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Allelopathic Effects of *Chrysanthemum boreale* on Seed Germination and Seedling Growth of the Selected Plants

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ABSTRACT: To examine the allelopathic potentials of *Chrysanthemum boreale*, aqueous extracts and essential oil of the plant were used in these experiments. Seed germination of the receptor species was inhibited by the aqueous extracts and the inhibitory effect was increased in proportion to the concentration of extracts. In contrast, seedling elongation showed varied results. *Achyranthes japonica, Bidens bipinnata, Raphanus sativus* var. *hortensis* for. *acanthiformis, Plantago asiatica, Pimpinella brachycarpa* and *Lactuca sativa* were inhibited by increasing concentration of the aqueous extract, while *Brassica campestris* subsp. *napus* var. *pekinensis* and *Echinochloa crus-galli* were stimulated by the extract. Dry weight was also inhibited proportionally by increasing concentration of the aqueous extract, while some species were stimulated by a lower concentration of the extract. The volatile substances of *C. boreale* did not affect the seed germination of receptor plants, but seedling elongation and dry weight of some species were inhibited dose-dependently. Root hair development of selected plants was inhibited along with the concentration of essential oil. The above mentioned results, therefore, confirmed that the natural substances from *C. boreale* had allelopathic potentials to other plants.

Key Words: Allelopathic effects, Aqueous extracts, *Chrysanthemum boreale*, Essential oil, Volatile substances

INTRODUCTION

Allelopathy is an important mechanism of plant interference mediated by the addition of plant-produced phytotoxins to the plant environment and competitive strategy of plants (Fischer *et al.* 1994, Langenheim 1994).

Allelopathic chemicals are released from plant tissues in a variety of ways, including leaching, root exudation and volatilization (Oleszek 1987, Pellissier and Souto 1999). Allelochemicals' action against higher plants is typically characterized as suppressing seed germination, causing injury to root and other meristems, or inhibiting seedling growth, photosynthesis and cell division (Bhowmik and Doll 1989, Inderjit *et al.* 1995, Yu *et al.* 2000). Virtually all plant parts have been shown to contain inhibitors. Leaves, stems, roots, rhizomes, flowers, fruits and seeds have been bioassayed and found to contain inhibitors. Leaves and roots are the most important sources (Rice 1974).

The purpose of the present study was to investigate the allelopathic potentials of *Chrysanthemum boreale* extracts by evaluating seed germination, seedling growth and root hair development of selected plants in various concentrations of the aqueous extracts and volatile substances from *C. boreale*.

MATERIALS AND METHODS

Preparation of *C. boreale* extract and essential oil

Plants of *C. boreale* were collected in November 1998 in the area surrounding Mt. Mireuk in Jeonbuk, Korea. Aqueous extract of *C. boreale* was made by keeping 100 g of dry shoot in 1 ℓ distilled water for 24 h at 21℃. The extracts were then filtered through a filter paper (Whatman No. 40) and diluted to 10 and 50% extract by mixing with distilled water. Undiluted filtrate was defined as 100% extract.

The essential oil of dry shoot of *C. boreale* was steam-distilled using a Karlsruker's apparatus (Stahl 1973) and stored in a deep freezer (-70℃) to minimize the escape of volatile compounds.

Germination and growth test

1) Aqueous extract test

Two sheets of filter paper were put in each Petri dish (12 cm in diameter) and moistened with 12 ml of an extract. Control was treated with distilled water. Thirty seeds of the eight receptor plants were spread out on the filter paper in Petri dishes, and then they were incubated at 20℃ / 15℃ (day / night). After 6 days, the germination percentage was determined, and shoot length, radicle length and dry weight were measured. Relative ratio of germination and elongation to the control were calculated according to the proposition of Rho and Kil (1986).

2) Volatile substances test

Two sheets of filter paper covering the bottom of a Petri dish were moistened with 12 ml distilled water and thirty seeds of test plants were scattered regularly in each dish. A small aluminum vial (diameter 5 mm, height 5 mm) was put in the center of the Petri dish containing 10~25 μl of the essential oil. An empty aluminum vial was used as the control. These were rapidly sealed with polyvinyl wrap and parafilm within 30 sec. to limit volatilization. Six days after sowing, relative germination ratio (RGR), relative elongation ratio (RER) and dry weight were evaluated.

Statistical analysis

Four replication bioassays were used. Statistical analyses of bioassays were performed using ANOVA. Differences between the controls and treatments were assessed by one-way ANOVA.

RESULTS AND DISCUSSION

It has been reported that aqueous extracts or leachate of leaves, roots and litter of plants inhibit seed germination and seedling growth of other plants, or cause the reduction of dry weight in plants (Moyer and Huang 1997, Robles *et al.* 1999, Singh *et al.* 1999).

The effects of *C. boreale* aqueous extracts on germination of receptor plants are shown in Fig. 1. Relative germination ratios (RGR) were reduced progressively in proportion to the increased extract concentration. Seedling elongation of *P. asiatica*, *R. sativus* var. *hortensis* for. *acanthiformis*, *B. bipinnata* and *A. japonica* was inhibited gradually according to the extract concentration gradient, and root elongation was more inhibited. Shoot elongation of *L. sativa* and *P. brachycarpa* was slightly stimulated in lower concentration of the extract, but was gradually inhibited at higher concentrations and their radicle elongation was also inhibited severely according to the extract concentration gradient. *B. campestris* subsp. *napus* var. *pekinensis* and *E. crus-galli* were stimulated by the extract (Fig. 2). From these results, it is confirmed that allelochemicals inhibit the growth of other plants, but they can stimulate the growth of some plants at low concentrations.

When the seeds are germinated in Petri dish, measurement of root elongation is difficult due to curling or other morphological alteration. But, measurement of dry weight make the relative

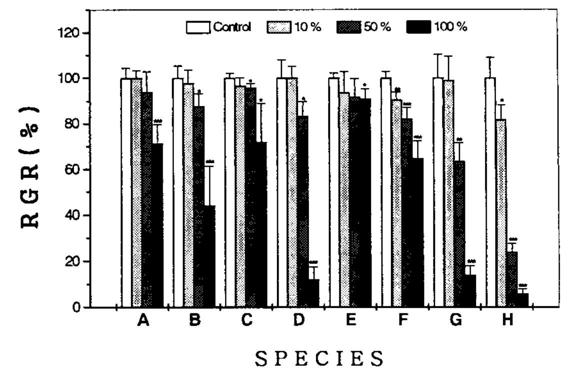

Fig. 1. Relative germination ratio (RGR) of receptor species grown at different concentrations of *Chrysanthemum boreale* aqueous extracts. Key to species: A, *Brassica campestris* subsp. *napus* var. *pekinensis*; B, *Echinochloa crus-galli*; C, *Lactuca sativa*; D, *Pimpinella brachycarpa*; E, *Plantago asiatica*; F, *Raphanus sativus* var. *hortensis* for. *acanthiformis*; G, *Bidens bipinnata*; H, *Achyranthes japonica*. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

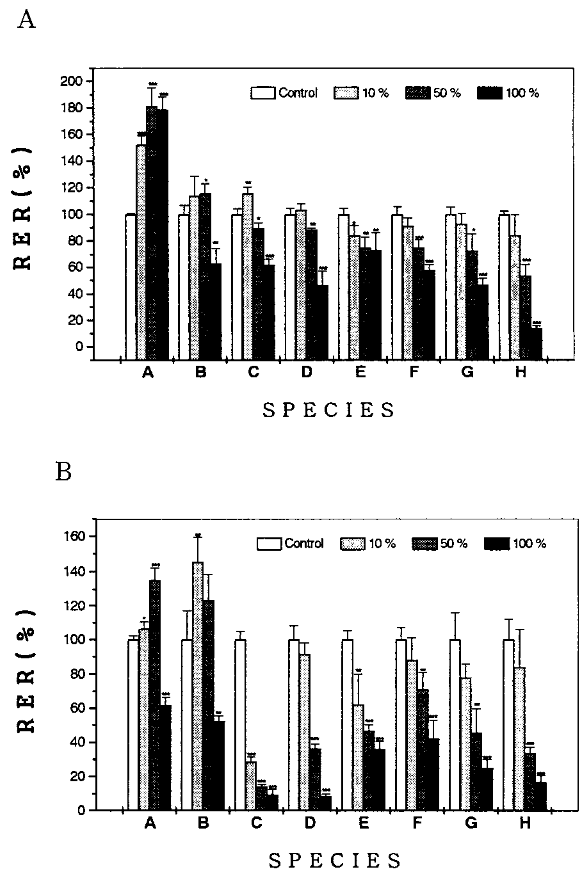

Fig. 2. Relative shoot elongation ratio (A) and relative root elongation ratio (B) of receptor species grown at different concentrations of *Chrysanthemum boreale* aqueous extracts. Key to species: same as in Fig. 1. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Table 1. Dry weights (mg) of receptor species grown at different concentrations of *Chrysanthemum boreale* aqueous extracts

Test species	Dry weights (Mean ± S. D.)			
	Concentration of aqueous extract (%)			
	0	10	50	100
Brassica campestris subsp. *napus* var. *pekinensis*	62.65± 5.160	65.85±2.901	63.58±4.760	50.70±1.890**
Echinochloa crus-galli	27.05± 5.060	28.90±1.230	24.48±1.480	13.17±7.501*
Lactuca sativa	22.20± 3.300	28.32±0.399***	21.70±1.140	20.40±1.520*
Pimpinella brachycarpa	26.48± 2.060	27.47±2.571	20.38±5.370	4.70±0.622***
Plantago asiatica	8.65± 0.858	7.70±1.280	7.30±0.469*	6.87±0.538*
Raphanus sativus var. *hortensis* for. *acanthiformis*	273.58±26.120	246.23±18.540	220.62±20.090*	170.83±18.371***
Bidens bipinnata	68.82±14.880	66.55±15.600	52.62±15.101	10.80±04.540***
Achyranthes japonica	31.50± 5.030	23.85±1.430*	7.47±3.020***	2.65±1.060***

Significant differences between control and treatments are indicated by * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

comparison of growth possible.

Dry weights of *P. asiatica*, *R. sativus* var. *hortensis* for. *acanthiformis*, *B. bipinnata* and *A. japonica* were decreased to the concentration of aqueous extracts. In contrast, dry weights of *L. sativa*, *P. brachycarpa*, *B. campestris* subsp. *napus* var. *pekinensis* and *E. crus-galli* were stimulated at lower concentration of 10%, and among them only *L. sativa* showed a significant difference (Table 1).

Volatile substances of *C. boreale* did not affect the germination of three receptor plants, but the germination of *B. campestris* subsp. *napus* var. *pekinensis* was significantly inhibited at the highest concentration (data not shown). Seedling elongation of *L. sativa* and *B. bipinnata* was inhibited and decreased gradually according to volatile substances concentration gradient, whereas that of *B. campestris* subsp. *napus* var. *pekinensis* was not affected by the volatile substances (Fig. 3). This was coincident with a previous study by Vokou (1992). He showed that the germination of *Hymenocarpus circinnatus*, *Astragalus hamosus* and *Medicago minima* was generally not affected by the quantities of tested oils, while seedling growth, particularly radicle elongation, was inhibited by volatile oils of *Rosmarinus officinalis*, *Coridothymus capitatus*, *Statureja thymbra* and *Teucrium polium*.

The dry weights of all receptor plants were reduced in proportion to the volatile substances concentration. Particularly, dry weight of *B. campestris* subsp. *napus* var. *pekinensis* was reduced significantly. However, its seedling elongation was not affected by volatile substances, therefore, it seems that inhibition of germination caused the dry weight to be reduced (Table 2).

Root hair development of receptor plants was

Table 2. Dry weights (mg) of receptor plants grown at different concentrations of *Chrysanthemum boreale* volatile substances

Test species	Dry weights (Mean ± S. D.)				
	Concentration of volatile substances (μl)				
	0	10	15	20	25
Brassica campestris subsp. *napus* var. *pekinensis*	70.77±2.950	60.50±4.100*	60.07±3.550*	61.27±3.250*	56.97±4.250**
Lactuca sativa	18.70±1.240	18.60±1.060	18.33±0.401	18.30±1.320	17.95±1.270
Bidens bipinnata	86.97±5.601	85.20±2.802	84.60±3.997	81.50±3.198	77.97±5.403

Significant differences between control and treatments are indicated by * $P<0.05$ and ** $P<0.01$.

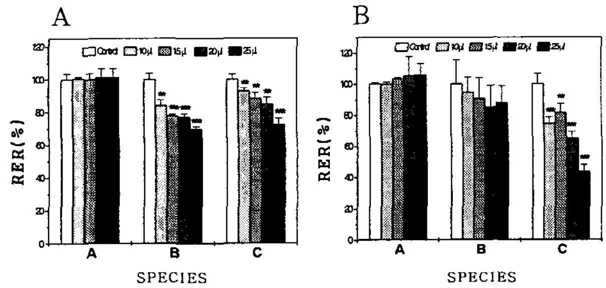

Fig. 3. Relative shoot elongation ratio (A) and relative root elongation ratio (B) of receptor species grown at different concentrations of *Chrysanthemum boreale* volatile substances. Key to species: A, *Brassica campestris* subsp. *napus* var. *pekinensis*; B, *Lactuca sativa*; C, *Bidens bipinnata*. ** P<0.01 and *** P<0.001.

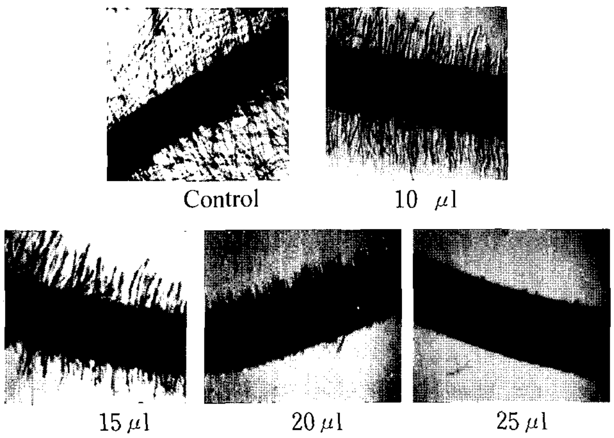

Fig. 4. Development of *Bidens bipinnata* root hairs treated with different concentrations of *Chrysanthemum boreale* volatile substances.

inhibited in proportion to the increased volatile substances concentration (Fig. 4). This result agrees with Kwak *et al.* (1999) which pointed out that development of root hairs of *L. sativa* and *B. campestris* subsp. *napus* var. *pekinensis* was severely inhibited by the essential oils of *Chamaecyparis obtusa*.

From these results described above in this study, it is suggested that the natural substances from *C. boreale* seemed to have a certain allelopathic potentials and useful for development of natural herbicide.

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