0971-4693/94 US $ 5.00
Allelopathy Journal 31 (1): 147-156 (2013) *International Allelopathy Foundation* 2013
Tables: 5, Figs : 2

Phenolic compounds in sorghum leaf extracts and their effects on weed control

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(Received in revised form: December 19, 2012)

ABSTRACT

The allelopathic potential of shoot extract of *Sorghum bicolor* (L.) Moench, was evaluated for sustainable weed management in organic farming. Its 2 to 15-days-old seedlings contained more total phenolic compounds than older plants. The ethyl acetate partitioned phenolic compounds from methanol extracts of sorghum leaf were better than other organic solvents test. Among the 6- physical methods of extraction used, the extract from grinding plus boiling provided good weed control, however, methanol extract of sorghum leaf proved the best. The sorghum leaf extracts may provide effective and environmentally sustainable weed control.

Keywords: *Amaranths retroflexus, Echinochloa crusgalli*, extraction methods, organic solvents, *Sorghum bicolor*, weed control.

INTRODUCTION

Allelopathy refers to any process involving secondary metabolites (allelochemicals) that influence the growth and development of agricultural and biological systems, including both positive and negative effects (23). In plants, allelochemicals are present in the leaves, bark, roots, root exudates, flowers and fruits (36). Allelochemicals (phenolics, terpenoids, alkaloids, coumarins, tannins, steroids, quinines) from plants are released into the environment through exudation from roots, leaching from leaves and other aerial plant parts, volatile emissions and the decomposition of plant material (14,36,40). Several factors (concentration, flux rate, age and metabolic state of plant, and prevailing climatic and environmental conditions) determine their toxicity (19,28,34,35). Their amount and production varies in quality and quantity with age, cultivar, plant organ and time of year (4,5). Environmental and human health concerns regarding current weed control methods have prompted worldwide research to reduce the heavy reliance on synthetic herbicides.

Sorghum (*Sorghum bicolor* L. Moench) is an allelopathic specie that suppresses the growth of weeds and even injures the succeding crops (3,15,29,30). Sorghum causes soil sicknesses and adversely affects the growth of other crops in rotation systems (18). Sorghum is sometimes used in integrated pest management systems as green manure or cover crop to suppress weeds (37) or as crop residue stubbles in no-tillage farming (1).

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The allelopathy has been suggested as one of the possible alternatives for sustainable weed management.

Many plant species produce and release biologically active compounds into the environment that suppress the growth of other plants. Sorghum vegetative parts are used for animal forage and grains as food for many people. It contains numberous allelochemicals, hence, has strong phytotoxic potential against weeds (15,37), itself (4) and other crops (2,27). This toxicity is due to the production and release of phenolic compounds [phenolic acids (2,4) and sorgoleone (16,39,17)]. Few studies have been done on sorghum phenolics metabolism and its variability in vegetative parts, but no attempt has been made to use sorghum shoot to control weeds. This study aimed to find the phenolic compounds in the shoot extract of sorghum and their effects on different weed species.

MATERIALS AND METHODS

Sorghum seeds (Variety SS-450) were collected from the National Institute of Animal Science, Sung Hwan, Korea. These were sown in an upland field at Experimental Farm Chungnam National University, Daejeon as per experimental treatments. To determine the optimum age of sorghum plants for maximum amount of phenolic compounds, at 15 days interval sorghum plants were harvested until 105 days after sowing. The first sample was taken on 15 May and continued upto 15 August. For other experiments 15 days old sorghum leaf samples were considered. Fresh leaves were allowed to dry in shade in vinyl house for one week. The dried leaves were chaffed with fodder cutter into 2-3 cm pieces and then ground in blender machine.

I. Sorghum leaf extraction

(i). Physical methods

Phenolic compounds were extracted from sorghum using 6-d physical methods (Table 1). After boiling, the leaves and extract were passed through a coarse mesh to remove plant residues and then filtered using filter paper. The filtered extract was evaporated under vacuum at 50°C.

Table 1. Physical methods used to extract phenolic componds from Sorghum leaves

Extraction Method	Treatment details
M1	Chaffed leaves (200 g) soaked in DW in 1:50 ratio (w/v) for 24 h at room temperature (25±2°C), then boiled for 2 h
M2	Ground leaves (200 g) soaked in DW in 1:50 ratio (w/v) for 24 h at room temperature, then boiled for 2 h
M3	Chaffed leaves (200 g) soaked in DW in 1:50 ratio (w/v) for 24 h at room temperature
M4	Ground leaves (200 g) soaked in DW in 1:50 ratio (w/v) for 24 h at room temperature
M5	Chaffed leaves (200 g) were placed in DW in 1:50 ratio (w/v) and immediately boiled for 2 h
M6	Ground leaves (200 g) placed in DW in 1:50 ratio (w/v) and immediately boiled for 2 h

DW: Distilled water,

(ii). Extraction with solvents

Ground sorghum leaves (200 g) were soaked separately in 6-solvents (methanol, chloroform, methylene chloride, ethanol, hexane, or ethyl acetate) in 1:50 (w/v) ratio for 24 h at room temperature. The leaves and organic solvent were passed through a coarse mesh to remove plant residues and then filtered using filter paper. The filtrate was evaporated under vacuum at 50°C.

II. Chemical analysis (phenolic compounds) of sorghum shoots at different growth stages

The sorghum leaves were collected from the crop field at 15,30,45,60,75,90 and 105 days after sowing. Collected leaves dried in shade in plastic-house and then ground using a mixture machine. Phenolic compounds were extracted from the ground material by soaking in methanol.

(i). Partitioning layer from methanol crude extract

Crude methanolic extract from 15 days old sorghum plants were used for partitioning with different organic solvents. The methanol extract of sorghum leaves was sequentially partitioned against *n*-hexane, ethyl acetate, or *n*-butanol (1:1 v/v). Filter extracts were evaporated under vacuum and phenolic compounds were measured using high-performance liquid chromatography (HPLC).

(ii). HPLC analysis of phenolic compounds

Dried extracts were dissolved in methanol (1 µg/mL) and filtered through a poly filter (pore size, 0.45 µm). Samples were then diluted 2-folds with methanol prior to analysis using HPLC. HPLC quantification of phenolic compounds was performed using a Futecs model NS-4000 HPLC apparatus (Daejeon, Korea). The analysis was monitored at 280 nm and performed using a C_{18} column (250 mm × 4.6 mm, 5 µm; RStech, Daejeon, Korea). The mobile phase was a gradient prepared from mixtures of acetonitrile and 0.15% acetic acid and the column was maintained at 30℃. The flow rate was set at 1.0 mL/min and the injection volume was 20 µL. The amounts were calculated using a standard curve. All samples were run in triplicate.

(iii). Effect of sorghum leaf crude extract on weeds

Seedling of *Amaranthus retroflexus* (broadleaf weed specie) and *Echinochloa crus-galli* (grass weed specie), were grown in small plastic pots (15 × 12 cm). Two-third of the pot was filled with soil and each pot was sown with 20 seeds of test weed species. Then, the remainder pot was filled with soil. Seedlings were grown in greenhouse at 25±5°C for 3-weeks. To determine the effect foliar application of sorghum leaf crude extract (extracted using different methods) on weed growth, a post-emergence application test was performed. Sorghum leaf extracts at 1 and 5 mg/ml water (w/v) were sprayed in 1000L/ha water using knap sack sprayer on foliage of test weed seedlings at 21 d after sowing. Three weeks after spraying, the shoots of each weed specie were collected to determine biomass.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect trial effects using the SAS Software release 9.2 and means were separated via Duncan Multiple Range Test.

RESULTS

Phenolic compounds Content

Three different phenolic compounds (*p*-hydroxybenzoic acid, *p*-coumaric acid, and *trans*-cinnamic acid) were identified in the extracts of sorghum leaves. Their amounts varied significantly with growth stage and decreased in shoot with increasing age of sorghum plants (Table 2). The *p*-hydroxybenzoic acid content in crude extracts of sorghum shoots ranged from 48.95 to 2.4 µg/mg with 15 DAS (Days after seeding) foliage to 2.4 µg/mg with 105 DAS foliage. The *p*-hydroxybenzoic acid content has changed very rapidly at 30 DAS, its content was 76.7% less at 30 DAS than 15 DAS, and afterwards that the variation was little. The *p*-hydroxybenzoic acid content in 15 day- old plants was 4.3 times that in 30-day-old plants and, 5.5, 8.8, 17.3, 18.8, and 20.4 times that in 45-, 60-, 75-, 90-, and 105-day-old plants, respectively. The other two phenolic compounds detected (*p*-coumaric acid, and *trans*-cinnamic acid) followed a similar trend to that of the *p*-hydroxybenzoic acid, as 15 day-old leaves contained the highest amount of these phenolic compounds. However, the variation of these two compounds (*p*-coumaric acid, and *trans*-cinnamic acid) was not higher than that of *p*-hydroxybenzoic acid.

Table 2. Effects of sorghum growth stages on phenolic compounds extracted in methanol

DAS	Phenolic compounds (µg/mg) in crude extract		
	p-Hydroxybenzoic acid	*p*-Coumaric acid	Ferulic acid
15	48.95 a[a]	3.45 a	0.81 a
30	11.40 b	3.26 a	0.32 b
45	8.84 c	3.03 b	0.34 b
60	5.54 d	2.62 c	0.28 bc
75	2.83 e	2.41 c	0.21 d
90	2.60 e	1.78 d	0.22 cd
105	2.40 e	1.55 e	0.20 d

Mean values indicated by the same letter in a column did not differ significantly at the 5% level using Duncan Multiple Range Test. DAS: Days after sowing.

Partitioning of crude methanol extract

Five phenolic compounds (*p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, *trans*-cinnamic acid, and kaempferol) were identified in the partitioned layers of crude sorghum extract (Table 3). The ethyl acetate-soluble layer of crude extract contained maximum amount of all isolated chemicals. The concentration of *p*-hydroxybenzoic acid ranged from 380.62 to 0.38 µg/mg crude extract. The ethyl acetate layers contained 58-times more *p*-hydroxybenzoic acid than butanol layer and 1001 times more than hexane layer. The concentration of *p*-coumaric acid ranged from 27.51 to 0.06 µg/mg crude extract. The ethyl acetate layer contained 28 -times more *p*-coumaric acid than butanol layer and

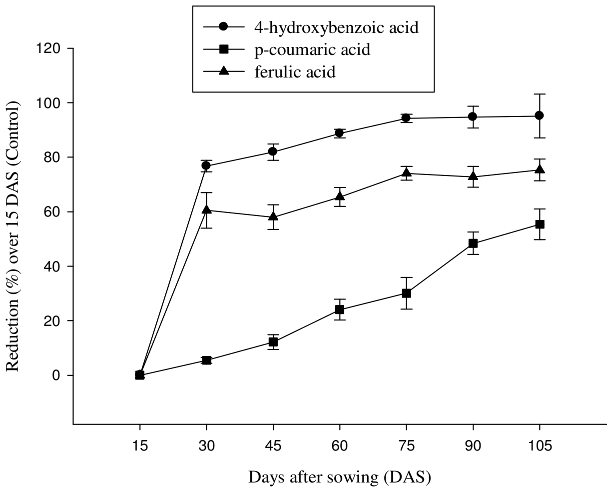

Figure 1. Effects of sorghum growth stages on phenolic compounds extracted in methanol

Table 3. Partitioning of phenolic ciompounds in crude extract of sorghum leaves in various organic solvents

Organic solvent	Phenolic compounds(µg/mg) crude extract				
	p-Hydroxybenzoic acid	*p*-Coumaric acid	Ferulic acid	trans-Cinnamic acid	Kaempferol
Ethyl acetate	380.62 a[a]	27.51 a	8.58 a	1.53 a	2.05 a
Butanol	6.58 b	0.99 b	0.62 b	0.02 b	0.09 c
Hexane	0.38 c	0.06 c	0 c	0.17 b	0.42 b

[a]Mean values indicated by the same letter in a column did not differ significantly at 5% level using Duncan Multiple Range Test.

459-times more *p*-coumaric acid than hexane portioned layer. The range of ferulic acid in ethyl acetate and butanol was 8.58 to 0.62 µg/mg crude extract. The concentration of *trans*-cinnamic acid ranged from 1.53 to 0.02 µg/mg crude extract. The ethyl acetate layer contained 9.0 times more trans-cinnamic acid than the hexane layer and 77 times the amount detected in butanol-partitioned layer. Kaempferol displayed a similar trend with concentrations ranging from 2.05 to 0.09 µg/mg crude extract. The ethyl acetate layer contained 5- times the amount of kaempferol detected in the butanol-partitioned layer.

Herbicidal activity of crude extracts of sorghum shoots

The foliar spray of all extracts proved phytotoxic against *A. retroflexus* and *E. crus-galli* (Table 4). The foliar application of shoot extract reduced the weeds growth and the broadleaf weed species showed greater susceptibility to extract than grass weed species. Application of extracts obtained by grinding plus boiling of sorghum leaves (M6) at 1 mg/mL concentration suppressed the growth of *A. retroflexus* and *E. crus-galli* by 79.2 and 53.6%, respectively. The M5 extract at 5 mg/mL caused 100% suppression of broadleaf weed species and 51.4 suppression in grass weed spp than control. Application

Table 4. Effects of various physical extraction methods and sorghum leaf extracts concentration on weeds control efficacy (%) in greenhouse

Extraction methods	*A. retroflexus*		*E. crus-galli*	
	1 mg/mL*	5 mg/mL*	1 mg/mL*	5 mg/mL*
M1[Cutting + soaking (24 h) + boiling (2 h)]	54.6 c[b]	70.7 d	23.4 d	35.5 d
M2 [Grinding + soaking (24 h) + boiling (2 h)]	70.1 b	83.9 bc	30.8 c	42.8 c
M3 [Cutting + soaking (24 h)]	70.6 b	86.2 b	15.3 e	55.0 b
M4 [Grinding + soaking (24 h)]	78.3 a	79.9 c	41.2 b	55.8 b
M5 [Grinding + soaking (24 h)]	66.8 b	100 a	29.7 c	51.4 b
M6 [Grinding + boiling (2 h)]	79.2 a	100 a	53.6 a	72.9 a

[b]Mean values indicated by the same letter in a column did not differ significantly at the 5% level using Duncan Multiple Range Test.

of sorghum extracts obtained by cutting plus soaking and grinding plus soaking resulted in similar degree of inhibition in both broadleaf and grass weed species. Extracts obtained using these two methods resulted in the second highest (after M6) inhibition of weed growth.

All tested chemical solvent extracts of sorghum leaves significantly inhibited the weeds growth ($P < 0.05$). Sorghum leaf extracted caused maximum reduction in weeds biomass (Table 5). Methanol extract inhibited 83.6% growth of *A. retroflexus* growth at 1 mg/mL and 35.7% inhibition in *E. crus-galli* growth. The broadleaf weed species was 100% suppressed with 5 mg/mL of methanol extract, while the grass weed species was suppressed by 60.1% (Figure 2).

Table 5. Effects of various organic solvents and sorghum leaf extracts concentration on weeds control efficacy (%) in greenhouse

Organic solvents	*A. retroflexus*		*E. crus-galli*	
	1 mg/mL*	5 mg/mL*	1 mg/mL*	5 mg/mL*
Ethanol	74.5 b[a]	80.8 b	38.8 a	53.0 b
Methanol	83.6 a	100 a	35.7 b	60.1 a
Methylene chloride	62.1 c	72.6 c	29.3 c	51.7 b
Ethyl acetate	66.7 c	83.5 b	15.8 d	42.1 c
Chloroform	63.8 c	100 a	13.2 e	42.0 c
Hexane	34.7 d	58.3 d	5.3 f	21.3 d

[a]Mean values indicated by the same letter in a column did not differ significantly at 5% level using Duncan Multiple Range Test.

DISCUSSION

Allelopathic interactions can be utilized directly or indirectly to manage weeds through the use of allelochemicals as alternatives to herbicides (12,13). Common allelochemicals from crop plants are generally secondary metabolites and includes phenolics, terpenoids, alkaloids, coumarins, tannins, flavonoids, steroids, and quinines (14).

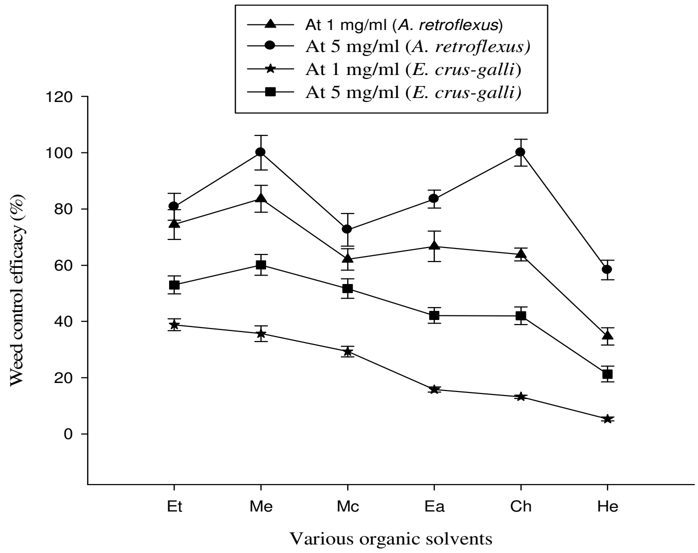

Figure 2. Effects of various organic solvents and sorghum leaf extracts concentration on weeds control efficacy (%) in greenhouse. Et, ethanol; Me, methanol; Mc, Methylene chloride; Ea, Ethyl acetate; Ch, Chloroform; He, Hexane

Methylene chloride is most common solvent (10,25,41) to extract sorgoleone from roots, although chloroform has also been used (11). Methanol extracts contained more sorgoleone than other test solvents, followed by methylene chloride and chloroform (33). In this study, methanol extracts of sorghum shoot caused the greatest inhibition of weeds growth, hence, methanol proved best extraction solvent to obtain the maximum yield of phenolic compounds from leaves. This result is similar to Uddin *et al*. (33).

Extracts of 15-day-old sorghum leaves yielded the highest concentration of phenolic compounds and the content of phenolic compounds decreased sharply with plant age. This agrees with the findings of other researchers, who reported that 7-days old sorghum-sudangrass hybrid (sudex) shoot tissue was most inhibitory to seedling growth. Besides, the dhurrin concentration (9) and tissue toxicity (22) are highest in sorghum seedling. In sudex shoot tissue, toxicity is correlated with the breakdown products of dhurrin, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid. Sudex shoot tissues also contains high concentrations of *p*-hydroxybenzoic acid and aldehydes that are correlated with the allelopathic potential of shoot tissues (38). In our study, the concentration of *p*-hydroxybenzoic acid was highest than other phenolic compounds identified. Foliar application of sorghum leaf extract significantly reduced the growth of test weed species. We visually observed the burning and growth inhibition immediately after extract application in sensitive species and significant reduction in weed growth after 21 days. Sorghum residues release sorgoleone, cyanogenic glycosides-dhurrin and many breakdown products of phenolics that caused weed suppression (26,38). Foliar sprays of 50 and 100% sorghum extracts significantly reduced the root and shoot biomass of *Parthenium* (20). Two foliar sprays of sorghum extract significantly decreased weed dry biomass (15-53%) and increased wheat yield by 14% (8). Likewise, two foliar sprays of sorghum extract significantly reduced the weeds density and biomass in cotton (7). The effects of sorghum allelochemicals are species specific and concentration dependent (6). The use of sorghum water extract (sorgaab) as a foliar weed inhibitor has been reported in mung bean (24), soybean (21), horsepurslane (31), and wheat (8).

The methanol extract of sorghum shoot contains plant growth inhibitors, hence, effectively inhnbited the growth of broadleaf weed species. However, further studies are required to evaluate the herbicidal efficacy of sorghum shoot extract against different weed species under field conditions before its recommendation to farmers.

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