Allelopathic potential of *Sorghum bicolor* L. Root Exudates on Growth and Chlorophyll Content of Wheat and Some Grassy Weeds

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Abstract. *Sorghum bicolor* produces root exudate contain allelochemical compound known as sorgoleon. A greenhouse experiment was carried out at Grdarasha farm/ Agricultural Engineering Sciences College/ Salahaddin University- Erbil to evaluate the allelopathic effects of root exudates of *Sorghum bicolor* on some growth parameters and chlorophyll content of *Triticum aestivum* L., *Triticum durum* Desf., *Hordeum spontaneum* K. Koch., *Avena fatua* L. and *Phalaris minor* Retz. in a modified stair step tool to eliminate any competition interaction between the donor and receiv er plants by factorial completely randomized design (Factorial C.R.D) with three replicates. The results indicated that *Phalaris minor* Desf. was the most sensitive species among all test plants for instance its total length and dry weight were (41.68 cm , 294.87 mg), also its chlorophyll content recorded minimum value were (31.65, 36.85, 36.08, 37.68) for all time intervals, comparing to the chlorophyll content of other plant species. Furthermore, all recorded parameters of other plant species were affected significantly by root exudate of *Sorghum bicolor*. Finally, root exudates of *Sorghum bicolor* L. may act as a bioherbicide to control grassy weeds in wheat fields.

1. Introduction

The allelochemicals release into the environment from plant parts by leaching, stem flow, root exudation, volatilization and residue decomposition [1, 2]. Sorghum species as a cultivated plant have suppressing weed growth behaviour [3,4]. Sorghum's root exudes several natural products, both hydrophilic and hydrophobic, that have allelopathic potential [5]. Sorghum root exudates compound identified as 2-hydroxy-5-methoxy-3-[(Z,Z)-8',11',14'-pentadecatriene]-p-hydroquinone which is hydrophobic component which oxidizes readily into the quinone, and three minor p-benzoquinones that are structurally similar, sorgoleone is stable quinone that led to root growth inhibition of lettuce and some weed species [6]. Secondary metabolites are natural products that have importance role in biological and ecological system and interference between neighboring plants and environment [7]. Recently, there has been an increased interest study in the effect of application of sorgoleon and sorghum root extract on the germination and growth of crop and weed plants [8]. The growth of duckweed (*Lemna minor*) was inhibited by 50 μM sorgoleone in liquid culture [9]. As well as the seedling growth of *Amaranthus retroflexus*, *Echinochloa crusgalli*, and *Setaria viridis* weeds were reduced by 10 μM sorgoleone added to the nutrient culture. They concluded that sorgoleone has biological activity at extremely low concentrations. There is suggestion that the mechanism of action of sorgoleone is growth inhibition so disrupt mitochondrial function [10].

Growth of *Pisum sativum* was reduced by sorgoleone because it had an inhibitory effect on photosynthesis. They showed that 10μM of sorgoleone inhibited oxygen evolution more than 50%; also it noticed that sorgoleone acted as a CO₂-dependent oxygen evolution inhibitor [11].

Soybean seedlings and H+-ATPase activity in corn root microsomal membranes was decreased by the presence of 10, 50, or 100μM concentrations of sorgoleone in the nutrient solution so, sorgoleone act as growth inhibition through impairment of essential plant processes, such as solute and water uptake, driven by proton pumping across the root cell plasma lemma [12]. Sorgoleone restrict several molecular target site through inhibition of photosynthesis in seedling, therefore, the mode of action of sorgoleone is photosynthesis inhibitor in young seedling and target molecular inhibitor in the older plants. Sorgoleone is hydrophobic compound so it is strongly absorbed in soil, which increases its persistence, but microorganism mineralizes it over time [13].

This research was conducted to evaluated the allelopathic potential of root exudates and their impact on plants grown in the vicinity.
2. Materials and Methods

Greenhouse experiment was carried out in the 23rd Jan/ 2017 at Grdarasha farm/ Agricultural Engineering Sciences College/ Salahaddin University- Erbil. A modified stair step was prepared in order to eliminate competition effect between plants and to insure the consistency of root exudates flow from donor plants Sorghum bicolor L. Moench to the receiver plants: Triticum aestivum L., Triticum durum Desf., Hordeum spontaneum K. Koch., Avena fatua L. and Phalaris minor Retz. [14]. The prepared modified stair step consists of four layers (Fig. 1) as describe below:

2.1 First layer

It was located at the top of the stair. A five liter that were poured with nutrient solution (Alanfal Fert. Industry Company in Amman / Jordan). The concentrations of the solution differs according to the plant age in the first 15 days 1.00 ml.L-1, then 1.15 ml.L-1 after 15 days, followed by 1.25 ml.L-1 after another 15 days, and finally 1.50 ml.L-1 at the last 25 days.

2.2 Second layer

S. bicolor seeds were planted as a donor plants in pots that were filled with cleaned and washed coarse sand [15] with 1% HCl for 24 hours to remove any organic matter then rewashed again with tap water for 48 hours to remove any acidic residue, and finally rinsed with distilled water.

2.3. Third layer

Receiver plant pots were filled with coarse sand that managed as mentioned above then sowed with receiver plants seeds Triticum aestivum L., Triticum durum Desf., Hordeum spontaneum K. Koch, Avena fatua L. and Phalaris minor Retz.

2.4 Fourth layer

It was located at the bottom of stair and the collection containers of this layer collect the nutrient solutions after passing through the donor and receiver plant and transferred when they were filled again to first layer containers. This process has taken place within 12 hours. There were addition of nutrient solution each 15 days due to reduction in size of the nutrient solution which been consumed by the developing plants according to the needs of plants during such period and the doses that were detailed on the label of the nutrient solution. Plants were irrigated with the nutrient solution and monitored carefully to insure the movement of the solution during the period of the experiment. Finally, after 70 days from planting, the experiment was ended and plant shoot parts were cut above ground and cleaning the root parts by washing the roots with tap water over a sieve. Following that the shoot and root part samples were put and dried by electrical oven (FAITHFUL GX-45BE) 70°C for 72 [14, 16].

3.5. Recorded Data

1. Shoot length (cm)

   It was measured the longest and nearest shoot in a centimetre [17].

2. Root length (cm)

   It was measured the longest and nearest root in a centimetre [17].

3. Total length (cm) (S.L.)

   It is a total sum of Shoot and root length [18].

4. Shoot and root dry weight (mg)

   The samples were placed in an electronic oven 70°C for 72 hours then weighed plant parts dry weights by sensitive electronic balance (DENVER INSTRUMENT) [19].

5. Total dry weight (mg)

   It is a total dry weight of shoot and root of plant [19].

6. Shoot growth inhibition percentage

   It has calculated according to the following equation based on shoot dry weight [20].

   \[
   \text{Shoot Growth Inhibition} \% = \left(1 - \frac{\text{Shoot dry weight under stress}}{\text{Shoot dry weight under non stress}}\right) \times 100
   \]

7. Root growth inhibition percentage

   It was calculated according to the following equation based on root dry weight [20].

   \[
   \text{Root Growth Inhibition} \% = \left(1 - \frac{\text{Root dry weight under stress}}{\text{Root dry weight under non stress}}\right) \times 100
   \]

8. Plant growth inhibition percentage (S.G.I. %)
It was calculated according to the equation provided by [21].

\[
\text{Plant Growth Inhibition} \% = \left(1 - \frac{\text{Total seedling dry weight under stress}}{\text{Total seedling dry weight under non stress}}\right) \times 100
\]

9. Root/ shoot ratio (R./Sh.)

It was calculated according to the equation shown below [19].

\[
\text{Ratio} = \frac{\text{Shoot dry weight}}{\text{Root dry weight}}
\]

10. Chlorophyll content

Chlorophyll content were measured at four times in the (12/3, 19/3, 26/3, 2/4 – 2017) with one week interval between them, the "atLEAF+" instrument were used.

Statistical Analysis

This study was conducted out under with a factorial completely randomized design (Factorial C.R.D) with three replicates. The data was subjected to standard analysis of variance and means were compared at significant 1% level by Tukey test using SPSS version 20 computer analysis according to [22].

Fig. 1. The *Sorghum bicolor* L. Moench exude experiment using the modified stair step. A is planted with donor plant sorghum; B is not planted with donor plant sorghum (control treatment).

3. Results and Discussions

3.1 Cereal growth parameters under sorghum root exudates

The donor plant *S. bicolor* root exudates significantly affected (P<0.01) all recorded growth parameters except root/shoot ratio, whereas the shoot, root and total length reduced in control treatment from (30.53 cm, 27.37 cm, 57.91 cm) respectively to (22.76 cm, 18.95 cm, 41.71 cm) respectively in test treatments (Fig. 2). As well as, the shoot, root and total dry weight decreased form (299.22 mg, 202.00 mg, 503.66 mg) respectively in control treatment to (227.66 mg, 155.49 mg, 380.70 mg) respectively in test treatments (Fig. 3). Shoot, root and plant growth inhibition % were in highest levels (24.54 %, 25.31 %, 25.02 %) respectively in test treatments while the minimum measures (0.00 %, 0.00%, 0.00%) respectively in control treatments (Fig. 4). Root/shoot ratio parameter that was not affected significantly was (Fig. 5). These results are in agreement with [23] that reported shoot and dry weight of large crabgrass, barnyard grass, velvetleaf and ivy leaf morning glory were decreased by increasing concentration of sorgoleone from (0.00 to 200 µM). Germination, shoot dry weight, leaf area and chlorophyll content of *Amaranthus*...
dubius L. and Solanum melongena L. were inhibited by root exudate of Tithonia diversifolia, also allelochemicals mode of action was target independent on the plant species and concentration levels thus, allelopathic potential differ from one target plant to another plant species [24].

Fig. 6 illustrates the significant (P≤0.01) differences for all chlorophyll content that recorded at different dates except the three weeks after first count treatment. Data of the highest chlorophyll content for first count (FC), one week after first count (1WAFC) and two weeks after first count (2WAFC) were reported in control treatment (43.81, 44.46, 41.93) respectively, but the lowest values (40.49, 41.29, 40.28) were observed in test treatment.
Figure 4. Effect of Sorghum bicolor L. root exudates on shoot, root and total growth inhibition (%) of cereal species.

Figure 5. Effect of sorghum bicolor L. root exudates on root/shoot ratio of cereal species.
3.2. Cereal growth parameters under cereal species.

The donor plant *Sorghum bicolor* L. root exudates significantly (P≤0.01) affected plant species growth parameters (Table 1). Among tested plant species, bread wheat recorded highest values for shoot, root and total length were (29.17 cm, 25.79 cm, 54.96 cm) respectively; but wild barley recorded highest values for shoot, root and total dry weight were (359.04 mg, 210.56 mg, 569.59 mg) respectively; while wild oat registered maximum values for root and plant growth inhibition (22.06 %, 18.02 %) respectively; but the largest rates of shoot growth inhibition and root/ shoot ratio (19.68%, 0.92) were reported with canary grass (Table 1).

Allelopathic potential of root exudate of different allelopathic crops have been studies previously on growth of crops and weeds such as (*S. bicolor* L.) and (*Oryza sativa* L.) that they have phytotoxic activity against various crops or weeds those share the same environment [25, 26]. It has been reported that the growth of weed plant were reduced due to allelochemicals that release form their roots especially sorgone (hydrophobic phytotoxic compound) from *S. bicolor* L. which it is a potent photosynthesis inhibitor that block electronic transportation [27, 28]. Sorgoleone could have pre- or post-emergence inhibitor to control grassy or broad leaf weeds [10].

In this study, the root exudates of *S. bicolor* L. plant inhibited the growth of five plant species by different degrees. While the most sensitive plant was canary grass then followed by wild oat because canary grass recorded minimum values (21.78 cm, 19.90 cm, 41.68 cm, 155.59 mg, 139.28 mg, 294.78 mg, 19.68 %) respectively for shoot, root and total length, and shoot, root and total dry weight, and shoot growth inhibition (Table 1). The growth of *Rumex japonicas* and *Portulaca oleracea* inhibited by 80% also shoot biomass of weed were reduced by using sorgoleone as a pre-emergence treatment at (0.4 kg. a.i. ha\(^{-1}\)) and reported they were the most sensitive weed among various weeds that were tested [29]. Fig (7) shows that the aqueous extract of *S. bicolor* displayed significant differences (P≤0.01) at all dates that where counted chlorophyll for all plant species. The maximum values (50.43, 45.82, 42.13, 50.19) respectively for first count, and the three consecutive weeks after first count (WAFC) for chlorophyll in durum wheat. While, the minimum records (31.65, 36.85, 36.08, 37.68) respectively were measured in canary grass.
Table 1. Effect of cereal species on growth parameters of cereal species.

| Cereal species | Shoot length (cm) | Root length (cm) | Total length (cm) | Shoot dry weight (mg) | Root dry weight (mg) | Total dry weight (mg) | Shoot growth inhibition % | Root growth inhibition % | Plant growth inhibition % | Root/shoot ratio |
|----------------|-------------------|------------------|-------------------|-----------------------|----------------------|----------------------|-------------------------|-------------------------|--------------------------|------------------|
| Bread wheat    | 29.17             | 25.79            | 54.96             | 310.2                 | 168.33               | 478.6                | 14.59                   | 14.89                   | 14.69                    | 0.54             |
|                | ±3.35             | ±3.95            | ±7.28             | ±23.9                 | ±13.52               | ±37                  | ±6.61                   | ±6.7                    | ±6.59                    | ±0.01            |
| Durum wheat    | 28.05             | 22.19            | 50.25             | 228.2                 | 198.89               | 427.0                | 3.91                    | 8.21                    | 5.96                     | 0.87             |
|                | ±0.93             | ±1.79            | ±2.61             | ±5.51                 | ±8.89                | ±13.6               | ±1.78                   | ±3.72                   | ±2.77                    | ±0.02            |
| Wild barley    | 27.83             | 24.61            | 52.45             | 359.0                 | 210.56               | 569.5                | 8.09                    | 2.82                    | 6.21                     | 0.59             |
|                | ±1.93             | ±2.09            | ±4.02             | ±17.62                | ±2.81                | ±19.56              | ±3.73                   | ±1.33                   | ±2.80                    | ±0.02            |
| Wild oat       | 26.39             | 23.32            | 49.71             | 264.0                 | 176.67               | 440.7                | 15.08                   | 22.06                   | 18.02                    | 0.65             |
|                | ±1.58             | ±0.86            | ±2.44             | ±21.6                 | ±25.39               | ±46.19               | ±6.85                   | ±9.87                   | ±8.07                    | ±0.03            |
| Canary grass   | 21.78             | 19.9             | 41.68             | 155.5                 | 139.28               | 294.8                | 19.68                   | 15.30                   | 17.67                    | 0.92             |
|                | ±1.86             | ±1.37            | ±3.21             | ±19.59                | ±12.7                | ±30.02               | ±8.81                   | ±6.85                   | ±8.04                    | ±0.03            |

*Within each column values followed by similar letters are not significant differences at 1% probability level by Tukey test.

3.3. Cereal growth parameters under sorghum root exudates and cereal species interaction.

Table (2) shows the significant (P≤0.01) differences of all growth characters of five receiver plant species that included in this study. Data of the highest shoot, root and total length were reported in control treatment of bread.
wheat plants (36.22 cm, 34.32 cm, 70.54 cm) respectively, while lowest data (18.11 cm, 17.74 cm, 35.58 cm) were in canary grass treated with sorghum root exudates treatment. Maximum records of shoot and total dry weight (390.64 mg, 607.31 mg) respectively were observed in control treatment of wild barley. While the maximum value for root dry weight were measured in control treatment of wild oat, while the minimum values (117.46 mg, 114.46 mg, 231.57 mg) of shoot, root and total dry weight were registered in canary grass under sorghum root exudates (Table 2). The major record (0.98) for root/ shoot ratio was in canary grass treated with sorghum root exudates treatment but minor values (0.54, 0.54) respectively reported in control and exudate treatment of bread wheat. The largest values (44.12%, 36.04%) respectively for root and plant growth inhibition were noticed in wild oat when treated by sorghum root exudates but the maximum value (39.37%) for shoot growth inhibition were registered in canary grass when treated with sorghum root exudates, while the minimum value (0.00%) for shoot, root and plant growth inhibition were documented in all control treatment with all plant species (Table 2). The (P≤0.01) impacts of combination effects between plant species and sorghum bicolor root exudates on chlorophyll content that measured at different time intervals. Whereas, the highest values (50.64, 47.80 atLEAF) were reported for fist count (FC) and one week after first count of chlorophyll content (1WAFC) in control treatment of durum wheat, while the highest record (45.12 atLEAF) chlorophyll content at two weeks after first count (2WAFC) were recorded in control treatment for wild oat while the maximum data (50.31 atLEAF) of chlorophyll content at two weeks after first count (2WAFC) respectively for first count (FC), one week after firsts count (1WAFC), two weeks after first count (2WAFC) and three weeks first count were reported in canary grass that treated with sorghum root exudates (Table 3). The general objective of this experiment was demonstrating the S. bicolor L. allelopathic activities by which may represent finding source of eco-friendly herbicide [30]. In this experiment growth inhibition of tested plant species, explain the truth that S. bicolor L. root exudates decrease the growth characters of the receiver plant comparing with control treatment [31, 32].
| Cereal species X Treatment | Shoot length (cm) | Root length (cm) | Total length (cm) | Shoot dry weight (mg) | Root dry weight (mg) | Total dry weight (mg) | Shoot growth inhibition (%) | Root growth inhibition (%) | Plant growth inhibition (%) | Root/shoot ratio |
|---------------------------|-----------------|-----------------|------------------|---------------------|-------------------|---------------------|---------------------------|---------------------------|-----------------------------|---------------|
| **Bread wheat**           |                 |                 |                  |                     |                   |                     |                           |                           |                             |               |
| Control                   | 36.22 ± 2.11    | 34.32 ± 4.11    | 70.54 ± 6.19     | 363.28 ± 1.38       | 197.78 ± 4.88     | 561.06 ± 4.13       | 0.00                      | 0.00                      | 0.00                         | 0.00 ± 0.02   |
| Treat                     | 22.11 ± 1.31    | 17.26 ± 2.33    | 39.37 ± 2.94     | 257.29 ± 4.81       | 138.89 ± 6.79     | 396.18 ± 2.34       | 29.17 ± 0.00              | 29.78 ± 0.00               | 29.38 ± 0.01                          | 0.54 ± 0.02   |
| **Durum wheat**           |                 |                 |                  |                     |                   |                     |                           |                           |                             |               |
| Control                   | 29.22 ± 1.42    | 25.93 ± 2.68    | 55.15 ± 5.92     | 237.48 ± 2.68       | 16.67 ± 3.85      | 254.14 ± 2.12       | 0.00                      | 0.00                      | 0.00                         | 0.00 ± 0.00   |
| Treat                     | 26.89 ± 0.99    | 18.46 ± 0.67    | 45.35 ± 1.67     | 218.92 ± 0.67       | 181.11 ± 3.85     | 399.03 ± 1.67       | 7.81 ± 0.00               | 16.41 ± 0.00              | 11.92 ± 0.00                          | 0.83 ± 0.02   |
| **Wild barley**           |                 |                 |                  |                     |                   |                     |                           |                           |                             |               |
| Control                   | 31.89 ± 1.47    | 29.09 ± 1.34    | 60.98 ± 13.78    | 390.64 ± 12.90      | 204.44 ± 12.00    | 594.08 ± 21.90       | 0.00                      | 0.00                      | 0.00                         | 0.00 ± 0.00   |
| Treat                     | 23.78 ± 0.22    | 20.13 ± 0.41    | 43.91 ± 19.04    | 327.43 ± 17.99      | 216.67 ± 2.05     | 533.72 ± 2.05       | 16.18 ± 0.00              | 5.64 ± 0.00                | 12.42 ± 0.00                          | 0.63 ± 0.01   |
| **Wild oat**              |                 |                 |                  |                     |                   |                     |                           |                           |                             |               |
| Control                   | 29.89 ± 0.49    | 25.2 ± 0.41     | 55.09 ± 0.89     | 310.97 ± 24.04      | 226.66 ± 27.57    | 537.64 ± 27.57      | 0.00                      | 0.00                      | 0.00                         | 0.00 ± 0.00   |
| Treat                     | 22.89 ± 0.22    | 21.45 ± 0.21    | 44.34 ± 0.43     | 217.18 ± 10.75      | 126.67 ± 2.73     | 343.85 ± 2.73       | 30.16 ± 0.00              | 44.12 ± 0.00              | 36.04 ± 0.00                          | 0.58 ± 0.03   |
| **Canary grass**          |                 |                 |                  |                     |                   |                     |                           |                           |                             |               |
| Control                   | 25.45 ± 0.78    | 22.33 ± 1.46    | 47.77 ± 20.86    | 193.72 ± 11.6       | 164.44 ± 22.30    | 358.17 ± 22.30      | 0.00                      | 0.00                      | 0.00                         | 0.00 ± 0.03   |
| Treat                     | 18.11 ± 1.79    | 17.47 ± 1.73    | 35.58 ± 5.46     | 117.46 ± 6.19       | 114.11 ± 1.47     | 231.57 ± 1.47       | 39.37 ± 0.00              | 30.61 ± 0.00              | 35.35 ± 0.00                          | 0.98 ± 0.02   |

*Within each column values followed by similar letters are not significant differences at 1% probability level by Tukey test.*
Table 3. Effects of cereal species and *Sorghum bicolor* L. root exudates interaction on chlorophyll content.

| Cereal species X Treatment | Chlorophyll (FC) | Chlorophyll (1WAFC) | Chlorophyll (2WAFC) | Chlorophyll (3WAFC) |
|----------------------------|------------------|---------------------|---------------------|---------------------|
| **Bread wheat**            |                  |                     |                     |                     |
| Control                    | 45.78 ab ±3.25   | 46.00 ab ±1.50      | 43.21 ab ±0.88      | 50.31 a ±1.07       |
| Treat                      | 42.83 abc ±2.48  | 41.21 ab ±2.27      | 39.8 abc ±1.49      | 47.6 abc ±0.37      |
| **Durum wheat**            |                  |                     |                     |                     |
| Control                    | 50.64 a ±1.13    | 47.80 a ±1.68       | 42.57 abc ±0.96     | 50.21 ab ±0.27      |
| Treat                      | 50.22 a ±0.97    | 43.84 ab ±0.58      | 41.69 abc ±0.82     | 50.17 ab ±1.16      |
| **Wild barley**            |                  |                     |                     |                     |
| Control                    | 49.03 a ±0.66    | 46.51 ab ±2.03      | 42.29 abc ±1.26     | 49.3 abc ±1.23      |
| Treat                      | 41.83 abc ±1.14  | 43.48 ab ±0.75      | 41.57 abc ±0.34     | 48.68 abc ±0.49     |
| **Wild oat**               |                  |                     |                     |                     |
| Control                    | 40.16 abc ±3.60  | 43.15 ab ±2.52      | 45.12 a ±0.13       | 45.55 bc ±0.23      |
| Treat                      | 37.88 abc ±1.53  | 43.07 ab ±2.20      | 42.63 abc ±2.09     | 44.71 c ±0.73       |
| **Canary grass**           |                  |                     |                     |                     |
| Control                    | 33.44 bc ±2.28   | 38.86 ab ±1.74      | 36.45 bc ±0.04      | 37.98 d ±0.16       |
| Treat                      | 29.68 c ±4.32    | 34.85 b ±2.83       | 35.71 c ±1.83       | 37.38 d ±0.92       |

*Within each column values followed by similar letters are not significant differences at 1% probability level by Tukey test.*

4. Conclusion

*Sorghum bicolor* L. Moench root exudates in controlled environment (without competition between plants) had an obvious allelopathic potential on shoot, root and total plant length and dry weight of tested plant species especially the three grassy weeds. Thus, allelopathic interaction could be the key factor that affects the relationship between plant species in vicinity.
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