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Phytotoxic Effects of *Xanthium occidentale* Extracts and Residues on Seedling Growth of Several Plant Species

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ABSTRACT

Compositae plants are known to contain biologically active substances that are allelopathic to agricultural crops as well as weed species. Aqueous or methanol extracts and plant residues from leaves of *Xanthium occidentale* were assayed against alfalfa (*Medicago sativa*) to determine its allelopathic effects, and the results showed that the extracts applied onto filter paper significantly inhibited seed germination as well as root growth of alfalfa. Aqueous leaf extracts of 40 g L^{-1} were completely inhibitory on root growth of alfalfa, while root growths of barley (*Hordeum vulgare* L.) and soybean (*Glycine max* L.) were less sensitive. Leaf residue incorporation at 100 g kg^{-1} into soil on seedling growth of barnyard grass (*Echinochloa crus-galli* Beauv. var. oryzicola Ohwi) inhibited both shoot and root fresh weights of barnyard grass by 94 and 96 %, respectively. Methanol extracts from BuOH and EtOAc fractions of X. *occidentale* reduced alfalfa root growth more than did those from hexane and water fractions. The results based on bioassay of extracts and residues show that X. *occidentale* had potent an allelopathic activity against other plant species.

Key words : allelopathy, aqueous extracts, bioassay, fractionation, residue incorporation, *Xanthium occidentale*.

INTRODUCTION

Allelopathy could be one of alternative control measures, since the principle from plants contributes to weed management strategies. Allelopathy was defined as any direct or indirect harmful or beneficial effect of one plant on another through the production of chemical compounds that escape into the environment (Rice, 1984). Most assessments of allelopathy involve bioassays of plant or soil extracts based on seed germination and seedling growth. Generally germination is less sensitive to the extracts than is seedling growth, especially root growth (Miller, 1996).

Allelopathic effects of several Compositae plant extracts or residues on agronomic crops and weeds have been reported. Inam *et al.* (1987) found that aqueous extracts of X. *strumarium* from different plant parts reduce germination, early growth and dry weight of B*rassica compestris, Lactuca sativa, and Pennisetum americanum*. Especially, *Parthenium hysterophours* is known to be very allelopathic to wheat (Kanchan and Jayachandra, 1979), soybean, and corn (Mersie and

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Singh, 1987). Their extracts and residues significantly reduced germination and shoot and root dry weight of the test plants. 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. C. arvense litter reduced the growth of *Amaranthus retroflexus* L. and *Setaria viridis* L. more than that of cucumber (*Cucumis sativus L.*) or barley (Stachon and Zimdal, 1980). In their field experiment, high densities of *C. arvense* reduced the incidence of annual weeds growing in the same vicinity of *C. arvense*.

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. The major biosynthetic pathways leading to the production of allelochemicals are probably shikimic acid or acetate pathways (Rice, 1984). Phenolic acids such as *p*-hydroxybenzoic, vanillic, *p*-coumaric, syringic and ferulic acids are a main category of allelochemicals. These phenolic acids have been identified as allelopathic agents in natural and agroecosystems (Guenzi and McCalla, 1966; 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 soybean (Einhellig and Stille, 1979). Numerous studies have also shown that many phenolics are inhibitory (allelopathic) to germinating seeds or growing plants (National Academy of Sciences, 1971). Chon *et al.* (2003) reported that the individual compounds identified in *X. occidentale* were mainly coumarin, trans-cinnamic acid, and p-chlorogenic acid; trans-cinnamic acid was detected as the greatest amount in ethylacetate fraction.

The objective of this research was to determine allelopathic effects of aqueous and methanol extracts or residues from *X. occidentale* plants on seedling growth of test plants. This research will promote a better understanding of allelopathy mechanisms in the natural- and agro-ecosystems through bioassay of the causative allelochemicals in plant extracts or residues.

MATERIALS AND METHODS

Sampling and preparation of plant materials

X. occidentale plants grown in pastures of the Suncheon area, Korea were harvested at a vegetative stage in May 2001. The plants were separately sampled into leaves, stems, and roots. The samples were immediately oven-dried at 60℃ for 5 days, ground with a Wiley mill to pass through a 1-mm screen, and stored in a refrigerator at 2℃ until required. Forty grams of dried leaves, stems and roots were separately extracted by soaking in 1L distilled water at 24℃ for 24 h in a shaker to give a concentration of 40g dry tissue L^{-1} (hereafter referred to as '$g\ L^{-1}$'). The extract was filtered through two layers of cheesecloth to remove the fibre debris, and centrifuged at 5000 rpm (x 4530g) 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 quantification of the causative allelochemicals.

Effect of aqueous plant extracts on root growth of alfalfa, barley and soybean

To know the response of upland crops to aqueous leaf extracts, leaf extracts of *X. occidentale* were prepared. Two layers of Whatman No. 1 filter paper were placed in each 9-cm-diameter plastic Petri dish.

Five milliliters of diluted extract were pipetted to the filter paper. Imbibed seeds of alfalfa (cv. Vernal), barley (cv. Saessalbori), and soybean (cv. Gwangan) were separately placed on filter paper wetted with aqueous plant extract concentrations of 0, 10, 20, 30, and 40 g L^{-1}. Bioassay procedures and conditions were same to the previous work. Root lengths on all seedlings were measured at 144 hours after transfer of seed on filter paper. Data were transformed to percent of control for analysis as used. There were two experiments, each with four replications.

Effect of residue incorporation on seedling growth of barnyard grass

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 cm3 pot, by vigorously shaking the components in plastic bags. The amount of plant residues in a soil medium used were; 0, 12.5, 25, 50, and 100 g kg^{-1}. After mixing, pots were filled with the medium 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 under greenhouse temperatures at 28℃ day/22℃ night. All plants were harvested to determine plant height, root length, shoot and root fresh weights 15 d after seeding. Data were transformed to percent of control for analysis.

Bioassay of 4 fractions from methanol extracts

Ground leaf samples of *X. occidentale* 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 using a rotary evaporator (N-1000V-W, Eyela, Japan). The yield of dried crude extracts from the original plant leaves was 10-15%. For fractionation, crude methanol extracts mixed with distilled water and n-hexane to collect hexane extracts, were shaken for 2 h. After hexane collection, the distilled water fractions were added with ethylacetate (EtOAc) to obtain EtOAc extracts in the same way. The same procedure was used in preparing *n*-butanol (BuOH) and water extraction. The fractions were taken to dryness on a rotary evaporator at 40-50℃.

The four dried samples, hexane, EtOAc, BuOH, and water fractions were dissolved in 95% 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 to Whatman No. 1 filter paper in Petri dish and evaporated to dryness for 24 h at 24℃. After evaporation, four milliliters of distilled water was pipetted to the filter paper and then 15 imbibed seeds of alfalfa were placed on the paper and grown for 6 d. Bioassay procedures and conditions were same to the previous work. Root length of alfalfa was measured for all seedlings in each Petri dish. The data were transformed into % of control and analyzed.

RESULTS AND DISCUSSION

Effect of aqueous plant extracts on root growth of alfalfa, barley and soybean

Root length of alfalfa was more reduced by the extracts treatments than was that of barley or soyabean. At highest extract concentrations of 40 g L^{-1}, the root growth of alfalfa was markedly reduced above 95%, while root growth of barley and soybean was less reduced, ranging from 40 to 60% reduction (Fig. 1). Allelopathic effects of several Compositae plant extracts or residues on some agronomic crops and weeds have been reported. Inam *et al.* (1987) reported that aqueous extracts of *Xanthium strumarium* from different plant parts reduce germination, early growth

and dry weight of *Brassica compestris, Lactuca sativa, and Pennisetum americanum. Parthenium hysterophours* is also known to be very allelopathic to wheat (Kanchan and Jayachandra, 1979), soybean, and corn (Mersie and Singh, 1987).

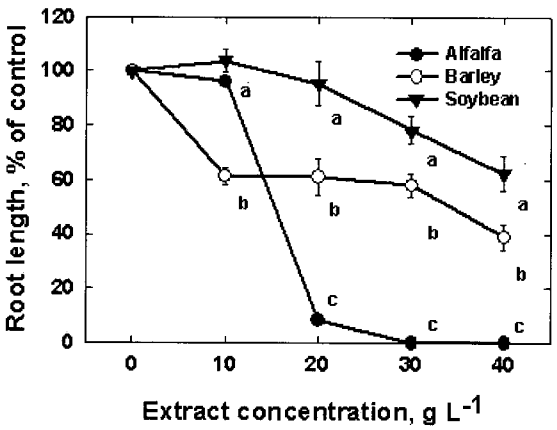

Fig. 1. Effect of aqueous *X. occidentale* extracts on root lengths of alfalfa, barley and soybean at 6 d after placing on filter paper wetted with extract. Within an extract concentration, means followed by the same letter are not significantly different at $p<0.05$. Each bar represents standard error of the mean.

Effect of residue incorporation on seedling growth of barnyard grass

The residue incorporation with dry materials significantly affected barnyard grass growth. The degree of inhibition increased with increasing the amount of residue incorporation (Fig. 2). Residues from *X. occidentale* at the highest amount of 100 g kg $^{-1}$ reduced shoot and root fresh weights of barnyard grass by 94 and 97%, respectively. The results also indicate that any inhibition of weed growth should be due primarily to the presence of toxic compounds or excessive solutes within the ground plant top growth. Cochran *et al.* (1982) and Elliott *et al.* (1981) reported that crop or weed residue toxicity to plant seedling was likely caused by an allelopathic chemical, especially residue inhibition of seedling growth was enhanced if crop residue was incorporated before planting.

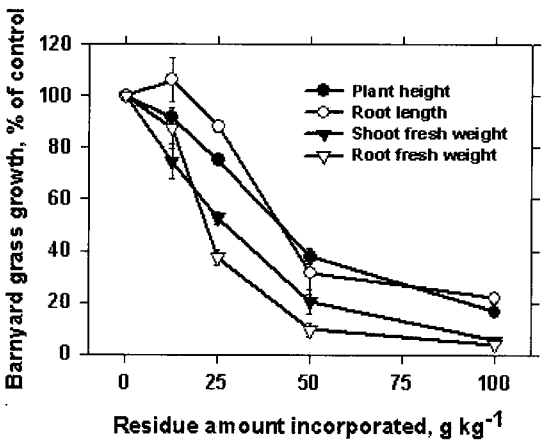

Fig. 2. Effect of residue incorporation with ground *X. occidentale* leaf materials on seedling growth of barnyard grass 15 days after seeding or treatment. Each bar represents standard error of the mean.

Bioassay of 4 fractions from methanol extracts

Methanol extracts of *X. occidentale* by fraction were also assayed against alfalfa. No significant difference was observed between the two controls, only between methanol solution and the distilled water (Data not shown). Methanol extracts from BuOH and EtOAc fractions of *X. occidentale* reduced alfalfa root growth more than did those from hexane and water fractions. Methanol extracts from BuOH and EtOAc fractions at 25 g L^{-1} reduced root growth by above 95%, while treatments at same concentration of hexane and water fractions reduced root growth by 45 and 40%, respectively (Fig. 3). The result suggests that BuOH and EtOAc fractions had more allelochemicals that are phytotoxic on seedling growth of alfalfa than were hexane and water fractions. The analysis of quantitative determination by means of HPLC shows that major allelochemicals are detected as the highest amounts in water and EtOAc fractions (Chon *et al.*, 2003).

In conclusion, bioassays on allelopathic effects using aqueous or methanol extracts and residues demonstrated that the *X. occidentale* plants had potent herbicidal activity and various allelochemicals, showing inhibitory

effects on early seedling growths of several plant species. Different compounds that cause allelopathy could be produced with different types and amounts of causative allelochemicals depending on fractionation method. Such differences also might be related to specific allelopathic compounds being produced in larger quantities in certain fractions, imparting a higher level of allelopathy. Our results suggest that the allelopathy of *X. occidentale* as a Compositae plant species may be a valuable alternative mean of weed control based on natural plant extracts or residues.

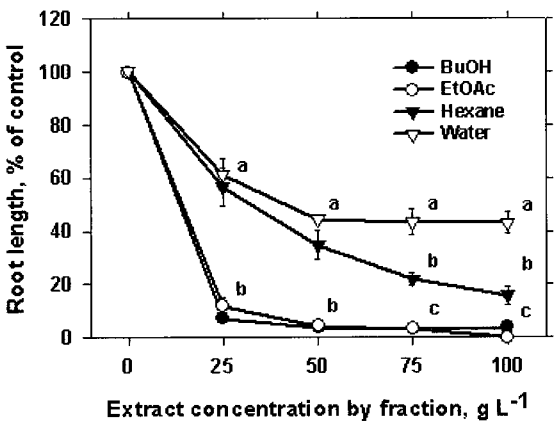

Fig. 3. Effects of methanol extracts from 4 fractions of *X. occidentale* on root length of alfalfa 6 days after seeding. Within an extract concentration, means followed by the same letter are not significantly different at $p<0.05$. Each bar represents standard error of the mean.

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