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Scientia Horticulturae 106 (2005) 309–317
SCIENTIA HORTICULTURAE
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Allelopathic potential in lettuce (*Lactuca sativa* L.) plants

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Received 8 July 2004; received in revised form 16 March 2005; accepted 25 April 2005

Abstract

Lettuce (*Lactuca sativa* L.) is known to contain water-soluble substances that are allelopathic. Aqueous or methanol extracts and residues from leaves of lettuce cultivar ''Cheongchima'', which showed the most inhibitory effects, were assayed to determine their allelopathic effects on seed germination and early seedling growth of several plant species. The aqueous extracts applied to filter paper significantly inhibited seed germination of alfalfa with increasing of extract concentration. Methanol extracts from hexane fraction of lettuce plants showed the most inhibition on alfalfa root growth and followed by ethylacetate, butanol and water fractions. Incorporation with leaf residues of 100 g kg $^{-1}$ into soil significantly inhibited shoot and root fresh weights of barnyard grass by 79 and 88%, respectively. These results suggest that extracts or residues from lettuce plants had potent allelopathic activity and that the activity differed depending on cultivar, extract or fraction.

Keywords: Allelopathy; Bioassay; Lettuce; Plant extracts; Residue incorporation

1. Introduction

Allelopathy, defined by Molisch (1937), is the chemical interaction between plants, including stimulatory as well as inhibitory influences. Allelopathy plays an important role

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0304-4238/$ – see front matter
doi:10.1016/j.scienta.2005.04.005

in both natural and agro-ecosystems. Suitable manipulation of the allelopathy towards improvement of crop productivity and environmental protection through eco-friendly control of weeds, pests, crop diseases, conservation of nitrogen in crop land and synthesis of novel agrochemicals based on natural products have gained prominent attention of scientist engaged in allelopathy research. Especially, allelopathy has potential in integrated weed management. Crop plants have the capability to produce and exude allelochemicals into their surroundings to suppress weeds in their vicinity.

Lettuce is an annual herbaceous plant of compositae, one of the largest and most diverse families of flowering plants. Major weeds including barnyard grass (*Echinochloa colonum*), common purslane (*Portulaca oleracea*), smooth pigweed (*Amaranthus hybridus*), shepherd's purse (*Capsella bursa-pastoris*) and common lambsquaters (*Chenopodium album*) are known to interfere with lettuce (Haar and Fennimore, 2003; Santos et al., 2003; Fennimore and Umeda, 2003). Weed control for the weeds in lettuce has based on chemical control method. Santos et al. (2003) reported, in their greenhouse study on mechanism of interference of weeds that smooth pigweed interfere with lettuce primarily through light interception by its taller canopy. Few studies on allelopathic effects of several compositae plant extracts or residues on some agronomic crops and weeds have been reported. Chon et al. (2003) reported that aqueous extracts of lettuce leaf were completely inhibitory to root growth of alfalfa and barnyard grass. 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.

To identify and quantify compounds contained in plant extracts or residues is an important part of the process of discovering agents of allelopathy. Plants contain thousands of natural products, but not all are implicated as being allelopathic (Bell and Charlwood, 1980; Rice, 1984). The major biosynthetic pathways leading to the production of allelochemicals are known to be shikimic acid or acetate pathways (Rice, 1984). Phenolic acids in the literature on allelopathy are often mentioned as putative allelochemicals and are perhaps the most commonly investigated compounds among potential allelochemicals. They are found in a wide range of soils or plants and their phytotoxic potential against various test plants has been demonstrated under controlled conditions. Phenolic compounds are among the most abundant groups of secondary metabolites in plants (Harborne, 1980). Penolics bear hydroxlated aromatic rings including simple phenols, phenolic acids, phenylpropanoids, coumarins, quinones, flavonoids, tannins and other miscellaneous phenols (Harborne, 1980). They are known to be of significance in allelopathy (Inderjit, 1996).

Phenolic acids such as *p*-hydroxybenzoic, vanillic, *p*-coumaric, syringic and ferulic acids are main category of allelochemicals. These phenolic acids have been identified as allelopathic agents in natural and agroecosystems (Blum et al., 1991; Ben-Hammouda et al., 1995). Einhellig et al. (1970) reported that a coumarin derivative, scopoletin, inhibited dry matter production, leaf area expansion and photosynthesis in tobacco (*Nicotiana tabacum* L.), sunflower (*Helianthus annuus* L.) and *A. retroflexus*. Ferulic acid and *p*-coumaric acid have known to reduce leaf water potential and stomatal diffusive conductance in grain sorghum (*Sorghum bicolor* (L.) Moench.) and soyabean (*Glycine max* L.) (Einhellig and Stille, 1979). Chon et al. (2003) reported that the individual compounds

identified from the various fractions of lettuce extracts were mainly coumarin, *trans*-cinnamic acid and chlorogenic acid; chlorogenic acid was detected as the greatest amount in ethylacetate fraction.

The objective of this research was to determine allelopathic effects of aqueous extracts or methanol extracts and residues from lettuce plants through Petri dish and pot tests. This research will promote a better understanding of allelopathy mechanisms in the natural and agricultural ecosystems through investigating the allelopathic effect and quantification of causative allelochemicals.

2. Materials and methods

2.1. Plant sampling and preparation

Lettuce cultivars, ''Cheongchima'', ''Ddukseom'', ''Hoehyang'' and ''Jeokchima'' were grown in plastic pots (20 cm diameter × 15 cm high) filled with silt-loam soil in greenhouse at a field of Experimental Farm, Dongshin University in 2002. The plants were harvested at a vegetative stage 40 days after planting. The plant tissues were clipped by hand 1 cm above the soil and directly oven-dried at 60 °C for 5 days. The dried samples were ground with a Wiley mill to pass a 1-mm screen and then stored in a refrigerator at 2 °C. Forty grams of dried leaves were extracted by soaking in 1 l-distilled water at 24 °C for 24 h in a shaker to give a concentration of 40 g dry tissue l^{-1} (hereafter referred to as '$g\ l^{-1}$'). The extract was filtered through two layers of cheese cloth to remove the fibre debris and centrifuged at 5000 rpm (×4530 *g*) for 2 h. The supernatant was vacuum filtered again through Whatman No. 42 paper.

Methanol extracts from ground plant samples were used for the following bioassay and fractionation. Ground leaf samples of lettuce 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 about 10%. 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.

2.2. Phytotoxic effects of aqueous extracts from four lettuce cultivars

Each stock extract was diluted appropriately with sterile distilled water to give the final concentrations of 10, 20, 30 and 40 g l^{-1}. Distilled water was the control. A Whatman No. 1 filter paper was placed in each 9-cm diameter plastic Petri dish. Four milliliters of diluted extract were pippetted to the filter paper. Root length of alfalfa was measured on all seedlings in each Petri dish at 6 days after placing seeds on the medium. Data were

transformed to percent of control for analysis. Extract concentrations resulting in 50% inhibition of root length (IR_{50}) of controls were determined by interpolation.

2.3. Effect of aqueous plant extracts on alfalfa seed germination

Aqueous leaf extracts from cultivar ''Cheongchima'' showed the highest inhibitory effect were used to evaluate seed germination rate of alfalfa. Alfalfa (cv. ''Vernal'') seeds were surface sterilized with 0.525 g l^{-1} sodium hypochlorite for 15 min. The 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. Leaf extracts from cultivar ''Cheongchima'', showed the most inhibitory effect, were pipetted on to filter paper with the concentrations of 0, 10, 20, 30 and 40 g l^{-1}. Fifty imbibed seeds of alfalfa were separately placed on the filter paper. The Petri dishes were covered, sealed by wrapping in parafilm and placed flat in a growth chamber at 24 °C during the 14-h light period and 22 °C during the 10-h dark period. Plates were illuminated with 400 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR), provided by a mixture of incandescent and fluorescent lamps. Cumulative germination was determined by counting the number of germinated seeds at 12-h intervals over a 132-h period and transformed into percent germination.

2.4. Phytotoxic effects of 4 solvent fractions

The four dried samples concentrated from hexane, EtOAc, BuOH and water fractions were again dissolved in MeOH to compare their phytotoxic effects. Four milliliters of each of these fraction solutions at 25, 50, 75 and 100 g l^{-1} were placed in a 9-cm plastic Petri dish lined with one Whatman No. 1 filter paper and evaporated to dryness for 24 h at 24 °C. For the distilled water control, 4 ml of methanol applied to Petri dishes. After evaporation, 4 ml of distilled water was added onto 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 percentage of control.

2.5. Phytotoxic effect of residue incorporation

For incorporation treatment, residues of lettuce plants were mixed with a high organic matter-potting medium (Hanter 21, Seoul, South 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 amount of plant residues incorporated in a soil medium was 0, 12.5, 25, 50 and 100 g kg^{-1}. The pots were filled with the soil and residue mixture and five barnyard grass seeds per pot were 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 greenhouse for 15 days at 28/22 °C day/night temperature. All plants were harvested to determine plant height, root length and seedling weights of barnyard grass15 days after seeding. Data were transformed to percent of control for analysis.

2.6. Statistical analysis

All experiments had four replications. Data were subjected to analysis of variance. When F test was significant ($p < 0.05$), means were separated based on the least significant difference (LSD) at the 0.05 probability level.

3. Results

3.1. Phytotoxic effects of aqueous leaf extracts of four lettuce cultivars

Aqueous leaf extracts from cultivar ''Cheongchima'' showed the highest inhibitory effect on alfalfa root growth ($IR_{50} = 14.9$) and followed by ''Ddukseom'' ($IR_{50} = 17.4$), ''Hoehyang'' ($IR_{50} = 26.3$) and ''Jeokchima'' ($IR_{50} = 32.1$). The result showed that phytotoxic effects of extracts from four cultivars differed depending on cultivar and that the extracts from cultivar ''Cheongchima'' with green color leaves were more inhibitory than those from three cultivars with purple leaves. Leaf extracts from cultivar ''Cheongchima'' above 20 g l^{-1} reduced alfalfa root length by 100%. The degree of inhibition increased with increasing the extract concentration (Fig. 1).

3.2. Effect of aqueous plant extracts on alfalfa seed germination

Controls germinated in 60 h, plant extracts of lettuce significantly delayed seed germination with increasing of extract concentration. However, highest extracts at 40 g l^{-1} apparently inhibited germination post imbibition, i.e., between inception of the germination process and emergence of the radicle from the seed coat. Germination on each extract was complete in 60 h at 0 g l^{-1}, but was delayed until 132 h at lower concentration of 10 and 20 g l^{-1} and inhibited over 132 h at 30 and 40 g l^{-1} extract concentrations (Fig. 2).

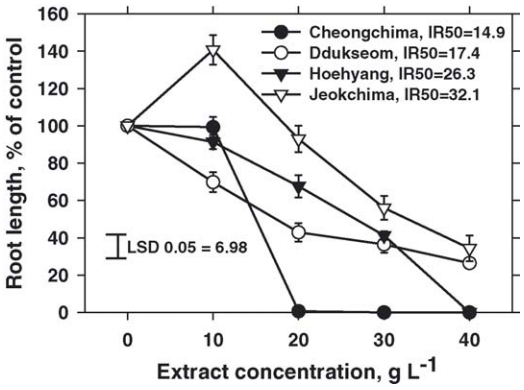

Fig. 1. Effects of aqueous leaf extracts from four lettuce cultivars on root length of alfalfa at 6 days after seeding on filter paper wetted with the extracts. Each bar represents standard error of the mean.

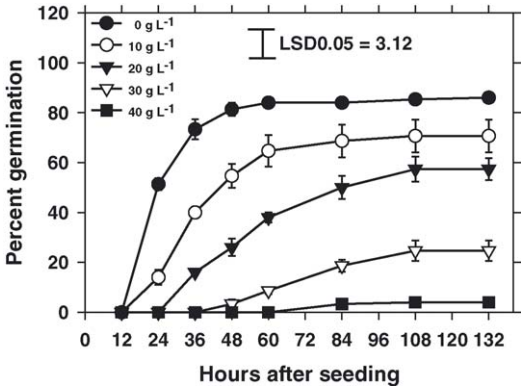

Fig. 2. Effect of aqueous lettuce leaf extracts on seed germination of alfalfa. Each bar represents standard error of the mean.

3.3. Phytotoxic effects of four solvent fractions

With root growth of alfalfa, all fractions exhibited significant biological activity compared with the control. The greatest phytotoxicity was associated with the hexane fraction, the least with the butanol fraction. Methanol extracts at all concentrations from hexane and EtOAc fractions of lettuce reduced alfalfa root growth more than those of BuOH and water fractions. Methanol extracts at 50 g l^{-1} from hexane and EtOAc fractions reduced root growth by 94 and 84%, respectively, while a treatment at same concentration of BuOH and water fractions reduced root growth by each 63%, respectively (Fig. 3).

3.4. Phtyotoxic effect of residue incorporation

The residue incorporation with dry materials of lettuce plant significantly affected barnyard grass growth compared with the control. The degree of inhibition increased with

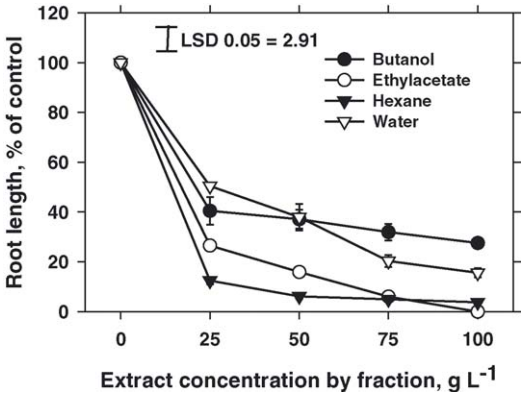

Fig. 3. Effects of methanol extracts from various fractions of lettuce leaves on alfalfa root length 6 days after seeding. Each bar represents standard error of the mean.

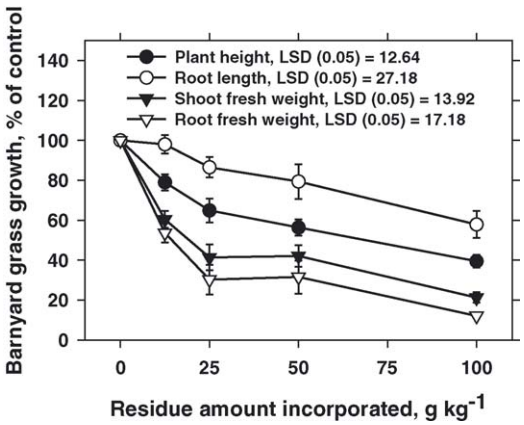

Fig. 4. Effect of residue incorporation with ground samples from lettuce leaves on seedling growth of barnyard grass 15 days after seeding or treatment. Each bar represents standard error of the mean.

increasing the amount of residue incorporation (Fig. 4). Residues from lettuce plants at the highest amount of 100 g kg^{-1} reduced shoot length, root length, shoot fresh weight and root fresh weight of barnyard grass by 60, 42, 79 and 88%, respectively.

4. Discussions

The result showed that phytotoxic effects of extracts from four cultivars differed depending on cultivar and that the extracts from cultivar ''Cheongchima'' with green color leaves were more inhibitory than those from three cultivars with purple leaves. Leaf extracts of lettuce significantly delayed seed germination with increasing of extract concentration. Delayed seed germination by an allelopathic extract could be confounded with osmotic effects on rate of imbibition, delayed initiation of germination and especially cell elongation (Black, 1989), the main factor that affects root growth before and after the tip penetrates the seed coat (Bewley and Black, 1978). More recently, Chon et al. (2004) concluded from studies using aqueous alfalfa leaf extract that delayed seed germination and, especially, reduced root elongation were due mainly to toxic factors of the leaf extract.

In our previous study (Chon et al., 2003), the major allelopathic substances present in the lettuce plant were analyzed by HPLC using standard compounds and the individual compounds identified were coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid and chlorogenic acid; *p*-coumaric acid was found in the greatest amount at ethylacetate fraction. Chon and Kim (2002) reported, in their study on phytotoxic effects of causative allelochemicals that coumarin at 10^{-3} M was most inhibitory on root growth of alfalfa and followed by *trans*-cinnamic acid and *o*-coumaric acid.

Methanol extracts from hexane and EtOAc fractions of lettuce reduced alfalfa root growth more than those of BuOH and water fractions. The result suggests that phytotoxic substances 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. Methanol extracts from fractions were more inhibitory effect on alfalfa seedling growth than did aqueous or

methanol extracts without fractionation procedure, indicating improvement of bioassay sensitivity.

Growth characteristics of barnyard grass were all significantly lower with dry-residue incorporation than in the control. The residue incorporation with dry materials significantly affected barnyard grass growth. 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, a bioassay on allelopathic effects of different solvent extracts or residues from lettuce plant demonstrates that lettuce had potent herbicidal activity on seed germination and early seedling growths of test plants and that the activity differed depending on cultivar, extract or fraction. Such differences might be related to specific allelopathic compounds being produced in larger quantities in certain cultivar or fraction, imparting a higher level of allelopathy. The allelopathic potential in extracts and residues of lettuce may be a valuable mean of biological weed control based on natural plant extracts in lettuce cropping system.

Acknowledgements

This research was supported by Korea Research Foundation grant (KRF-2001-003-G00024). Appreciation is expressed to my colleagues at Biotechnology Industrialization Center, Dongshin University, Naju, Korea, for their technical assistance.

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