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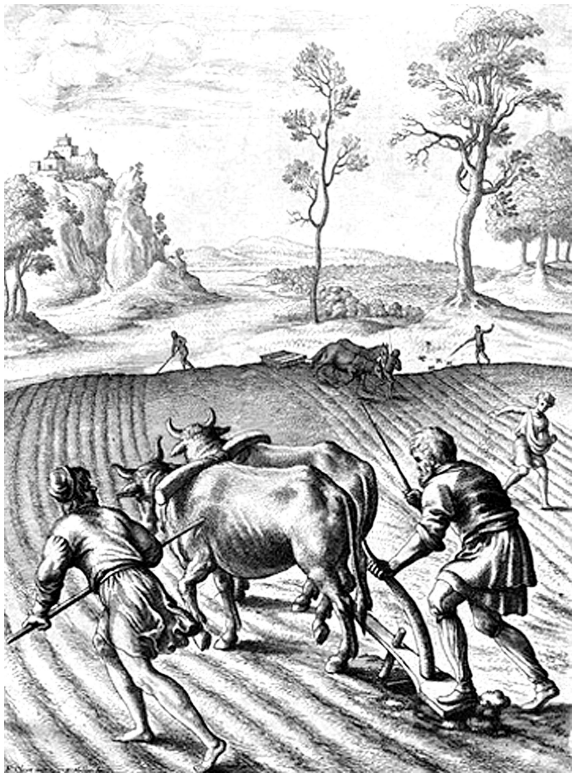

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Inhibitory Effects of Aqueous Extracts of Black Mustard on Germination and Growth of Lentil

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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 and dry weights of hypocotyl, and radicle length of 8-d old lentil seedlings over a range of extract concentrations. Increasing the aqueous extract concentrations of separated *Brassica nigra* plant parts significantly inhibited lentil germination, seedling length and weight. Radicle length was more sensitive to extract source than seed germination or hypocotyl length. Based on 8-d-old lentil 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. 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.

Key words: Allelopathy, *Brassica nigra* L., black mustard, lentil, germination, seedling growth

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 *allelochemics* (Whittaker and Feeny, 1977) or, more commonly, *allelochemicals*. Allelochemicals usually are called secondary plant products or waste products of the 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 focused on the effect of interactions among weed species (Narwal, 1994), weeds and crops (Rice, 1984), and crop species (Hegde and Miller, 1990). *Brassica* species is a wild plant, which naturally grows on the plains and hilly areas of north Jordan and neighboring countries. In North America and Europe, *Brassica* species are important oil seed crop, and have potential for use as green manure crops (Grodzinsky, 1992). Members of Brassicaceae have frequently been cited as allelopathic crop (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). In a natural grassland community, allyl-isothiocyanates (ITC) isolated from black mustard [*Brassica nigra* (L.), W.J.D. Koch] residues inhibited establishment of grass species. Benzyl-ITC, a break-down product of white mustard (Josefsson, 1968; Tollsten *et al*., 1988) was phytotoxic to velvetleaf, 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 compounds like isoprenoid and benzenoid released from *Brassica* tissue degradation may suppress weed growth (Tollsten *et al*., 1988). It was also found in many studies 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 effects 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 relatively a 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 extract concentration of various black mustard (*Brassica nigra* L.) plant parts on lentil 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 black mustard (*Brassica nigra* L.) 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} concentration of tissue per 100mL of water. These solutions were filtered through four layers of cheesecloth to remove the fiber debris and centrifuged at a low speed (3000-revolution min^{-1}) for 4 h. The supernatant was filtered again using a 0.2-mm filterware unit. Ten milliliter aliquots from each plant part extract were mixed together to evaluate whole-plant extracts.

Seed bioassay: Germination tests were conducted for each of the respective plant part extract as follows: 100 lentil seeds (cv. Jordan 1) were surface- sterilized with 10:1 water/ bleach (5.25 5 w/v NaOCl) solution and were evenly placed on filter paper in sterilized 9-cm petri dishes. Ten milliliters of extract solution 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. Treatments were arranged in a completely randomized design with four replications. Germination was determined by counting the number of germinated seeds at 24-h intervals over a 6-d period and expressed as total percent germinated. Radicle and hypocotyl lengths were determined 8-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.

Water uptake: One- gram samples of lentil seeds were soaked for 8, 16, 24 and 48 h in *Brassica nigra* leaf aqueous extracts of 4, 8, 12, and 16g 100 mL^{-1} water. After an 8-h interval, seeds were taken from the solution, blotted for 2 h between two folds of filter paper , and weighed. The water uptake was calculated by subtracting the original seed weight from the final seed weight.

Distilled water was used as the control.

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 (*Brassica nigra* L.) plant leaves, flowers, roots and mixture solutions showed inhibitory effects on seed germination. The degree of inhibition increased with the extract concentration. At the highest extract concentration (16 g kg^{-1}), all aqueous extracts significantly reduced seed germination compared with distilled water control (Table 1). Chang and Miller (1995) support these findings; they found that, degree of inhibition increased with increased extract concentration. Leaf extract was the most inhibitory at all concentrations, while the extract of root was the least inhibitory. The results found in this study are inconsistent with Ballester *et al*. (1979), who reported that the most inhibitory effect of allelopathic plants was produced by flower extracts.
The degree of reduction increased as the extract concentration progressively increased from 4 to 16 g kg^{-1}. The effect of flower extracts was statistically similar to those of leaf extracts at 12 and 16g kg^{-1} concentrations. Mixture extract reduced proportion germinated seed by 8.7, 10.5, 12.8, and 15.9 % as compared with control, at 4, 8, 12, and 16 g kg^{-1} concentrations, respectively.

Hypocotyl length: Hypocotyl length was not affected by stem extracts at 4, 8 and 12g kg^{-1} concentrations and slightly decreased 11.6 % at 16g kg^{-1}concentration. At 16g kg^{-1} concentration, the flower and leaf extracts caused the greatest reduction in hypocotyl length (32.6 and 30.2 % respectively), when compared with other part extracts. The mixture of all extracts significantly reduced hypocotyl length of all concentrations when compared with control. The results of this study support the previous studies by Chung and Miller (1995), who found that mixture of all the extracts significantly reduced hypocotyl length at all concentrations when compared with the control.

Radicle length: Radicle length was relatively more sensitive to autotoxic allelochemicals than was hypocotyl length. These results are in agreement with earlier studies reporting that water extracts of allelopathic plants were more pronounced effects 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 lentil seedlings (Table 3). An especially high degree of inhibition occurred with leaf, flower and mixture of all plant parts extracts at the highest concentrations. Besides inhibiting radicle elongation, other morphological abnormalities occurred as many of the extracts caused twisted radicle growth. The most severely twisted roots were observed in seeding 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: leaf, flower, mixture of all plant parts, root and stem. These results are consistent with those of Chung and Miller (1995).

Seedling weight: All aqueous extracts significantly inhibited lentil seed germination and seedling growth when compared with distilled water control (Tables 1 and 3). Chang and Miller (1995) support this finding. The leaf extracts reduced hypocotyl dry weight significantly more than extracts from other plant parts at 12 and 16g kg^{-1} extract concentrations (Table 4). These results are in agreement with those of Chung and Miller (1995), who found that leaf and flower extracts did significantly inhibit seedling growth. Radicle dry weight tended to decrease as the extract concentration increased (Table 5). Compared with the control,

Table 1: Influence of various concentrations of different aqueous extracts made from *Brassica nigra* plant parts on proportion of germinated lentil seeds after 8 days of inhibition at 24 C°.

	% germination seeds, by extract conc., (g kg^{-1})				
Extracting	4	8	12	16	LSD (0.05)
Leaf	88.0	84.3	80.0	76.5	3.3
Stem	97.0	96.5	96.3	96.0	2.1
Flower	91.5	86.3	80.3	77.0	3.1
Root	96.0	93.0	90.5	88.3	2.2
Mixture	89.0	87.3	85.0	82.0	2.3
Control = 97.5					
LSD (0.05)	3.3	2.0	4.1	3.7	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing 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 of 8-d old lentil seedlings.

	Hypocotyl length by extract conc., (g kg^{-1})				
Extracting	4	8	12	16	LSD (0.05)
Leaf	3.7	3.5	3.3	3.0	0.2
Stem	4.2	4.2	4.1	3.8	0.3
Flower	3.8	3.4	3.2	2.9	0.3
Root	4.3	4.1	4.1	3.9	0.2
Mixture	4.1	4.0	3.8	3.6	0.2
Control = 4.3 cm					
LSD(0.05)	0.2	0.2	0.3	0.2	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing 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 of 8-d old lentil seedlings.

	Radicle length by extract conc., (g kg^{-1})				
Extracting	4	8	12	16	LSD (0.05)
Leaf	2.3	2.1	1.7	1.4	0.2
Stem	2.8	2.8	2.7	2.6	0.2
Flower	2.4	2.1	1.8	1.5	0.2
Root	2.7	2.5	2.5	2.4	0.1
Mixture	2.3	2.0	1.7	1.6	0.2
Control = 3.2 cm					
LSD(0.05)	0.1	0.2	0.1	0.2	

Leaf, stem, and root extracts, obtained from vegetative plants; flower extract obtained from reproductive plants. The mixing 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 dry weight of hypocotyl 8-d old lentil seedlings.

	Hypocotyl dry wt., by extract conc., (g kg^{-1})				
Extracting	4	8	12	16	LSD (0.05)
Leaf	0.84	0.76	0.70	0.66	0.05
Stem	0.95	0.92	0.91	0.86	0.04
Flower	0.83	0.77	0.73	0.70	0.05
Root	0.96	0.93	0.92	0.88	0.04
Mixture	0.93	0.91	0.86	0.82	0.04
Control = 1.05g					
LSD(0.05)	0.03	0.05	0.05	0.06	

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

Table 5: Influence of various concentrations of different aqueous extracts made from *Brassica nigra* plant parts on the dry weight of the radicle 8-d old lentil seedlings.

	Radicle dry wt., by extract conc., (g kg^{-1})				
Extracting	4	8	12	16	LSD (0.05)
Leaf	0.38	0.32	0.24	0.20	0.06
Stem	0.56	0.52	0.46	0.44	0.05
Flower	0.48	0.42	0.37	0.33	0.04
Root	0.53	0.51	0.46	0.40	0.05
Mixture	0.44	0.40	0.36	0.30	0.04
Control = 0.98 g					
LSD(0.05)	0.04	0.06	0.05	0.06	

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

Table 6: Total water uptake by lentil seeds treated with different concentration of aqueous extracts of vegetative stage leaves at different soaking periods

	Water uptake, by soaking time (h)				
Concentration (g Kg^{-1})	4	8	12	16	LSD (0.05)
0.0	1.12	1.20	1.24	1.24	0.03
4.0	0.78	0.80	0.82	0.88	0.03
8.0	0.72	0.74	0.83	0.78	0.02
12.0	0.68	0.75	0.78	0.76	0.04
16.0	0.61	0.70	0.72	0.62	0.05
LSD (0.05)	0.02	0.03	0.04	0.02	

radicle dry weight (Table 5) was significantly inhibited by leaf, flower, root and mixture of all plant parts by all concentrations. The leaf extract was the most inhibitory at 4, 8, 12 and 16g kg^{-1} concentration and reduced radicle dry weight of seedlings by 61.2, 67.3, 75.5 and 79.6 %, respectively.

Water uptake: Many enzymatic functions important to plants are inhibited by the presence of allelochemicals (Rice, 1984). In high protein seeds like lentil, proteases play an important role in the hydrolysis of proteins during germination. To a large extent the activity of these enzymes is primarily related to water imbibition by the seeds. Although enzyme activity was not investigated in this study, an indirect association between lowers seed germination and allelopathic inhibition may be the consequence of the inhibition of water uptake and enzyme activity (Table 6). Increasing the concentration of aqueous leaf extracts significantly inhibited the water uptake by germination lentil seeds. These matters of direct observation are in transaction to those of Chung and Miller (1995). The greatest inhibition in water uptake occurred at the 16g kg^{-1} extract concentration for seeds soaked for 8 h.

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. 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.

References

Ballester, A., A.M. Vieitez and E. Nieitez, 1979. The allelopathic potential *of Erica austrials* L. and *E. arborea* L. Bot. Gaz. (Chicago) 140: 433-436.

Bell, D.T. and C.H. Muller, 1973. Dominance of California annual grasslands by *Brassica nigra*. Am. Midland Nat., 90: 227-299.

Bialy, Z., W. Oleszek, J. Lewis and G.R. Fenwick, 1990. Allelopathy potential of glucosinolates (mustard oil glysides) and their degradation products against wheat. Plant and Soil, 129: 277-181.

Brown, P.D. and J.M. Morra, 1993. Fate of ionic thiocyanate (SCN-) in soil. J. Agric. Food Chem., 41: 978-982.

Brown, P. D., J.M. Morra J.P. McCaffery, D.L. Auld and L.I. Williams, 1991. Allelochemicals produced during glucosinolate degradation in soil. J. Chem. Ecol., 17: 2021-2034.

Chung, I. M. and D.A. Miller, 1995. Natural herbicide potential of alfalfa residues on selected weed species. Agron. J., 87: 920-925.

Grodzinsky, A.M., 1992. Allelopathic effects of cruciferous plants in crop rotation. P. 77-85 in S. J. H. Rizvi and V. Rizvi, ed., Allelopathy: Basic and Applied Aspects. Chapman and Hall Press, London.

Hall, M. H. and P.R. Henderlong, 1989. Alfalfa autotoxic fraction characterization and initial separation. Crop Sci., 30: 1255-1259.

Hegde, R.S. and D.A. Miller, 1990. Allelopathy and autotoxicity in alfalfa: Characterization and effects of preceding crops and residue incorporation. Crop Sci., 30: 1255-1259.

Josefsson, E., 1968. Method for quantitative determination of p-hydroxybenzel isothiocyanate in digests of seed meal of *Sinapis alba* L. J. Sci. Food Agric., 19: 192-194.

Kuiters, A.T., 1989. Effects of phenolic acids on germination and early growth of herbaceous woodland plants. J. Chem. Ecol., 15: 467-479.

Muehlchn, A. M., R.E. Rand and J.L. Parke, 1990. Evaluation cruciferous green manure crops for controlling Aphanomyces root rot of peas. Pl. Dis., 64: 651-654.

Narwal, S. S., 1994. Allelopathy in crop production. Scientific Publishers, Jodhpur, pp: 288.

Newman, E. I. and A.D. Rovira, 1975. Allelopathy among some British grassland species. J. Ecol., 63: 727-737.

Oudhia, P., S.S. Kolhe and R.S. Tripathi, 1998. Allelopathic effect of *Blumea lacera* L. on rice and common Kharif weeds. Oryza, 35: 175-177.

Rice, E.L., 1984. Allelopathy., 2nd ed. Academic Press, New York

Tollsten, L. and G. Bergstrom, 1988. Headscape volatiles of whole plant and macerated plant parts of *Brassica and Sinapis*. Phytochemistry., 27: 4013-4018.

Vaughan, J.G. and J.S. Hemingway, 1959. The utilization of mustard. Econ. Bot., 13: 196-204.

Whittaker, D.C. and P.P. Feeny, 1977. Allelochemicals: Chemical interactions between species. Science (Washington, DC) 171: 757-770.