathic plants were more pronounced on radicle growth than on hypocotyl growth (Kimber, 1973). Such an outcome might be expected, because it is likely that roots are the first to absorb the allelochemicals or autotoxic-compounds from the environment. All extracts caused a marked reduction in radicle length of wheat seedlings (Table 3). An especially high degree of inhibition occurred with leaf and flower extracts at the highest concentrations. Besides inhibiting radicle elongation, other morphological abnormalities as were also induced by many of the extracts twisted radicle growth. The most severely twisted roots were observed in seedlings treated with leaf and flower extracts. Based on significant radicle length reactions to aqueous extracts, the toxicity may be classified in the following order of decreasing inhibition: leaves, flowers, mixture of all plant parts, root and stem (Table 3). These results are consistent with those of Chung and Miller (1995).

Total seedling length: Total seedling length has been probably viewed as being generally more sensitive to inhibitory compounds than seed germination (Hall and Henderlong, 1989; Hedge and Miller, 1990). Thus, overall seedling growth may be the indicator of sensitivity to allelochemicals (Rietveld, 1983). Total seedling length was more inhibited by the flower extracts at 16 g kg^{-1} concentration than was germination percentage: 66 and 47 %, respectively (Tables 1 and 4). At 4, 8, 12 and 16 g kg^{-1} concentrations, flower extracts inhibited the total seedling length by 40, 45, 55 and 66 %, respectively (Table 4). Comparing all other extractions at 16 g kg^{-1} concentration, total seedling length was more inhibited than germination percentage in descending order respectively: flower, 66 vs.47; leaf 65 vs.45; mixture 59 vs.44; stem 59 vs. 19 and root 57 vs.21 % (Table 4). These results are in agreement with earlier studies reporting that water extracts of allelopathic plants have more pronounced effects on seedling growth than on germination (Hedge and Miller, 1990).

Seedling weight: All aqueous extracts significantly inhibited the wheat seed germination and seedling growth when compared with distilled water (Tables 4 and 1). The flower extracts reduced hypocotyl dry weights significantly more than extracts from other plant parts at all extract concentrations (Table 5). These results are in agreement with those of Chung and Miller (1995), who found that flower extracts did significantly inhibit the seedling growth. Radicle dry weight tended to decrease as the extract concentration increased (data not presented). Compared with the control, total dry weight was significantly inhibited by leaf, flower, stem, root and mixture of all plant parts at all concentrations (Table 6). The leaf extract was the most inhibitory at 4 g kg^{-1} concentration and reduced total dry weight of seedlings by 25 % and the flower extracts at the 16 g kg^{-1} concentration reduced total seedling dry weight by 57%.

It is difficult to apply our results to a production situation directly, because the concentration of inhibitory substances in aqueous extracts is probably greater than what would be observed under natural conditions. Depending on the amount of black mustard (*Brassica nigra* L.) residue and the stage of growth when tillage occurs, allelopathic activity will vary with reseeding of black mustard (*Brassica nigra* L.). Further investigations are also needed to determine the influence of seasonal and cultivar variations, and to identify the active compounds involved in black mustard (*Brassica nigra* L.) autotoxicity and allelopathy.

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