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Growth Promotion Effects of Plant Extracts on Various Leafy Vegetable Crops

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Abstract

Though the mechanisms are not yet fully understood, the macro- and micro-nutrients, amino acids, and hormone-like growth substances present in plants (whole plants and their extracts) may lead to biostimulatory activities when applied to crops. This study was conducted to determine the growth promotion effects on lettuce (*Lactuca sativa* L.) of plant extracts obtained by different extraction methods (water, boiling water, fermentation, and ethanol) from 31 agricultural materials. We also determined the growth promotion effects of three other leguminous crop extracts on lettuce. In total, these 31 different agricultural materials produced 82 different extracts. The most effective extracts were water and boiled water extracts of Chinese chive (*Allium tuberosum*), water extracts of soybean leaves (cvs. Daewon and Haepum), and water, fermentation, and ethanol extracts of soybean stems (cv. Daewon). These induced a 31 - 45% increase in shoot fresh weight of lettuce compared with control plants. Chinese chive, soybean leaf, and soybean stem extracts had a greater impact on shoot fresh weight of lettuce when applied at 20 days after seeding (DAS) than when applied at 30 and 40 DAS. In addition, when extracts from Chinese chive and soybean leaf and stem were used, the shoot fresh weight of lettuce was higher at 7 days after treatment (DAT) than at 14 and 21 DAT. The shoot fresh weight of lettuce was 13 - 15% higher when the selected extracts were applied twice instead of once. Moreover, the growth promotion effects of the selected plant extracts were also observed in endive and broccoli, but not in pak choi and kale. Additionally, the growth promotion of lettuce was higher in response to soybean leaf and stem extracts than extracts of other leguminous crops (cowpea, mung bean, and red bean). The selected extracts showed higher growth promotion effects on lettuce than those of urea and a commercial extract. Overall, the results indicate that the selected plant extracts can be used for growth promotion in organic cultivation of various crops.

Additional key words: agricultural material, broccoli, endive, lettuce, organic agriculture, traditional agriculture

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Introduction

Organic agricultural practices are becoming more popular because of consumers' increased interest in and demand for safer agricultural products. Organic agriculture is the environmentally friendly response to traditional agriculture, which uses chemical pesticides and fertilizers (Chae et al., 2008). In organic agriculture, treatments are made from natural ingredients, and the use of synthetic chemicals is forbidden. Additionally, organic agriculture is a production system that uses crop rotation, fallow fields, and green manure cultivation. Organic matter and agricultural by-products are used to improve soil fertility and plant extracts can be used to control crop pests (Chae et al., 2008). Nutrients in organic agriculture are basically supplied through compost and green manure crops, but there are many cases in which nutrients are insufficient during the crop growth period (Chae et al., 2008; An et al., 2011). Therefore, organic farmers use various organic materials to supplement their crops' nutrient needs. These include animal by-products (blood, bone meal, fish meal, fish, etc.), plant by-products (mushrooms, rice bran, and soybean meal), and minerals such as ash. Organic materials such as these can be used to create liquid fertilizers through a process of fermentation. These fertilizers also activate beneficial microorganisms in the soil (Park et al., 2001).

Such fertilizers are also beneficial to crop growth particularly in promoting root protection and production (Kai et al., 1990; Elad and Shtienberg, 1994). Soybean meal oil cakes, in particular, are fast-acting fertilizers often used as crop supplements in organic agriculture (Im et al., 2015). When the aforementioned organic fertilizers are used, yields of rice, red pepper, and red mustard are comparable to or higher than yields of crops fertilized with conventional materials (Kim et al., 2012). However, the effects of farm-made organic liquid fertilizers were not consistent, even if they were made from the same materials, because of differences in manufacturing methods and fermentation conditions (An et al., 2011).

In Korea, a total of 1,642 eco-friendly agricultural materials are registered for soil improvement, growth promotion, and control of diseases and insects, 45 of which are plant extracts (RDA National Academy of Agricultural Science, 2018).

In Korean agriculture books dating back to the 1920s, there are farming systems which emphasize the importance of utilizing readily-available natural resources for growth promotion (Guh et al., 2011). These ancient organic methods may help to expand current organic farming technologies. However, a number of the agricultural organic materials used in traditional farming have not been scientifically investigated for their effects on growth promotion.

In Korea, lettuce (*Lactuca sativa* L.) is commonly used as a "wrap vegetable" for various dishes and is eaten along with perilla (*Perilla frutescens*) (Park and Lee, 2006). We selected the lettuce plant for our study because it is widely consumed in many different countries.

Thus, this study was conducted to evaluate the growth promotion effects that 31 agricultural materials had on lettuce crops using various application and extraction methods. We also compared the growth promotion effects of these materials on other leafy vegetable crops and determined the growth promotion effects of extracts of three other leguminous plants.

Materials and Methods

Extraction Materials

Thirty-one traditional agro-materials (Table 1), in which potential effects of crop growth promotion have been documented, were used in this study. These materials were used in traditional Korean farming and are alluded to in agricultural books that are nearly 100 years old (Guh et al., 2011). Some agricultural materials, such as rice hull, rice bran, and barley bran, were collected as by-products of the milling process. Additionally, pine leaf powder, charcoal powder, sesame oil cake, and ashes from oak trees, bamboo, and pine trees, and chicken, pork, and duck meat were purchased from the Korean Material Cooperation (Suncheon, South Korea). We used all above-ground parts of *Chrysanthemum zawadskii* var. latilobum KITAMURA, Chinese chive (*Allium tuberosum*), and reed (*Phragmites communis* Trinius) purchased from Chonnam

Table 1. Effect of various traditional agro-materials at 5% concentration on the shoot fresh weight of lettuce at 7 days after treatment

Growth increase (%)	Extraction method			
	Water	Ethanol	Boiled water	Fermentation
0 - 10	Rice hull charcoal (cv. Hwangeumnodle), Barley hull (cv. Huinchalssalbori), Sesame oil cake, Oak tree ash, Bamboo ash, Pine tree ash, Pine tree stem, Reed, Duck meat	Barley hull (cv. Youhyo), Barley hull (cv. Huinchalssalbori), Sesame oil cake, Oak tree ash, Bamboo ash, Pine tree ash, Chinese chive (cv. Sanbuchu), Soybean leaf (cv. Daewon), Chrysanthemum, Reed	Barley hull (cv. Youhyo), Barley hull (cv. Huinchalssalbori), Sesame oil cake, Oak tree ash, Bamboo ash, Pine tree ash, Pine tree stem, Soybean leaf (cv. Daewon), Soybean stem (cv. Daewon), Soybean leaf (cv. Haepoom), Soybean stem (cv. Haepoom), Chicken, Pork, Duck meat, Chrysanthemum	Pork, Duck meat, Chicken + Chinese chive (cv. Sanbuchu), Pork + Chinese chive (cv. Sanbuchu)
11 - 20	Rice hull (cv. Hwangeumnodle), Barley hull (cv. Youhyo), Pork	Rice straw (cv. Boramchan), Rice hull charcoal (cv. Hwangeumnodle), Rice bran (cv. Kanto504), Pine needle, Soybean leaf (cv. Haepoom)	Rice straw ash (cv. Hwangeumnodle), Rice hull (cv. Hwangeumnodle), Rice hull charcoal (cv. Hwangeumnodle), Reed	Chicken, Duck meat+Chinese chive (cv. Sanbuchu)
21 - 30	Rice straw (cv. Hwangeumnodle), Rice straw (cv. Boramchan), Rice straw (cv. Baekokchal), Rice straw ash (cv. Hwangeumnodle), Rice straw ash (cv. Baekokchal), Rice bran (cv. Kanto504), Chrysanthemum, Chicken, Soybean stem (cv. Haepoom)	Rice straw (cv. Hwangeumnodle), Rice straw (cv. Baekokchal), Rice straw ash (cv. Hwangeumnodle), Rice straw ash (cv. Baekokchal), Rice hull (cv. Hwangeumnodle), Soybean stem (cv. Haepoom)	Rice straw (cv. Hwangeunnodle), Rice straw (cv. Boramchan), Rice straw (cv. Baekokchal), Rice straw ash (cv. Baekokchal), Rice bran (cv. Kanto504)	Soybean leaf (cv. Haepoom), Soybean stem (cv. Haepoom), Soybean leaf (cv. Daewon)
31 - 40	Soybean leaf (cv. Haepoom), Soybean leaf (cv. Daewon), Soybean stem (cv. Daewon)	Soybean stem (cv. Daewon)	Chinese chive (cv. Sanbuchu)	Soybean stem (cv. Daewon)
41 - 50	Chinese chive (cv. Sanbuchu)			

Hanyaknonghyup Corporation (Hwasun, South Korea). Additionally, leaves and stems of soybean, mung bean, cowpea bean, and red bean were provided from the Jeollanamdo Agricultural Research and Extension Service and dried in an oven at 40°C for 5 days.

Effect of Various Traditional Agro-materials Obtained by Different Extraction Methods on Growth Promotion of Lettuce

We used 50 g of each agricultural material. With the exception of the meats, pine leaf powder, and charcoal powder, all other agricultural materials were dried in a drying oven at 40°C for 5 days and were ground using a coffee grinder (Proctor Silex E160B, Southern Pines, NC). The chicken, pork, and duck meat were ground fresh in the coffee grinder. Pine leaf powder and charcoal powder were used in their original form. The extraction methods of water, boiled water, and ethanol were the same as those described in a previous study (Jang and Kuk, 2018). Although some details of our extracts varied, we always extracted from 50 g ground agricultural material. This material was always first mixed using a homogenizer. In our water extract, the agricultural material was mixed with 1,000 mL distilled water for 24 h. In our ethanol extract, we followed the same process but instead used 1,000 mL ethanol for 24 h. In our boiled extract, the agricultural material was mixed with 1,000 mL distilled water and then boiled at 100°C for 30 min. In our fermentation extract, agricultural material was placed in 500 mL of distilled water and stored at room temperature for 14 days under dark conditions.

Lettuce (cv. Cheonghacheongchima) seeds were sown in pots (200 mL) filled with potting mix (Hungnong-Bio Soil, South Korea) in a greenhouse under light conditions of 14 h light/10 h dark at a day/night temperature of 30 ± 2°C / 20 ± 3°C, 70% relative humidity, and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. At the 4- to 5-leaf stage of lettuce, the seedlings were treated by foliar spray with an aqueous solution (5 mL per pot) of the traditional agro-materials at 0, 1, 3, and 5% concentrations with a hand sprayer. However, data at 1 and 3% concentrations was not provided because the growth promotion effects were too low. Leaf injury (data not shown), plant height, and shoot fresh weight were determined 7 days after treatment (DAT). Data were converted using the equation of 100*[1 - (treated/nontreated)], expressed as a percentage of increase compared to the control.

Growth Promotion Effect of the Plant Extracts with Different Application Methods on Lettuce

Three water extracts of Chinese chive (*Allium tuberosum* Rottler; cv Sanbuchu), soybean leaf (cv, Daewon), and soybean stem (cv, Daewon) were selected to determine their growth promotion effect on lettuce. The water extracts of Chinese chive, soybean leaf, and soybean stem had a pH range of 5.2 to 5.8 and an EC range of 2.0 to 4.3 $mS \cdot cm^{-1}$.

In order to confirm the cultivar-specific response of lettuce, seedlings (cvs. Jeogchima, Heugssamchima) of lettuce at the 4-leaf stage were treated by foliar spray with an aqueous solution (5 mL per pot) of the water extracts at 0, 1, 3, and 5% concentrations with a hand sprayer. Additionally, to determine the optimum application time, seedlings (cv. Jeogchima) were treated at 20 days (4-leaf stage), 30 days (6-leaf stage), and 40 days (10-leaf stage) after seeding by foliar spray with an aqueous solution (5 mL per pot) of the plant extracts at 0, 1, 3, and 5% concentrations with a hand sprayer. Plant height and shoot fresh weight were determined 7 DAT. To determine effective persistence, the seedlings at the 4-leaf stage were treated by foliar spray with the same aqueous solutions mentioned above. Plant height and shoot fresh weight were determined 7, 14, and 21 DAT. In addition, to determine the optimal application frequency, the seedlings at the 4-leaf stage were treated once or twice in a 7-day interval by foliar spray with the same aqueous solutions mentioned above. Leaf injury, plant height, and shoot fresh weight were determined 14 days after the first treatment. For our soil application experiment, pots received powdered extracts mixed to a depth of 5 cm by hand at 0, 50, 100, and 200 $g \cdot m^{-2}$ in pots (200 mL) filled with commercial potting soil (Pungnong NPKO, Seoul, Korea). Thereafter, lettuce was transplanted at the 4-leaf stage at a density of one plant per pot. Leaf injury (visual rate; 0 - 100%, 0 = no damage), plant height, and shoot fresh weight were investigated at 10 DAT.

Growth Promotion Effect of the Plant Extracts on Other Vegetable Crops

In order to confirm the growth promotion effect on endive (cv. Riccia cuore dore), pak choi (cv. Da cheong chae), Keil (cv. Gwijogkeil), and Broccoli (cv. Ekuseu), 3-leaf-stage lettuce seedlings were treated by foliar spray with an aqueous solution (5 mL per pot) of the plant extracts at 0, 1, 3, and 5% concentrations with a hand sprayer. Other procedures were the same as those described in the previous section.

Growth Promotion Effect of Extracts of Other Leguminous Plants on Lettuce

In order to confirm the growth promotion effects of extracts of three other leguminous crops, lettuce seedlings at the 4-leaf stage were treated with an aqueous solution (5 mL per pot) of the water extracts of leaves and stems of mung bean (cv. Dahyeon), cowpea (cv. Seowon), and red bean (cv. Hong-eon) at 0, 1, 3, and 5% concentrations with a hand sprayer. Other procedures were the same as those described in the previous section.

Comparison of the Growth Promotion Effects of the Plant Extracts and Commercial Materials on Lettuce

To compare the effectiveness between commercial urea or soybean fermented extract (Bullosu) and the plant extracts used in this study, the 4-leaf-stage seedlings were treated by foliar spray of urea at 0.2, 0.4, 0.6, 0.8, and 1% or soybean fermented extract at 0.4% (recommended rate). Leaf injury, plant height, and shoot fresh weight were determined 7 DAT.

Statistical Analysis

Data were expressed as a percentage of the untreated control to standardize comparisons between treatments. Significant differences were determined using analysis of variance (ANOVA). Analyses were performed using Statistical Analysis Systems (SAS, 2000) software. In the case of significant differences, means were separated using Duncan's multiple range test at $p \leq 0.05$.

Results and Discussion

Effect of Various Traditional Agro-materials Obtained by Different Extraction Methods on the Growth of Lettuce

Adverse effects of synthetic fertilizers on the environment are encouraging investigation of natural sources of fertilizers, biostimulants, and soil ameliorants. Therefore, in this study, the growth promotion effects of various traditional agro-material extracts obtained by various extraction methods (water, boiling water, fermentation, and ethanol) on the growth of lettuce were investigated in a greenhouse experiment (Table 1). The traditional agro-material extracts induced various levels of growth promotion in lettuce. Fifty-eight of the 82 extracts led to 0 - 10% increases in plant height, while 24 induced 11 - 20% increases in plant height in lettuce compared with control plants (data not shown). On the other hand, 38 of the extracts showed 0 - 10% increases in the shoot fresh weight of lettuce when compared with control plants. Moreover, 14 of the extracts induced 11 - 20% increases in lettuce shoot fresh weight relative to control plants, while 23 of the extracts induced 21 - 30% increases in shoot fresh weight of lettuce compared with control plants.

There were seven extracts which were particularly effective. These extracts were water and boiled water extracts of Chinese chive (cv. Sanbuchu), water extract of soybean leaves (cvs. Daewon, Haepum), and water, fermentation, and ethanol extracts of soybean stems (cv. Daewon). These extracts produced 31 - 45% increases in shoot fresh weights of lettuce compared with control plants. These findings indicate that the chemical compositions of the extracts differed by the extraction method and species. In another study, the effects of biostimulants were found to differ among species and cultivars. Environmental factors, dosage amount, and time of application have also been found to influence the effectiveness of extract applications (Kunicki et al., 2010).

Soybean leaf extracts have been shown to contain higher levels of flavonoids such as salicylic, 4-hydroxybenzoic, valillic, 4-hydrooxycinnamic, ferulic, caffeic, gentisic, and quercetin (Porter et al., 1986). There is also a large amount of serine, alanine, and histidine, as well as substances related to antioxidant activity, in soybean leaf extracts (Stutte and Park, 1973). Chinese chives are also known to contain relatively higher contents of β-carotene, vitamin C, sulfur-containing compounds, and many kinds of flavonoids (Moon et al., 2003). Due to their effectiveness in our studies, we selected water extracts of Chinese chive and soybean leaves and stems for further analysis.

Effect of Different Application Methods of the Plant Extracts on Lettuce Growth

To verify the growth promotion effects on lettuce, extracts of Chinese chive and soybean leaves and stems were applied to seedlings by foliar spray under greenhouse conditions (Fig. 1). The shoot fresh weight showed a greater increase compared to plant height in response to the extracts. Moreover, plant height did not differ significantly between Chinese chive extracts at 1, 3, and 5% and the control. However, plant height increased 11 - 25% in response to treatment with the water extracts of soybean leaves and stems relative to the control. Moreover, the shoot fresh weight of lettuce increased 31 - 46% in response to the extracts of Chinese chive and soybean leaves and stems when compared with the control. There were no significant differences in the effect on plant height and shoot fresh weight between the two soybean cultivar extracts (cvs. Daewon, Haepoom). Numerous studies have revealed a wide range of beneficial effects of applications of seaweed extract on plants, such as improved early seed germination and establishment, improved crop performance and yield, and elevated resistance to biotic and abiotic stresses (Beckett and Van Staden, 1989; Hankins and Hockey, 1990; Norrie and Keathley, 2006).

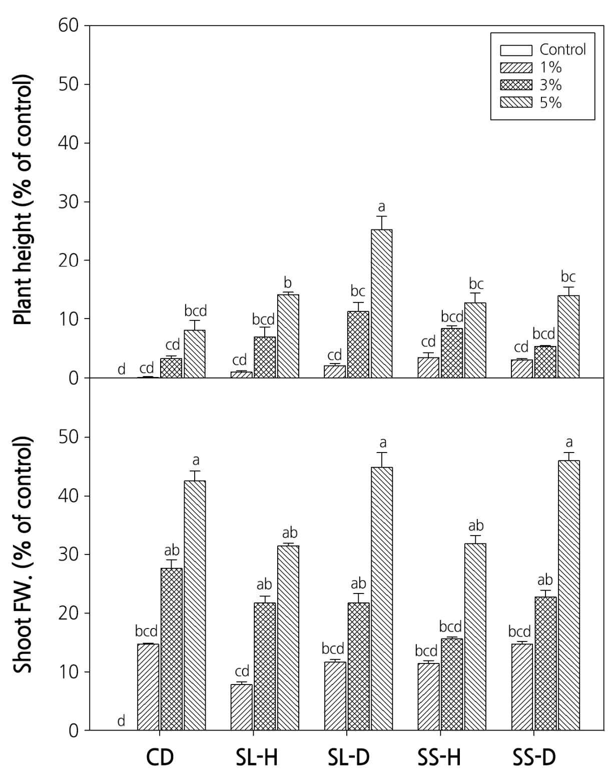

Fig. 1. Effects of the plant extracts [CC, Chinese chive, SL-H, soybean leaf (cv. Haepoom), SL-D, soybean leaf (cv. Daewon), SS-H, soybean stem (cv. Haepoom), SS-D, soybean stem (cv. Daewon)] on the growth promotion of lettuce grown in a greenhouse. Parameters were measured at 7 days after treatment. Means within bars followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.

To determine the cultivar-specific responses of lettuce, the different plant extracts were applied to seedlings of cvs. Jeogchima and Heugssamchima (Fig. 2). The plant height of cv. Jeogchima was not increased by treatment with the plant extracts when compared with the control. However, in the case of cv. Heugssamchima, plant height was increased 12 - 18% by Chinese chive and soybean leaf and stem extracts when compared with the control. On the other hand, the shoot fresh weight of cv. Jeogchima increased 21 - 40% in response to Chinese chive and soybean leaf and stem extracts when compared with the control. The shoot fresh weight of cv. Heugssamchima also increased 17 - 40% in response to Chinese chive and soybean leaf and stem extracts when compared with the control. Thus, growth promotion was observed in two different lettuce cultivars.

To determine the best application timing, the plant extracts were applied to seedlings of cv. Jeogchima at 20, 30, and 40 DAS (Fig. 3). The plant height of lettuce increased 14% in response to soybean stem extract at 5% when applied at 20 DAS. Additionally, the plant height of lettuce increased 12% in response to Chinese chive extract at 3% when applied at 30 DAS. However, the plant height of lettuce did not differ significantly in response to other extracts and application times when compared with the control. The shoot fresh weight of lettuce increased 35, 19, and 25% in response to Chinese chive extracts applied at 20, 30, and 40 DAS, respectively, when compared with the control. Furthermore, the shoot fresh weight of lettuce increased 42, 20, and 14% in response to soybean leaf extracts applied at 20, 30, and 40 DAS, respectively, relative to the control. Similar to the Chinese chive and soybean leaf extracts, the shoot fresh weight of lettuce increased 31, 20, and 15% in response to soybean stem extracts when applied at 20, 30, and 40 DAS, respectively, compared with the control. These results indicate that the shoot fresh weight of lettuce increased more in response to extracts of Chinese chive and soybean leaves and stems when applied at 20 DAS than when applied at 30 and 40 DAS.

To determine the effectiveness of our treatments over time, the seedlings were treated with the plant extracts at the 4-leaf

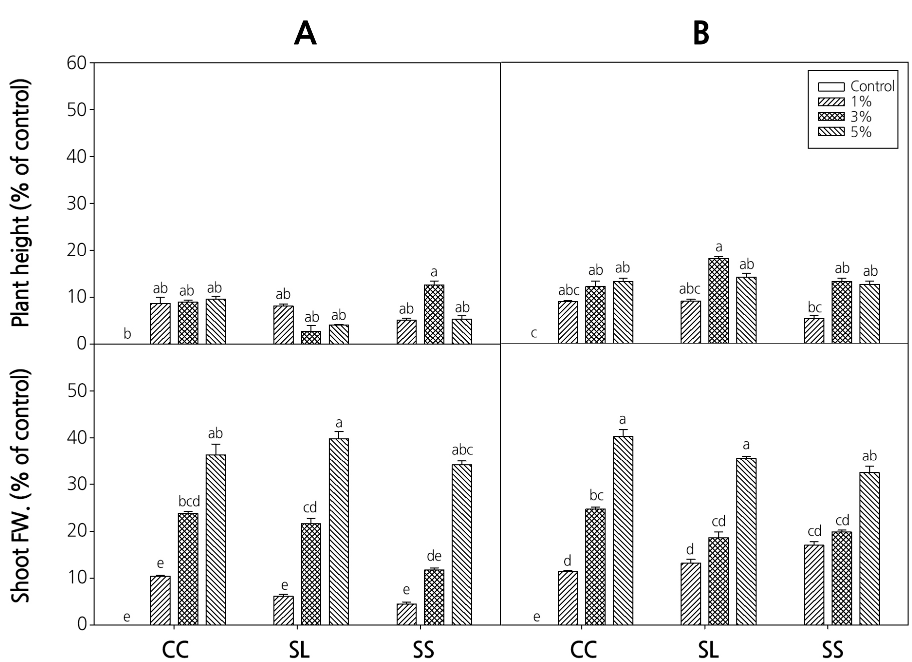

Fig. 2. Effect of the plant extracts (CC, Chinese chive; SL, soybean leaf; SS, soybean stem) on the growth promotion of different cultivars (A, Jeogchima; B, Heugssamchima) of lettuce plants. Parameters were measured at 7 days after treatment. Means within bars followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.

stage and then plant height and shoot fresh weight were measured 7, 14, and 21 DAT (Fig. 4). The plant height of lettuce increased 14 - 18%, 10 - 11%, and 4 - 7% at 7, 14, and 21 DAT, respectively, with Chinese chive and soybean leaf and stem extracts at the tested concentrations when compared with the control. Additionally, the shoot fresh weight of lettuce increased 46%, 22%, and 19% at 7, 14, and 21 DAT with the Chinese chive extract at 5%, respectively, when compared with the control.

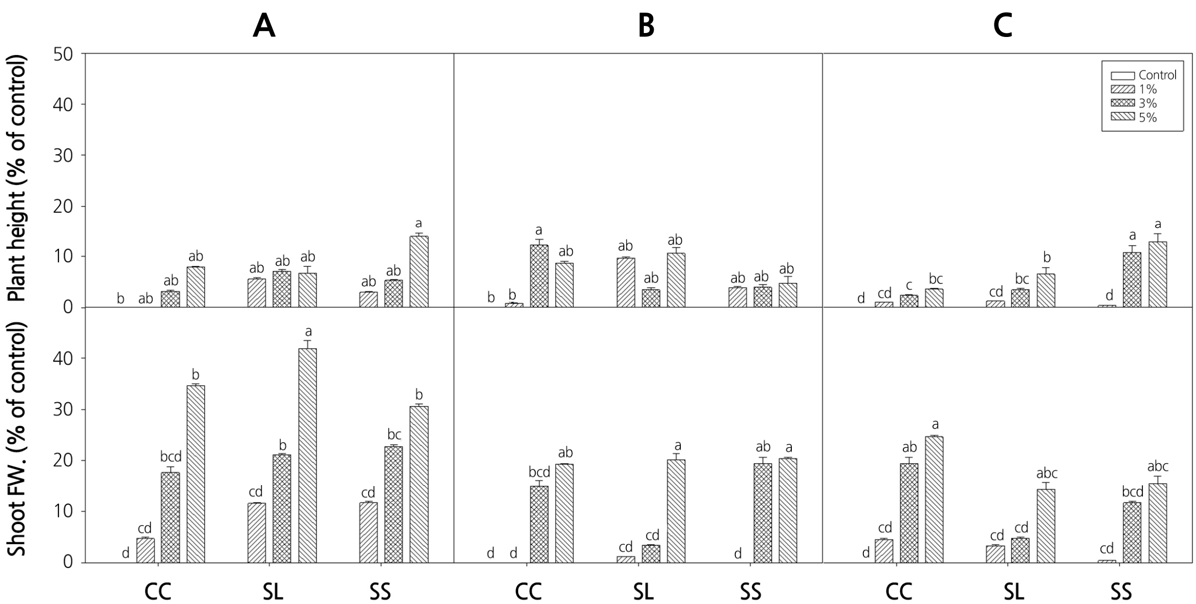

Fig. 3. Effects of the plant extracts (CC, Chinese chive; SL, soybean leaf; SS, soybean stem) at different growth stages (A, 20 days after seeding (DAS); B, 30 DAS; C, 40 DAS) on the growth promotion of lettuce grown in a greenhouse. Parameters were measured at 7 days after treatment. Means within bars followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.

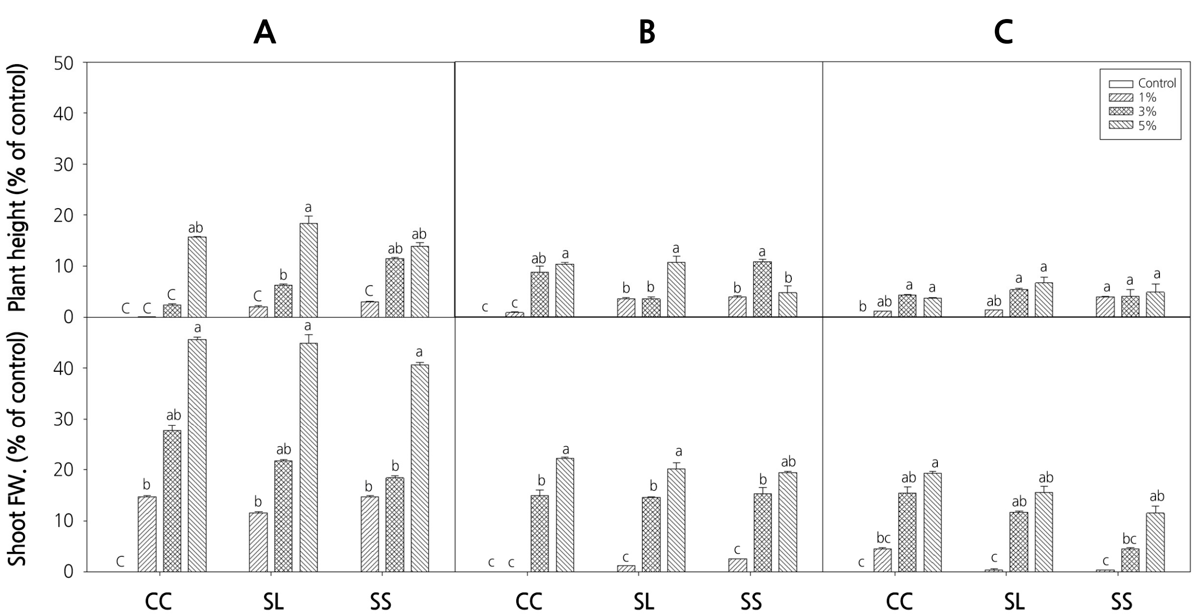

Fig. 4. Effects of the plant extracts (CC, Chinese chive; SL, soybean leaf; SS, soybean stem) on the growth promotion of lettuce at 7 (A), 14 (B), and 21 (C) days after treatments in a greenhouse. Means within bars followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.

The shoot fresh weight of lettuce was also increased 45%, 20%, and 16% at 7, 14, and 21 DAT with soybean leaf extract at 5%, respectively, when compared with the control. Similarly, the shoot fresh weight of lettuce increased 41%, 19%, and 12% at 7, 14, and 21 DAT with 5% soybean stem extract, respectively, when compared with the control. In general, the shoot fresh weight of lettuce increased with increasing concentrations of all extracts tested, regardless of investigation times (7, 14, and 21 DAT). Based on these results, the shoot fresh weights of lettuce at 7 DAT with Chinese chive and soybean leaf and stem extracts were higher than those at 14 and 21 DAT. Thus, the selected extracts needed to be applied two or more times to obtain higher growth promotion in lettuce.

To verify the growth promotion effects of the plant extracts, extracts were applied to the lettuce seedlings several times and results were measured after both the first and second treatments (Table 2). The plant height of lettuce increased 9 - 11% in response to soybean stem extracts when applied only once, but not in response to the extracts of Chinese chive and soybean leaf. However, plant height of lettuce increased 10 - 17% in response to all extracts when applied twice. Additionally, the shoot fresh weight of lettuce increased 42%, 45%, and 34% in response to Chinese chive and soybean leaf and stem extracts when applied once, relative to the control. However, when extracts were applied twice the shoot fresh weight of lettuce increased 60%, 58%, and 48% in response to Chinese chive and soybean leaf and stem extracts, respectively, when compared with the control. Thus, the shoot fresh weight of lettuce was 13 - 15% higher when the extracts were applied twice instead of once.

Table 2. Effects of selected plant extracts (CC, Chinese chive; SL, soybean leaf; SS, soybean stem) with application frequency (once or twice) on the growth promotion of lettuce grown in a greenhouse. Parameters were measured at 14 days after treatment

Application number	Extract conc. (%)		Leaf injury (%)	Plant height (% of control)	Shoot fresh wt (% of control)
	Control		0	0.0 e[z]	0.0 i
1st	CC[y]	1	0	4.5 cde	13.2 gh
		3	0	6.8 b-e	20.7 e-h
		5	0	7.2 b-e	41.7 bc
	SL	1	0	3.4 ed	18.1 fgh
		3	0	8.6 bcd	35.0 cd
		5	0	3.4 ed	45.0 bc
	SS	1	0	5.8 cde	15.5 efg
		3	0	9.1 a-d	27.3 def
		5	0	11.2 a-d	34.1 cd
2nd	CC	1	0	5.5 cde	12.6 ghi
		3	0	10.0 a-d	34.0 cd
		5	0	11.4 a-d	60.3 a
	SL	1	0	11.9 a-d	8.6 hi
		3	0	12.9 abc	32.9 cde
		5	0	11.1 a-d	57.9 a
	SS	1	0	4.5 cde	10.0 hi
		3	0	17.2 a	24.5 d-g
		5	0	15.2 ab	48.1 ab

[z]Means within a column followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.
[y]CC, Chinese chive; SL, soybean leaf; SS, soybean stem.

To confirm the effects of soil incorporation of the plant materials on growth promotion of lettuce, we applied dried Chinese chive, soybean leaves, and soybean stems at 50, 100, and 200 g·m^{-2} to soils 3 days before transplanting lettuce (Fig. 5). Plant height was not affected by incorporation with Chinese chive or soybean leaf or stem materials, regardless of treatment rates, although shoot fresh weight was increased 14 - 20% by Chinese chive at 100 and 200 g·m^{-2}. Furthermore, shoot fresh weight increased 18 - 38% and 17 - 23% in response to soybean leaves and stems, respectively, when compared with the control. However, there were no significant differences among the 50, 100, and 200 g·m^{-2} treatments for any material. Biostimulants can act directly on plant physiology and metabolism or by improving soil conditions (Nardi et al., 2009). Moreover, biostimulants in soils affect the macroflora and may influence plant growth, even though this was not investigated in the present study. Additionally, compost tea can provide nutrients when applied to the soil or directly to the plant as a foliar spray. When tea is mixed with soil, it improves nutrient retention, which can stimulate plant growth (Azza et al., 2010). This is because the increasing amount of nutrients that become available to the root system leads to stronger and healthier plants. Indeed, Azza et al. (2010) reported that soluble nutrients in compost tea and their extracts enhanced plant growth. In conclusion, the growth promoting effects of a foliar application of Chinese chive and soybean leaf and stem extracts on lettuce were greater than those observed in response to soil application of dried plant materials.

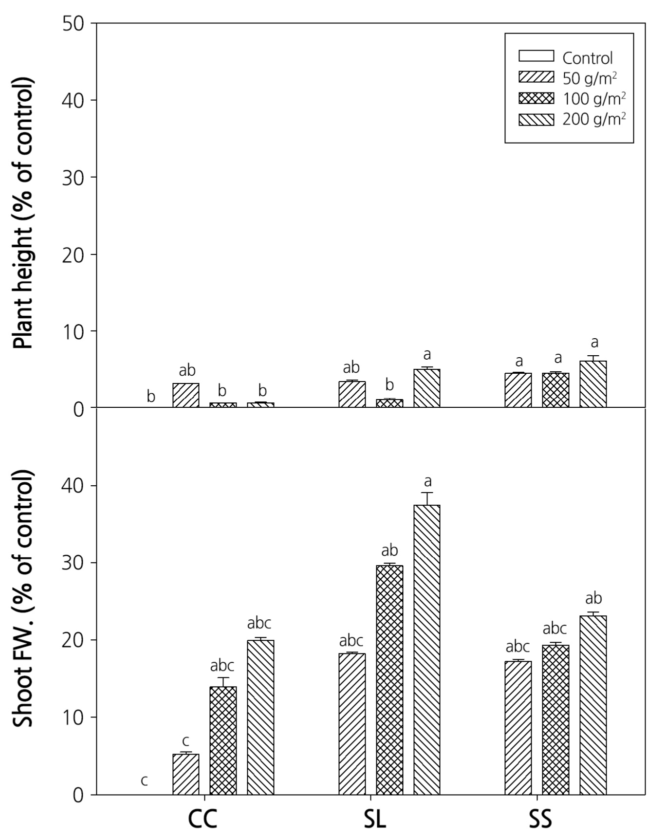

Fig. 5. Effect of soil application of plant materials (CC, Chinese chive; SL, soybean leaf; SS, soybean stem) on the growth promotion of lettuce grown in a greenhouse. Parameters were measured at 10 days after treatment. Means within bars followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.

Table 3. Effects of the plant extracts on the growth promotion of various leafy vegetable seedlings grown in a greenhouse. Parameters were measured at 7 days after treatment

Plant species	Extract conc. (%)		Plant height (% of control)	Shoot fresh wt. (% of control)
Endive	Control		0.0 a[z]	0.0 d
	CC[y]	1	0.0 a	10.6 d
		3	1.3 a	18.3 cd
		5	7.5 a	45.0 b
	SL-D	1	0.0 a	47.6 ab
		3	4.8 a	56.1 ab
		5	0.8 a	67.3 a
	SS-D	1	2.2 a	27.7 bc
		3	6.8 a	35.5 abc
		5	3.4 a	45.2 b
Pak choi	Control		0.0 c	0.0 b
	CC	1	2.8 abc	0.0 b
		3	3.1 abc	12.9 ab
		5	1.3 abc	4.7 b
	SL-D	1	4.5 abc	9.0 b
		3	6.7 abc	11.9 ab
		5	8.4 ab	13.6 ab
	SS-D	1	6.5 abc	9.6 ab
		3	11.7 a	6.2 b
		5	7.2 abc	16.8 a
Kale	Control		0.0 a	0.0 a
	CC	1	0.8 a	3.5 a
		3	1.8 a	3.7 a
		5	5.2 a	2.2 a
	SL-D	1	0.0 a	0.0 a
		3	0.0 a	2.7 a
		5	0.0 a	1.5 a
	SS-D	1	0.0 a	0.4 a
		3	0.0 a	3.0 a
		5	4.1 a	6.8 a
Broccoli	Control		0.0 e	0.0 e
	CC	1	4.1 de	11.2 cd
		3	6.5 e	11.0 cd
		5	13.0 cd	21.4 b
	SL-D	1	15.2 bcd	12.0 cd
		3	16.6 bcd	15.4 bcd
		5	18.0 ab	30.5 a
	SS-D	1	7.5 d	8.4 d
		3	15.4 bcd	23.9 b
		5	21.9 b	28.1 ab

[z]Means within a column followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.
[y]CC, Chinese chive; SL-D, soybean leaf (cv. Daewon); SS-D, soybean stem (cv. Daewon).

Growth Promotion Effect of the Plant Extracts on Other Leafy Vegetable Crops

To confirm the growth promotion effects of our extracts, we applied them to other leafy vegetable crops (Table 3). Endive plant height was not affected by Chinese chive or soybean leaf or stem extracts, regardless of treatment concentrations. However, endive shoot fresh weight increased 45% in response to the Chinese chive extract at 5%, as well as 48 - 67% in response to soybean leaf and 28 - 45% in response to soybean stem extracts at 1, 3, and 5% when compared with the control. Thus, the increase in shoot fresh weight in response to the soybean leaf extract was higher in endives than in lettuce. The plant height of pak choi was not increased by Chinese chive or soybean leaf or stem extracts, except for the soybean leaf extract at 5% and the soybean stem extract at 3%. In addition, the shoot fresh weight of pak choi increased 17% in response to the soybean stem extract at 5%, but not at other levels. However, plant height and shoot fresh weight of kale were not increased by any of the extract treatments. The plant height of broccoli increased 13% in response to the Chinese chive extract at 5% when compared with the control, but not in response to the other concentrations. However, the plant height of broccoli increased 15 - 18% in response to the soybean leaf extract at 1, 3, and 5% when compared with the untreated control. Similar to plant height, the shoot fresh weight of broccoli increased 21%, 24 - 35%, and 24 - 28% in response to extracts of Chinese chive at 5%, soybean leaf at 1 and 5%, and soybean stem at 3 and 5%, respectively, when compared with the control. In conclusion, our plant extracts produced growth promotion in endive and broccoli, but not in kale. Components of soybean leaves and stems and Chinese chive, such as macro- and micronutrients, amino acids, and free sugars, may affect cellular metabolism in treated crops, leading to enhanced growth and yield. In another similar study, treatment with the seaweed *Agave sisalana* and *Yucca schidigera* stimulated seedling vigor and increased the dry weight of sorghum plants by 35% (Andersen et al., 2015). Seaweed extracts improve nutrient uptake by roots, resulting in root systems with improved water and nutrient use efficiency, thereby causing enhanced plant growth and vigor (Crouch et al., 1990).

Comparison of the Growth Promotion of Lettuce by the Plant Extracts and Commercial Materials

The growth promotion effect of the plant extracts were compared with those of urea and a commercial extract (Table 4). Foliar application of urea is used during the cultivation of many crops as a supplement for nitrogen deficiency (Bahr, 2007). Injury to lettuce appeared in response to urea treatments at 0.8% and 1%. The plant height of lettuce increased significantly by 4 - 9% in response to urea treatments at 0.6, 0.8, and 1%, but the shoot fresh weight increased 22% in response to treatment with urea at 0.8%. Additionally, plant height and shoot fresh weight showed no significant difference between the soybean

Table 4. Effects of urea and a commercial material (Bullosu) on the growth promotion of lettuce grown in a greenhouse. Parameters were measured at 7 days after treatment

Material	Conc. (%)	Leaf injury (%)	Plant height (% of control)	Shoot fresh wt. (% of control)
Control		0.0 c[z]	0.0 b	0.0 b
Urea	0.2	0.0 c	3.0 ab	6.0 ab
	0.4	0.0 c	4.9 ab	9.4 ab
	0.6	5.2 bc	3.7 a	16.1 ab
	0.8	10.8 b	8.9 a	22.4 a
	1.0	18.5 a	4.7 a	10.4 ab
Bullosu	0.4	0.0 c	0.7 ab	10.0 ab

[z]Means within a column followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.

fermented extract (Bullosu), which is registered for use in organic cultivation (RDA National Academy of Agricultural Science, 2018), and the control. In conclusion, the extracts used in this study showed a higher growth promotion effect on lettuce than that of urea and a commercial extract (Bullosu). Thus, the plant extracts tested in this study can be used to promote the growth of lettuce during organic cultivation.

Growth Promotion Effect of Extracts of Other Leguminous Crops on Lettuce

To confirm the growth promotion effects of extracts of three other leguminous crops in the same family, mung bean, cowpea, and red bean, we applied the extracts to lettuce seedlings (Table 5). Plant height and shoot fresh weight of lettuce were significantly increased by extracts of the three other leguminous crops when compared with the control. However, mung bean and cowpea extracts produced negligible effects on the plant height and shoot fresh weight of lettuce. Red bean extracts proved to be more effective. Plant height and shoot fresh weight of lettuce increased 10 - 15% and 6 - 24%, respectively, in response to extracts of red bean leaves and stems when compared with the control. Taken together, these results indicate that the growth promotion of lettuce was higher in response to soybean leaf and stem extracts than to extracts of other leguminous crops. In another study, the shoot fresh weight of lettuce was increased by 9 - 27% in response to extracts of soybeans left over from the production of cheongguk-jang (Hong et al., 2006). The mechanism by which plant extracts improve the growth of plants has been difficult to elucidate as extracts contain numerous bioactive compounds, the complexity of which has not been

Table 5. Effects of leguminous crop extracts on the growth promotion of lettuce plants grown in a greenhouse. Parameters were measured at 7 days after treatment

Plant species	Plant part	Extract conc. (%)	Leaf injury (%)	Plant height (% of control)	Shoot fresh wt. (% of control)
Control			0	0.0 e[z]	0.0 e
Mung bean	Leaf	1	0	0.0 e	3.3 cd
		3	0	0.0 e	1.5 de
		5	0	2.8 de	2.2 cde
	Stem	1	0	4.5 c	3.8 cd
		3	0	8.7 bc	10.8 b
		5	0	11.8 b	4.9 c
Cowpea	Leaf	1	0	6.3 bc	2.8 cde
		3	0	8.5 bc	11.0 b
		5	0	4.3 c	11.0 b
	Stem	1	0	2.5 de	2.5 cde
		3	0	4.6 c	4.8 c
		5	0	4.9 c	6.2 c
Red bean	Leaf	1	0	10.3 b	12.8 b
		3	0	14.2 a	12.8 b
		5	0	14.6 a	23.8 a
	Stem	1	0	10.3 b	5.7 c
		3	0	14.2 a	10.2 b
		5	0	14.6 a	11.8 b

[z]Means within a column followed by the same letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.

fully researched or understood. However, it is believed that macro- and micronutrients, amino acids, and hormone-like growth substances present in selected plants (whole plants and their extracts) may lead to the biostimulatory activities. Thus, further studies are needed to determine the mechanisms by which plant extracts promote crop growth.

Conclusions

Out of the 82 extracts used in this study, Chinese chive and soybean leaf and stem extracts induced the highest rate of growth promotion in lettuce. When these extracts were used, we observed a 31 - 45% increase in shoot fresh weight of lettuce compared with control plants. The growth promotion effects of our plant extracts were observed in two cultivars of lettuce. The shoot fresh weight of lettuce increased more in response to Chinese chive and soybean leaf and stem extracts when applied at 20 DAS than when applied at 30 and 40 DAS. At 7 DAT, we observed a higher shoot fresh weight than at 14 and 21 DAT. Plants that received two applications of the extracts fared better than plants that received only one, and the foliar application of extracts was more effective than the soil application of dried plant materials. The growth promotion effects of the plant extracts were observed in endive and broccoli, but not in pak choi and kale. Additionally, the growth promotion of lettuce was higher in response to soybean leaf and stem extracts than in response to extracts of other leguminous crops (cowpea, mung bean, and red bean). Moreover, the extracts showed higher growth promotion effects in lettuce than those of urea and a commercial extract. Overall, the results indicate that the plant extracts tested in this study can be used for growth promotion in the organic cultivation of various crops.

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