

Effect of Sorghum [*Sorghum Bicolor* (L.) Moench] Aqueous Extract on Germination and Seedling Growth of Wheat, Wild Oat, Wild Barley and Canary Grass

Krmanj Y. Naby*, Kawa A. Ali

Department of Field Crops, College of Agriculture, Salahaddin University-Erbil, Kirkuk Road, Erbil City.

Correspondence: Krmanj Y. Naby, Department of Field Crops, College of Agriculture, Salahaddin University-Erbil, Kirkuk Road, Erbil City.

Email: krmanj.naby@su.edu.krd

ABSTRACT

Weed control is a necessary tool in crop production because of remarkable economic losses in crop yield. A bioassay experiment was conducted in order to evaluate phytotoxic activity of Sorghum bicolor shoot water extract on germination and some parameters of *Triticum aestivum* L., *Triticum durum* L., *Hordeum spontaneum*, *Avena fatua* and *Phalaris minor* seeds. The concentration levels were (control, 15, 30, 45, 60%) in this study, shoot aqueous extract of Sorghum bicolor were applied to 20 seeds of each species in sterilized petri dishes during the 10 days incubated period at 22 °C and the experimental units were laid out in randomized complete design. The quantification and determination of allelopathic substances were done by using high performance liquid chromatography (HPLC). Results indicated that sorghum shoot aqueous extract significantly affected germination percentage, inhibition of germination percentage, total dry weight, seedling growth inhibition, plumule and radicle dry weight, seedling length, seedling vigor index, plumule and radicle length, plumule and radicle elongation velocity. Moreover the five tested plant species were responded significantly in different ratio to sorghum aqueous extracts. In addition, all concentration levels had on germination and all growth parameters especially higher concentration levels. The germination percentage of wild barley was eliminated due to apply 45 and 60% concentrations. The HPLC results showed that the shoot of sorghum plant contained syringic acid, vanillic acid and ferulic acid. The outcomes proposed that the sorghum shoot concentrates can be recommended as a bioherbicide to inhibit weed germination.

Keywords: Allelopathy, Aqueous extract, Bioherbicide, Phenolic compounds, Sorghum

Introduction

Allelopathy is a term that expresses a natural occurrence of a plant liberated chemical inhibitors that inhibits the plant growth

in vicinity. ^[1] Years later allelopathy was defined as inhibitory or stimulatory and effects of a plant on another including microorganisms. ^[2] Allelochemicals are secondary plant compounds which, releases into the environment leaching, root exudation, volatilization and plant residues in soil. ^[3, 4] Allelochemicals might affect plant metabolism pathways such as mineral absorption, photosynthesis, cellular respiration and water uptake.

Indicating a new biochemical compound produced by plants that potentially act as herbicides for weed control in crop fields is an ecofriendly process. ^[5] Previous study indicated the suppressive impact of sorghum plants extracts on some weed residues on different crop and weed plants *Chenopodium album*, *Convolvulus arvensis* *Melilotus parviflora* and *Rumex dentatus*. ^[6]

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Convolvulus arvensis, *Rumex dentatus* and *Triticum aestivum* Germination and growth were inhibited by different water extracts of sunflower (*Helianthus annuus*) sorghum (*Sorghum bicolor*), and johnson grass (*Sorghum halepense*).^[7]

Sorghum (*Sorghum bicolor* L. Moench) from poaceae family imposed allelopathic effect on different studied plants due to release allelochemicals such as phenolic compounds.^[8, 9] Almaghrabi, (2012) revealed that germination percentage of wild oat seed significantly decreased by using four synthetic phenolic compound salicylic acid, hydroxybenzoic acid ferulic acid and hydroxyphenyl acetic acid was the most effective.^[10] Dry matter and growth of soybean was inhibited significantly when it treated with some phenolic acids like chlorogenic acid, caffeic acid, p- coumaric acid, gallic acid, ferulic acid, vanillic acid and p-hydroxybenzaldehyde.^[11]

This study evaluated the allelopathic potential of *Sorghum bicolor* shoot extract on germination and growth characteristics of two crops bread and durum wheat (*Triticum aestivum* L., *Triticum durum* L.) and three grass weed plants (*Hordeum spontaneum*, *Avena fatua* and *Phalaris minor*) as a first step toward ecofriendly bio-herbicides.

Material and Methods

Seed Sources

Three Crop seeds of sorghum (*Sorghum bicolor* L. Moench) Var. Enqaz, bread wheat (*Triticum aestivum* L.) Var. Aras, durum wheat (*Triticum durum* L.) Var. Hawler 1 and three grassy weed seeds of wild oat (*Avena fatua*), wild barley (*Hordeum spontaneum*) and canary grass (*Phalaris minor*) were received from Erbil Research Center.

Extract Preparation

Sorghum seeds were sown at Grdarasha fields/ College of Agriculture/ Salahaddin University Lat. 36.4° N, Long. 44.1° E in the 2nd of July 2016. Sorghum plant herbage harvested when plants were reached to maturity stage. Plant was cut to 2-3 cm pieces then stored under shade till dryness, next it was grind by electrical mill instrument (JFSO-100). After that, shoot extract was prepared by soaking the powder into distilled water with the ratio of (20:100 w/v) after that it was placed in a shaker (LAB SK-180-Pro) with 120 rpm for 24 hours at 25°C. Later, it was filtered through four layers of cheesecloth then by Whatman filter paper No.1. Then adding distilled in order to complete volume to 100 ml. Finally, the extract kept in dark glass bottles and stored in deepfreeze at (-20°C) till implementation of experiment.^[12]

Bioassay

The experiment was conducted at Agriculture college laboratories in the October 2017. A series of sorghum aqueous extract concentrations (control, 15, 30, 45, 60%) were prepared by using distilled water.^[13, 14] Twenty seeds of each species placed between pair of filter paper #1 in 9 cm petri dishes then each of them were treated with 8ml of sorghum

shoot extract; then they were sealed by Para-film tape and placed in growth chamber machine under (20°- 25°C) and continued in darkness. Germination percentages of each petri dish were determined on (3rd, 5th, 7th and 10th day). Finally, data of Plumule, radicle and seedling lengths were calculated in centimeter (cm), as well as radicle, plumule and total dry weight were recorded in milligram (mg) after placing seedlings in electronic oven at (40°C) for 72 hours or until fixed weight.

Recorded Data

They were consisted of germination percentage (G%), inhibition of germination percentage (I.O.G.%), seedling growth inhibition percentage (S.G.I.%);^[15, 16] seedling length (S.L.), radicle length (R.L.), radicle elongation velocity (R.E.V.), plumule length (P.L.), plumule elongation velocity (P.E.V.)^[17]; radicle dry weight (R.D.W.), plumule dry weight (P.D.W.), total dry weight (T.D.W), seedling vigor index (S.V.I.)^[18, 19] according to equations shown below.

$$G. \% = T.G.S. / T.T.S. * 100 \quad (1)$$

$$I.O.G. \% = \{(G.P.C. - G.P.T.) / G.P.C.\} * 100 \quad (2)$$

$$S.V.I. = \{S.L. (cm) * G \% \} / 100 \quad (3)$$

$$P.E.V. (cm/ day) \text{ or } R.E.V. (cm/ day) = \{P.L. (cm) \text{ or } R.L. (cm)\} / T.D.W. * 100 \quad (4)$$

$$S.G.I. \% = [G. \% * S. L. (cm)] / 100 \quad (5)$$

Where T.G.S = total number germinated seeds, T.T.S. = Total number tested seeds, G.P.C. = germination percentage of control, G.P.T. = germination percentage of treatment.

Chromatography Analysis

Sorghum shoot aqueous extract were prepared as mentioned by Singh et al., (1989) and Alsaadawi et al. (2005).^[20, 21] Ten grams of sorghum shoot residue were soaked in (100 ml) of distilled water for 72 hours at (25 °C) then it was filtered to separate lipids and glycosides with petroleum ether and diethyl ether respectively. Identification of some phenolic acids was used (HPLC) UV Detector L-7400 and Pump L-7100, monitoring wave length 225 nm and column (c¹⁸) 0.5 mish. Mobile phase of acetonitrile was 30% and deionized water was 70% and 10 ml of solution for injection were used to isocratic elution.^[22] Standards were used as standard library for poly phenolic compounds were caffeic acid (3,4-Dihydroxycinnamic acid), chlorogenic acid (3-Caffeoylquinic acid), ellagic acid (Benzoic acid), ferulic acid (4-Hydroxy-3-methoxycinnamic acid), gallic acid 0.02% (3,4,5-Trihydrobenzoic acid), P-hydroxi benzoic acid (4-hydroxybenzoic acid), protocatechuic acid (3,4-Dihydroxybenzoic acid), syringic acid (4-Hydroxy-3,5-Dimethoxybenzoic acid), vanillic acid (4-Hydroxy-3-methoxybenzoic acid).

Statistical Analysis

The design of this experiment was factorial in completely randomized (Factorial C.R.D) with three replications. The factors were five plant species and five concentration levels of shoot part of sorghum aqueous extract. The data were analyzed by using SPSS computer analysis that means were compared at (1%) significant level by Tukey test and standard error of mean were found. [23, 24]

Result and Discussion

Influence of sorghum shoot aqueous extract on plant species

The significant impact of *Sorghum bicolor* aqueous extract on germination percentage, inhibition of germination, total dry weight, seedling growth inhibition, plumule dry weight and radicle dry weight is elucidated in (Fig. 1). The highest value of germination percentage and plumule dry weight (56.33%, 34.97mg) respectively were recorded for durum wheat, while inhibition of germination and seedling growth inhibition maximum record (74.58%, 51.17%) respectively were observed with wild barley but the highest record of radicle and total dry weight were (32.13mg, 66.22mg) respectively at bread wheat. On the other hand lowest measurements were

with germination percentage (25.33%) in wild barley, inhibition of germination (40.20%) with durum wheat, seedling growth inhibition (22.28%) at wild oat and the radicle, plumule and total dry weight (17.68 mg, 27.40 mg, 45.08 mg) respectively in wild oat. Identical results were indicated by Shahid et al., (2006), Mubeen et al., (2012) and Anwar et al., (2019). [7, 25, 26] Metabolic processes during early growth stages of plants may inhibit by allelopathic weed plant extracts because of the phenolic compounds presence. [27]

Results indicated that there were significant difference $p < 0.01$ between all studied plant parameters (Fig. 1). The highest record for seedling length, seedling vigor index, plumule length and plumule elongation velocity of durum wheat were (17.35 cm, 11.30, 11.13 cm, 1.11 cm/ day) respectively and the maximum data (6.30 cm, 0.63cm/ day) of length and velocity elongation of radicle noted with bread wheat, however the minimum values of seedling length, seedling vigor index, radicle length and radicle elongation velocity were (12.14 cm, 5.66, 3.07 cm, 0.31 cm/ day) with canary grass but the lowest data for plumule length and plumule elongation velocity were (8.29 cm, 0.83 cm/day) respectively for wild barley (Fig. 1). Previous studies showed the similar when different plant species exposed to radish water extracts, [28] also various sensitivity of plant species toward allelopathic plants were found due to genetic variation properties of plant species. [29]

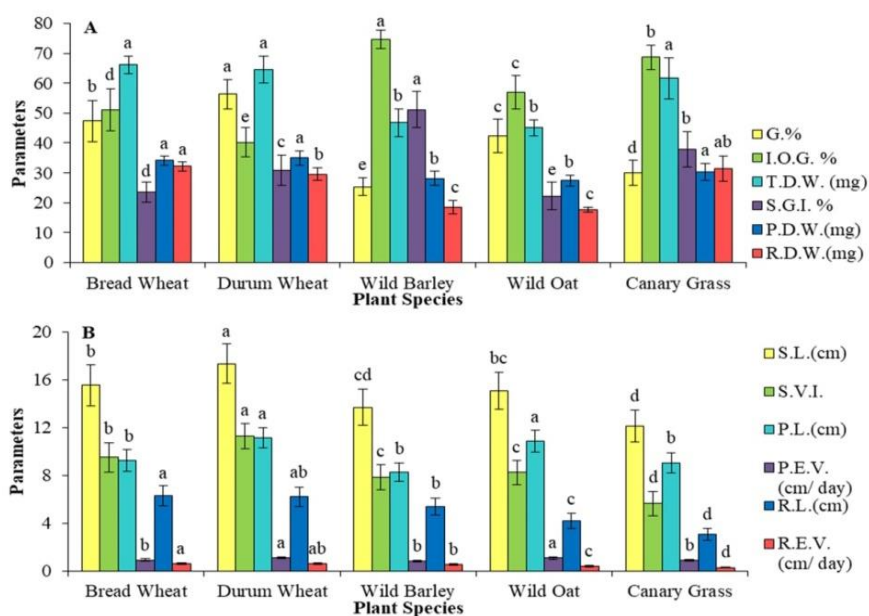


Figure 1: (A, B) The effect of sorghum shoot aqueous extract on germination and some seedling growth parameters of some plant species. Common letter means that there was non-significance difference at probability level (1%) by Tukey test.

Influence of different concentration levels of sorghum aqueous extract

Increasing sorghum water extract concentration levels caused significant effect on all studied criteria (Fig. 2). The highest record for germination percentage, radicle, plumule and total dry weight were (94.33%, 40.37 mg, 46.33 mg, 86.70mg)

respectively with control treatment but the minor values for mentioned parameters were (10.33%, 14.82 mg, 17.38 mg, 32.21 mg) with 60% concentration level that means by increasing concentration levels lead to decreasing the mentioned parameters but in contrast with increasing concentration levels induced to increasing inhibition of germination and seedling growth inhibition from (5.67%,

0.00%) to (84.94%,60.98%) respectively (Fig. 2). These results are in agreement with the other results where they were revealed that growth and germination of *Lactuca sativa* seedling were significantly inhibited by *C. papaya* extract.^[30] Another study has reported that small doses of secondary compounds may stimulate growth but the large doses have to be toxic impacts.^[31] As well as seed germination of *Vigna radiata* were inhibited by different concentration of *Lantana camara* and *Chromolaena odorata* leaf extracts.^[32] All tested plant criteria significantly affected by different concentration levels of sorghum aqueous extract indicated in (Fig. 2). It was obvious a reverse relationship between all tested plant parameters and

concentration levels which decreased the data of seedling length, seedling vigor index plumule length, plumule elongation velocity, radicle length and radicle elongation velocity from (26.48 cm, 25.05, 15.49 cm, 1.55 cm/ day, 10.99 cm, 1.10 cm/ day) to (5.40 cm, 0.72, 4.42 cm, 0.44 cm/ day, 0.98 cm, 0.10 cm/ day) respectively. Javaid et al. (2006) named this phenomenon as concentration dependent effect which may lead to a new bioherbicide.^[33] As well as, Mustafa et al., (2019) showed the same reduction in percentage of germination, root and shoot growth of some plants due to rising concentration levels of some allelopathic weed plant aqueous extract.^[34]

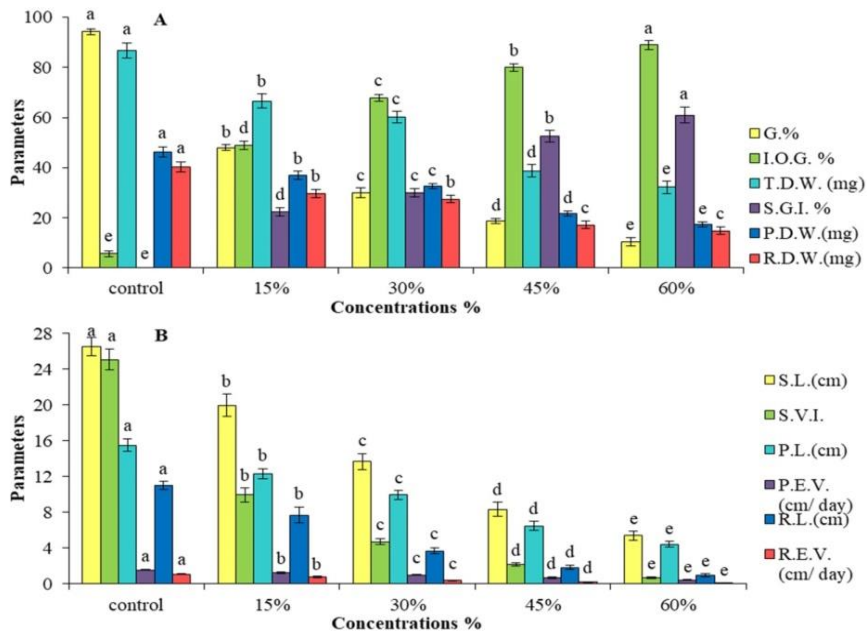


Figure 2: (A and B) The effect of concentration levels of sorghum shoot aqueous extract on germination and some seedling growth parameters of some plant species. Common letter means that there was non-significance difference at probability level (1%) by Tukey test.

Isolation and Identification of polyphenols from sorghum shoot part

Results indicated that the sorghum shoot extract has three peaks syringic acid, vanillic acid and ferulic acid form studied standard curves (Fig. 3). The quantities of each phenolic acid compounds of sorghum shoot part were (0.06%, 0.06% and 0.064%) for Syringic acid, Vanillic acid and Ferulic acid respectively (Fig. 4). Syringic acid is a natural phenolic acid found in Barley (*Hordeum vulgare*) and rice. It was reported that syringic acid is shown to

have auto toxicity and act as allelochemical compound and affect growth of wheat.^[35, 36] Vanillic acid is a natural hydroxycinnamic acid. It was noticed that vanillic acid inhibited the growth of rice, bluegrass and wheat, also inhibited the germination and seedling growth of lettuce.^[37, 38] Ferulic acid is also hydroxycinnamic acid which is distributed in the plant kingdom. It was showed that ferulic acid acts as chemical inhibitor on soybean germination and growth because it has inhibitory impact on synthesis of protein. The exact behavior was noticed on lettuce and cucumber plant.^[39, 40]

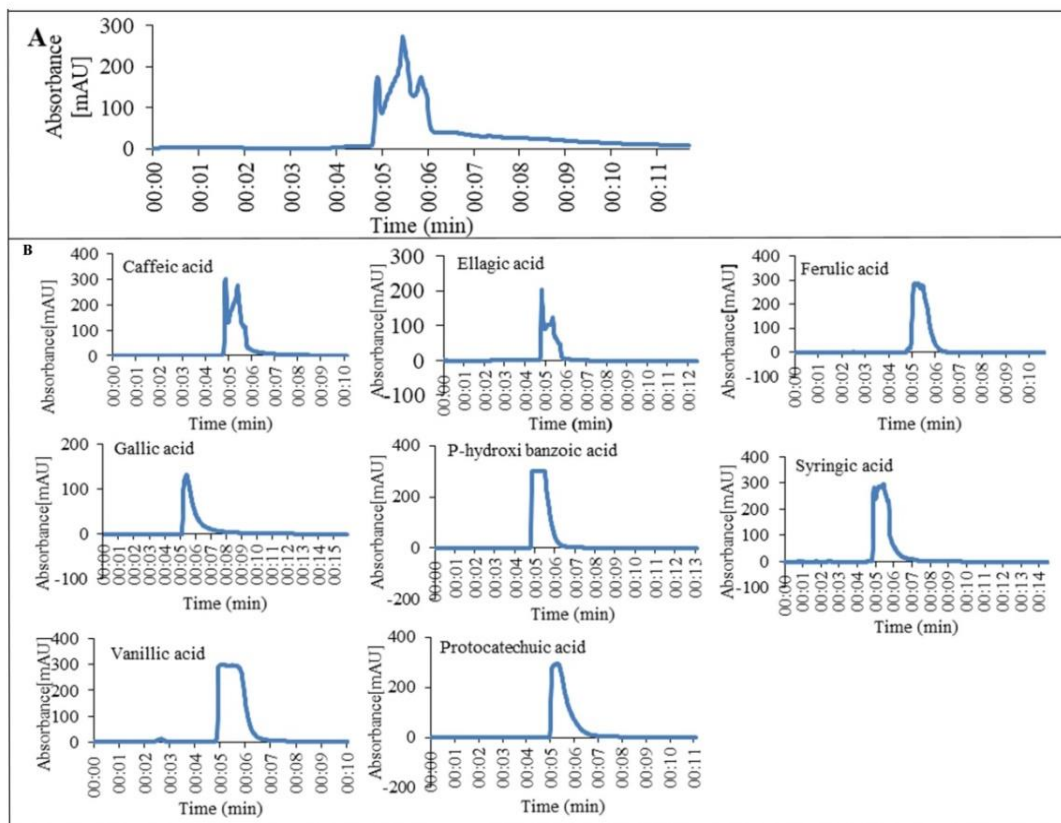


Figure 3: HPLC chromatogram of (A) sorghum shoot aqueous extract (B) standards of phenolic acids expected in sorghum shoot aqueous extract.

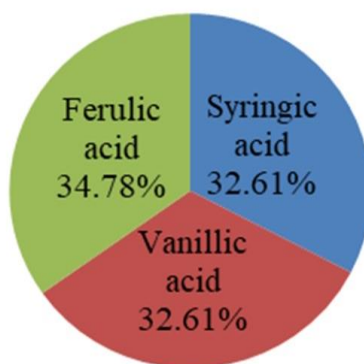


Figure 4: The amount phenolic acid in sorghum shoot aqueous extract.

Conclusion

Results of this study revealed that application the sorghum shoot extract suppressed germination and some seedling growth parameters due to three effective phenolic acid compounds syringic acid, vanillic acid and ferulic acid which they acts as biochemical herbicide. Higher concentration levels 45 and 60% of sorghum aqueous extract imposed maximum reduction for germination. In addition the three studied grass weeds were more sensitive comparing bread and durum wheat. Therefore, these results may allow the evolution of selective biosynthesized herbicides to control weeds in sustainable crop production.

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