An Assessment of Allelopathic Potential of Korean Black Soybean Plant Parts

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ABSTRACT: A series of aqueous extracts and residues from leaves, stems, roots, pods and seeds of Korean black soybean (*Glycine max* (L.) Merr.) were assayed against alfalfa (*Medicago sativa*) and barnyard grass (*Echinochloa crus-galli*) to determine their allelopathic activities through petri-dish and greenhouse experiments, and the results showed highest inhibition in the extracts or residues from the seeds, and followed by pods. The extracts of 40 g dry tissue L^{-1} applied on filter paper in petri-dish bioassay significantly inhibited root growth of alfalfa, and especially extracts from seeds and pods reduced root length of alfalfa more than those from leaves, stems, or roots. Plant height, root length, shoot and root dry weights of barnyard grass were reduced significantly by residue incorporation of seeds and pods as the incorporated amount increased. These results suggest that black soybean plants had herbicidal potential, and their activities were exhibited differently depending on plant parts.

Keywords: Black soybean, aqueous extracts, residue incorporation, plant parts, bioassay, allelopathy

Allelopathy, defined by Molisch (1937), is the chemical interaction between plants, or sometimes between microbes and plants, including stimulatory as well as inhibitory influences. Allelopathy plays a key role in both natural and manipulated ecosystems. In the former it plays an active role in terrestrial as well as plant succession, vegetation patterning, imparting dormancy to the seeds and preventing the seed decay, while in the latter it results in a number of direct or indirect effects (Kohli *et al.*, 1998).

Most of the studies on allelopathy have focused on its negative impacts. Several weeds, crops, and trees have been shown to exert allelopathic influence on the crops, thus affecting their germination and growth adversely. There are many examples of allelopathic activity by plants in the agro-ecosystems. Allelopathic interactions include weed-weed, weed-crop, and crop-crop interrelationships. Phytotoxic compounds have been found in oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and grain sorghum (*Sorghum bicolor* (L.) Moench)(Guenzi and Norstadt, 1967). Maun (1977) grew barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) in competition boxes with 'Vansoy' soybeans and found that 6- to 10-week-old soybean plants reduced both height and dry matter production of barnyard grass 94%. Weed species such as velvetleaf (*Abutilon theophrasti* Medic.)(Gressel and Holm, 1964) and quackgrass (*Agropyron repens* (L.) Beauv.)(Gabor and Veatch, 1981) have been shown to possess the ability to inhibit competing plant growth by release of chemicals into the soil environment.

Soybean is an increasingly important human food source, animal feed, and new functional agent, mainly due to its high protein and oil contents (Pazdernik *et al.*, 1997). Soybean cultivars also possess the ability to inhibit growth of other plants by chemical exudates. Guenzi and McCalla (1962) found that ground soybean hay inhibited wheat root growth 30% and wheat shoot growth 45%. Massantini et al. (1997) also found evidence for allelopathy in several soybean lines. Soybeans have various seed coat colors such as yellow, green, brown or black. The pigmentation of such colors is due to anthocyanins of breakdown products of these pigments (Carlson and Lernsten, 1987; Todd and Vodkin, 1993). Black soybeans, which have been widely utilized as foodstuffs and oriental medicinal materials contain anthocyanins in the seed coat (Chung, 1998). The black pigmentation is due to accumulation of anthocyanins in the epidermis palisade layer in the seed coat (Todd and Vodkin, 1993).

A few workers (Dawson, 1964; Eaton *et al.*, 1973; Knake and Slife, 1962; Wicks *et al.*, 1973) have evaluated the ecological effects of a crop on the weed species growing with it, especially a single weed species. Dawson (1964) showed that field beans (*Phaseolus vulgaris* L.) significantly suppressed the growth of weeds that germinated late in its life cycle. Wicks *et al.* (1973) concluded that the growth of grassy weeds is at its maximum between 4 and 8 weeks after the emergence of onions. Detailed studies on the suppression of a weed by the associated crop could lead to conclusions similar to the ones arrived at by determining the effect of a weed on a specific crop.

It has been accepted that water extracts of top growth (especially leaves) produce more allelochemicals 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 and Miller,

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1992; Chung and Miller, 1995). 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 highest inhibition against three test plants, alfalfa, lettuce (*Lactuca sativa*), and Chinese cabbage (*Brassica chinensis*).

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. Bae *et al.* (1997) reported that the phenolic acids identified by TLC and HPLC analyses in ethyl acetate fraction were p-coumaric, p-hydroxy benzoic, trans-cinnamic, syringic, ferulic and salicylic acids.

The objective of this research was to compare allelopathic effects among extracts or residues from leaves, stems, roots, pods, and seeds of black soybean through laboratory and greenhouse experiments. This research will promote a better understanding of the mechanisms of allelopathy in soybean cropping system.

MATERIALS AND METHODS

Preparation of Plant Samples

Black soybean (cv. 'Geomjeong I') plants were harvested at reproductive stage in 2001. The plant samples were separated into leaves, stems, roots, pods, and seeds. The five samples were immediately oven-dried at 60°C for 5 days (Chon and Nelson, 2001), ground with a Wiley mill to pass through a 1-mm screen, and stored in a refrigerator at 2°C until used. Forty grams of dried samples 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 (× 4530 g) for 2 hours. The supernatant was vacuum filtered again through Whatman No. 42 paper. EC (electrical conductivity), pH, and osmotic potential (Boyer and Knipling, 1965) were measured on stock extracts 2 days after extraction. Aqueous extracts or ground samples of each plant part were used for alllelopathy bioassay.

Phytotoxic Effects of Aqueous Plant Extracts against Alfalfa and Soybean

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 used as a control. 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. In the same way, another Petri dish bioassay was followed to determine autotoxic effects of pod and seed extracts on soybean 'Gwangan' seedling growth. The petri dishes were covered, sealed by wrapping in parafilm, 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 with 400 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) provided by a mixture of incandescent and fluorescent lamps. Root and hypocotyl lengths were 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.

Effects of Plant Residue Incorporation against Barnyard Grass

Residues of each plant part of black soybean 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 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°C day/22°C night. All plants were harvested to determine plant height, root length, and fresh and dry weights 15 days after seeding. Data were transformed to percent of control for analysis.

RESULTS AND DISCUSSION

Phytotoxic Effects of Aqueous Plant Extracts against Alfalfa and Soybean

EC, pH, and osmotic potential of thistle extracts measured at 40 g L^{-1} were ranged from 0.38 to 1.31 S/m, from 5.41 to 7.01, and from -0.085 to -0.126 MPa, respectively (Table 1). It was thought that EC, pH, and osmotic potential of the plant extracts did not affect seedling growth of test plants, indicat-

Table 1. Electrical conductivity (EC), pH, and osmotic potential of aqueous black soybean extracts at 40 g L^{-1} by different plant parts.

Plant part	EC (S/m)	pH	Osmotic potential (-MPa)
Leaves	1.313	7.06	0.095
Stems	0.826	7.07	0.085
Roots	0.379	5.41	0.096
Pods	1.078	5.50	0.115
Seeds	1.015	5.93	0.126

ing allelopathic effects of black soybean extracts could go beyond the osmotic effects. Our experience, in another study, demonstrated that no significant growth reduction was observed at all concentrations of polyethylene glycol (PEG), corresponding to same osmotic potential of alfalfa leaf extracts. Osmotic potential less than -0.20 MPa of PEG has little effect on root growth at concentrations of extract normally used. 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 entirely due 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.

Extracts from different plant parts of black soybean inhibited root length of alfalfa differently (Fig. 1). Seed or pod extracts had greater inhibitory effect on the root growth than stem, root or leaf extracts. The degree of inhibition increased with increasing the extract concentration. At highest extract concentration of 40 g L^{-1}, seed and pod extracts reduced root length 78% and 74%, respectively, while leaf and root extracts reduced root length 51% and 27%, respectively. However, hypocotyls length was much less sensitive to the extracts than was root length. At an extract concentration of 40 g L^{-1} from seed and pod sample, the hypocotyl growth of alfalfa was completely inhibited (Fig. 1). This result corroborates earlier reports that root growth is more sensitive to extracts than is seed germination or hypocotyl growth (Hedge and Miller, 1990; Chung and Miller, 1995; Chon *et al.*, 2000). Different compound(s) that caused autotoxicity could be produced with different amount from different plant parts. These results differed the other report (Chung and Miller, 1995) that autotoxic effects of water extracts of plant part of alfalfa were ranked in order of leaf (greatest autotoxicity), seed, root, and stem (least autotoxicity). However, it is not clear if the same chemical(s), which causes autotoxicity, contributes to heterotoxicity, or vice versa. Qualification and quantification of the compounds in the extracts from different plant parts are needed to examine if the same compound causes autotoxicity, or if autotoxic effects of various plant extracts are dependent on the amount of the compound.

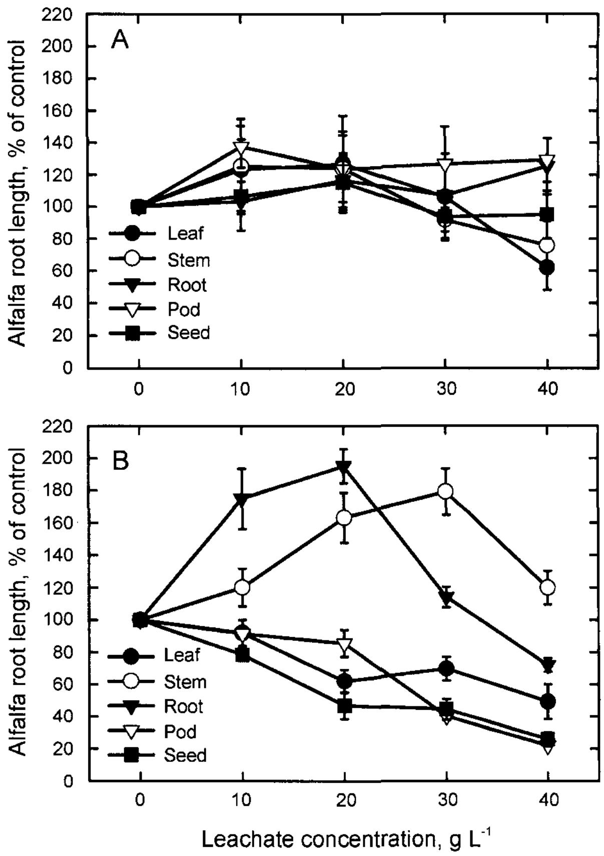

Fig. 1. Effects of black soybean leaf, stem, root, pod and seed extracts on shoot (A) and root lengths (B) of alfalfa as affected by different concentrations. The seedling growth was determined at 6 days after seeding on the filter paper wetted with the various extracts.

On the other hand, at pod and seed extracts at 40 g L^{-1} root length averaged 12.7 and 23.8% of the control, respectively (Fig. 2). The results were similar to a previous experiment (Fig. 1) regarding on alfalfa seedling growth response, the root growth of soybean was markedly reduced with increasing of the extract concentration. The results suggest that black soybean could contain water-soluble substance(s) that are autotoxic to the same species as well as allelopathic to other plant species.

Effects of Plant Residue Incorporation against Barnyard Grass

The residue incorporation from different plant parts of

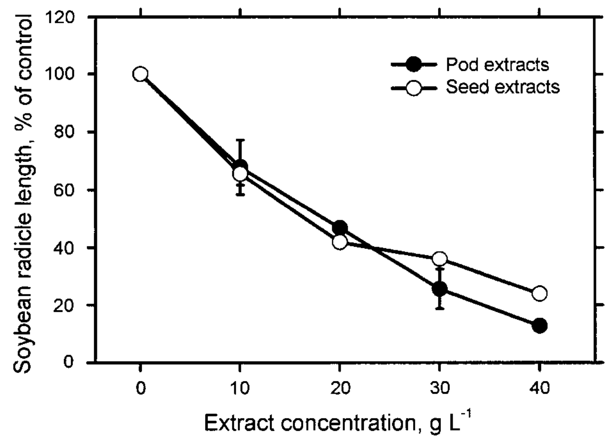

Fig. 2. Effects of black soybean pod and seed extracts on root length of soybean 'Gwangan' as affected by different concentrations. The seedling growth was determined at 6 days after seeding on the filter paper wetted with the various extracts.

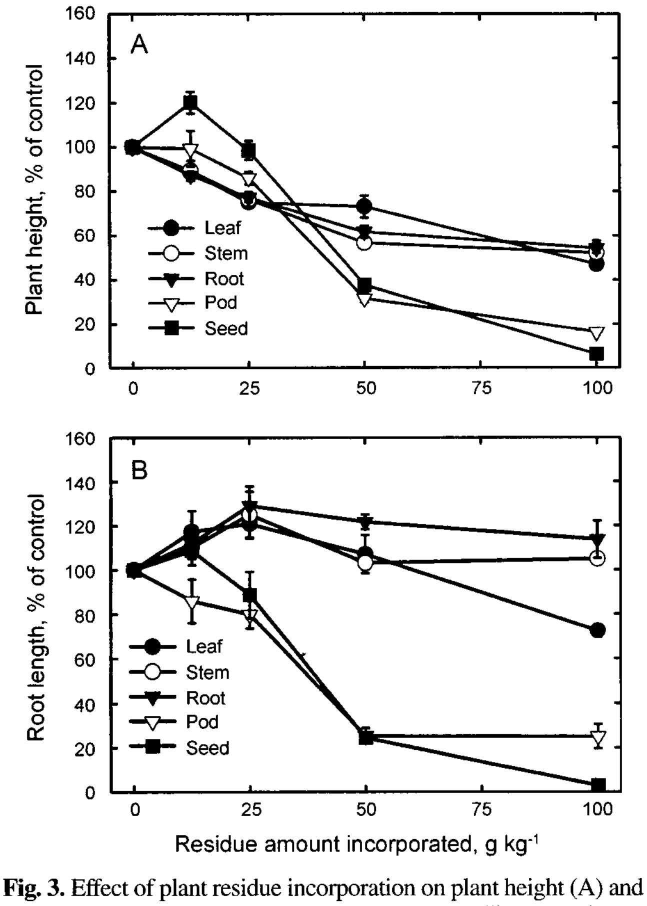

Fig. 3. Effect of plant residue incorporation on plant height (A) and root length (B) of barnyard grass. The seedling growth was determined at 15 days after seeding on potting medium.

black soybean significantly affected barnyard grass growth. Seed residues had greatest inhibitory effect on barnyard grass growth, and followed by pod, leaf, stem, and root residues of black soybean. The degree of inhibition increased

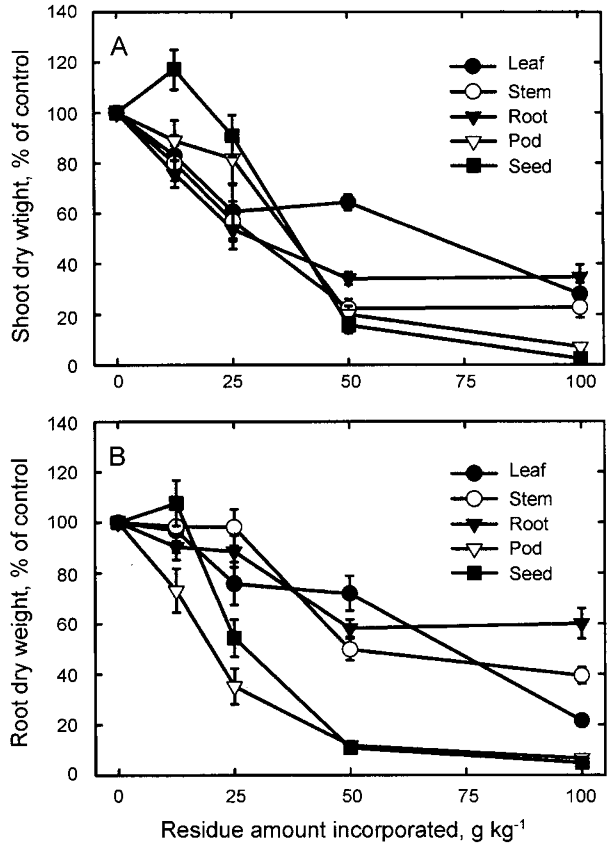

Fig. 4. Effects of plant residue incorporation on dry shoot (A) and root weights (B) of barnyard grass. The seedling growth was determined at 15 days after seeding on potting medium.

with increasing the amount of residue incorporation (Fig. 3 and 4). Seed and pod residues at the highest amount of 100 g kg^{-1} reduced shoot length of barnyard grass 94 and 84%, respectively, while leaf, stem, and root residues reduced the shoot length 53, 48, and 46%, respectively. In the residues of leaf, stem and root at 12.5 to 50 g kg^{-1}, however, root growths of barnyard grass were stimulated up to 21, 25, and 29% over the control, respectively (Fig. 3).

Incorporation with 100 g kg^{-1} soybean residues into the soil in the greenhouse reduced fresh and dry weight of barnyard grass 98-99% and 95-98% (Fig. 4), respectively. Incorporation of residues of leaf, stem and root, however, showed less effect on fresh and dry weight of shoots and roots than the seed or pod dry matter. Seed residues showed the greatest ability to inhibit seedling weights of barnyard grass and followed by pod residues, showing the same tendency to plant height and root length. These results support the result of greenhouse study by Rose *et al.* (1984) that incorporation of 1% ground soybean dry matter into soil inhibited germination and dry weight of greenhouse grown velvetleaf an average of 46% each. The results also indicate that any inhibition of weed growth should be primarily due to the presence of toxic compounds or excessive solutes within the ground black soybean top growth.

SUMMARY AND CONCLUSIONS

This study demonstrates allelopathic effects, partly autotoxic effects, of black soybean plant extracts or ground dry matters on early seedling growths of alfalfa and soybean as crop plants or barnyard grass as a weedy plant species through Petri dish and greenhouse bioassay. Allelopathic potentials of black soybean were ranked in order of seed (greatest), pod, leaf, stem, and root (least). Different compounds that cause allelopathy could be produced with different amount from different plant parts. Such differences might be related to allelopathic compounds being produced in larger quantities in certain tissue, imparting a higher level of allelopathy. The results suggest that black soybean plants had allelopathic potentials against several plant species, sometimes also against same species. Therefore, the results may have important values for a mean of biological weed in soybean cropping system. However, further research on qualification and quantification of the compounds from different plant parts are needed to confirm if the same compound causes allelopathy, or if the effects of various plant part extracts or residues are dependent on the amount of the causative compound (s). The potential uses and benefits of allelopathic soybeans in agricultural situations are many. One might be the isolation of the toxic chemical and subsequent use or synthesis of new herbicides or growth regulators. The management of phytotoxic soybean residue would have to be considered in a crop rotation.

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