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Article

Allelopathic Potential of Sweet Sorghum Root Exudates and Identification of the Relevant Allelochemicals

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Abstract: This study determined the influence of cattle manure compost, chemical fertilizers, and mulch on the growth of weeds, sugar content, and growth of sweet sorghum (*Sorghum bicolor* (L.) Moench). The inhibitory potential of root exudates from two sweet sorghum cultivars (A; K1151 and B; K3351) was also evaluated. Chemical fertilizers increased the plant height, stem weight, biomass production, and sugar content of sweet sorghum. The total phenolic contents in the root exudates were 22.93 mg gallic acid equivalent per g dry weight (GAE/g DW) for cultivar A and 15.66 mg GAE/g DW for cultivar B. The total flavonoid contents in the root exudates were 14.77 mg rutin equivalent per g dry weight (RE/g DW) for cultivar A and 12.44 mg RE/g DW for cultivar B. The leaf extracts contained a higher amount of total phenolics and flavonoids than that of the stem and root. The inhibitory level of the root exudates from cultivar A on the seed germination and shoot growth of lettuce was greater than for cultivar B. Six phenolic acids, including protocatechuic, *p*-hydroxybenzoic, syringic, sinapic, *p*-coumaric, and benzoic acids, were detected from root exudates, root, stem, and leaf of both cultivars. The amount of *p*-coumaric acid in root exudates was greater than the other plant parts; however, protocatechuic acid was only found in the root exudates. *p*-Coumaric and protocatechuic acids may play an important role in the allelopathy of sweet sorghum to help reduce the dependence on synthetic herbicides in agricultural practice. This study indicates that cultivation methods and fertilization are important to increase both agronomic and economic values of sweet sorghum in agricultural production.

Keywords: allelopathy; phenolic acids; sweet sorghum; total flavonoids; total phenolics; weeds

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1. Introduction

The world energy crisis and increasing deterioration of the ecological environment have forced people to develop and utilize bio-energy [1]. Fuel ethanol is a clean energy of an infinite closed circuit, and is an eternal renewable energy [2]. As a raw material for fuel ethanol, sweet sorghum is listed as the first source of biological liquid fuel in the renewable energy development worldwide [3]. Sweet sorghum is a cultivated crop, mainly used as a forage and sugar crop due to its sugar-rich stems [4]. Recently, researchers focused on ethanol and bio-energy production from sweet sorghum [5]. The growth and yield performances of sweet sorghum is affected by several factors, including fertilizer application, cultural practices, and weed management [6,7].

On the other hand, the allelopathic potential of sweet sorghum has attracted the attention of scientists for weed management due to its competitive structure and morphology [8,9]. Allelopathy is a biological phenomenon in which an organism produces one or more bio-chemicals that influence the growth, survival, and reproduction of other organisms [10,11]. Allelopathy plays an important role in the agro-ecosystem, leading to a

wide array of interactions. Several crop species, including sweet sorghum, have exhibited allelopathic interactions that play a significant role in the complex environment of the agro-ecosystem [12]. Among the plant parts, roots play important role in allelopathy [13]. Plant root exudates are the general term in the process of growth communication and signaling [14]. The root system secretes huge amounts of substances to the growing medium to inhibit and suppress nearby plants [15]. It is proposed that root exudates secrete carbohydrates, amino acids, organic acids, and phenolics to the rhizosphere, which interfere with the growth of nearby plants [16,17]. These secretions are known as allelochemicals and can have beneficial or detrimental effects on the target organisms and plant growth [18].

Among the allelochemicals, phenolics are aromatic organic compounds, and thousands of them are known for their potential allelopathic properties [19]. Phenolic substances are important for stabilizing lipids against per-oxidation and for preventing several human diseases including diabetes [20]. They are used in the pharmaceutical industries as anti-oxidants, anti-inflammation, anti-aging, and anti-proliferative agents [21]. Phenolic compounds play crucial roles in plant allelopathy and defense systems, and are among the secondary metabolites [22,23]. They are also applied in the formation and formulation of synthetic herbicides [24]. However, the genetic background and agronomic practices including fertilizer application and cultural practices have effects on sweet sorghum growth and allelopathy [25].

To increase productivity and valuable allelochemicals of sweet sorghum, it is required to select adequate fertilizer applications and cultivation practices. Thus, the aims of this study are to (1) determine the influence of fertilizers and mulching practices on the growth, biomass production, and sugar content of sweet sorghum; (2) evaluate phytochemicals and the allelopathic potential of root exudates; and to (3) identify and quantify phenolic acids in root exudates, as well as the roots, stems, and leaves of the crop.

2. Results

2.1. Growth Parameters of Sweet Sorghum and Weeds

The growth parameters and dissolved sugar contents (brix %) of the two sweet sorghum cultivars under different mulching and fertilizer application practices are illustrated in Table 1. Generally, cultivar A exhibited a better performance related to plant height, stem weight, and biomass production, but was lower for sugar content. The best growth was received by the mulch practice across all of the treatments. The mulch with synthetic fertilizer (M-CF) showed the best performance for plant height, stem weight, biomass production, and sugar content, followed by cattle manure compost (M-CMC) and the control (C). This order was also followed in non-mulch treatments (Table 1). The sugar content differed between the two cultivars among the mulch and fertilizer application. The highest sugar content was observed in cultivar A using M-CF treatment (12.26%) and the lowest was for the C treatment (4.51%).

Table 1. Growth parameters and sugar content (brix%) of sweet sorghum cultivars under mulching and fertilizer application.

Cultivar	Treatment	Growth Parameters			
		Plant Height (cm)	Stem Weight (kg)	Biomass (kg)	Brix (%)
A	M-CMC	205.33 ± 8.01 bcd	0.26 ± 0.02 c	0.36 ± 0.04 c	8.24 ± 0.14 e
	CMC	183.70 ± 4.93 de	0.21 ± 0.02 cd	0.30 ± 0.03 cd	6.57 ± 0.10 g
	M-CF	257.30 ± 4.42 a	0.46 ± 0.03 a	0.60 ± 0.04 a	12.26 ± 0.21 a
	CF	211.56 ± 8.08 bc	0.35 ± 0.03 b	0.48 ± 0.04 b	11.00 ± 0.23 bc
	M-C	150.91 ± 6.92 fg	0.17 ± 0.02 cde	0.25 ± 0.04 cde	7.68 ± 0.08 ef
	C	124.15 ± 3.72 h	0.06 ± 0.01 f	0.11 ± 0.01 f	4.51 ± 0.07 h

Table 1. *Cont.*

Cultivar	Treatment	Growth Parameters			
		Plant Height (cm)	Stem Weight (kg)	Biomass (kg)	Brix (%)
B	M-CMC	180.93 ± 6.46 de	0.10 ± 0.01 ef	0.15 ± 0.02 ef	9.98 ± 0.10 d
	CMC	143.74 ± 3.79 gh	0.05 ± 0.01 f	0.08 ± 0.01 f	7.39 ± 0.20 f
	M-CF	230.41 ± 4.09 b	0.16 ± 0.01 de	0.23 ± 0.01 de	11.67 ± 0.16 ab
	CF	189.41 ± 5.84 cde	0.09 ± 0.01 ef	0.14 ± 0.01 ef	10.95 ± 0.18 c
	M-C	172.81 ± 3.90 ef	0.06 ± 0.01 f	0.10 ± 0.01 f	9.61 ± 0.07 d
	C	139.48 ± 3.11 gh	0.04 ± 0.01 f	0.07 ± 0.01 f	6.26 ± 0.05 g

Data are presented as mean (n = 10) ± SE (standard errors). Different letters within a column indicate significant differences at $p < 0.05$. M, mulch; CMC, cattle manure compost; CF, chemical fertilizer; C, control. Brix shows the dissolved sugar contents.

The weed emergence was determined, including plant height and dry weight, which are presented in Table 2. Sweet sorghum showed a stronger inhibition on the weed germination and growth in CF than for the CMC and C treatments. The CMC treatment had large number and diversity of weeds, followed by the C and CF treatments in both cultivars (Table 2). However, the height of the weeds was the maximum but the dry weight of the weeds was the lowest among treatments compared with CF.

Table 2. Growth performance of weeds in non-mulch treatments.

Cultivar	Treatment	Weed Growth	
		Height (cm)	Weight (g)
A	CMC	56.40 ± 1.52 ab	9.33 ± 1.61 b
	CF	64.68 ± 2.05 a	2.85 ± 0.94 c
	C	38.30 ± 3.46 d	7.90 ± 1.61 bc
B	CMC	47.53 ± 1.15 c	15.83 ± 2.32 a
	CF	54.81 ± 1.58 bc	6.76 ± 1.38 bc
	C	36.63 ± 1.84 d	10.45 ± 0.95 ab

Data are presented as mean (n = 10) ± SE (standard errors). Different letters within a column indicate significant differences at $p < 0.05$. CMC, cattle manure compost; CF, chemical fertilizer; C, control.

2.2. Inhibitory Activity of Sweet Sorghum Root Exudates

Sweet sorghum roots might secrete certain allelochemicals during seed germination and the first growth period of 10 days, thus these chemicals inhibited the germination and growth of lettuce (Table 3). The root exudates of the A and B cultivars decreased the germination rate of the lettuce seeds by 43% and 24% over the control, respectively. The shoot elongation of lettuce was also decreased at 55% and 35%, respectively. In general, cultivar A exhibited a stronger inhibition on the seed germination and shoot height of lettuce than B (Table 3).

Table 3. Inhibitory potential of root exudates on the seed germination and shoot height of lettuce.

Group	Emergence		Reduction over Control (%)	
	Germination (%)	Shoot Height (cm)	Germination	Height
A	46.67 ± 3.33 c	1.43 ± 0.09 c	43.00	55.00
B	63.33 ± 3.33 b	2.07 ± 0.09 b	24.00	35.00
Control	83.33 ± 3.33 a	3.17 ± 0.09 a	-	-

Data are presented as mean (n = 18) ± SE (standard errors). Different letters within a column indicate significant differences at $p<0.05$. - means not measured.

2.3. Total Phenolic and Flavonoid Contents

The total phenolic content (TPC) and total flavonoid content (TFC) in the root exudates, root, stem, and leaf extracts are shown in Table 4. Generally, TPC was higher

in the leaf extracts, followed by the stem, root, and root exudates. In general, cultivar B showed a higher TPC than cultivar A. Cultivar A showed a higher TPC in the root exudates (22.93 mg gallic acid equivalent per g dry weight (GAE/g DW)), which had lower values than cultivar B (Table 4), while cultivar B showed a higher amount of TPC in the root (98.53 mg GAE/g DW), stem (83.91 mg GAE/g DW), and leaf extracts (337.05 mg GAE/g DW) (Table 4).

Table 4. Total phenolic and total flavonoid contents in sweet sorghum extracts.

Cultivar	Plant Parts	TPC (mg GAE/g DW)	TFC (mg RE/g DW)
A	Root exudates	22.93 ± 0.91 f	14.77 ± 0.25 f
	Root	52.55 ± 2.11 e	17.61 ± 0.8 e
	Stem	76.33 ± 3.18 d	61.91 ± 3.72 d
	Leaf	220.89 ± 8.43 b	192.45 ± 3.25 b
B	Root exudates	15.66 ± 0.63 f	12.44 ± 0.16 f
	Root	98.53 ± 5.71 c	71.38 ± 1.34 c
	Stem	83.91 ± 5.89 cd	75.36 ± 3.99 cd
	Leaf	337.05 ± 6.22 a	273.02 ± 3.55 a

Data are presented as mean (n = 9) ± SE (standard errors). Different letters within a column indicate significant differences at $p < 0.05$. TPC, TFC, GAE, RE, and DW are total phenolic contents, total flavonoid contents, gallic acid equivalent, rutin equivalent, and dry weight, respectively.

No significant difference in the quantity of TFC was found between cultivars A and B. Generally, cultivar B contained higher TFC than cultivar A. The leaf extract recorded the highest TFC, followed by the stem, root, and root exudates. Cultivar B had the greater amounts of TFC in the leaf (273.02 mg rutin equivalent per g dry weight (RE/g DW)), stem (75.36 mg RE/g DW), and root (71.38 mg RE/g DW) than cultivar A (Table 4).

2.4. Identification and Quantification of Phenolics by HPLC (High Performance Liquid Chromatography)

Six phenolic acids, including protocatechuic, *p*-hydroxybenzoic, syringic, sinapic, *p*-coumaric, and benzoic acids, were identified and quantified using HPLC (Table 5). In cultivar A, the root and stem had a greater number of phenolic acids (protocatechuic, *p*-hydroxybenzoic, sinapic, *p*-coumaric, and benzoic acids), followed by leaf (syringic, *p*-coumaric, and benzoic acids), and root exudates (protocatechuic and *p*-coumaric acids). Among them, the amount of *p*-coumaric acid was the greatest. For cultivar B, protocatechuic acid was uniquely observed in the root exudates (2.01 mg/g DW), while *p*-hydroxybenzoic acid was presented in all extracts (Table 5). The number of phenolic acids was the most abundant in stem extracts (*p*-hydroxybenzoic, syringic, sinapic, *p*-coumaric, and benzoic acids), followed by the root (*p*-hydroxybenzoic, sinapic, *p*-coumaric, and benzoic acids), leaf (*p*-hydroxybenzoic, syringic, *p*-coumaric, and benzoic acids), and root exudates (protocatechuic, *p*-hydroxybenzoic, and *p*-coumaric acids). Similar to cultivar A, the quantity of *p*-coumaric acid was also the greatest (Table 5).

Table 5. Phenolic compounds in different extracts of sweet sorghum.

Cultivar	Plant Parts	Phenolic Acids (mg/g DW)					
		Pt Acid	*p*-Hy Acid	Sy Acid	Si Acid	*p*-Co Acid	Be Acid
A	Root exudates	2.16 ± 0.01 L	nd	nd	nd	3.34 ± 0.03 f	nd
	Root	0.81 ± 0.01 u	0.40 ± 0.02 v	nd	0.78 ± 0.03 u	1.57 ± 0.01 pq	0.75 ± 0.01 u
	Stem	2.17 ± 0.01 L	1.33 ± 0.01 s	nd	2.90 ± 0.01 h	4.43 ± 0.01 d	2.41 ± 0.01 j

Table 5. *Cont.*

Cultivar	Plant Parts	Phenolic Acids (mg/g DW)					
		Pt Acid	***p*-Hy Acid**	**Sy Acid**	**Si Acid**	***p*-Co Acid**	**Be Acid**
B	Root exudates	2.01 ± 0.01 m	1.71 ± 0.01 n	nd	nd	2.52 ± 0.01 i	nd
	Root	nd	1.12 ± 0.01 t	nd	2.22 ± 0.01 kL	4.23 ± 0.01 e	1.63 ± 0.01 op
	Stem	nd	1.70 ± 0.01 no	1.52 ± 0.01 q	3.07 ± 0.01 g	5.34 ± 0.01 c	2.52 ± 0.01 i
	Leaf	nd	1.18 ± 0.01 t	1.12 ± 0.01 t	nd	7.22 ± 0.01 b	2.24 ± 0.03 k

Pt acid, protocatechuic acid. *p*-Hy acid, *p*-hydroxybenzoic acid. Sy acid, syringic acid. Si acid, sinapic acid. *p*-Co acid, *p*-coumaric acid. Be acid, benzoic acid. Nd, not detected. Values represent as means (n = 4) ± SE. Different letters indicate significant differences at $p < 0.05$ across all portions. DW means dry weight.

3. Discussion

In this study, the chemical fertilizer (CF) showed a greater growth and sugar content (brix%) than the cattle manure compost (CMC) and control (C) treatments. It was reported that the absorption and utilization efficiency of the chemical fertilizer was greater than for CMC [26]. In this study, mulch with CF treatment increased the content of sugar in the sweet sorghum biomass (Table 1). It has been reported that a high sugar content in sweet sorghum is an important trait for ethanol production and sweet industries [27]. During the growth of sweet sorghum, there is competition for nutrients and water absorption between sweet sorghum and weeds, which may affect the compounds secreted from the roots of sweet sorghum. The growth performance of sweet sorghum in CF treatment was better than CMC; thus, weed growth was negatively influenced and their weight was decreased (Table 2). Sweet sorghum might secrete certain allelochemicals in CF to exhibit a stronger inhibition on weeds. Glab et al. [28] reported that sweet sorghum with a better growth performance suppressed growth of surrounding weeds significantly.

Root exudates play an important role in sweet sorghum allelopathy, in which principal compounds are phenolic acids, which can reduce weed interference [29]. Root exudates of both cultivars decreased the germination rate of lettuce by 43% (cultivar A) and 24% (cultivar B), and the shoot growth by 55% (cultivar A) and 35% (cultivar B) (Table 3), respectively. Cultivar A showed a stronger inhibitory potential than cultivar B. Phenolics produced by plants are their natural defense system and can enhance agricultural productivity and food safety [30,31]. The phenolic and flavonoid contents were higher in the root exudates of cultivar A than cultivar B; thus, they strongly inhibited the seed germination and shoot growth of lettuce, which is in line with previous studies [29,30].

Sorghum is among the most studied allelopathic crops with its relevant allelochemicals to inhibit suppression of weeds [32]. In this study, six phenolic acids were detected in different parts of sweet sorghum; of them, protocatechuic and *p*-coumaric acids may play an important role in the allelopathic activities (Table 5). Hussain et al. [13] identified benzoic, *p*-hydroxybenzoic, vanillic, ferulic, chlorogenic, *m*-coumaric, *p*-coumaric, gallic, and caffeic acids, *p*-hydroxybenzaldehyde, dhurrin, sorgoleone, *m*-hydroxybenzoic, and protocatechuic acids in different plant tissues of sorghum and root exudates. Sorgoleone is considered to be a principal allelochemical in sorghum [13]. Ayeni and Kayode [33] stated that the *Euphorbia heterophylla* L. germination rate, dry shoots, and root weights were stunted when sorghum residues were increased from 0 g to 50 g in 5600 g soil.

It has been reported that sorghum allelopathy is affected by soil properties, microorganisms, and fertilizer application and nutrient availability in its residues [34,35]. This study states that mulch and chemical fertilizer application improved the growth, sugar content, and phytochemical capacity of sweet sorghum crop, while weed emergence was reduced. Protocatechuic and *p*-coumaric acids may play an important role in the allelopathic activities of sweet sorghum (Table 5).

Sweet sorghum also has potential for bioethanol production because of its fast growth and adaptability to high temperatures and elevated CO_2 [36]. However, the cultivated conditions including sowing times, densities, and soils may strongly affect the biomass and ethanol yield of sweet sorghum [37]. The ideal soil, with a pH of 5.5 and fertiliza-

tion of Al and Zn of 39.4 and 0.6 g kg^{-1}, was the best condition to provide an ethanol yield of >5000 L ha^{-1}. However, while the soil pH increased > 6.0, both the biomass and ethanol yield of the plant were significantly reduced due to the decrease in zinc content in soil [37]. In this research, fertilization was also found to be correlated to biomass, weed suppression, allelochemicals, and phytochemicals, but the affects from soil pH micronutrients were not investigated.

The great biomass from sweet sorghum has been considered as an alternative source of biomass energy for electricity generation in order to minimize CO_2 emissions worldwide [38]. Therefore, fertilization and cultivation methods are important to determine its biomass, as investigated in this study and in the literature [37,38]. The energy gain of the sweet sorghum biomass varies from 170 to 226 GJ ha^{-1}, depending on either low-input or high-input technology [38]. Sweet sorghum biomass is also useful for the development of silage for cattle, which was reported considering the growth performance, carcass traits, and meat quality of lambs [39]. Therefore, fertilization and cultivation methods strongly increase both the agronomic and economic values of this crop [38,39].

Crop allelopathy can effectively manage weed emergence and thus reduces the dependence on synthetic herbicides [40]. Among the many allelopathic crops, sorghum shows a strong allelopathic potential to suppress weed growth and thus increases crop productivity [41]. The allelopathic potential of crops has been known to be variable among crop varieties [41–43], thus the development of new crop cultivars with a strong potential for weed reduction is required. In addition, the genetic correlation between the biomass and contents of allelochemicals and beneficial phytochemicals such as antioxidants should be exploited to effectively breed new sorghum cultivars for sustainable agricultural production.

4. Materials and Methods

4.1. Experiment Design and Materials

Experiments were conducted in the research field of Saijo Agriculture High School, Higashi Hiroshima City, Hiroshima Prefecture, Japan, in an area of 0.04 ha. The field was plowed and prepared for cultivation at the beginning of May 2015. The experiment was carried out in a completely randomized design with three replications. Two famous and widely cultivated sweet sorghums, including cultivars A; K1151 and B; K3351, were purchased from Snow Brand Seed Co., Ltd (Hokkaido, Japan). They were cultivated under mulch (M) and no-mulch conditions with three different fertilizations, including cattle manure compost (CMC), chemical fertilizer (CF), and no fertilizer (C). Vinyl film was used as a mulching practice. The concentration of nitrogen (N), phosphorus (P), and potassium (K) were separately adjusted to 150 kg/ha, 100 kg/ha, and 150 kg/ha from both the application of CMC and CF. In order to observe the interaction between the weeds and sweet sorghum, a control group was set up. The control was mulched with a layer of vinyl film used to prevent the weed interference.

Three seeds of each cultivar were sown and then thinned to be one seedling per hill after two weeks. The cultivation season started in June and was harvested at the end of September 2015. After harvest, the plant height, stem weight, biomass production, sugar concentration (brix %), and weed height and weight were measured. The whole biomass of weeds was dried and weighted and the height was measured.

4.2. Sugar Content of Sweet Sorghum

After harvest, sweet sorghum stalks were harvested and used for the production of juice using a juice presser squeeze. Juice drops were placed on a refractometer (Aichose, Medicare Products Inc., New Delhi, India) to measure the sugar concentration (brix %). In order to ensure the accuracy of the data and to reduce the impact of external temperature, the measurement was made at 25 °C and repeated thrice.

4.3. Reagents and Standards

Reagents and standard compounds including gallic acid, rutin, potassium persulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3), Folin–Ciocalteu reagent, hydrochloric acid (HCl), aluminum chloride hexahydrate ($AlCl_3$ $6H_2O$), sodium hypochlorite (NaOCl), sodium hydroxide (NaOH), and sodium acetate ($C_2H_3NaO_2$) were of analytical grade and were purchased from Fujifilm Wako Pure Chemical Co., Osaka, Japan. The solvents for extraction and isolation, as well as acetonitrile, were acquired from Junsei Chemical Co., Ltd., Tokyo, Japan and Fisher Scientific Co., Hampton, New Hampshire, USA. The remaining chemicals were procured from Kanto Chemical Co., Inc., Tokyo, Japan.

4.4. Sample Extraction

Samples from the root, stem, and leaf were separately collected and kept in an incubator (Biotron NC system, Nippon Medical and Chemical Instrument, Co., Ltd., Osaka, Japan) for 3 days at 40 °C in order to obtain dried samples. Then, the samples were converted into powder. First, 1 g of powder from the roots, stems, and leaves were separately added into polystyrene bottles. The bottles were filled with 100 mL of 99.5% ethanol and shaken for 24 h at room temperature. The obtained extracts were filtered using 90 nm filter papers twice. The filtrated extracts were adjusted into a rotary evaporator (Rotavapor R-300, Nihon Buchi K.K., Tokyo, Japan) to increase their concentrations. The concentrations of the achieved extracts were adjusted to 10 mg/mL and kept at a low temperature for future use in a dark room. Root exudates were achieved by removing the germinated sweet sorghum seeds from the agar solution. The obtained materials were mixed with ethanol, shaken and centrifuge in 5000 r/min for 5 min, and consequently the above extraction procedures were followed.

4.5. Determination of Total Phenolic and Flavonoid Contents

The Folin–Ciocalteu method was used to measure the total phenolic contents (TPC) of the samples following [44]. Briefly, 0.2 mL of the sample extract was mixed with 1 mL 10% of Folin–Ciocalteu. After three min, 0.8 mL 7.5% Na_2CO_3 solution was added. The mixture was kept at room temperature for 40 min. Gallic acid was used as a standard to obtain a calibration curve. The absorbance of the resulting blue color was measured at 765 nm using a spectrophotometer (HACH DR/4000U-Japan). TPC is illustrated as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW).

The total flavonoid contents (TFC) of the samples were measured following the method of Djeridane et al. [45]. Briefly, 1 g of the sample extract was mixed with 1 mL 2% $AlCl_3$. The mixture was kept at room temperature for 15 min. The absorbance was measured at 430 nm using the spectrophotometer. Rutin was used for the calibration curve and the TFC was illustrated as mg rutin equivalent per g dry weight (mg RE/g DW).

4.6. Inhibitory Activities of Extracts

A 12-well plate was used to measure the inhibitory potential of the extracts. Initially, each well received 300 mL of 5% agar solution. Lettuce (*Lactuca sativa* L.) was used as an indicator plant. Before seed sowing, lettuce seeds were soaked in 0.1% NaOCl solution for 20 min and washed well with distilled water. The seeds were sown on the agar solution and managed for one week. Each extract was used in different concentrations to obtain the IC_{50} value. The IC_{50} value illustrated as the concentration of extract showed 50% inhibition and was calculated by a previously described method [46,47]. A control group with ethanol was used to compare the effects of extracts on lettuce germination and growth inhibition. Data related to germination rate and shoot height of lettuce were collected. Germination rate was measured by counting the germinated seeds in all replications. Shoot height was recorded by measuring the length of the shoot from the base node until the tip of the shoot.

4.7. Identification and Quantification of Phenolic Compounds

High-performed liquid chromatography (LC-Net II/ADC, UV-2075 Plus and PU-2089 Plus system) was used for the identification and quantification of the phenolic compounds. Column (Waters Cooperation, Milford, MA, USA) was used for this measurement. The column temperature was kept at 25 °C. The mobile phase was composed of 99.8% methanol and 0.1% acetic acid. An amount of 5 µL from each sample extract was injected to the system and measured at 254 nm. Gradient elution was adjusted and run with a 1 mL/min flow-rate applying the following time gradients: 5% B (0–2 min), 5–70% B (2–12 min), 100% B (12–16 min) and maintained for 6 min, 100-5% B (22–24 min), and another 10 min for equilibration. As standard compounds, 15 phenolic acids, including benzoic, catechol, cinnamic, chlorogenic, ellagic, ferulic, caffeic, gallic, *p*-coumaric, *p*-hydroxybenzoic, protocatechuic, sinapic, syringic, vanillic, and vanillin acids, were measured and their retention times and peak areas were collected.

4.8. Statistical Analysis

All of the data were analyzed by Minitab 17.0 statistical software. One-way analysis of variance (ANOVA) was performed to calculate the mean differences and standard error (SE) of the treatments. Significant differences were compared using Tukey's test at ($p < 0.05$) level.

5. Conclusions

Cattle manure compost, synthetic fertilizers, and mulching provided different effects on sweet sorghum growth, sugar content, and weed emergence. Among the identified phenolic acids, *p*-coumaric acid was present in all parts of the two cultivars, and its quantity was the maximum in leaves. Protocatechuic acid was found in the root exudates of both cultivars A and B. It is therefore proposed that *p*-coumaric and protocatechuic acids may play an important role in reducing weed growth. Further trials should be further improved by cultivation methods and fertilization to enhance the agronomic and economic values of this crops in sustainable agricultural production.

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