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Allelopathic Effect of Sweet Corn and Vegetable Soybean Extracts at Two Growth Stages on Germination and Seedling Growth of Corn and Soybean Varieties

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Abstract: A study was conduct to assess the allelopathic effect of sweet corn and vegetable soybean on two varieties of each corn and soybean. Aqueous extracts from shoot and root of the two crops at two crop growth stages were used and compared distilled water as control. Sweet corn and vegetable soybean shoot extract from 35 Days Old (DO) plants significantly retarded the germination of all tested varieties compared to control, but in soybean the same effect was also noticed from 60 DO plants, while 60 DO corn had variable effect on germination. Similarly, the shoot extract of 35 DO corn and soybean significantly reduced number of roots and total root length per seedling of corn varieties. The root extract of soybean and corn also retarded both number of roots and total root length, but the magnitude was lower than that of shoot effect. Sixty days old corn shoot and root extracts had stimulatory effect on number of roots and total root length of both corn varieties. On the other hand soybean shoot extract reduced number of roots per seedling as well as total root length per seedling compared to control. With regard to soybean root extract had no significant influence on the number of roots per seedling, but enhanced the total root length per seedling. Shoot extract from 35 DO soybean and corn reduced shoot and hypocotyl length of corn and soybean respectively, while root extract as well as both shoot and root extract from 60 DO plants had variable effect. Five compounds (R_f =0.80, 0.70, 0.50, 0.48 and 0.40) were separated from soybean shoot and root, three (R_f = 0.84, 0.50 and 0.40) and one (R_f = 0.84) from corn shoot and root, respectively. Soybean and corn shoot extract from 35 days old plants deformed root and hypocotyl tip of corn and soybean varieties, respectively.

Key words: Allelopathy, corn, soybean, shoot, root, extract, germination, growth

INTRODUCTION

Sustainable crop production over long period of time is currently emphasized rather than maximizing yield in a short term (Narwal, 1998). To achieve sustainable production, there is a need to understand ecological interactions occurring with in a given agro-ecosystem (Narwal, 1998). In crop associations or natural stands, besides nutrient, water, light and space competition, there is allelopathic interference (Rice, 1984), which is inhibitory or stimulatory effects of one plant on another (Rice, 1984). Moreover, research findings indicate that a given crop species only some accessions or varieties have allelopathic effects as reported in barley, oats, rice, wheat and alfalfa (Chon and Kim, 2002, Ebana *et al*., 2001; Kato-Noguchi and Ino, 2001). Additionally, some crop plants have auto-toxicity, which is atypical type of allelopathy in which plants have inhibitory effect on other plants of the same species (Jensen *et al*., 1981). Similarly different plant organs of the same species differ in their allelopathic composition. Tawaha and Turk (2003) reported that leaf extract of black mustard was more inhibitory than other plant organs on wild barley plant, while Tefera (2002) noticed that leaf extract of *Parthenium hysterophorus* was more inhibitory than stem extracts, whereas Chung *et al*. (1997) noticed hull extract of rice to contain more water soluble substances toxic to barnyard grass than stem or leaf extract. On the other hand within a given species, varieties differ in their allelopathic constituent of leaves, stems and flowers. Chung *et al*. (2003) reported that certain rice varieties straw extract was more inhibitory, while others leaf extract was more retardant.

Maize and soybean are two crops, normally grown in association. However, there are view research results indicating the allelopathic effect of corn (Minorisky, 2002) and soybean (Jimenez *et al*., 1983). Moreover, to the best of our knowledge allelopathic interference of corn and soybean and also within corn and soybean have not been studied. Hence, since allelopathy is part of crop-crop

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interference, it is time to investigate, whether there is allelopathic effect of sweet corn and vegetable soybean on each other for the better under standing of the system, bearing this in mind this research was undertaken using water as extracting medium because allelochemicals are often water soluble and escape into the environment by means of root exudation, leaching by dews and rains or decaying of plant tissue (Tawaha and Turk, 2003) with the following objectives.

- To see whether the shoot and toot extract of sweet corn and vegetable soybean have allelopathic effect on each other
- To see which part of the plant has more inhibitory effect
- Separation of allelopathic compounds from corn and soybean plant extracts

MATERIALS AND METHODS

The experiment was conducted in 2004 (27th July to 30th September) and 2005 (1st May to 28th July) at the experimental farm (field two) and plant nutrition laboratory of the department of crop science, Faculty of Agriculture, University Putra Malaysia (101°42' E, 3°00' N with an altitude of 30 m above mean sea level).

Growing of the crops: Sweet corn variety Masmadu and vegetable soybean variety MKS1 were grown in polythene bags containing 50 kg of top soil from muchong soil series (Very fine, kaolinitic, isohyperthermic), lime was applied to adjust the pH at 6 and 15 days after lime application, two plants of sweet corn or four plants of vegetable soybean were grown per bag on 27th July 2004. Recommended fertilizer rate was applied. For dry sample extract of allelopathic substances plants were harvested 35 days after panting, while for fresh plant extracts plants were harvested 60 days after planting.

Experimental design: The experimental design was CRD in factorial arrangement, with two factors; namely extracts [Corn shoot (Cs), corn root (Cr), soybean shoot (Ss), soybean root (Sr) and distilled water as control (W)] and test varieties [Msmadu, PutraJ-58, MKS1 and AGS190].

Preparation of extracts

Extracts from oven dried samples: Thirty five days old plants of sweet corn and vegetable soybeans were harvested and separated each into shoot and roots, then dried at 70°C for three days in forced draft oven and ground to pass 1 mm screen. Ten grams of either shoot or root were placed in 250 mL conical flask and added distilled water till the volume became 200 mL (giving concentration of 5%, or 50 g L^{-1}, as recommended by Wardle *et al.* (1992). The mixture was stirred for ten minutes and left in at room temperature for 48 h. The extracts were filtered with two layers of cheese cloth followed by whatman No.1 filter paper. Extracts were kept at 4°C in the refrigerator till use. The filtrates were taken out of the refrigerator 24 h before being used experimentally, in order to achieve 25°C room temperature.

Extracts from fresh plants: Sweet corn and vegetable soybean were harvested 60 days after planting. Each species were separated into shoot and root. Then cut into small pieces of less than 2 cm in length. Twenty grams of shoot (in proportion of leaf, stem and cob for corn and leaf stem and pod for soybean) and root were added to 200 mL of distilled water (giving the ratio of 1:10, w/v), the mixture was shaken for 10 min and then left at room temperature for 48 h. Filtration was like that of oven dried samples.

Germination and seedling growth bioassay: In this study, one sweet corn variety Masmadu, grain corn variety Putra J-58, vegetable soybean MKS1 and grain soybean AGS190 were used as test crops. The seeds were surface sterilized with 10:1, distilled water and sodium hypochlorite for 15 min and then rinsed with distilled water for four times. Ten seeds based on treatments were placed evenly in sterile 9 cm Petri dishes lined with two layers of filter papers. Then each Petri dish was applied to 10 mL of extract (shoot or root extract of either sweet corn or vegetable soybean) and water was used as control. The experiment was conducted under dark condition with minimum exposure to light during data collection. Four days after treatment application, germination was determined by counting the number of seeds germinated in each Petri dish and expressed in percentage. The 5th day, number of roots/seedling, total root length and shoot length of corn and hypocotyls length of soybean was measured from five randomly selected seedlings. The percentage of inhibition was calculated following the formula forwarded by Chung *et al.* (2003)

% inhibition = [(control-extracts)/control]×100, by which negative sign indicates stimulation and positive sign shows inhibition.

Extraction and separation of allelopathic compounds: The extractions of allelopathic compounds were carried out in May to July, 2005. Five grams of dried plant samples from 35 days corn or soybean (root and shoot of corn and soybean) were finely grounded and placed in a 250 mL conical flask containing 100 mL of 80% of High Performance Liquid Chromatography (HPLC) grade of methanol and stirred for 24 h at room temperature. Then the extract was filtered through whatman No. 42 filter paper. The filtrate was evaporated at 40°C to dryness using rotary evaporator. This extraction procedure is that of Chon and Kim (2004).

The dried material was diluted in 80% of HPLC grade of methanol. The compounds were separated using Thin Layer Chromatography (TLC) silica gel chromatofolios and the eluting solvent was Butanol: Acetone: Water (80:20:10 by volume). The plates were developed in glass champers lined up in all the sides with filter papers and saturated with the mobile phase solvent by pouring over the lining paper one hour prior to the development (as recommended by Touchstone, 1992). The glass champers was covered with watch glass during the development. Seven standards were used which were coumarin, trans-cinamic acid, o-coumaric acid, p-coumric acid, caffeic acid, ferulic acid and salicylic acid. The samples and standard of 0.3 μL was applied on spots on the silica gel plates 1.5 cm from the bottom, based on the distance traveled by spots and the mobile phase R_f value was calculated as follow (Jimenez *et al*., 1983; Touchstone, 1992).

$$R_f = \frac{\text{Distant spot traveled}}{\text{Distant solvent traveled}}$$

Spot colours were observed using UV light at 253 nm wave length.

Statistical analysis: The bioassay experiment was designed in factorial CRD with two factors and replicated four times. One factor was extracts: Shoot and root extract of sweet corn or vegetable soybean and water as control, the other factor was two corn variety and two soybean varieties. The analysis of variance was carried out using SAS program (version 9.1). Mean values were separated based on Least Significant Difference (LSD) at 0.05 probability level (Gomez and Gomez, 1984)

RESULTS

Germination percent: Effect of corn and soybean shoot and root extracts from 35 and 60 Days Old (DO) plants on germination are presented in Table 1 and 2, respectively. Extracts from 35 DO corn shoot significantly ($p<0.05$) inhibited germination percentage compared to control by 95, 66.7, 59.0, 52.7% for AGS190, Masmadu, MKS1 and

Table 1: Effect of sweet corn and vegetable soybean plant part extracts on germination percentage (average of ten seeds) of corn and soybean seeds (35 old plant extracts)

	Corn varieties				Soybean varieties			
	Masmadu		Putra J-58		MKS1		AGS190	
Tissue extracted	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)
Corn shoot	17.50*	66.7	42.50*	52.7	22.50*	59.0	2.50*	95
Corn roots	35.00*	33.3	77.50	13.8	52.50	4.5	57.50	30
Soybean shoot	30.00*	42.8	32.50*	63.9	10.00*	81.8	12.50*	75
Soybean root	35.00*	33.3	65.00*	27.8	55.50		50.00	
Control	52.50		90.00		55.00		50.00	
$LSD_{(0.05)}$	16.28		21.75		13.20		14.03	
SE±	2.86		5.54		4.58		4.85	

Values in the same column followed by* are significant different from the control at probability level of p<0.05,-sign indicates stimulatory, +indicates inhibitory

Table 2: Effect of sweet corn and vegetable soybean plant part extracts on germination percentage (average of ten seeds) of corn and soybean seeds (60 days old plant extract)

	Corn varieties				Soybean varieties			
	Masmadu		Putra J-58		MKS1		AGS190	
Tissue extracted	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)
Corn shoot	87.50*	-45.8	60.00*	17.2	12.50*	80.76	67.50	-28.57
Corn roots	65.00	-8.3	92.50*	-27.6	87.50*	-34.6	95.00*	-80.95
Soybean shoot	45.00*	25.0	35.00*	51.7	45.00*	30.76	20.00*	61.90
Soybean root	77.50*	-29.2	85.00*	-17.2	30.00*	53.85	45.00	14.28
Control	60.00		72.50		65.00		52.50	
$LSD_{(0.05)}$	10.65		10.65		17.18		17.18	
SE±	3.63		4.86		6.43		6.13	

Values in the same column followed by* are significant different from the control at probability level of p<0.05,-sign indicates stimulatory, + indicates inhibitory

Table 3: Effect of sweet corn and vegetable soybean plant part extracts on root number and total root length(average of five seedlings) of two corn varieties (35 days old plant extract)

Tissue extracted	No. of roots/seedling		Total root length/seedling (cm)	
	Masmadu	Putra J-58	Masmadu	Putra J-58
Corn shoot	1.20*	2.95*	0.98*	2.70*
Corn roots	2.45*	4.45*	13.37*	28.37*
Soybean shoot	1.60*	0.95*	2.02*	0.85*
Soybean root	2.05*	4.65*	11.85*	32.20*
Control	3.60	5.50	19.60	45.74
$LSD_{(0.05)}$	0.85	0.75	3.36	5.55
SE±	0.22	0.38	1.68	4.08

Values in the same column followed by* are significant different from the control at probability level of p<0.05,-sign indicates stimulatory, +indicates inhibitory

Table 4: Effect of sweet corn and vegetable soybean plant part extracts on root number and total root length of two corn varieties (average of five seedlings) (60 days plant extract)

Tissue extracted	No. of roots/seedling		Total root length/seedling (cm)	
	Masmadu	Putra J-58	Masmadu	Putra J-58
Corn shoot	4.45*	3.60*	14.25*	8.51*
Corn roots	3.05	4.10*	9.37*	10.83*
Soybean shoot	1.15*	0.65*	1.85	0.77*
Soybean root	3.05	3.75	13.00*	17.38*
Control	2.90	2.90	4.32	4.42
$LSD_{(0.05)}$	0.62	0.65	3.21	3.05
SE±	0.25	0.30	1.18	1.36

Values in the same column followed by* are significant different from the control at probability level of p<0.05,-sign indicates stimulatory, +indicates inhibitory

Table 5: Effect of sweet corn and vegetable soybean extracts on shoot length of corn and hypocotyl length of soybean (average of five seedlings). 35 days old plant extracts

Tissue extracted	Shoot length/ seedling (cm)		Hypocotyl length/ seedling (cm)	
	Masmadu	Putra J-58	MKS1	AGS190
Corn shoot	0.785*	1.325*	1.275*	0.4700*
Corn roots	2.695	3.725*	2.525	2.750
Soybean shoot	1.295*	1.050*	0.532*	0.537*
Soybean root	2.725	4.200*	2.200	2.525
Control	3.000	5.425	2.475	2.100
LSD $_{(0.05)}$	0.917	0.940	0.631	0.612
SE±	0.236	0.408	0.197	0.239

Values in the same column followed by* are significant different from the control at probability level of p<0.05,-sign indicates stimulatory, +indicates inhibitory

Putra J-58 respectively. Corn root extract only significantly retarded germination of Masmadu by 33.3%. Similarly soybean shoot extract significantly reduced germination percentage by 81.8, 75, 63.9 and 42.8% for MKS1, AGS190, Putra J-58 and Masmadu, respectively. On the other hand soybean root extract inhibited germination of Masmadu (33.3%) and Putra J-58 (27.8).

With regard to extracts from 60 DO plants, corn shoot extract was significantly inhibitory to germination of MKS1 (80.76%) and Putra J-58 (17.2%), while it was significantly stimulatory to germination of Masmadu (45.8%), but not significantly influenced by AGS190, though stimulatory influence of 28.6% was recorded. On the other hand soybean shoot extract significantly retarded germination compared to control by 61.9, 51.7, 30.76 and 25% for AGS190, Putra J-58, MKS1 and Masmadu, respectively. Soybean root extract had variable effect, which were stimulatory to Masmadu (29.2%) and Putra J-58(17.2), inhibitory to MKS1 (53.8%) and no significant effect to AGS190. Corn root extract had stimulatory effect to all test varieties.

Seedling growth

Number of roots and total root length of corn: Effect of sweet corn and vegetable soybean shoot and root extracts on seedling roots (35 and 60 DO plants respectively) are presented in Table 3 and 4. Extracts from 35 DO shoots of corn significantly reduced the number of roots and total root length of both recipient corn varieties, the magnitude of root length reduction was three and two folds for Masmadu and Putra J-58, respectively compared to control. While the total root length of Masmadu (0.98 cm) and Putra J-58 (2.7 cm) was reduced significantly due to corn shoot extract compared to control (19.6 and 45.74 cm for Masmadu and Putra J-58, respectively). Corn root extract also reduced significantly both number of roots per seedling and total root length of both corn varieties compared to control, but the extent was not like that of corn shoot. Soybean shoot and root extract significantly inhibited number of roots and total root length, though shoot extract had more inhibitory effect than root.

Sixty DO corn plant's shoot and root extracts had stimulatory effect on number of roots and total root length of Masmadu and PutraJ-58. On the other hand soybean shoot extract reduced number of roots per seedling as well as total root length per seedling compared to control. With regard to soybean root extract it had no significant influence on the number of roots per seedling, but enhanced the total root length per seedling.

Shoot length of corn and hypocotyls length of soybean: Thirty five DO corn plant's shoot extract significantly reduced the shoot length of Masmadu (73.8%) and Putra J-58(75.6%), as well as hypocotyl length of MKS1 (48.5) and AGS190 (77.6%) compared to control (Table 5). Corn root extract had significantly reduced the shoot length of Putra J-58(31%), but not Masmadu or hypocotyl length of soybean. Similar to the corn shoot, soybean shoot extract (60 DO) also retarded shoot length of Masmadu (56.8%) and Putra J-58(80.6%), as well as hypocotyl length of MKS1 (78.5%) and AGS190 (74.4%). Soybean root had significantly inhibited only shoot length of Putra J-58 (22.6%) (Table 6).

Separation of allelopathic compounds: The compounds separated from corn and soybean plant extracts are

Table 6: Effect of sweet corn and vegetable soybean plant part extracts on shoot length of corn and hypocotyl length of soybean, 60 days old plant extracts

Tissue extracted	Shoot length/ seedling (cm)		Hypocotyl length/ seedling (cm)	
	Masmadu	Putra J-58	MKS1	AGS190
Corn shoot	3.00	2.90	0.65	5.47*
Corn roots	3.47	4.37	6.67*	7.47*
Soybean shoot	2.67	1.60*	3.45	1.22
Soybean root	3.87	5.00*	2.32	2.47
Control	3.35	3.62	2.20	2.22
LSD $_{(0.05)}$	0.82	1.19	1.90	1.62
SE±	0.14	0.31	0.52	0.58

Values in the same column followed by* are significant different from the control at probability level of $p<0.05$,-sign indicates stimulatory, +indicates inhibitory

Table 7: R_f value of allelopathic compounds from plant extract and standards

Sn	Compound	R_f value
Standards		
1	Ferulic acid	0.72
2	Salicylic acid	0.60
3	o-Coumaric acid	0.78
4	Tms-cinnamic acid	0.74
5	Caffeic acid	0.85
6	p-Coumaric acid	0.77
7	Coumarin	0.77
Soybean root		
1	Compound 1	0.80
2	Compound 2	0.70
3	Compound 3	0.51
4	Compound 4	0.48
5	Compound 5	0.40
Soybean shoot		
1	Compound 1	0.80
2	Compound 2	0.70
3	Compound 3	0.51
4	Compound 4	0.48
5	Compound 5	0.40
Corn shoot		
1	Compound 1	0.84
2	Compound 2	0.50
3	Compound 3	0.40
Corn root		
	Compound 1	0.84

presented in Table 7, based on their R_f value. The compounds separated from both soybean and corn did not match with the standards used as indicated by R_f value.

DISCUSSION

Germination percentage: The inhibitory effect of soybean shoot extract from both 35 and 60 DO plants on germination could be that soybean maintained sufficient inhibitory concentration of allelochemicals at both crop growth stages and it was also reported that some crops release allelopathic substances even at later crop growth period like barley (Ben-Hammouda *et al*., 2001), while variable effect of corn shoot extract might be due to reduction of allelopathic effect with age of the crop as most allelopathic plants release allelopathic substances at early stage of crop growth (Dekker and Meggitt, 1983; Economou *et al*., 2002).With regard to variable effect of extracts (shoot or root extract) on different varieties might be due to differential susceptibility of recipient varieties. In this regard Ismail and Chong (2002) reported that aqueous extract of *mikania* plant leaves retarded germination of tomato and Chinese cabbage, but did not affect germination of corn and long bean, similarly Wu *et al*. (1998) noticed that wheat extract affected differently the germination of ryegrass weed, this indicates crops and varieties have different susceptibility to allelopathic effect and in this study Putra J-58 and MKS1 seem to be more sensitive to allelopathic effect as both young and old plant extracts inhibited their germination. On the other hand, the variable effect of corn and soybean shoot might be attributed to change in concentration or composition. Because the result indicates this as we have seen reduced germination or no effect at 35, but enhanced germination at 60 days. In soybean root which at initial stage (35 days) retarded germination of corn but not soybean and later stage (60 days old) enhanced corn germination might be explained that the compounds affecting corn and soybean are different or the concentration level inhibitory to corn and soybean are different. Moreover, it was also reported that allelopathic effect is species specific and concentration dependent (Einhelling, 1996)

Number of roots and total root length: Corn and soybean shoot extract from both 35 days old plants indicated the presence of inhibitory level of allelochemicals due to reduced number and total root length of corn varieties. But at 60 days, the variable effect could be due to reduced concentration of allelochemicals in corn but not in soybean, because in soybean the shoot extracts of both 35 and 60 days old plants were both inhibitory. Root extract's effect seems to change also with the age of the crop, which might be decreasing as crop age increases. So at early stage (35 days) both corn and soybean plants had sufficient quantity of allelochemicals which can retard root growth, but later on this concentration might be reduced as indicated by enhanced or no effect on root growth, it was also reported that lower concentration of allelochemicals stimulate growth (Einhelling, 1996). Other workers indicated that most allelochemicals are released at early stage of crop growth (Dekker and Meggitt, 1983; Economou *et al*., 2002). On the other hand soybean shoot and root contained 5 compounds, while corn had 3 compounds in shoot and one in root extract; hence soybean seems to have more inhibitory allelopathic compounds than corn. Meanwhile shoot extract which contains leaf parts was more phytotoxic than root, other workers confirmed higher allelopathic effect of leaves. For instance Economou *et al.* (2002) noticed that leaves of *Conyza albida* had more allelopathic effect than stems, additionally and it was reported that leaves of congress weed (Tefera, 2002) and alfalfa (Chon and Kim, 2002) had more allelopathic effect than stem and roots. Soybean may also contain allelopathic substances lasting long. Moreover, it was reported that some plants can release allelopathic compounds later in the crop growth (Ben-Hammouda *et al.*, 2001) or even from their crop residue (Bhowmik and Doll, 1984). Sensitivity of root to allelopathic effect could be attributed to its direct contact with the extract during bioassay. The mechanism of growth inhibition by allelopathic substances might be, as a result of reducing cell division and elongation.

Shoot length of corn and hypocotyl length of soybean: Different effect of corn shoots extract to corn and soybean varieties could be explained that, the extract might contain different substances, some of which are inhibitory to one variety while others might be stimulatory to other variety. Hypocotyls and shoot growth are less sensitive to allelopathic effect compared to roots (Tefera, 2002). On the other hand stimulatory effect of corn root extract on soybean hypocotyl length might be due to lower concentration of allelochemicals in corn root extract and this lower concentration is stimulatory to growth. It was also reported that root extracts have stimulatory effect (Tefera, 2002). Numerous research results indicate that different plant parts or extracts of the same plant parts can influence different crops differently. For instance, Ebana *et al.* (2001) reported that aqueous extract two rice varieties differed in their allelopathicity against growth of lettuce.

Allelopathic compounds of corn and soybean extracts: oybean seems to have more allelopathic activity which could be the presence of more compounds compared to corn, on the other hand soybean root which contain the same number of compounds to soybean shoot, its variable effect could be change in concentration. Abenavoli *et al.* (2001) noticed concentration dependent effect of coumarin on wheat root histology. Higher allelopathic effect of corn shoot compared to corn root might be more compound present in corn shoot (3) compared to roots 1 compound). Abenavoli *et al.* (2001) noticed that higher concentration of coumarin resulted in swollen root tips, similar to the effect of corn and soybean shoot extract on corn root tip in our experiment, but coumarin was not found in our extract which means there might be some other compounds having similar effect to coumarin.

CONCLUSIONS

Corn and soybean shoot extracts from 35 days old plants and soybean from 60 days old plants inhibited germination, number of roots and total root length of all tested varieties. While that of 60 days old corn had variable effect. This indicates that soybean had higher allelopathic effect as shown by more compounds and deformation of root and hypocotyl tips. Corn root extract from 35 days crop had inhibitory effect on germination of Masmadu and soybean root extract reduced germination of both corn varieties, but not soybean. This means roots had lesser allelopathic activity which might be lower concentration in case of soybean and both lower concentration and viewer number of compounds in case of corn. However, there is a need to exactly ascertain the compounds present in the extract, might be with the help of mass spectrophotometer which does not require standards and identifies compound by giving mass charge ratio, since in the present work compounds were different from the standards.

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