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Allelopathic Effects of Aqueous Extracts of *Sinapis arvensis* on Growth and Related Physiological and Biochemical Responses of *Brassica napus*

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Abstract

Charlock (*Sinapis arvensis*) is frequently found growing in colza (*Brassica napus*) crops and is a serious weed in the fields. There is much evidence that allelochemicals released from certain weeds into the soil. It was observed when colza fields infested with charlock. In present research, samples of colza and charlock were collected from field. Etiolated seedling of colza were cultured in Hoagland's culture with or without shoot and root of charlock (in 5-6 foliar leaf stage) aqueous extraction at 0.5% and 1.5% concentration of stock solution (10 gr dried material extracted in 100 ml DW). Shoot extract reduced shoot and root length, leaf area and fresh weight of colza, but root extract were only at 1.5% significantly affected. Shoot extract at 1.5% reduced chlorophyll *a*, and total chlorophyll of colza compared to control, but root extract at the same concentrations reduced chlorophyll *b*. In addition shoot extracts reduced Hill reaction. Shoot and root extracts of charlock increased soluble and decreased non-soluble carbohydrate content in colza. Charlock aqueous extraction reduced protein and increased proline content in leaves. Shoot and root extracts of charlock at 1.5% concentration increased catalyzed oxidation of Guaiacol (peroxidase activity) of leaf extract of colza.

Keywords: *Allelopathy, Chlorophyll, Hill reaction, Carbohydrate, Peroxidase, Charlock, Colza (or Canola).*

Introduction

Allelopathy is the direct influence of chemicals released from one plant on the development and growth of another plant (Olofsdotter 1998). Plants produce a large number of chemical compounds which vary in their chemical compositions and concentrations. The protein, carbohydrate and chlorophyll contents of plants are considerably influenced by allelochemicals (Kohli 1987). Allelopathy as a mechanism of plant interference in agro-ecosystems offers an opportunity to manage weeds in a crop sequence, but could also adversely affect crop yields and influence choice of rotation (Moncef *et al.* 2001). Previous studies have shown that sorghum (*Sorghum bicolor* L.) vegetation possesses a variety of potential inhibitors such as dhurrin, a cyanogenic glycoside (Haskins *et al.* 1984) and phenolics (Moncef 1994), which are potentially allelopathic to weeds (Alsaadawi *et al.* 1986) with a maximum of inhibitory activity at harvest. Bioassays of germination, radicle growth and coleoptile growth are used to test the allelopathic potential of a crop species (Kimber 1973). Chlorophyll reduction was observed in soybean plants treated with aqueous extract of velvelt leaf (Colton & Einhellig 1980). It has also been suggested that some allelopathic compounds may interfere with the synthesis of porphyrin, precursor of chlorophyll biosynthesis (Rice 1984).

Colza (*Brassica napus*) is a valuable and widely used crop. Charlock (*Sinapis arvensis*) is frequently found growing in colza fields and is a serious weed in agriculture. Nilsson and Hallgren (1991, 1992) found the white mustard meal as herbicide for control of *Matricaria indora*, *Galium aparine* and *Chenopodium album*. Johnson (1992) found the weed control in vegetables with mustard cake.

Chou and Lin (1976) found the aqueous extracts of decomposing rice residues in soil contained five phenolics and several unknown compounds and that extracts inhibited the radicle growth of lettuce and rice seeds and the growth of rice seedlings.

The objective of the present study was to assess the effects of aqueous extract of *Sinapis arvensis* on growth and some biochemical parameters of *Brassica napus*, because the aqueous extracts of decomposing *Sinapis arvensis* residues in soil may be unknown compounds inhibiting growth of *Brassica napus*.

Material and Methods

Collection and preparation of plant material: Samples of canola (or colza) (*Brassica napus*) and *Sinapis arvensis* were collected from the field. Seeds and a herbarium vouchers were deposited at the herbarium of the Gorgan University of Agricultural Sciences and Natural Resources. To break the dormancy of the seeds of charlock, they were submerged in gibberilic acid (1000 ppm) for 24 h. Charlock seeds were sown in sandy-clay soil.

Charlock plants were pulled out of the fields at two growth stages (stage 1= five to six leaves, stage 2 = flowering). They were washed with distilled water and were dried using paper towel and then separated into roots, stems, leaves and flowers. All plant components were chopped into 1 cm long pieces and dried at 60ºC for 24 h. Ten grams of dried plant material was extracted in 100 ml cold distilled water. Samples were transferred into a 500 ml flask and placed on a horizontal shaker for 24 h. Extracts were passed through cheese cloth and centrifuged at 3500 g for 15 min and supernatant (as a main stock) stored at under 5ºC until bio-assayed.

Plant material and growing conditions: For germination bioassays, seeds of *Brassica napus* were surface sterilized with 10% aqueous solution of sodium hypochloride for 30 sec, rinsed 5 times with distilled water and dried with paper towel and were placed in a plastic pots containing fine sand and distilled water. After 10 days when the main leaf was beginning to expand, the plants were removed from the sand, their roots were washed, and the plants were then transferred to plastic pots and sub-irrigated with aerated Hoagland's solution (Hoagland & Arnon 1950). For the growth experiments, 0, 0.5, 1.5, percent of stock solution of organs (shoot and root) of charlock as an allelopathic compounds were used. Each concentration was replicated three times. The pH of the Hoagland's solution was maintained at 5.5.

Analysis of growth dries production: At the 4-foliar stage of colza, leaf areas and dry weight of shoot and root were determined.

Chlorophyll and Hill reaction determination: The concentration of Chlorophyll *a* and *b* was determined using the spectrophotometer method of Arnon (1965). Absorbance was measured with a Shimadzu-160A UV-VS spectrophotometer at 645nm and 663nm. The Hill reaction was determined according to the method of Bregman (1996). Four grams of fresh colza leaf was weighted and then major veins were removed, cutting the leaves into small pieces and placed in a chilled mortar with 15 ml of ice-cold Tris-NaCl buffer and a sprinkling of purified sand. Grinding the tissue with a chilled pestle for 2 min was the next step. The suspension filtered through four layers of cheese cloth into a chilled 15-ml conical centrifuge tube and centrifuged at 200 g for 1 min for the preparation of the Chlorophyll suspension. The supernatant decanted into a clean, chilled centrifuge tube and spin at 1300g for 5 min. Hill reaction measured with 0.5 ml chlorophyll suspension, 0.5 ml distilled water, 0.5 ml 4×10-4 DCPIP (2,6-dichloropheno-lindophenol sodium) and 3.5 ml Tris-NaCl buffer. The tubes warmed to 20˚C and illuminated with a 100-watt frosted incandescent lamp and absorbance was measured with spectrophotometer Shimadzu-160A at 600 nm.

Carbohydrate content: Carbohydrate contents (water soluble, non-soluble) were measured using phenol sulfuric acid method of Chapin and Kenedy (1987); 5 ml ethanol (70%) was added to 50 mg of powdered dried material of colza (shoot and root) and decolorated with charcoal and was kept for one week, then centrifuged at 1800 g for 15 min. The reaction mixture contained 0.5 ml supernatant, 1.5 ml distilled water, 1 ml phenol (5%) and 5 ml sulfuric acid. After 30 min absorbance was measured with a Shimadzu-160A UV-VS spectrophotometer at 485 nm and the concentration was determined with standard plot. For determination of non-soluble sugar, after centrifuging at 1800 g for 15 min to get pellet, add 10 ml DW and placed in 100°C bath for 15 min and used above method.

Proline content: Proline was extracted from the shoots and roots of colza and estimated following Bates *et al*. (1973). Homogenate of the 0.5 g fresh weight of the samples were prepared in 10 ml of 3% sulphosalicylic acid. Pink color was developed by a reaction with glacial acetic acid and ninhydrin. The color was separated in toluene layer by vortex and centrifugation of the sample. The intensity of the color was measured at 520 nm.

Protein content and peroxidase activity: The extraction of protein was according to Kar and Mishra (1976) method. One hundred milligrams of colza leaf was homogenized with 5 ml of phosphate buffer pH 6.8 and centrifuged at 2˚C, 15000 rpm for 10 min. The pellet was washed successively with 10% (W/V) cold trichloro-acetic acid (twice), ethyl alcohol (once), ethyl alcohol-chloroform (3:1, V/V twice), ethyl alcohol-ether (3:1, V/V, once), and finally with ether (once). The pellet was dried. The protein was solublized by boiling in 1 N NaOH for 15 min in a water bath. It was centrifuged at 15000 rpm for 15 min and was taken for protein determination (Lowry *et al.* 1951).

Peroxidase assay: Colza leaf tissues were homogenized in 100 mM sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar to extract enzymes. The homogenate was centri-fuged at 7000 rpm for 10 min and the resulting supernatant was used for determination of POD activity. The whole extraction procedure was carried out at 4 °C. Measuring POD activity was after Mc-Adam *et al.* (1992). Guaiacol was used as the substrate. POD activity was measured in a reaction mixture (3.15 ml) that contained 50 µl enzyme extract, 50 µl H2O2 3%, 50 µl Guaiacol 0.2 M and 3 ml phosphate buffer 0.1 M (pH 6.8). Absorbance was measured at 436 nm for 3 min (15 sec interval).

Experimental design and statistical analysis: The data subjected to Duncan test. Analysis of variance was conducted using SAS (SAS Institute 1985) and Fisher's protected LSD at the 0.05 level of probability (Steel & Torrie 1980).

Results

Analysis of growth, chlorophyll content, Hill reaction and carbohydrate content at 1.5% concentration of root or shoot of charlock extract, but both 0.5% and 1.5% reduced shoot of colza shoot of charlock (*Sinapis arvensis*) extract at 0.5 and 1.5% (stock solution) con-centration reduced shoot and root length, leaf area and fresh shoot weight of colza, but root of charlock only at concentration significantly affected (LSD, P=0.05). Fresh root weight of colza was reduced only 1.5% dry weight compared to control (LSD, P=0.05) (Tab. 1).

Shoot of charlock extract at 1.5% concentration reduced chlorophyll *a* and total chlorophyll of colza compared to control. However, charlock root extract (LSD, P=0.05) reduced chlorophyll *b* at the same concentration (no significant at LSD, P=0.05). In addition to affecting chlorophyll, shoot of charlock extract at 1.5% concentration reduced Hill reaction in colza leaf at 4-folial stage compared to control (LSD, P=0.05). Other treatment had no significant effect (Fig. 1).

Tab. 1. Effect of charlock (*Brassica arvensis*) aqueous extract on growth parameters of colza (*B. napus*)[a].

Change→ Sources ↓	F	Shoot length (cm)	Root length (cm)	Leaf area (cm^2)	Shoot FW (g)	Root FW (g)	Shoot DW (g)	Root DW (g)	Total FW (g)	Total DW (g)
Treatment*	4	49.422	323.65	1027.853	7.037	0.681	0.063	0.015	11.959	0.138
Error	10	0.967	1.883	12.985	0.171	0.015	0.002	0.001	0.241	0.003
CV (%)		9.30	5.59	13.39	15.35	14.9	15.08	14.93	13.93	13.41

[a] Analysis of variance (qui-square).
* Values are not significantly different at the 0.01 probability level.

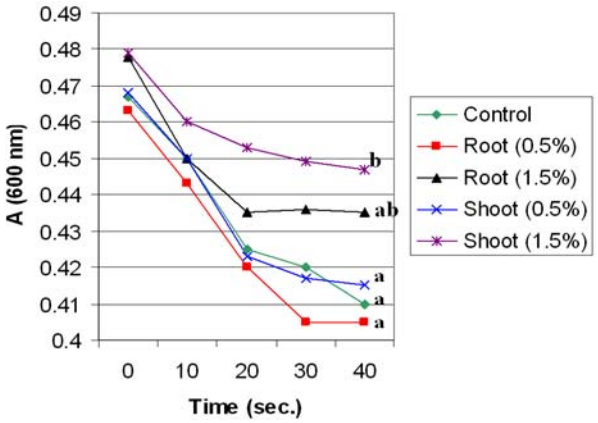

Fig. 1. Effect of various charlock aqueous extract on the Hill reaction in colza leaf extract. Within each plot values sharing the same letter are not significantly different at the 0.5 probability level.

Charlock root extract in 1.5% concentration increased shoot and root of colza soluble carbohydrate content but had no effect in 0.5% concentration (LSD, P=0.05) compared to control. However, charlock shoot extract in 0.5% and 1.5% concentrations had significantly more effect (Tab. 2). Meanwhile extractions of shoot and root of charlock in 1.5% concentration decreased shoot and root of colza insoluble carbohydrate content (LSD, P=0.05) (Tab. 2).

Discussion

The observed reduction in photosynthesis was associated with the significant reduction in shoot and root length, leaf area, fresh and dry weight, chlorophyll and carbohydrate content and Hill reaction in colza treated by aqueous extract of charlock. Chlorophyll reduction has been observed in soybean plants treated with aqueous extract of velvetleaf (Colton and Einhellig, 1980). It has also been suggested that some allelopathic compounds may interfere with the synthesis of porphyrin, precursors of chlorophyll biosynthesis (Rice, 1984).

Chou and Lin (1976) found the aqueous extracts of decomposing rice residues in soil contained five phenolics and several unknown compounds and that inhibit the growth of radicle in rice seeds and the growth of rice seedlings. According to these reports, shoot and root of charlock decomposing residues may have growth inhibitors for colza. Besides, the compounds released from charlock may have these effects.

Protein content, proline content and peroxidase activity: Charlock shoot aqueous extraction at 0.5% and 1.5% concentration showed highest reduction of protein content in colza than charlock root at 1.5% (LSD, P=0.05). All the treatments increased proline content in colza leaf, but had no significant effect on root and shoot of charlock extraction at 0.5% (LSD, P=0.01, see Tab. 3).

Tab. 2. Effect of charlock (*Brassica arvensis*) aqueous extract on the photosynthetic parameters of colza (*B. napus*).

Parameter → / Treatment↓	Hill reaction (dA/min)	Chl. a mg/gFW	Chl b mg/gFW	Chlo. T[1] mg/gFW	S.Su[2] (shoot) µg/gdw	S.Su[2] (root) µg/gdw	in.su[3] (shoot) µg/gdw	in.su (root) µg/gdw
control (0%)	0.058[a]	0.723[c]	0.279[b]	1.041[c]	6.007[c]	4.737[b]	7.51[a]	5.997[a]
root (0.5%)	0.056[a]	1.25[a]	0.506[a]	1.756[a]	6.31[bc]	4.703[b]	7.71[a]	6.247[a]
root (1.5%)	0.044[a b]	0.919[b c]	0.351[b]	1.27[bc]	7.487[a]	4.097[b]	6.037[b]	3.393[c]
shoot (0.5%)	0.057[a]	1.046[a b]	0.459[a]	1.505[a b]	7.207[a]	4.207[b]	7.377[a]	4.133[b]
shoot (1.5%)	0.036[b]	0.478[d]	0.174[c]	0.652[d]	7.913[a]	5.84[a]	4.803[b]	2.43[d]

[a] Within each column, values sharing the same letter are not significantly different at the 0.05 probability level
[b] Total Chlorophyll,
[c] Soluble Sugar
[d] Insoluble Sugar

Tab. 3. Effect of charlock (*Brassica arvensis*) aqueous extract on the biochemical parameter of colza (*B. napus*).

Parameter* Treatment	POD Activity dA/min	Protein mg/gFW	Proline µM/gFW
control	0.078[c]	7.203[a]	143.028[c]
root (0.5%)	0.082[c]	7.14[a]	187.1[bc]
root (1.5%)	0.171[b]	5.17[b]	260.62[b]
shoot (0.5%)	0.165[b]	4.96[bc]	201.2[bc]
shoot (1.5%)	0.205[a]	4.11[c]	408.422[a]

* Within each column, values sharing the same letter are not significantly different at the 0.05 probability level
[a] Total Chlorophyll,
[b] Soluble Sugar
[c] Insoluble Sugar

Kao (1980) reported that protein breakdown precedes chlorophyll loss during rice leaf senescence. Charlock decomposing residue may produce compounds such as glucosinolates or thiocyanates as a senescence effectors for colza. Protein breakdown may produce glutamate. Guerrier (1998) reported that glutamate is a main amino acid for proline biosynthesis as a result of increased proline content in colza leaf. In addition, protein breakdown produce proline, an amino acid as an osmotic protectant.

Peroxidase activity: Extraction of shoot and root of charlock at 1.5% concentration increased catalyzed oxidation of Guaiacol (2-methoxy phenol) of leaf extract of colza (LSD, P=0.05; see Fig. 2).

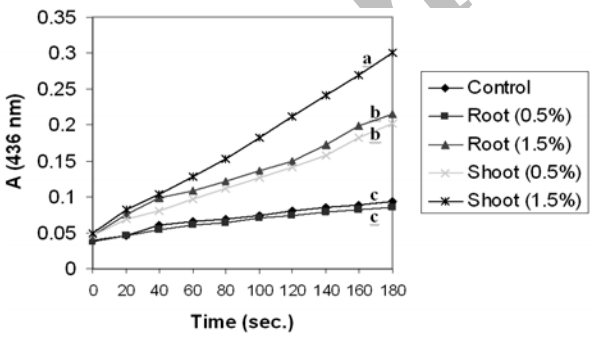

Fig. 2. Effect of various charlock aqueous extract on the initial velocity of peroxidase reaction in colza leaf extract. Within each plot, values sharing same letter are not significantly different at the 0.05 probability level.

In cell-free extracts and purified preparations, many plant-derived phenolic compounds have been found to serve as substrates peroxidase reactions (Badiani *et al.,* 1990). Peroxidases catalyze the inter-conversion, degradation and polymerization of polyphenols and some isoflavons (Barz 1977). Ozan *et al.* (1996) reported ρ-hydroxy-benzoic acid and naringin caused a slight stimulation of Guaiacol oxidation and vanillic acid enhanced Guaiacol oxidation considerably, but chlorogenic acid, quercetin and catechin decreased the rate.

According to those reports our experiments suggest that cell-free extracts of charlock shoot and root may have various naturally occurring plant phenolic compounds that increase peroxidase activity of leaf extracts of colza.

We conclude that some of allelochemical compounds in charlock aqueous extracts significantly reduced the net photosynthetic rate of colza leaflet, dry mass, leaf area, non-soluble carbohydrate, protein contents, increased proline and soluble carbohydrate contents. Increase in proline content which has a role in leaf water stress may be partially responsible for the reduction of photosynthesis. The data indicated that charlock extracts which includes some phenolic compounds were able to increase the rate of peroxidase activity. Phenolic compounds play an important role in polyphenol degradation and polymerization (Barz 1977). Available evidence supports the view that inhibitory substances of charlock can have significant role in weed-crop (colza) interact-tions.

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