



A chemical basis for differential allelopathic potential of root exudates of *Sorghum bicolor* L. (Moench) cultivars on companion weeds

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ABSTRACT

Study was conducted to test if the variation in weed population and biomass between the stands of Enkath and Rabeh sorghum cultivars, which was observed in the field, was due to differences in their allelopathic potential. Field experiment revealed that Enkath cultivar significantly suppressed weed density and dry weight biomass over Rabeh cultivar by 34 and 29% after 35 days after sowing (DAS), and 22 and 24% after 65 DAS. Stair case experiment indicated that root exudates of Enkath cultivar showed more suppression to weeds than Rabeh giving additional evidence for the superiority of Enkath indicated that in its allelopathic weed suppression. Chemical analysis revealed that sorgoleone and several phenolic acids were present in higher concentrations in root exudates of Enkath compared to Rabeh. The results recommend screening more sorghum cultivars in order to offer a potential source of allelopathic germplasm that could be manipulated to enhance weed suppression in an effective and environmentally sustainable approach.

Keywords: Allelopathy, sorghum cultivars, weeds, root exudates, phytotoxins.

Introduction

During the past four decades considerable research work has been focused on the possible use of allelopathic crops in weed control with the aim of reducing the rely on synthetic herbicides (Alsaadawi et al., 2007; Cheema et al., 2002; Dilday et al., 1998; Weston, 1996). Different strategies have been adopted including the use of root exudates of allelopathic plants (3, 26). Sorghum Allelopathy has been well documented, and studies performed in the field have revealed that sorghum phytotoxicity varies with genotype, plant part, plant age, environmental factors, and the target weed species (25,27). The residues of sorghum provide short-term weed suppression, which appears to be due to phenolic compounds released from decomposing sorghum shoot residues (4,14,26). Most of these phenolic compounds are relatively simple in structure and are quickly metabolized in the soil rhizosphere (26). Also, sorghum produced root exudates containing phytotoxic chemicals. The exudates were

reported to contain two major components, which were identified as 2-hydroxy-5-methoxy-3-[(80Z, 110Z)-80,110,140-pentadecatriene]-p-benzoquinone (sorgoleone) and its p-hydroquinone reduced form named xenognosin (6,8). Sorgoleone was found to inhibit photosynthesis by blocking the flow of electron in electron transport system and reported to be more effective as photosystem II inhibitor than the herbicide atrazine (11,13,24).

Field observations indicated that growth and population of companion weeds are variable between the stands of two local sorghum cultivars (Enkath and Rabeh). The suppressive ability increased with increased planting density (1). This suggests that allelopathy might be the mechanism responsible for the variation in weed growth and population, and the differences among stands could be attributed to the variable allelopathic potential of the two cultivars. The aim of this study was to determine the suppressive potential of root exudates of these cultivars and to isolate, identify and quantify allelochemicals in their root exudates.

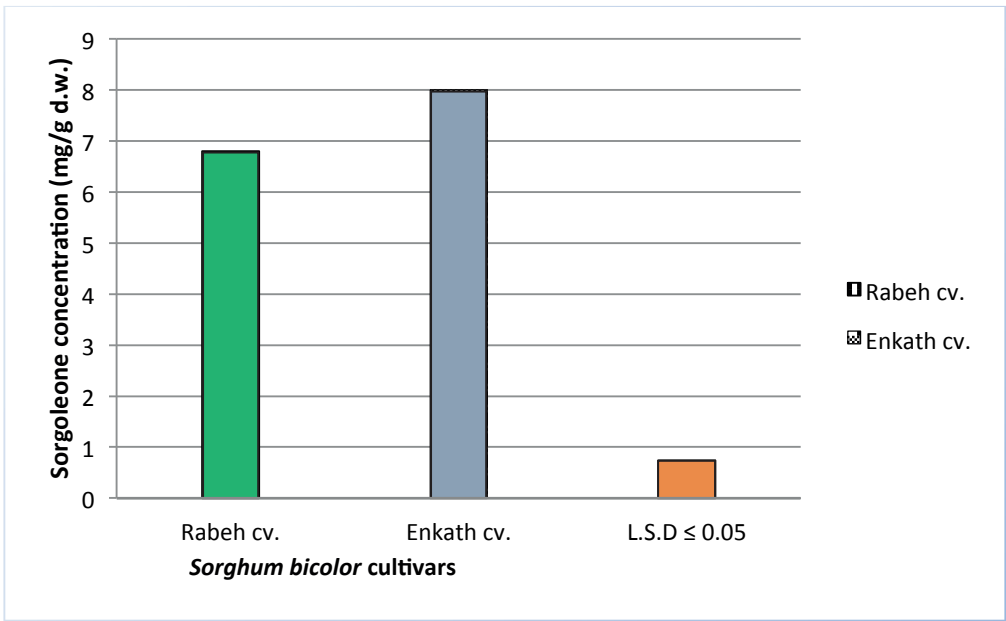


Figure 1. Sorgoleone collected from root exudates of two *Sorghum bicolor* cultivars.

Materials and methods

Field experiment

Field experiment was conducted in 2011 at Experimental Station of Field Crop Department in Abu-Ghraib, State Board of Agricultural Researches, Ministry of Agriculture, Baghdad, Iraq to determine the allelopathic potential of the test sorghum cultivars on companion weeds. The site was located at 33°19' 29.12" N, 44° 12' 56" E. The field is characterized by calcareous silt clay loam soil with pH 7.4 and electrical conductivity 2.7 dS m⁻¹; average annual rainfall was less than 50 mm and day/night temperatures during the growing season were 17-45/ 14-38 °C. Plots (7 × 9 m) were randomly made in the first of April 2011 in a field where previous field history showed heavy weed infestation. Seeds of sorghum cultivars were sown in their respective plots in 75 cm apart rows with a distance of 20 cm between seeds in order to achieve target density of 6.6 plants per m². Plots without crops were used as a control. The experiment was laid down in a randomized complete block design (RCBD) with three replicates. Fertilizers used were nitrogen as urea (46% N) at 240 kg ha⁻¹ and phosphorus as triple super phosphate (46% P₂O₅) at 160 kg ha⁻¹, which were applied as recommended for this crop; the whole phosphorous and half of the nitrogen were applied at planting while the remaining half of the nitrogen was applied at flowering stage.

For weed measurements, weeds density and dried biomass were taken after 35 and 65 days of sowing of sorghum crop. Weeds biomass was recorded after clipping the weeds at the soil surface and oven dried at 70°C for 72 h.

The statistical analysis was performed using analysis of variance test (ANOVA) by averages with GENISTAT computer software package. Differences among treatment averages were compared using LSD at 0.05 probability level (22).

Root exudates bioassay

This experiment was run under greenhouse conditions to test the differential allelopathic potential of the test sorghum cultivars against weeds by eliminating the competition for light, moisture and minerals between sorghum and weeds. The assay was made using stair case devise described by Alsaadawi and Rice (2) with little modification. Seeds of sorghum cultivars and the weed *Echinochloa colonum* L., one of the companion weeds found in sorghum fields, were planted separately in trays filled with acid washed sand to prepare seedlings for the assay devise. Two uniform seedlings of Rabeh and Enkath cultivars and *E. colonum* L. were transplanted in their respective plastic pots each containing 1.5 kg acid washed sand. Treatment series consisted of pots of sorghum cultivars alternated with pots of *E. colonum* L. in the stair case system. A control series consisted of pots

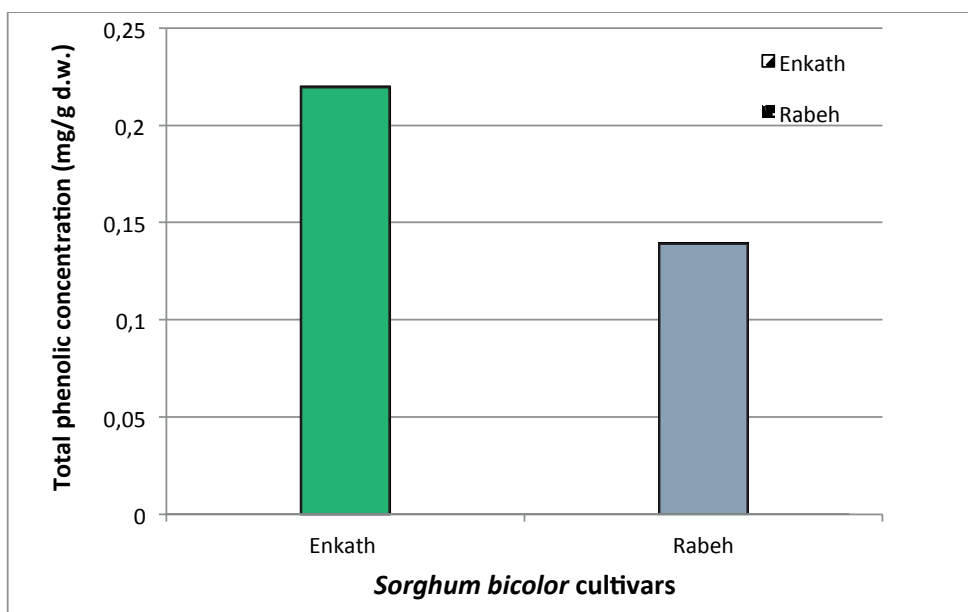


Figure 2. Total phenolics collected from root exudates of two *Sorghum bicolor* cultivars. Difference between the test cultivars was statistically significant at 0.05 level by ANOVA test.

of *E. colonum* L. alternated with pots containing acid washed sand only. Complete nutrient solution (15) was stored at reservoirs fixed at the top of each series and allowed to pass the pots of each series and finally accumulated at the bottom reservoirs of each series, where it recycled every day. The solution was changed biweekly. *Echinochloa colonum* plants were allowed to grow for 60 days, then harvested, separated to roots and shoot and compared on the basis of oven dried weight. All treatments were replicated three times and were arranged in a randomized design. The data were normal and there was homogeneity of variance. The average values were compared using Least Significant Differences (LSD) at 0.05 probability level.

Isolation and characterization of root exudates

Collection of sorgoleone

Seeds of Enkath and Rabeh sorghum cultivars were surface-sterilized by soaking for 20 min in 10% bleach (0.6% sodium hypochlorite) and rinsed with copious amount of deionizer water (9). Twenty seeds were placed in 20 × 100 mm sterile Petri dishes over the surface of sterile Whatman #1 filter paper (90 mm diameter). Three milliliters of sterile water were added to each dish, and the seeds were covered with a second sterile filter paper. The top lid was placed upside down to provide some mechanical

pressure over the top of the developing seedlings. The dishes were sealed by paraffin film and incubated in the dark at 30 °C. Each treatment was replicated three times. Sorgoleone was extracted from 7-day-old seedlings. All steps of procedure were executed under low-intensity light (9) to prevent the formation of anthocyanins by sorghum roots and photo degradation of the samples during extraction.

For sorgoleone extraction, roots were excised from sorghum seedlings and immersed in 12 ml of CHCl_3 and stirred the roots for three minutes. Roots and root debris were removed and the organic solvent was collected in a clean tube and concentrated under N_2 jet at 40 °C. The weight of the total crude extract was recorded prior to compositional analysis by HPLC. The roots were transferred to pre-weighted pieces of aluminum and oven-dried three days at 60°C. The roots with the aluminum were weighted, and then the dry weight of roots was calculated.

Identification of sorgoleone by HPLC

Sorgoleone in the root exudates for the two cultivars was identified and quantified by HPLC system (Shimadzo HPLC system, a Model LC-2010A HT autosampler) equipped with a column of Hyper Clone 5 μm CN 120A (4.6 × 250 mm I.D) phenomenex C_{18} reversed phase; the column temperature was set at 25°C. Mobile phase consisted of 0.1% phosphoric acid and acetonitrile (50:50 v/v); the flow rate was 0.5

Table 1. Effect of root exudates of sorghum cultivars on total dry biomass and population of companion weedsat 35 and 65 days after sowing (DAS).

Sorghum cultivars	Total weeds dry weight	Reduction (% of control)	Total weeds number (plant/ m ²)*	Reduction (% of control)
35 DAS				
Enkath	214.3	41.2	51.2	46
Rabeh	302.0	17.1	78.1	17
Control	364.3	0.0	94.6	
LSD ≤ 0.05	7.6		8.0	
65 DAS				
Enkath	74.11	51.0	56.3	42
Rabeh	98.15	35.1	72.0	26
Control	151.26	0.0	97.2	0
LSD ≤ 0.05	11		7.3	

mlmin⁻¹. The injection volume for each sample was 1 µl and each injection lasted for 20 min. The amount of sorgoleone in each extract was quantified at280 nm based on a calibration curve obtained with standard kindly provided by Dr Franck Dayan, US Department of Agriculture, Agricultural Research Service (USDA-ARS), Natural Products Utilization Research Unit. Each treatment was replicated three times.

Collection and quantification of total phenolics

Total phenolics of root exudates were collected and extracted from living sorghum seedlings of the test cultivars using a direct resin adsorption method described by Kong et al. (16). Five uniform seedlings at the 3rd leaf stage were transplanted into previously autoclaved pot (10 × 7 cm) containing 75 g XAD-8 resin and 300mL of 1mM Mes-Tris buffer (pH 5.5) +0.5mM CaSO₄ solution, and the

pots were placed in a sterile environment growth chamber. The seedlings were grown at 25±1°C with a 12h photoperiod (light intensity), and the pots were watered once a day to maintain the volume of the solution the same during the course of the experiment. After 14 days, the seedlings were carefully separated from the resin, and then the resin and the solution were transferred to a column (5×20 cm). The water was first allowed to drain from the column and the resin was washed with distilled water and then eluted three times with 50 ml methanol. The methanol fractions were pooled and evaporated to dryness under vacuum by rotary evaporator at 40°C and the residues were taken up by2 ml methanol and kept in deep freeze until use.

For determination of total phenolics, the residues collected from elution of XAD-8 resin were used to quantify phenolics by the Folin-Denis method as described by Ben-Hammouda et al.(4).

Table 2. Effect of root exudates of sorghum cultivars on growth of *Echinochloa colonum* weed.

Treatments	Dry weight (g)		
	Roots	Shoot	Whole plant
Control	0.191	0.126	0.317
Enkath	0.093	0.038	0.131
Rabeh	0.164	0.064	0.228
LSD ≤ 0.05	0.03	0.024	0.102

Table 3. Isolation and quantification of phenolic acids collected from root exudates of two *Sorghum bicolor* cultivars.

Compounds	Concentration µg/ml	
	Cultivars	
	Enkath	Rabeh
protocatecheuic acid	54.66	33.16
p-hydroxybenzoicacid	79.20	15.55
vanillic acid	129.66	44.81
syringic acid	254.64	209.81
p-coumaric acid	191.17	108.74
ferulic acid	400.35	84.11
sinapic acid	350.40	172.72
Total	1460.08	668.9

Identification of phenolic acids by HPLC analysis

Separation and quantification analysis of allelochemicals in root exudates was also performed using Fast Liquid Chromatographic Apparatus, Shimadzu 2010 LC equipped with binary delivery pump model 2010 Shimadzu with a C₁₈ reversed-phase column (50 × 2.0 mm I.D), 3 µm particle size. Mobile phase was methanol-deionized water (30:70, v/v), eluted at a flow rate of 1.0 mlmin⁻¹. The injection volume of samples was 20µl. All samples were filtered through a 0.25-µm nylon syringe before analysis, and the eluted peaks were monitored by UV-Vis 2010 SPD spectrophotometer detected at 352 nm.

Results and discussion

Weed flora appeared in the sorghum field during the study consisted mainly of *Sorghum halepense* L., *Echinochloa colonum* L. *Convolvulus arvensis* L. *Portulaca oleracea* L., *Xanthium strumarium* L., *Amaranthus retroflexus* L., *Malva rotundifolia* L., and *Chenopodium album* L. Both sorghum cultivars significantly suppressed total number and dry biomass of these weeds at 35 and 65 DAS (Table 1). However, Enkath recorded the highest inhibition of total weed number and dry biomass with a reduction of 46 and 41% of control, respectively at 35 DAS and 42 and 51% at 65 DAS. The differential inhibition of weeds between the two cultivars could be attributed mainly to the difference in exudates production and not to competition on light, moisture or nutrients since field measurements exhibited no differences in plant height and above ground biomass between the test cultivars (data not shown). Differential allelopathic potential among cultivars of several allelopathic crops

including grain sorghum have been documented previously (4,5,20). The staircase experiment supports the results of field experiment as the root exudates of both cultivars significantly inhibited root and shoot growth of the test weed (Table 2), with the superiority of Enkath over Rabeh in the suppression of roots, shoots and whole plant of the test weed by 43, 40 and 43%, respectively.

Chemical analysis indicated the presence of sorgoleone compound in root exudates of both cultivars with significant increase in Enkath cultivar (8 mg/g d.w.) compared to cultivar Rabeh (6.8 mg/g D.W) (Fig. 1). Other studies have indicated differences in root exudates production among different sorghum accessions grown under the same conditions (19,24). This compound has been reported to be phytotoxic to broadleaf and grass weeds at concentrations as low as 10 µM in hydroponic assays (11,19), and broadleaf weed species are more susceptible than grass weed species (23). Sorgoleone exerts both pre-emergence and post-emergence effects on different weeds (24,8).

The results of this study showed that sorgoleone was not the only phytotoxin exists in root exudates but phenolics are also present in sizeable amounts (Fig.2). These compounds appeared to be higher in Enkath (0.22 mg/g) than in Rabeh (0.14 mg/g) giving additional evidence for the higher allelopathic potential of Enkath. Chemical analysis on these phenolics by HPLC indicated the presence of several allelochemicals as protocatecheuic, hydroxybenzoic, vanillic, syringic, p-coumaric, ferulic and sinapic acids and four unidentified compounds (Table 3). Almost all these compounds were drastically higher in Enkath than in Rabeh cultivar. The inhibitory activity of the identified allelochemicals against

weeds has been reported and most of them are water soluble and when imbibed by the germinating weed seeds, hampered their germination and subsequent seedling growth, thus contributing to overall decline in the density, vigor and stand establishment of the weed community (12,16).

In conclusion, the variation in weed suppressive ability between the test sorghum cultivars was attributed to their differences in the allelochemicals content of root exudates. The phytotoxicity of root exudates was not restricted to sorgoleone alone but with phenolic acids. These results encourage conducting more work in order to offer a potential source of germplasm that could be manipulated to enhance weed suppression in an effective and environmentally sustainable approach.

References

- Al- Bedairy, N. R. L.; Alsaadawi I. S. and Shati R. K. 2012. Combining effect of allelopathic *Sorghum bicolor* L. (Moench) cultivars with planting densities on companion weeds. Archives of Agronomy and Soil Science. <http://dx.doi.org/10.1080/03650340.2012.697995>
- Alsaadawi, I. S., and Rice. E. L. 1982. Allelopathic effects of *Polygonum aviculare* L. I. Vegetational patterning. J. Chem. Ecol. 8: 933-1009.
- Alsaadawi, I. S. and Dayan, F. E. 2009. Potential and prospect of sorghum allelopathy in agroecosystem. Allelopathy J. 24:255-270.
- Alsaadawi, I. S., Al-Ekeelie, M. H. S., and Al-Hamzawi, M. K. 2007. Differential allelopathic potential of grain sorghum genotypes to weeds. Allelopathy J. 19:153-160.
- Ben-Hmmouda, M., Robert, J. K., and Harry, C. M. 1995. Phytotoxicity of extracts from sorghum plant components on wheat seedlings. Crop. Sci. 35:1652-1656.
- Chang, M., Netzly, D. H., Butler, L. G., and Lynn, D. G. 1996. Chemical regulation of distance: characterization of the first natural host germination stimulant for *Striga asiatica*. J. Am. Chem. Soc. 106:7858-7860.
- Cheema, Z. A., Khaliq, A., and Ali, K. 2002. Efficacy of sorgaab for weed control in wheat grown at different fertility levels. Pak. J. Weed. Sci. Res. 8:33-38.
- Czarnota, M. A., Paul, R. N., Dayan, F. E., Nimbal, C. I., and Weston, L. A. 2001. Mode of action, localization of production, chemical nature and activity of sorgoleone a potent PSII inhibitor in *Sorghum* spp. root exudates. Weed Technol 15:813-825.
- Dayan, F. E. 2006. Factors modulating the levels of the allelochemicals sorgoleone in *Sorghum bicolor*. Planta 224:339-346
- Dilday R. H, Yan G. W, Moldenhauer K. A. K, and Gravois K. A. 1998. Allelopathic activity in rice for controlling major aquatic weed. In Olofsdotter M Allelopathy in rice. Proc. Workshop on Allelopathy in Rice; Manila, Philippines. pp. 25-27. November 1998. IRRI, Manila, Philippines .
- Einhellig, F. A. and Souza, I. F. 1992. Phytotoxicity of sorgoleone found in grain sorghum root exudates. J. Chem. Ecol. 18:1-11.
- Gallandt, E.R., Libeman, M. and Huggins, D.R. 1999. Improving soil quality: implications for weed management. J. Crop Prod. 2: 95-121.
- Gonzalez, V. M., Kazimir, J., Nimbal, C. I., Weston, L. A., and Cheniae, G. M. 1997. Inhibition of a photosystem II electron transfer reaction by the natural product sorgoleone. J. Agric. Food Chem. 45:1415-1421.
- Guenzi, W. D., McCalla, T. M., and Norstadt, F. A. 1967. Persistence of phytotoxic substances in wheat, oat, corn, and sorghum. Agron. J. 59:163-165.
- Hoagland D. R, and Arnon D. I. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular No. 347. Berkeley (CA): University of California.
- Khanh, T. D., Chung, M. I., Xuan, T.D. and Twta, S. 2005. The exploitation of crop allelopathy in sustainable agriculture. J. Agron. Crop Sci. 191: 172-184.
- Kong, C. H., Hu F., Wang, P., and Wu, J.L. 2008. Effect of allelopathic rice varieties combined with cultural management options on paddy field weeds. Pest Manag. Sci. 64: 276-82.
- Netzly, D. H. and Butler, L. G. 1986. Roots of sorghum exude hydrophobic droplets containing biologically active components. Phytochemistry 59:775-778.
- Nimbal, C. I., Weston, L. A., Pedersen, J., and Yerkes, C. 1996. Phytotoxicity and distribution of sorgoleone in grain sorghum germplasm. J. Agric. Food Chem. 44:1343-1347
- Reberg-Horton, S. C, Burton, J. D, Danehower, D. A, Ma, G., Monks, D. W, Murphy, J. P, Ranells N. N, Williamson J. D, and Creamer, N. G. 2005. Changes over time in the allelochemicals content of ten cultivars of rye (*Secale cereale*). J Chem Ecol. 31:179-192.
- Rimando, A. M., Dayan, F. E., Czarnota, M. A., Weston, L. A., and Duke, S. O. 1998. A new photosystem II electron transfer inhibitor from *Sorghum bicolor*. J. Nat. Prod. 61:927-930.
- Steel, R.G.D., Torrie, J.H. and Dickey, D. 1997. Principles and Procedures of Statistics: A Biometrical Approach (3rd ed.). McGraw Hill Book Co. Inc., New York, USA. pp. 172-177.
- Uddin, M.R., Kim, Y.K., Park, S.U. and Pyon, J.Y. 2009. Herbicidal activity of sorgoleone from grain sorghum root exudates and its contents among sorghum cultivars. Kor. J. Weed Sci. 29:229-236.
- Weston, L. A. and Czarnota, M. A. 2001. Activity and persistence of sorgoleone, a long-chain hydroquinone produced by *Sorghum bicolor*, pp. 363-377, in R. Kohli et al. (eds.), Allelopathy and Agroecosystems. The Hayworth Press, London.

- Weston, L. A. 1996. Utilization of allelopathy for weed management in agroecosystems. *Agron. J.* 88:860-866.
- Weston, L. A., Alsaadawi, I. S. and Bearson, S. R. 2013. Sorghum allelopathy: from ecosystem to molecule. *J. Chem. Ecol.* DOI 10.1007/s10886-013-0245-8
- Weston, L. A., Harmon, R., and Mueller, S. 1989. Allelopathic potential of sorghum-sudangrass hybrid (Sudex) (*Sorghum bicolor* × *Sorghum sudanese*). *J. Chem. Ecol.* 15:1855-1865.

RECEIVED June 15, 2014

ACCEPTED June 26, 2014
