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Weed Control Efficacy of Sorghum Shoot Extract Extracted with Various Solvents

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The application of allelopathy in sustainable agriculture was suggested as an environmentally friendly tool to manage weed infestation. This study aims to determine the phenolic compounds in the sorghum shoot extracts by liquid:liquid extraction using various solvents (ethanol, ethanol–chloroform, ethanol–hexane, ethanol–ethyl acetate, and ethanol–methylene chloride) and to assess weed control efficacy of the sorghum extracts. Analysis of the phenolic compounds indicated that ethanol–ethyl acetate fraction contained highest phenolic compounds. Bioassay using four weed species, *Abutilon avicennae*, *Digitaria sanguinalis*, *Amaranthus retroflexu*, and *Echinochloa crus–galli* indicated that the extracts significantly inhibited the germination and growth of four weed species. In addition, the sorghum extracts had post–emergence activity for the weed species. Based on field studies, the sorghum shoot extract at 1 g/mL concentration and three times of applications at 7, 14, and 21 days after tillage controlled weeds more than 90% of untreated control. Based on the high herbicidal efficacy of the sorghum shoot extract extracted with ethanol–ethyl acetate, the sorghum extract is a promising material and might be used as a natural herbicide in the organic farming system.

Key words: Natural herbicide, Phenolic compound, Sorghum shoot, Weed control

INTRODUCTION

Modern agriculture focuses on sustainable development and weeds have always been a major challenge in crop production (Hwang *et al*., 2017). If weeds are not adequately controlled, they can cause substantial losses in agricultural production. Annual worldwide losses by weeds were estimated at 10–15% of attainable production among the principal food sources (Abouziena and Haggag, 2016). The abuse of chemicals for weed control in agricultural production has caused serious damages to crops and the environment like unsafe agricultural foods and human health problems (Blair *et al*., 1992; Nazarko *et al.*, 2003; Hien *et al.*, 2015). In addition, the over–use of herbicides can induce resistant plants, which can reproduce and become dominant in the population (Bo *et al.*, 2017).

Recently, allelopathy has emerged as one of the possible alternatives to existing herbicides for sustainable weed management. Allelopathy, which was described the stimulatory or inhibitory impact of any biochemical interaction between plants (Rice, 1984; Molisch, 1937). The application of allelopathy in agriculture was suggested as an appropriate tool to manage weed infestation in agricultural production (Purvis *et al.*, 1985; Cheema 1988). Allelopathy offers potential for biorational weed control through the production and release of allelochemicals from leaves, stems, roots, flowers, and seeds of living or decomposing plant materials (Salhi Nasrine *et al*., 2011). Under appropriate conditions, allelochemicals may be released in quantities that can suppress developing weed seedlings and often exhibit a selectivity similar to that of synthetic herbicides (Weston, 1996). The suppression of weeds by allelopathy of crops can be exploited to improve effectiveness and sustainability of weed management in crop production (Amb and Ahluwalia, 2016).

Among the plants with allelochemical contents, sorghum [*Sorghum bicolor* (L.) Moench] is an allelopathic species that suppresses the growth of weeds (Won *et al*., 2011). The allelopathic potential of sorghum was reported and well documented by several investigators (Panasiuk *et al*., 1986; Einhellig and Souza, 1992). Chemical constituents of sorghum include tannins, phenolic acids, anthocyanins, phytosterols, and policosanols (Awika and Rooney, 2004). Among these chemicals, phenolic acids have been most frequently identified as phytotoxins (Blum, 1996). A review of literature indicated that water extracts of different plant parts of potential allelopathic crops had significantly suppressive effects on the growth of weeds (Ashraf and Akhlaq, 2007). Water extract of mature sorghum plants were used by (Cheema and Khaliq, 2000) and they reported that foliar application of this extract reduced weed biomass by 35–40%, and increased wheat yield by 10–21%. Sorghum root exudates reduced growth of various weed species at a very low concentration (Roth *et al*., 2000).

Therefore, in this study we optimized extraction

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method for sorghum shoot using various solvents and to explore the possibility of using it as a weed control agent in agriculture.

MATERIALS AND METHODS

Plant materials

Sorghum cultivar named 'Hwangumchal' was collected from the National Institute of Crop Science, JeonJu, Korea; then it were dried at farm of Chungnam National University and chaffed into 2–3 cm using fodder and crushed and powderized by blender machine.

Sorghum shoots extraction

Firstly, 100 g sorghum powder were soaked and shaken with 500 ml of ethanol for 24 hours at room temperature, forming a solid–liquid mixture. The solid residue was then filtered out and the remaining solution was divided into five equal–volumes and the solvents were subsequently evaporated. Secondly, the mixture remaining post evaporation was extracted by dissolving them into various solvent solutions and distilled water, eluted at 50%:50% including chloroform ($CHCl_3$), hexane (C_6H_{14}), ethyl acetate (C_2H_5–OOC–CH_3), and methylene chloride (CH_2Cl_2). These solutions were separated into two liquid phases (polar and non–polar fractions), from which the polar phase was removed. The remaining non–polar phase was evaporated completely and subsequently dissolved into distilled water for quantitative analysis of the total phenolic content by HPLC. Finally, extracts were obtained in five solvent systems, which referred to as 'ethanol', 'ethanol–chloroform', 'ethanol–hexane', 'ethanol–ethyl acetate', and 'ethanol–methylene chloride', respectively.

High–performance liquid chromatography (HPLC) analysis of phenolic compounds

The sorghum extracts obtained by using five various solvent systems including 'ethanol', 'ethanol–chloroform', 'ethanol–hexane', 'ethanol–ethyl acetate', and 'ethanol–methylene chloride' were analyzed by HPLC on C18 column (250 mm×4.6 mm), using acetonitrile and acetic acid as mobile phase. The flow rate was set at 1.0 mL/min and the injection volume was 20 μL. All samples were run in triplicate.

Application of sorghum extracts on weeds

Germination studies under laboratory conditions

Four weed species including: *Echinochloa crus–galli, Digitaria sanguinalis, Abutilon avicennae*, and *Amaranthus retroflexus* were used to study the effectiveness of sorghum shoot extract for germination inhibition. Inhibition of germination was evaluated under treatment with various concentrations (1 g/mL; 0.5 g/mL; 0.25 g/mL) (1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract) of the extracts. The experiment was conducted as follows: Weed seeds were placed on filter papers (Hyundai 10, 50 mm) in a petri dish (60×15 mm) and then sorghum extract (5 mL) was put in a petri dish. The control received only distilled water. Treatments were placed in a growth chamber (set at: 25°C, 4000 lux, illumination period: 7:00 – 19:00). After seven days, plant numbers were determined. Three replicates were done.

Herbicidal activity of sorghum shoot extracts under greenhouse conditions

This bioassay, which involved two experiments (Different sorghum extracts from five solvents and various sorghum shoot extract concentrations) was conducted in the greenhouse. First experiment five prepared extracts ('ethanol', 'ethanol–chloroform', 'ethanol–hexane', 'ethanol–ethyl acetate', and 'ethanol–methylene chloride') were tested herbicidal activity on four weed species (*A. avicennae, D. sanguinalis, A. retroflexus,* and *E. crus–galli*). Second experiment, the extract with the best herbicidal activity was identified and was applied with various concentrations in post–emergent studies. Then, this extract was set as original concentration (1 g/mL and its dilutions of 50%, 25%, 12.5%, and 6.25% were made (0.5, 0.25; 0.125; 0.0625 g/mL), respectively (1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract). Weeds were grown in the greenhouse at a temperature of 25 ± 5 °C, water irrigation was kept for moisture continuity. Three replicates were used. When the 2–3 weed leaf stage appeared, the sorghum extracts were sprayed on these weeds. After one week, the shoot of each weed specie was collected for biomass determination.

Field studies

This experiment was conducted in the Experimental Farm of Chungnam National University, Korea. The field was divided into plots with an area of 0.25 m^2. The arable land was tilled by conventional methods. A completely randomized design with three replicates was used. In addition, control plots were treated with distilled water only. The extract most efficient in controlling the weeds was identified and used as the treatment.

Study of soil application under field conditions

After soil tillage, 25 mL of sorghum shoot extract at 1 g/mL concentration was sprayed onto the soil (following the formula 1000 L/1 ha) (1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract). The treatments were as follows: one–time application, 7 days after tillage; sprayed two times, 7 days apart, at 7 and 14 days after tillage; sprayed three times, 7 days apart, at 7, 14, and 21 days after tillage. Twenty–eight days after treatment, weeds of each plot were collected and the effectiveness of sorghum extract to control the weeds from each treatment plot was assessed.

Study of foliar application under field conditions

For foliar application in the field, weeds were grown in the field. At the 2–3 leaf stage, sorghum extracts at various concentrations (1; 0.5; 0.25 g/mL) were sprayed on four weed plots (1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract). Twenty–eight days after treatment, weeds of each plot were collected and the effectiveness of sorghum extract to control the weeds from each treatment plot was evaluated.

RESULTS

Analysis of the phenolic compounds in sorghum extracts

Phenolics are the most common water–soluble allelochemicals and play a significant role in plant–plant interactions including allelopathy (Batish *et al*., 2007a, b; 2006b). In this study, upon HPLC analysis seven phenolic compounds including 4–hydroxybenzoic acid, chlorogenic acid, caffeic acid, p–coumaric acid, ferulic acid, benzoic acid, and kaempferol were identified from sorghum shoot. All these phenolic compounds are known as phytotoxins and widely implicated in allelopathic studies, and even effected the growth of weeds (Dharmraj, 1998). The results showed that the sorghum extract using 'ethanol–ethyl acetate' had the highest amount of phenolic compounds compared to other solvents (Table 1).

Effect of sorghum shoot extract on weeds

Germination studies under laboratory conditions

Results from the present study (Table 2) indicated that sorghum shoot extracts have inhibitory activity on weed seed. The ethanol–ethyl acetate fraction contained the compounds with the greatest herbicidal activity. The extract from ethanol–ethyl acetate inhibited root

Table 1. Identification and quantification of phenolic compounds in sorghum extracts using various solvents

Phenolic compounds	Solvents				
	Ethanol	Ethanol – Chloroform	Ethanol – Hexane	Ethanol – Ethyl acetate	Ethanol – Methylene chloride
4–hydroxybenzoic acid	2.34±0.28	0.92±0.09	0.91±0.02	5.19±0.75	1.76±0.11
Chlorogenic acid	0.66±0.04	0.51±0.01	0.51±0.01	0.57±0.07	0.45±0.04
Caffeic acid	0.05±0.00	0.05±0.01	0.06±0.00	0.09±0.01	0.06±0.00
p–coumaric acid	0.41±0.03	0.17±0.03	0.21±0.00	0.82±0.11	0.41±0.12
Ferulic acid	0.05±0.01	0.06±0.01	0.04±0.00	0.14±0.03	0.10±0.00
Benzoic acid	3.12±0.12	2.14±0.21	1.51±0.11	2.76±0.13	2.45±0.21
Kaempferol	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Total	6.64±0.28	3.89±0.30	3.26±0.12	9.58±0.71	5.26±0.45

The value are mean [mg/g] ± standard deviation

Table 2. Inhibition of germination by using various sorghum shoot extracts from 'ethanol', 'ethanol–chloroform', 'ethanol–hexane', 'ethanol–ethyl acetate', and 'ethanol–methylene chloride) on weed species

Solvents	Concentrations (g/mL)	Inhibition of germination on weed species			
		Abutilon avicennae	*Digitaria sanguinalis*	*Amaranthus retroflexu*	*Echinochloa crus–galli*
Ethanol	0.25	53.3±1.15	59.3±0.58	69.0±1.00	30.0±1.00
	0.5	93.3±1.15	81.5±1.15	100.0±0.00	46.7±1.53
	1	100.0±0.00	100.0±0.00	100.0±0.00	86.7±0.58
Ethanol – Chloroform	0.25	33.3±1.15	55.6±1.00	58.6±1.73	26.7±0.58
	0.5	83.3±0.58	66.7±1.00	100.0±0.00	46.7±2.08
	1	93.3±0.58	100.0±0.00	100.0±0.00	86.7±1.15
Ethanol–Hexane	0.25	30.0±2.65	51.9±1.53	58.6±1.00	30.0±1.73
	0.5	83.3±1.15	55.6±1.00	100.0±0.00	43.3±1.53
	1	96.7±0.58	100.0±0.00	100.0±0.00	70.0±1.00
Ethanol – Ethyl acetate	0.25	56.7±1.15	63.0±0.58	75.9±1.53	40.0±1.00
	0.5	93.3±0.58	88.9±0.00	100.0±0.00	50.0±1.00
	1	100.0±0.00	100.0±0.00	100.0±0.00	90.0±1.00
Ethanol – Methylene chloride	0.25	43.3±0.58	63.0±1.53	55.2±0.58	23.3±2.08
	0.5	83.3±0.58	70.4±1.53	100.0±0.00	46.7±0.58
	1	96.7±0.58	100.0±0.00	100.0±0.00	80.0±1.00

1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract
The values are mean % ± standard deviation

growth at concentrations as low as 0.25 g/mL, and inhibition increased at higher extract concentrations (0.5 g/mL and 1 g/mL) (1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract). Thus, the allelochemicals extracted from sorghum shoot may effectively be bio–herbicides. To go into more detail, at 1 g/mL, inhibition of weed species (*A. avicennae*; *D. sanguinalis*, *A. retroflexus*) from the ethanol–ethyl acetate fractions was almost 100% whereas it was 90% for *E. crus–galli*. In addition, percent germination inhibition at the lowest concentration of 0.25 g/mL inhibition of weed species was 56.7% for *A. avicennae*, 63.0% for *D. sanguinalis*, 75.9% for *A. retroflexus*, and 40.0% for *E. crus–galli*.

Among the phenolic compounds 4–hydroxybenzoic acid is known for being a very potent and effective allelochemical (Weston *et al*., 1989). In addition, Rimando *et al*., 2001 illustrated that p–coumaric acid is also a strong inhibitor of seed germination. This is consistent with our results from Table 2, in which the total amounts of 4–hydroxybenzoic acid and p–coumaric acid of the ethanol–ethyl acetate extraction were present in the highest concentrations. Based upon this observation, further isolations of the allelopathic compounds focused on the ethanol–ethyl acetate fraction.

Herbicidal activity of sorghum shoot extracts under greenhouse conditions

To further investigate allelopathic activities of sorghum extracts for potential weed management purposes, sorghum shoot extracts were tested on weeds as shown in Figure 1. From this result, we can easily select the extract that had the highest effect on weeds, which is the extract of sorghum by ethanol–ethyl acetate extraction. Specifically, the highest herbicide efficacy of weed control was 84.53% (*A. avicennae*) followed by 87.06% (*D. sanguinalis*), 87.79% (*A. retroflexus*), and 51.56% (*E. crus–galli*).

Various concentrations of sorghum shoot extract using 'ethanol–ethyl acetate' were used (1; 0.5; 0.25; 0.125; 0.0625 g/mL) (1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract). The total weed population was significantly reduced by all the treatments compared to control (Figure 2). The sorghum extracted by 'ethanol–ethyl acetate' showed the greatest effect on weeds at 1 g/mL concentration of the extract. Efficacy of the extract from ethanol–ethyl acetate fractions was 86.48% (*A. avicennae*), followed by 89.25% (*D. sanguinalis*), 90.62% (*A. retroflexus*), and 50.54% (*E. crus–galli*). At low concentration 0.0625 g/mL, the total weed population went down and the herbicide weed control efficacy also decreased by 32.23% (*A. avicennae*) followed by 33.51% (*D. sanguinalis*), 38.54% (*A. retroflexus*), and 20.43% (*E. crus–galli*). Results indicated that sorghum extract contain allelopathic potential and inhibited growth of weeds. The inhibitory degree of this species was proportional to the increase of applied concentration.

Field studies

For soil application, the extract of sorghum (by ethanol–ethyl acetate) with 1 g/mL concentration was sprayed on soil at three occasions at "7, 14, and 21 days" after tillage and was compared with control (1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract). The data shown in Table 3 are of the following treatments: '7DAT', '7–14DAT', and '7–14–21DAT', respectively. Sample '7–14–21DAT' showed the best preventive method in controlling the growth and development of weeds.

For foliar application, the extracts of sorghum (by ethanol–ethyl acetate) at various concentrations of 0.25; 0.5; 1 g/mL were sprayed on weeds at the 2–3 leaf stage. The data was collected 28 days after soil preparation. Herein, the treatment with the highest concentration (1 g/mL) was the most effective compared to other concentrations (Table 3).

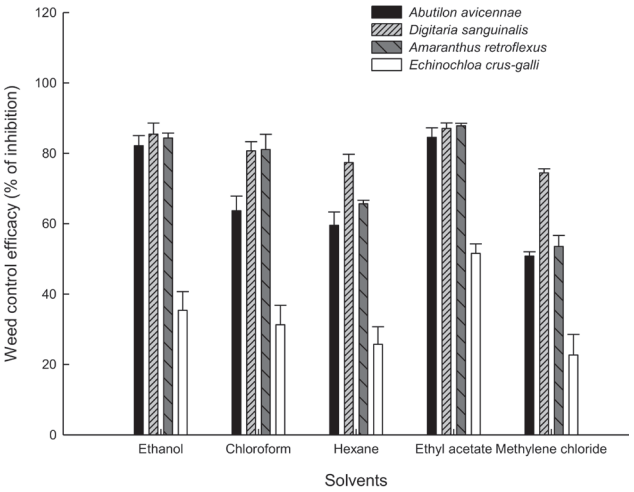

Fig. 1. Efficacy of sorghum shoot extracts extracted with various solvents at 1 g/mL concentration on the growth of four weed species following post–emergent studies under greenhouse conditions. The vertical bars represent the mean ± standard deviation. 1 g/mL: 1 g dry weight sorghum–equivalent per 1 mL extract.

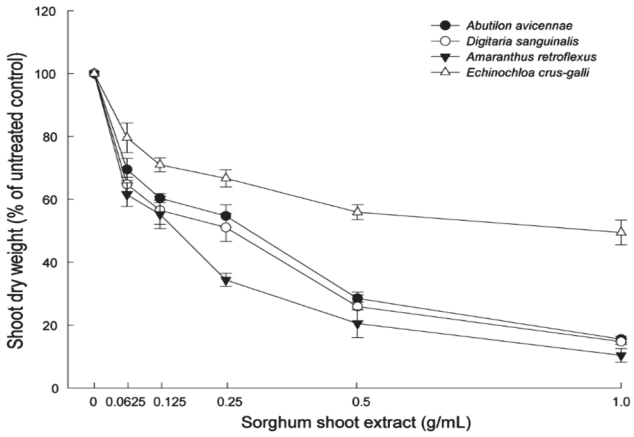

Fig. 2. Weeds response to increasing concentrations of sorghum shoot extract extracted with ethanol–ethyl acetate solvent following post–emergent studies under greenhouse conditions. The vertical bars represent the mean ± standard deviation. 1 g/mL: 1 g dry–weight sorghum–equivalent per 1 mL extract.

Table 3. Effects of sorghum extract with different times of treatment and concentration on emergence and growth of weeds in paddy soils under field conditions

Application	Treatments	Weed control efficacy (%)
Pre–emergence	7 DAT	37.3 ± 1.5
	7–14 DAT	86.0 ± 1.7
	7–14–21 DAT	100.0 ± 0.0
Post–emergence	0.25 g/mL	30.3 ± 2.5
	0.5 g/mL	70.33 ± 1.5
	1.0 g/mL	98.6 ± 2.3

DAT, Day after tillage
The data were collected 28 days after treatment by visual rating (7 DAT: spray once 7 days after tillage, 7–14 DAT: spray twice 7 and 14 days after tillage, 7–14–21 DAT: spray three times 7, 14 and 21 days after tillage).
The values are mean % ± standard deviation

DISCUSSION

This work was carried out to extract sorghum shoot (Hwangumchal cultivar) using various solvents (ethanol, ethanol–chloroform, ethanol–hexane, ethanol–ethyl acetate, and ethanol–methylene chloride). Among these solvents, shoot extract obtained by "ethanol–ethyl acetate' extraction performed the best in partitioning phenolic compounds (especially high contents of 4–hydroxybenzoic and p–coumaric acid).

Findings in this study confirmed that sorghum shoot had strong allelopathic activity, with significant inhibition on weed species (*Abutilon avicennae*, *Digitaria sanguinalis*, *Amaranthus retroflexus*, and *Echinochloa crus–galli*). For foliar application, both greenhouse experiments and field studies also pointed out high herbicidal activity of sorghum extract on weed species. In addition, using the sorghum shoot was extracted with ethyl acetate solvent at 1 g/mL concentration and three time of applications at 7, 14, and 21 days after tillage not only prevented the growth and development of weeds but also exhibited a strong inhibition on natural weed growth in paddy soil.

Hence, the study determined the basis for the allelopathic potential of sorghum in weed management. Furthermore, additional studies are required to evaluate the herbicidal efficacy of sorghum shoot extracts against various weed species before these methods can widely be applied and recommended to farmers for use. If proven to be an efficient method for weed management and adopted successfully by growers, it can lead to significant accomplishments in the development of sustainable agriculture.

AUTHOR CONTRIBUTIONS

Thi Hien LE and Wei Qiang JIA carried out substantial contribution to the concept and design on this paper. Ok Jae WON, and Jeung Ju Lee carried out analysis and interpretation of data. Yoshiyuki SHINOGI verified the data. Kee Woong PARK and Taek–Keun OH supervised the project and wrote the paper. All authors commented on the manuscript.

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