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Allelopathy and Quantification of Causative Allelochemicals in Sweet Potato

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ABSTRACT: Greenhouse and laboratory studies were conducted to determine the allelopathic potentials of extracts or residues from sweet potato (*Ipomoea batatas* L. (Lam). The extracts applied on filter paper in a Petri dish bioassay significantly inhibited root growth of alfalfa. Aqueous leachates at 40 g dry tissue L^{-1} (g L^{-1}) from leaves showed the highest inhibition against alfalfa, and followed by stems and roots. Alfalfa root growth was significantly inhibited by methanol extracts of the same plants as the concentration increased. The effect of residue incorporation into soil on seedling growth of corn, soybean, barnyard grass and eclipta was examined in the greenhouse, and results showed that the leaf residues at 200 g kg^{-1} by plant parts inhibited shoot dry and root dry weights of test plants by 60-80%. By means of HPLC, causative allelopathic substances present in plant parts of sweet potato "Sinyulmi" were identified as coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, and chlorogenic acid. Total content of these compounds for leaves extracts were detected as the greatest amount in EtOAc fraction, especially *trans*-cinnamic acid was the greatest component. These results suggest that sweet potato plants have herbicidal potentials, and that their activities exhibit differently depending on plant parts.

Keywords: Allelopathy, Aqueous extracts, Bioassay, HPLC, Residue incorporation, Methanol extracts, Sweet potato.

Allelopathy was defined by Molisch (1937) as a chemical interaction between plants (sometimes microbes and higher plants) including stimulatory as well as inhibitory influences. It plays a key role in natural as well as manipulated ecosystems such as agricultural areas. Allelopathic effects exhibited differently depending on plant parts of donor plant. It is accepted that water extracts of top growth (especially leaves) produce more allelopathy for seedlings than those from roots, stems, and crowns of alfalfa (*Medicago sativa* L.) (Chung and Miller, 1995; Miller, 1996), and that shoot extract from the reproductive stage was more inhibitory than from the vegetative stage under laboratory conditions (Chung and Miller, 1995; Hedge and Miller, 1992). Chung and Miller (1995) ranked autotoxic effects of water extracts of plant parts of alfalfa as leaf (greatest), seed, root, flower, and stem (least). Chou and Leu (1992) reported that extracts from flowers of *Delonix regia* (BOJ) RAF exhibited the highest inhibition against three test plants, alfalfa, lettuce (*Lactuca sativa*), and Chinese cabbage (*Brassica chinensis*).

Sweet potato has long been recognized as a very competitive crop against certain weeds (Villmayor and Perez, 1983), despite its prostrate growth habits. Taylorson (1967) first reported the occurrence of components of sweet potato that inhibited cucumber (*Cucumis sativa* L.) hypocotyl growth and yellow tuber germination. Decaying sweet potato tissues inhibited alfalfa (*Medicago sativa* L.) and yellow nutsedge growth (Harrison and Petersom, 1986; Walker and Jenkins, 1986). Walker *et al.* (1989) reported that sweet potato residues impaired the uptake of Ca, Mg, and S by cowpea. Sweet potato greatly reduced yellow nutsedge growth when the two species were grown together in a greenhouse system designed to eliminate sweet potato competition for light, water, and nutrients (Harrison and Peterson, 1991). Aqueous methanol extracts of sweet potato periderm tissue inhibited seed germination of nine weed species, and a high degree of selectivity among species was observed (Peterson and Harrison, 1991)

The major biosynthetic pathways leading to production of allelochemicals probably are shikimic acid or acetate pathways (Rice, 1984). Allelopathic properties have been demonstrated for the related species of sweet potato, *Ipomoea aquatica* (Forsk.) and *I. tricolor* (Cav.). *I. aquatica* contains terpenoid compounds that were inhibitory to pearl millet (*Pennisetum typhoideum* Rich.) seedling growth (Singhvi and Sharma, 1984), and *I. tricolor* contains a jalapolinic acid (11-hydroxyhexadecanoic acid)-glycoside complex that was inhibitory in seed germination and seedling growth bioassays (Anaya *et al.*, 1990).

The objectives of this study were to determine allelopathic potentials of three different plant parts of sweet potato and to quantify the causative allelochemicals in each plant part. Weed control using natural plant extracts from sweet potato may be a possible development.

MATERIALS AND METHODS

Sampling and preparation of plant materials

Whole parts of sweet potato "Sinyulmi" were harvested at

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the Experimental Farm of Dongshin University in October 2002. The plant samples were separated into leaves, stems, and roots. The three samples were immediately oven-dried at 60°C for 5 days (Chon and Nelson, 2001), ground with a Wiley mill to pass a 1-mm screen, and stored in a refrigerator at 2°C until used.

Effects of Aqueous Extracts on Alfalfa Root Growth

Forty grams of dried leaves, stems, and roots of sweet potato 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}). The extract was filtered through two layers of cheesecloth to remove the fibre debris, and centrifuged at 5000 rpm (x 4530 g) for 2 hours. The supernatant was vacuum filtered again through Whatman No. 42 paper.

Each stock extract from the plant parts of the sweet potato was diluted appropriately with sterile distilled water to give the final concentrations of 10, 20, 30, and 40 g L^{-1}. Four milliliters of the extracts were pippetted onto Whatman No. 1 filter paper in a Petri dish. Distilled water was the control. Alfalfa (cv. 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 swollen seeds were evenly placed on filter paper wetted with extract in each Petri dish. 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} PAR, provided by a mixture of incandescent and fluorescent lamps. Root length was measured on all seedlings in each petri dish 6 days after seeding on the filter paper. Data were transformed to percent of control for analysis as used.

Effects of Methanol Extracts on Alfalfa Root Growth

Ground samples of sweet potatoes were soaked with 95% methanol at room temperature. The samples were 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 sweet potato plants was around 10-15%. To compare the phytotoxic effects of methanol extracts of leaves, stems and roots from three cultivars at 25, 50, 75, and 100 g L^{-1} and only methanol solution (control) were pipetted to Whatman No.1 filter paper in Petri dish and evaporated to dryness for 24 hours at 24°C. 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 days. Root length was measured on all seedlings in each petri dish. Data were transformed to percent of control for analysis.

Effect of Residue Incorporation on Seedling Growth of Several Crops and Weeds

Leaf residues of sweet potato, which showed highest inhibitory against alfalfa among three plant parts in previous bioassay, were incorporated with a high organic matter potting medium that contained 30% sphagnum peat moss, 50% vermiculite, 18% perlite, and 2% sand (v/v) per 425 cm^3 pot by vigorously shaking the components in plastic bags. The amount (w/w) of plant residues used were; 0, 25, 50, 75 and 100 g kg^{-1}. After incorporation, five seeds of corn, soybean, barnyard grass and eclipta 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. The experiments were conducted in greenhouse for 20 days during the summer. The average temperature was 28°C. Fresh and dry weights of all plant roots were determined 20 days after seeding. All treatments were replicated four times using a randomized complete block design.

Identification and Quantification of Allelopathic Substances

Crude methanol extracts from white sweet potato mixed with distilled water and hexane, were shaken to collect hexane extracts 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 butanol and water extraction. The fractions were taken to dryness on a rotary evaporator at 40°C. The four dried hexane, EtOAc, BuOH, and water fractions were redissolved in HPLC grade MeOH to give 1,000 ppm for HPLC analysis.

The standard phenol compounds used for HPLC analysis were coumarin, trans-cinnamic acid, o-coumaric acid, p-coumaric acid, and chlorogenic acid (Aldrich Co., USA). All of chemicals were purchased as high purity standards and the used solvents were HPLC spectral grade. All solvents and distilled water were degassed before use. All solvent ratios were based on volume.

Allelopathic compounds were identified by a HPLC using SPP 10AVP (Shimadzu, Tokyo, Japan) with a flow rate of 1 mL min^{-1}, the column was CAPCELL PAK C18 SG120 (4.6×250 mm) and an autoinjector with a 10 μl sample loop

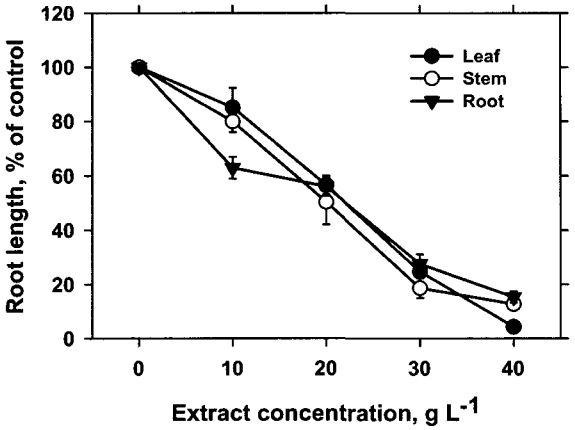

Fig. 1. Effects of aqueous extracts from 3 different plant parts of sweet potato on alfalfa root length 6 days after seeding. The seedling growth was determined at 6 days after seeding on the filter paper wetted with the various extracts. Bars represent standard error.

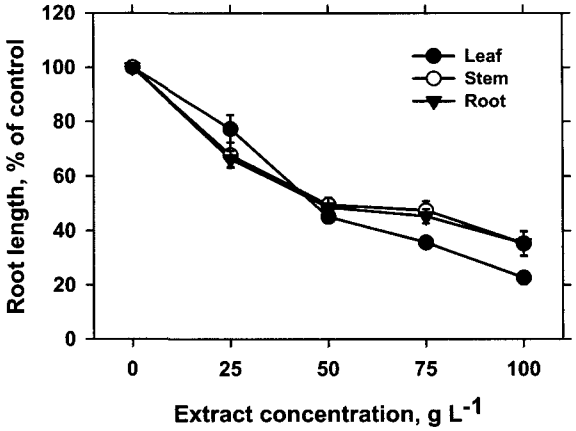

Fig. 2. Effects of methanol extracts of plant parts from sweet potato on root lengths of alfalfa. Four milliliters of methanol extracts at 0, 25, 50, 75, and 100 g L^{-1} were pipetted on filter paper, evaporated for 24 hours, and then added with distilled water of 4 mL. The root growth was determined at 6 days after seeding on the filter paper. Bars represent standard error.

was employed. The mobile phase consisted of water, methanol and acetic acid in the ratio of 12:15:1 volume, respectively. The UV detector wavelength was set at 275 nm. Standard compounds were chromatographed alone and as mixtures. Retention times for the standard compounds and the major peaks in the extract were recorded. Phenolic compounds were identified by retention times or standard addition, and amounts were calculated by comparing peak area with those of standards.

RESULTS AND DISCUSSION

Effects of Aqueous Extracts on Alfalfa Root Growth

Aqueous extracts from different plant parts of sweet potatoes inhibited root lengths of alfalfa differently. Leaf extracts of "Sinyulmi" had the greatest inhibitory effect on root growth of alfalfa. The degree of inhibition in each cultivar was increased with increasing the extract concentration. At highest extract concentration of 40 g L^{-1}, leaf extracts of "Sinyulmi" reduced root length by 96%, while the stem and root extracts reduced root lengths of alfalfa by 87% and 85%, respectively (Fig. 1). Allelopathic effects of aqeous extracts at 40 g L^{-1} from sweet potato were ranked in order of leaf (greatest), stem, and root (least). Such differences might be related to 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 and 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

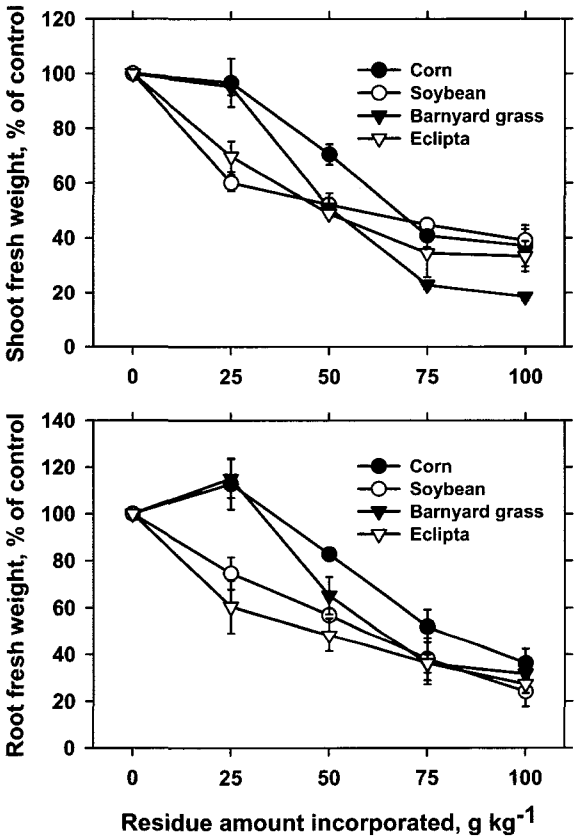

Fig. 3. Effect of residue incorporation with ground sweet potato leaves on shoot (A) and root fresh weight (B) of barnyard grass. The seedling growth was determined at 20 days after seeding on potting medium. Bars represent standard error.

amount of responsible allelopathic compounds found in *Delonix regia* are well correlated.

Effects of Methanol Extracts on Alfalfa Root Growth

Methanol extracts of sweet potato were assayed against alfalfa. No significant difference was observed between two controls, only methanol solution and the distilled water (Data not shown). Each methanol extract significantly reduced root length of alfalfa by 70-80% as the extract concentration increased, even though it shows less inhibitory effect than aqueous leachates. MeOH extracts of leaves at 75-100 g L^{-1} showed the most inhibitory and followed by stems and roots (Fig. 2). Taylorson (1967) and Harrison and Peterson (1986) also found that aqueous ethanol or methanol extracts of sweet potato periderm were inhibitory to yellow nutsedge root growth and tuber germination and cucumber hypocotyl.

Effect of Residue Incorporation on Seedling Growth of Several Crops and Weeds

The dry matter incorporation with leaf samples affected shoot and root growth of test plants. The degree of inhibition increased with increasing the amount of residue incorporation. Leaf residues at 75-100 g kg^{-1} had greatest inhibitory effect on shoot growth (around 80% inhibition) of barnyard grass (Fig. 3). Leaf residue at 100 g kg^{-1} reduced root fresh weight of 4 test plants by 60-75%. 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 sweet potato plants. Walker and Jenkins (1986) demonstrated that sweet potato field residues were inhibitory to sweet potato and cowpea (*Vigna unguiculata* (L.) Walp.) growth.

Identification and Quantification of Allelopathic Substances

The major allelopathic substances present in the methanol extracts from "Sinyulmi" leaf, stem and root were analyzed by HPLC using standard compounds and recorded as each or total phenol compounds in four fractions. The individual compounds identified were coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, and chlorogenic acid.

By means of HPLC analysis several phenolic compounds in leaves of sweet were detected as the highest amount in the EtOAc fraction (37.73 mg 100 g^{-1}). Of these, *trans*-cinnamic acid was present in all fractions as the greatest component (21.5 mg) and followed by p-coumaric acid and coumarin (Table 1). However, stem and root sample had the highest amount of phenolic compounds in the BuOH fraction at 19.7 and 6.2 mg, respectively. Chlorogenic acid in stem and root samples was the greatest component at 29.0 and 11.3 mg, respectively (Table 2 and 3). Taken together,

Table 1. Quantitative determination of HPLC analysis on some phenolic compounds present in leaves of sweet potato "Sinyulmi".

Compound	Fraction				
	Hexane	EtOAc	BuOH	Water	Total
			– mg 100 g^{-1} –		
coumarin	0.1495	9.1338	4.1347	0.1422	13.5600
trans-cinnamic acid	0.2611	20.8900	0.2809	0.0683	21.5000
o-coumaric acid	0.0615				0.0615
p-coumaric acid	7.7106	9.7051			17.4700
chlorogenic acid	0.1859		5.3763	2.1223	7.6800
Total	0.7090	37.7300	19.4970	2.3328	60.2729

Table 2. Quantitative determination of HPLC analysis on some phenolic compounds present in stems of sweet potato "Sinyulmi".

Compound	Fraction				
	Hexane	EtOAc	BuOH	Water	Total
			– mg 100 g^{-1} –		
coumarin	0.3090	0.1288	1.9602	0.1932	2.5912
trans-cinnamic acid	0.0272	0.0223	0.2010	0.0174	0.2679
o-coumaric acid	0.1017	0.0424	0.6443	0.0636	0.8520
p-coumaric acid					0.0000
chlorogenic acid	0.7158	8.8376	16.8902	2.5222	28.9658
Total	1.1537	9.0311	19.6957	2.7964	32.6769

Table 3. Quantitative determination of HPLC analysis on some phenolic compounds present in roots of sweet potato "Sinyulmi".

Compound	Fraction				
	Hexane	EtOAc	BuOH	Water	Total
			– mg 100 g^{-1} –		
coumarin	0.2977	0.4510		0.0528	0.8015
trans-cinnamic acid	0.0192	0.0366	0.0158	0.0040	0.0756
o-coumaric acid	0.0098	0.1484		0.0174	0.1756
p-coumaric acid		0.8388		0.0102	0.8490
chlorogenic acid	0.6501	1.1038	6.2288	3.3224	11.3051
Total	0.9768	2.5786	6.2446	3.4068	13.2068

leaf samples (60.3 mg) of sweet potato had the highest phenolic compounds and followed by stems (32.7 mg) and roots (13.3 mg). The results show that findings of the bioassay through petri-dish and greenhouse studies were highly correlated with the amount and type of causative allelochemicals found in plant extracts. The results corroborate the report of Sondiheimer (1958) that caffeic acid and also chlorogenic acid and two of its isomers were identified in sweet potato peelings.

In conclusion, bioassays on allelopathic effects of sweet potato extracts or residues against test plants demonstrated that sweet potato plants had potent allelopathy on several plant specie. Leaf extracts of "Synyulmi" had more inhibitory effect on test plants than stems or roots. HPLC analysis suggests that differential allelopathic effect by plant parts would be due to quantitative as well as qualitative matters of the causative chemicals, suggesting that types and amount of allelochemicals detected from different plant parts exhibited differently.

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