Comparison of allelopathic effects of some brassica species in two growth stages on germination and growth of sunflower

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ABSTRACT

Members of Brassicaceae have been frequently cited as allelopathic crop. The toxic effect of *Brassica* spp. may be caused by hydrolysis products of glucosinolates that occur in substantial amounts in the vegetative parts of *Brassica* spp. This study investigated the allelopathic potential of *Brassica napus*, *B. rapa* and *B. juncea* on the sunflower seed germination and seedling growth. Aqueous extracts of three species from two stages (full flowering and straw) of sampling were separately made with 0 (distilled water), 10, 20, 30 and 40% concentrations. This experiment was conducted in 2 × 3 × 5 factorial arrangement based on completely randomized design with five replications. There was a highly significant difference among different concentrations of extracts and also between two stages of extraction. All aqueous extracts significantly affected sunflower germination, germination rate, seedling root and hypocotyl length, fresh and dry matter weight when compared with distilled water control. The greatest concentration showed a stronger inhibitory effect. Root length was more sensitive to extracts than hypocotyl length.

Keywords: allelopathy; *Brassica*; *Helianthus annuus*; seedling growth

Allelopathy phenomenon was defined for the first time in the late 1930s by Hans Molisch as the influence of one plant on another through releasing of chemicals into the environment (Molish 1937). It was later explained as any direct or indirect harmful or beneficial effect of one plant (including microorganisms) on another through the production of chemical compounds that are released into the environment (Rice 1984).

The interaction of plants through chemical signals (allelopathy) has many possible agricultural applications (Nelson 1996). Decline in crop yields in cropping and agro-forestry system in recent years has been attributed to allelopathic effects. Crop rotations are practiced to eliminate the effect of monoculture, but the succeeding crop may be influenced by the phytotoxins released by the preceding crop (Reigosa et al. 2000). Among crop plants, *Brassica* species contain allelochemical compounds as glucosinolate that is, under special conditions, released to environment and affects seed germination and plant growth (Bones and Rossiter 1996). Glucosinolates are degraded by the enzyme myrosinase, which results in the release of various hydrolysis products such as isothiocyanates, nitriles and others. This specific plant defense determines various interactions with other organisms (Halkier and Gershenzon 2006) and might be one of the key factors underlying the invasion success of Brassicaceae species.

Sunflower planting after *Brassica* species may reduce sunflower germination and growth. The aim of this research was to study the effect of aqueous extracts of three species of *Brassica* in two stages (1) full flowering and (2) straw on the sunflower seed germination and seedling growth.

MATERIALS AND METHODS

Plant sampling and preparation of extracts. Three species of *Brassica* including *Brassica napus* (canola), *B. rapa* (turnip) and *B. juncea* (mustard) planted on field plots of the Seed and Plant Improvement Institute in September 2008. Aerial parts of three species were collected in full flow-

ering stage in April 2009. Samples were air dried in shade for three weeks. The dried samples were stored in plastic bags before used for experiments. Straws of three species were collected in June. All samples were separately ground to fine powder to pass through a 3-mm sieve. Ten gram of each species from two different stages were mixed with 100 ml distilled water and left for 24 h at 25°C in dark for extraction. Aqueous extracts were obtained as filtrate of the mixture and final volume was adjusted to 100 ml. The extracts were considered as stock solutions and a series of solution with different concentrations 0 (distilled water) 10, 20, 30 and 40% were prepared. This experiment was conducted in 2 × 3 × 5 (two stages, three *Brassica* species and five concentrations) factorial arrangement based on completely randomized design with five replications. Seeds of sunflower (*Helianthus annuus* L. cv. SHF81-85) were sterilized in 2% solution of sodium hypochlorite for 15 min and rinsed in distilled water four times. Ten uniform seeds of sunflower were kept for germination in sterilized 9 cm Petri dishes on filter paper (Whatman No. 1). The Petri dishes were maintained under laboratory conditions (temperature 24 ± 1°C and lighted room during day) for 6 days. Samples were moistened with 8 ml of different concentrations of extracts. Equal volume of distilled water was added in the dishes when moisture content of the filter paper declined. Number of seeds germinated was counted at 48 h intervals over a 6-day period to obtain germination rate. At the end of the test period germination percentage, germination rate, root and hypocotyl lengths, seedlings fresh and dry matter weight were determined. Germination percentage was determined by counting the number of germinated seeds after 6 days. Germination rate was calculated according to the following formula:

$$\text{Germination rate} = N_1/D_1 + N_2/D_2 + ...+ N_i/D_i$$

Where: N_i means the number of seeds that germinated in days i (D_i).

Germination rate is the speed of germination. High germination rate means that more seeds germinated in shorter time.

The root and hypocotyl lengths were measured with a ruler. After measuring the root and hypocotyl lengths, fresh weight was measured for all replications. Seedlings were oven dried at 70°C for 48 h to get dry weight.

Water uptake. One-gram samples of sunflower seeds were soaked for 24 and 48 h in aqueous extracts of 10, 20, 30 and 40% from two stages of three species and distilled water for comparison. After a 24 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.

Statistical analysis. Significance of the difference was tested and compared using the Analysis of variance. All statistical analyses were done using the MSTAT-C software.

RESULTS AND DISCUSSIONS

Seed germination. The analysis of variance for germination percentage showed that there was a highly significant difference between full flowering stage and straw aqueous extracts (Table 1). Different concentrations of aqueous extracts also showed highly significant difference (Table 1). Increasing the aqueous extract concentrations significantly reduced sunflower germination when compared with distilled water control (Figure 1).

Table1. Analysis of variance (ANOVA) in extraction stages, species and concentrations for different traits

Source	*df*	Mean square					
		seed germination	germination rate	root length	hypocotyl length	FW	DW
Extraction stage	1	31.740**	10.021*	129.438**	77.056**	5.576**	0.028**
Species	2	6.64^{ns}	6.158*	10.318**	5.308**	3.973**	0.011**
Extraction stage × species	2	29.540**	11.426**	7.331**	7.548**	5.008**	0.019**
Concentration	4	172.077**	117.060**	121.384**	24.676**	3.747**	0.082**
Extraction stage × concentration	4	8.190**	5.154*	1.053^{ns}	4.588**	0.356^{ns}	0.015**
Species × concentration	8	5.972*	2.820^{ns}	0.346^{ns}	1.129^{ns}	0.449^{ns}	0.002^{ns}
Extraction stage × species × concentration	8	5.815*	1.977^{ns}	1.817^{ns}	0.942^{ns}	0.667^{ns}	0.006**
Error	120	2.653	1.849	1.618	0.753	0.384	0.002

*$P < 0.05$; **$P < 0.01$; ^{ns}not significant

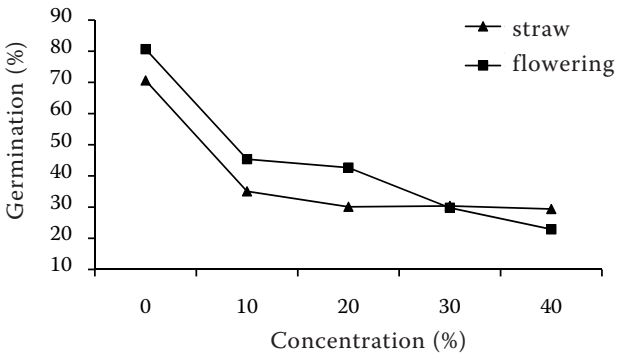

Figure 1. Effect of different concentrations of full flowering stage and straw aqueous extracts on sunflower seeds germination

Table 2. Effect of extraction stages and concentrations on different traits

Treatment	Extract (%)	Germination rate	Hypocotyl length (cm)
Full flowering	0	8.1[a*]	3.4[ab]
	10	4.5[c]	1.5[e]
	20	4.3[c]	0.9[f]
	30	3.0[de]	0.3[f]
	40	2.3[e]	0.2[f]
Straw	0	7.1[b]	3.5[a]
	10	3.5[cd]	2.8[bc]
	20	3.0[de]	2.7[cd]
	30	3.0[de]	2.41[cd]
	40	2.9[de]	2.0[de]

*, [a–c]means within a column for each trait that are followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

There was no significant difference among three species of *Brassica* for germination percentage (Table 1). Germination rate showed highly significant difference in different concentrations of extracts (Table 1). Aqueous extract of full flowering stage of the highest concentration (40%) highly reduced germination rate (Table 2) but totally increasing concentrations of straw aqueous extracts had a more reducing effect on germination rate (Table 2).

Potential of allelopathic compounds is often verified by testing their influence on seed germinability and seed viability. Inhibition or delay of seed germination and radicle growth by allelochemicals from many species for example sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), sunflower (*Helianthus annuus*) and rye (*Secale cereale*) were reported and reviewed (Wu et al. 1999, Inderjit and Callaway 2003, Inderjit and Duke 2003, Weston and Duke 2003).

Inhibition in lipid mobilization during germination of fat-storing seeds, in the presence of allelopathic compounds was detected in canola, sunflower and mustard (*Sinapis alba*) seeds (Levitt et al. 1984, Baleroni et al. 2000, Bogatek and Stepien 2003, Kupidlowska and Bogatek 2003).

Seedling growth. ANOVA showed a very significant difference ($P \leq 0.01$) for the stage of extraction, among three species and also different concentrations of aqueous extracts (Table 1). Root length and hypocotyl length declined with increasing concentration of the extract (Figures 2a,b). There were very significant ($P \leq 0.01$) differences of extraction stage × concentration interaction effect for hypocotyl length (Table 1). Full flowering stage extraction showed more reducing effects on root length and hypocotyl length (Table 3). Hypocotyl length was declined by higher concentrations (30% and 40%) of full flowering stage

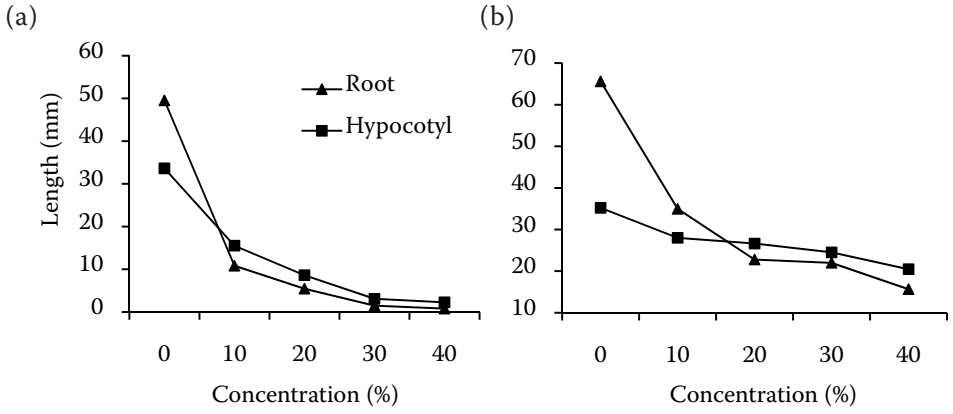

Figure 2. Root and hypocotyl length of sunflower seedlings in the presence of various concentrations of full flowering (a) and straw (b) aqueous extracts from three *Brassica* spp.

Table 3. Effect of extraction stages and species on different traits

Treatment	Species	Germination rate	Root length (cm)	Hypocotyl length (cm)	Fresh weight (g)
Full flowering	*B. napus*	4.2^{ab*}	1.4^{d}	1.1^{d}	1.6^{c}
	B.rapa	4.5^{ab}	1.4^{d}	1.3^{d}	1.8^{bc}
	B.juncea	4.5^{ab}	1.3^{d}	1.3^{d}	1.7^{c}
Straw	*B. napus*	4.81^{a}	4.1^{a}	3.4^{a}	2.7^{a}
	B.rapa	3.9^{bc}	3.2^{b}	2.7^{b}	2.1^{b}
	B.juncea	3.1^{c}	2.4^{c}	2.0^{c}	1.5^{c}

*, $^{a–c}$means within a column for each trait that are followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

extracts (Table 2). Similarly as it was observed on cress, lettuce, timothy and ryegrass in the presence of leaf extract of *Pueraria thunbergiana* or capsaicin (Kato-Naguchi 2003–4, Kato-Naguchi and Tanaka 2003–4). Detrimental effects of toxins from *Brassica* spp. on the next year's wheat, barley, or flax crops were reported (Horricks 1969, Vera et al. 1987, Gubbles and Kenaschuk 1989).

Root elongation was affected more than that of the hypocotyl (Figures 2a,b). It might be due to the direct contact of root with the extract and subsequently with inhibitory chemicals (Quasem 1995). There were very significant ($P \leq 0.01$) differences of extraction stage × species interaction effect for fresh and dry matter weight (Table 1). The study demonstrated that fresh matter weight of seedlings which were exposed to distilled water was very significantly higher than that of seedlings exposed to different concentrations of extracts (data not shown). Different concentrations of extracts also showed a very significant difference ($P \leq 0.01$) for fresh and dry matter (Table 1). Full flowering stage with aqueous extracts showed more reducing effect on fresh matter weight in comparison with straw aqueous extracts (Table 3).

One of the suggested explanations for disruption of seedling growth and development during allelopathy stress is modification in mitochondrial respiration leading to decreased supply of ATP for all energy demanding processes (Gniazdowska and Bogatek 2005). A great decrease in ATP/ADP level and energy charge was detected in *Sinapis alba* seeds treated by sunflower leaf allelochemicals (Bogatek et al. 2002). Ion uptake and growth are the most energy-consuming processes in plant cells (Van der Werf et al. 1988). Inhibition of seedling growth in allelopathy stress conditions may be therefore a result of decreased ion uptake. A root is the first organ to come into contact with allelochemicals in the rhizosphere, thus the effect of allelochemicals on ion uptake is particularly important. There is also much data on the effect of allelochemicals on membrane bound enzymes e.g. proton pumping ATPase localized in plasma membrane (H^+-ATPase). H^+-ATPase inhibition results in reduction in mineral and water uptake by roots and as a consequence leads to a strong effect on essential plant functions such as photosynthesis, respiration or protein synthesis leading finally to reduction of growth (Gniazdowska and Bogatek 2005).

Investigations showed that water uptake was also reduced by increasing the concentration of aqueous extracts (Figure 3). The greatest inhibition in water uptake occurred at 40% extract concentration for seeds soaked for 48 h (Figure 3).

The results demonstrated that sunflower seeds are sensitive to allelopathic compounds released by *B. napus*, *B. rapa* and *B. juncea*. Thus future plans are to conduct field experiments to assess their potential to reduce sunflower growth and also their persistence in soil.

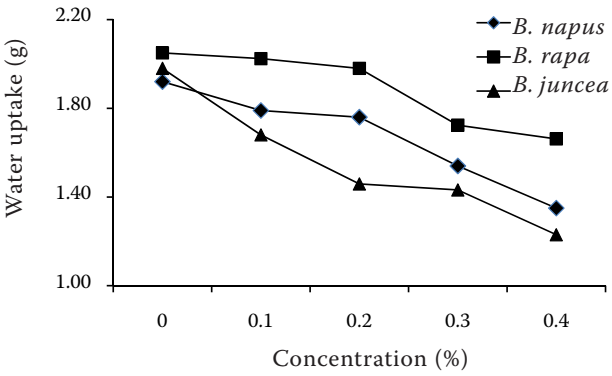

Figure 3. Effect of straw aqueous extracts from three *Brassica* spp. on sunflower seeds water uptake after 48 h

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Received on June 5, 2010

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