Korean J. Crop Sci. 51(1): 101~106(2006)

Bioassay on Natural Herbicidal Potential in Common Thistle (*Cirsium pendulum* Fisch.)

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ABSTRACT: Common thistle contains water-soluble substances that are phytotoxic to neighboring plant species. A series of aqueous extracts from leaves, stems, roots and flowers of common thistle (*Cirsium pendulum* Fisch.) were assayed against alfalfa (*Medicago sativa*) seedlings to determine their allelopathy, and the results showed highest inhibition in the extracts from flowers and leaves, and followed by stems, and roots. The extracts at 40 g dry tissue L^{-1} (g L^{-1}) applied on filter paper in a Petri-dish significantly inhibited root growth of test plant by 87%. Methanol extracts at 100 g L^{-1} from leaves inhibited root growth of alfalfa and barnyardgrass (*Echinochloa crus-galli*) by 89 and 98%, respectively. Hexane and ethylacetate fractions of common thistle reduced alfalfa root growth more than did butanol and water fractions. Incorporation into soil with the leaf residues at 100 g kg^{-1} inhibited shoot fresh weights of barnyardgrass and eclipta (*Eclipta prostrate*) by 88 and 58%, respectively, showing higher sensitivity in grass species. These results suggest that common thistle plants had allelopathic potential for eco-friendly vegetation management, and that especially their activities were differently exhibited depending on plant part.

Keywords: allelopathy, bioassay, common thistle, eco-friendly vegetation management, extracts, plant parts, residue incorporation

Allelopathy is the chemical interaction between including stimulatory as well as inhibitory influences (Molisch, 1937). Allelopathy plays an important role in both natural and agro-ecosystems, especially eco-friendly weed management. Most of scientific studies have been concentrated to exploit the positive significant roles, this phenomenon, if suitably managed, can play in enhancing crop pro-ductivity and use naturally occurring substances as natural replacements for synthetic pesticides from a number of plant resources. In addition, allelochemicals from higher plants can be successfully exploited for weed control. This method is safe and effective since natural products are biodegradable and unlike most of the synthetic herbicides do not accumulate in soil as persistent pollutants.

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Common thistle is a noxious perennial weed which causes serious yield losses in spring sown small grains row crops, and pastures (Hodgson, 1963 and 1968). Growth and germination of wheat (*Triticum aestivum* L.) and flax (*Linum usitatissimum* L.) were inhibited by aqueous extracts of Canada thistle (*Cirsium arvense* Scop.) roots and shoots (Helgeson & Konzak, 1950). Bendall (1975) studied water and ethanol extracts and residues in soil, and concluded that an allelopathic mechanism might be involved in the exclusion of some annual thistle, pasture, and crop species from Canada thistle areas. Stachon & Zimdal (1980) in their greenhouse experiments found that Canada thistle litter reduced the growth of redroot pigweed (*Amaranthus retroflexus* L.) and green foxtail (*Setaria viridis* L.) more than that of cucumber (*Cucumis sativus* L.) or barley (*Hordeum vulgare* L.). Chon *et al.* (2003) reported that the aqueous leaf extracts of 16 Compositae plant species, including common thistle, significantly inhibited hypocotyls and root lengths of alfalfa. In their earlier study, trans-cinnamic acid was found as the greatest amount at ethyl acetate fraction from methanol extracts of common thistle plant.

It is generally accepted that water extracts of top growth (especially leaves) produce more allelopathy for seedlings than those from roots and crowns of alfalfa (*Medicago sativa* L.) (Miller, 1996), and that shoot extract from the reproductive stage was more inhibitory than from the vegetative stage under laboratory conditions (Hedge & Miller, 1992; Chung & Miller, 1995). Chung & Miller (1995) ranked autotoxic effects of water extracts of plant parts of alfalfa as leaf (greatest), seed, root, flower, and stem (least). Chou & Leu (1992) reported that extracts from flowers of Delonix regia (BOJ) RAF exhibited highest inhibition against three test plants, alfalfa, lettuce (*Lactuca sativa*), and Chinese cabbage (*Brassica chinensis*).

Few studies on allelopathic effects of common thistle plant residues have been reported. Chon (2004) reported that the major phenol compounds identified and quantified by means of HPLC analysis were coumarin, *trans*-cinnamic acid, and chlorogenic acid. Of them, *trans*-cinnamic acid was found as the greatest amount at ethylacetate fraction. Bendall (1975) studied water and ethanol extracts and residues in soil and concluded that an allelopathic mechanism

might be involved in the exclusion of some annual thistle (*Carduus crispus* L.), pasture and crop species in areas infested with *Cirsium arvense* (L.) Scop. In addition, the aqueous and residues from common thistle leaves are known to be allelopathic to several agronomic crops including alfalfa, barley, and soybean (Chon, 2004).

Probable major biosynthetic pathways leading to production of allelochemicals have been known to be shikimic acid or acetate pathway (Rice, 1984). The objective of this research was to determine allelopathic effects of extracts or residues from leaves, stems, roots, and flowers of common thistle. This research will promote a better understanding of the mechanisms of allelopathy in manipulated ecosystem, and contribute to development of eco-friendly alternative weed control mean.

MATERIALS AND METHODS

Sampling and preparation of plant materials

Common thistle plants grown in a pasture of the Suncheon area, Korea were harvested at a reproductive stage in July 2001. The plant samples were separated into leaves, stems, roots and flowers. The four samples were directly oven-dried at 60 °C for 5 days, ground with a Wiley mill to pass a 1-mm screen, and stored in a refrigerator at 2 °C until used. Forty grams of dried leaves, stems, roots, and flowers were separately extracted by soaking in 1L distilled water at 24 °C for 24 hours in a shaker to give a concentration of 40 g dry tissue L^{-1} (hereafter referred to as '$g\ L^{-1}$') (Chon & Kim, 2002). The extract was filtered through two layers of cheesecloth to remove the fiber debris, and centrifuged at 5000 rpm (× 4530 *g*) for 2 hours. The supernatant was vacuum filtered again through Whatman No. 42 paper. EC, pH, and osmotic potential (Boyer & Knipling, 1965) were measured on stock extracts 2 days after extraction. The aqueous extracts, ground plant residues were used for allelopathy bioassay.

Phytotoxic effects of common thistle extracts

Each stock extract from different plant parts was diluted appropriately with sterile distilled water to give the final concentrations of 10, 20, 30, and 40 g L^{-1} (Chon *et al.*, 2003). Distilled water was the control. Root and hypocotyls lengths were measured on all seedlings in each Petri dish 6 days after placing seeds on the filter paper. Alfalfa 'Vernal' seeds were surface sterilized with 0.525 g L^{-1} sodium hypochlorite for 15 min. Seeds were rinsed four times with deionized water, imbibed in deionized water at 22 °C for 12 h, and carefully blotted using a folded paper towel. Twenty swelled seeds were evenly placed on filter paper wetted with extract in each Petri dish. The Petri dishes were covered, sealed by wrapping in parafilm to keep wetness, and placed flat in a growth chamber held at 24 °C during the14-h light period and 22 °C during the 10-h dark period. Plates were illuminated at 400 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) provided by a mixture of incandescent and fluorescent lamps. The experiments were duplicated, each with four replications.

To prepare methanolic extracts for a bioassay, ground leaf samples of common thistle were extracted with 95% methanol at room temperature. The extract was then filtered through a Whatman No. 1 filter paper. The collected filtrate was evaporated to dryness under vacuum at 40 °C using a rotary evaporator (N-1000V-W, Eyela, Japan). The yield of dried extracts from the original plant leaves was 10-15%. To compare phytotoxic effects of methanol extracts from common thistle, 4 mL of methanol extracts at 25, 50, 75, and 100 g L^{-1} and the methanol only solution (to determine methanol effect as a control) were pipetted to Whatman No. 1 filter paper in Petri dish and evaporated to dryness for 24 h at 24 °C. After evaporation, 4 milliliters of distilled water was pipetted to the filter paper and then 15 imbibed seeds of alfalfa (*Medicago sativa* L.) and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv) were placed on the paper and grown for 6 days. Root length was measured for all seedlings in each Petri dish.

Phytotoxic effects of 4 solvent fractions on alfalfa root growth

Methanol extracts from ground plant samples were used for the following fractionation and bioassay. For fractionation, crude methanol extracts were diluted with distilled water and hexane to collect hexane fraction using a separating funnel. After hexane collection, the distilled water fractions were added with ethylacetate (EtOAc) to obtain EtOAc fraction in the same way. The same procedure was used in preparing butanol (BuOH) and water fractions. The fractions were taken to dryness on a rotary evaporator at 40-50 °C, and transferred into vacuum freeze dryer to obtain dry matters.

The four dried hexane, EtOAc, butanol, and water fractions were dissolved in MeOH for bioassay. Four milliliters of methanol extracts at 25, 50, 75 and 100 g L^{-1} and the methanol only solution (control) were pipetted on Whatman No. 1 filter paper in Petri dish and evaporated to dryness for 24 h at 24 °C. After evaporation, 4 milliliters of distilled water was pipetted on the filter paper and then 15 imbibed seeds of alfalfa were placed on the paper and grown for 6 days. Bioassay procedures and conditions were same to the

previous work. Root length was measured for all seedlings in each Petri dish. The data were transformed into % of control and analyzed. When the F-test was significant ($P<0.05$) means were separated on the basis of least significant difference (LSD) (SAS Institute, 2000).

Effect of leaf residue incorporation on seedling growth of barnyardgrass and eclipta

Residues of each plant species were incorporated with a high organic matter-potting medium (Hanter 21, Seoul, Korea) that contained 30% sphagnum peat moss, 50% vermiculite, 18% zeolite, and 2% sand (v/v) per 200 cm^3 pot, by vigorously shaking the components in plastic bags. The amounts of plant residues in a soil medium used were 0, 25, 50, 75, and 100 g kg^{-1}. After mixing, the pots were filled with the medium mixture and barnyardgrass and eclipta (*Eclipta prostrate* L.) seeds per pot were separately planted. The pots were saturated with water by subsurface irrigation. During plant growth, the growing medium was maintained near field capacity by sub-irrigation without nutrition solution. The experiments were conducted in a greenhouse for 15 days at 28/22 (day/night). All plants were harvested to determine fresh and dry weights 15 days after seeding. Data were transformed to percent of control for analysis.

RESULTS AND DISCUSSION

Phytotoxic effects of aqueous and methanol extracts

Electrical conductivity (EC), pH, and osmotic potential of thistle extracts measured at 40 g L^{-1} were ranged from 0.15 to 1.53 S/m, from 5.45 to 6.00, and from -0.085 to -0.165 MPa, respectively (Table 1). It was thought that EC, pH, and osmotic potential of thistle extracts did not affect seedling growth of test plants, indicating allelopathic effects of common thistle extracts could go beyond the osmotic effects. Our experience in other study demonstrated that no significant growth reduction was observed at all concentrations of PEG 8000, corresponding to same osmotic potential of alfalfa leaf extracts. Osmotic stress less than –0.2 MPa of PEG 8000 is known to have little effect on root growth at concentrations of extract normally used (Chon *et al.*, 2004). Reduction of root length can be explained mainly by allelopathic effect from extracts, not by osmotic effect (Chon *et al.*, 2003). Although it is often assumed that the response of seeds or seedlings to plant extracts is due entirely to allelopathy, extract may also exert negative osmotic effects on the test species (Bell, 1974), and some investigators have qualitatively assessed the relative importance of the osmotic influence and allellopathic potential of plant extracts on seed germination (Wardle *et al.*, 1992). Wardle *et al.* (1992) concluded, in their study using aqueous leaf extracts of four pasture grass species that bioassays are more realistic when they are compared to control values that are adjusted to the same osmotic potential as the plant extract being tested.

Table 1. Electric conductivity (EC), pH, and osmotic potential of aqueous common thistle extracts at 40 g L^{-1} as affected by different plant parts at 2 days after extraction.

Plant part	EC (S/m)	pH	Osmotic potential (- M Pa)
Leaves	0.40	5.45	0.110
Stems	0.53	5.99	0.165
Roots	0.28	5.72	0.096
Flowers	0.21	5.64	0.085
Distilled water	0.00	6.71	0.001

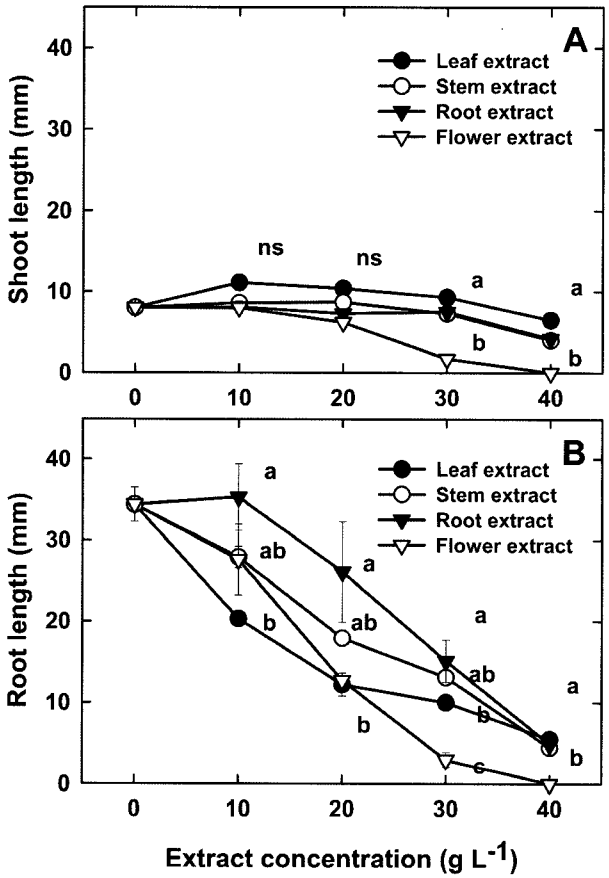

Fig. 1. Effects of common thistle leaf, stem, root and flower extracts on shoot (A) and root lengths (B) of alfalfa as affected by different concentrations. The seedling growth was determined at 6 days after seeding on the filter paper wetted with the various extract concentrations. Within an extract concentration, means followed by the same letter are not significantly different at $p<0.05$. Each bar represents standard error of the mean.

Aqueous flower extracts above 30 g L^{-1} had the greatest inhibitory effect on both the shoot and root growth of test plants while root extracts had the least effect. The degree of inhibition was increased with increasing the extract concentration. At highest extract concentration of 40 g L^{-1}, flower extracts reduced root length by completely, while the other extracts reduced root and shoot lengths of alfalfa by 83-87 % and 19-49%, respectively (Fig. 1). Allelopathic effects of water extracts at 40 g L^{-1} from common thistle were ranked in order of flower (greatest), leaf, stem, and root (least). Such differences might be related to specific allelopathic compounds being produced in larger quantities in certain tissue, imparting a higher level of allelopathy. Release of phytotoxic compounds could also be affected by tissue type. Chou & Leu (1992) reported that flowers among plant parts of *Delonix regia* had the highest inhibition against test plants by 70%. They also concluded that the findings of bioassay and the number and amount of responsible allelopathic compounds found in *Delonix regia* are well correlated.

No significant inhibition in root growth was observed in only methanol solution that used as a control, indicating that the methanol solution was completely evaporated and then was not toxic to seedling growth of test plants. Methanol leaf extracts, which showed most inhibition to aqueous extracts, were assayed against alfalfa and barnyardgrass. Their root growth was significantly inhibited by the extracts at 25, 50, 75, and 100 g L^{-1} compared to untreated controls (Fig. 2). Mixtures at all the concentrations significantly reduced root lengths of alfalfa and barnyardgrass, especially barnyardgrass were more sensitive to the extracts than alfalfa.

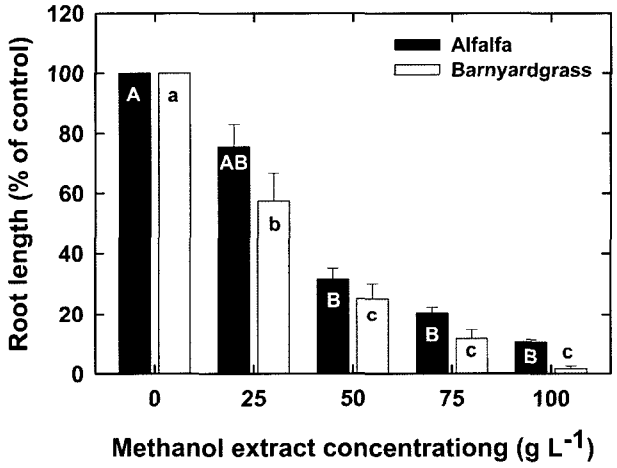

Fig. 2. Effects of methanolic leaf extracts from *Cirsium pendulum* on root lengths of alfalfa and barnyardgrass. Four milliliters of methanol extracts at 25, 50, 75, and 100 gL^{-1} were pipetted on filter paper, evaporated for 24 hours, and 4 mL distilled water was added. The root growth was determined at 6 days after seeding on the filter paper. Within a test plant, alfalfa (capital letter) and barnyardgrass (small letter), means followed by the same letter are not significantly different at $p<0.05$. Each bar represents standard error of the mean.

Phytotoxic effects of 4 solvent fractions on alfalfa root growth

Methanol extracts of common thistle by fraction were assayed against alfalfa. Methanol extracts through fractionation significantly reduced root lengths of alfalfa. Methanol extracts from hexane and EtOAc fractions of common thistle reduced alfalfa root growth more than those of BuOH and water fractions. Methanol extracts from hexane and EtOAc fractions at 50 g L^{-1} reduced root growth by each 97%, while treatment at same concentration of BuOH and water fractions reduced root growth by 77 and 59%, respectively (Fig. 3). Methanol extracts from hexane and ethylacetate fractions of common thistle reduced alfalfa root growth more than did those from butanol and water fractions. The result suggests that phytotoxic allelochemicals were more present in the hexane and EtOAc fractions than in the BuOH and water fractions, resulting in more inhibitory effects on the test plant. Chon (2004) reported that the major phenol compounds identified and quantified in common thistle leaf extracts were coumarin, *trans*-cinnamic acid, and chlorogenic acid. Especially, *trans*-cinnamic acid was found as the greatest amount at ethylacetate fraction. However, these phenolic acids from common thistle leaf extracts may not be major alleochemicals that cause phytotoxic effect.

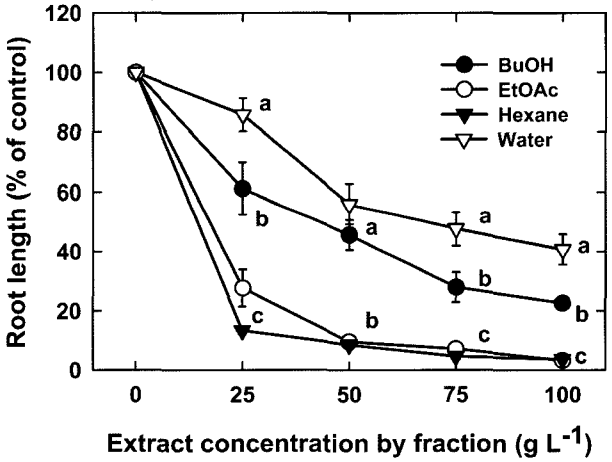

Fig. 3. Effects of four fractions from common thistle leaves on alfalfa root length 6 days after seeding on filter paper wetted with extracts. Each bar represents standard error of the mean. Within an extract concentration, means followed by the same letter are not significantly different at $p<0.05$. Each bar represents standard error of the mean.

Effect of leaf residue incorporation on seedling growth of barnyardgrass and eclipta

The residue incorporation with dry materials of common thistle significantly affected barnyardgrass growth. The degree of inhibition increased with increasing the amount of residue incorporation (Fig. 4). Residues from common thistle at the highest amount of 100 g kg^{-1} reduced shoot fresh weights of barnyardgrass and eclipta by 88 and 58%, respectively. The result shows that grass species was more sensitive to the residue incorporation than broadleaved species. The results show that any inhibition of weed growth should be due primarily to the presence of toxic compounds or excessive solutes within the ground plant samples. Cochran *et al.* (1980) and Elliott *et al.* (1981) reported that crop or weed residue toxicity to plant seedling was likely caused by an allelopathic substance, especially residue inhibition of seedling growth was enhanced if crop residue was incorporated before planting.

In conclusion, this study demonstrates allelopathic effects of common thistle plant extracts or residues on early seedling growths of test plants. Allelopathic effects of water extracts of common thistle above 30 g L^{-1} were ranked in order of flower (greatest), leaf, stem, and root (least). These results indicate that different compounds that cause allelopathic activity could be produced with different amount from different plant parts. Methanol extracts from hexane and ethylacetate fractions of common thistle reduced alfalfa root growth more than did those from butanol and water fractions. The result suggests that causative phytotoxic allelochemicals were more present in the hexane and EtOAc fractions than those in the BuOH and water fractions, resulting in more inhibitory effects on the test plant. The allelopathic potential in extracts and residues of common thistle may be a valuable mean of biological weed control based on natural plant extracts. However, qualification and quantification of the compounds from different plant parts are needed to confirm if the same compound causes allelopathic effect, or if the effects of various plant extracts are dependent on the amount of the causative compound (s).

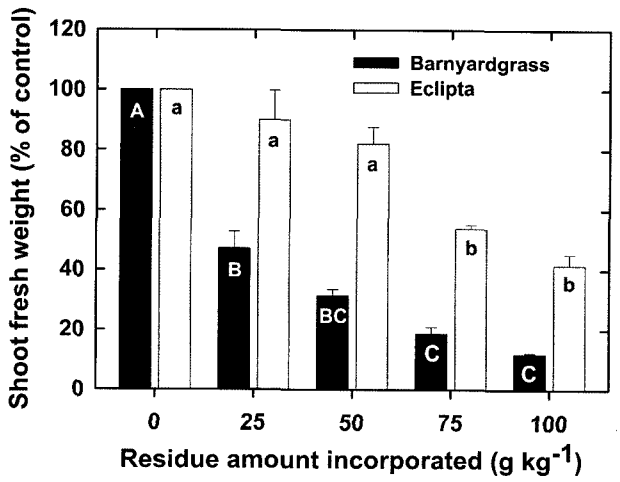

Fig. 4. Effects of residue incorporation with common thistle leaf materials into soil on shoot fresh weights of barnyard grass and eclipta 15 days after seeding or treatment. Within a test plant, barnyardgrass (capital letter) and eclipta (small letter), means followed by the same letter are not significantly different at $p<0.05$. Each bar represents standard error of the mean.

ACKNOWLEDGEMENTS

This research was supported by Korea Research Foundation grant (KRF-2001-003-G00024). Appreciation is expressed to Dr. Young-Min Kim, Dongeuinara Co. Ltd., Dongshin University, Naju, Korea, for his technical assistance.

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