

Research Article

## Allelopathic activity of *Sorghum bicolor* root parts and exudates on *Bipolaris sorokiniana*

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**Abstracts:** *Sorghum bicolor* L. releasing allelochemicals in the soil through their root exudation that functional their associated soil microorganisms and can help in building disease control strategy for increasing sustainability. The obtained results of sorghum rhizospheric exudates exhibited markedly effect on bacterial count in rhizosphere soil. The extract of root exudates profile was tested by two prepared concentrations; 500 ppm and 1000 ppm against the *Bipolaris sorokiniana* compared to untreated control. The reduction percentages were calculated after four and seven days of fungus growth, the results represented that the reduction over control were 17.53, 45.63% after four days, however after seven days the reductions over control were 17.28, 36.40%. For sorghum root parts, the reduction increase with increasing concentration ranged from 49.71 to 71.67%, the highest reduction was afforded by conc. 1600 ppm while the lowest reduction by 200 ppm. The analysis to identify sorghum allelochemicals was conducted by LC-MS/MS and FTIR afforded; proline, coumaric acid, cinnamic acid, hydroxycoumarin, benzoxazolone, ferulic acid and sorgoleone. While, sorghum root parts extract compounds were; coumaric acid, cinnamic acid, vanillic acid, luteolin3-Hydroxycoumarin, gallic acid, ferulic acid and sorgoleone. It could be used sorghum root exudates and root parts extract in disease bio-control due the effect of secreted biochemical molecules as step toward sustainable agriculture.

**Keyword:** Sorghum, allelochemicals, root exudates, rhizosphere, LC-MS/MS, FTIR

### Introduction

*Sorghum bicolor* commonly called sorghum and also known as great millet, *durra*, is a grass species cultivated for its grain, which is used for food, both for animals and humans, and for ethanol production. Sorghum is the world's fifth most important cereal crop after rice, wheat,

maize and barley. Sorghum (*Sorghum bicolor*) is popular cereals consumed by both adults and infants in Africa (Asiedu *et al.*, 1993). The phytochemicals present in the root exudates of plants mediate several types of communication processes in the rhizosphere, such as root-root, root-microbe and root-insect interactions (Walker *et al.* 2003; Bais *et al.* 2004). Rhizosphere, the layer of soil influenced by plant roots plays pivotal role in plant growth and development (Hrynkiewicz and Baum, 2012).

The antibiotic activity of bio-control is based on secretion of molecules that kill or

Handling Editor: Vahe Minassian

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Received: 1 November 2017 Accepted: 16 June 2018

Published online: 13 July 2018

reduce the growth of the target pathogen (Dowling and O’Gara, 1994; Whipps, 2001; Lugtenberg and Kamilova, 2009). Antibiotics encompass a heterogeneous group of organic, low-molecular-weight compounds that are deleterious to the growth or metabolic activities of other microorganisms (Duffy, 2003). Peroxidases from root exudates of sorghum *Sorghum bicolor* L. Moench are involved in the rhizosphere degradation of polycyclic aromatic hydrocarbons (PAHs) which Sorghum peroxidase oxidizes anthracene and phenanthrene (Dubrovskaya *et al.*, 2016). Sorigoleone production in root exudates of Australian and African Sorghum species was compared and contrasted; results suggest that certain Australian genotypes produce unique metabolic profiles with little to no sorigoleone. Differential sorigoleone production may be the result of long-term evolutionary adaptation to diverse climates after continental drift (Zhu *et al.* 2017). Root exudates from some plant species can inhibit potential soil-borne pathogens by releasing allelochemicals (Li *et al.*, 2013). This work aimed to find bioactive compounds against plant pathogenic microbes, the secondary metabolites of both sorghum exudates and sorghum root parts were extracted and bioassayed against *Bipolaris sorokiniana*, then subjected to the identification with spectroscopic techniques.

## Materials and Methods

### Sorghum root exudates collection and its effects

#### Rhizosphere effect (R/S)

The rhizosphere effect of the selected plants was calculated according to Curl and Truelove (1986), using the following formula, R/S ratio.

R = Total count of microbes in rhizospheric soil (CFU/ml).

S = Total count of microbes in non-rhizospheric soil (CFU/ml).

The ratio was calculated for any microorganism using the mean population.

### Collection and extraction of sorghum root exudates

Sorghum seeds were placed on static Murashige and Skoog (MS) basal medium and allowed to germinate for 7 days. Then seedlings with only submerged roots were transferred to tissue culture tubes containing 5 mL (MS) liquid medium. After 28 days exudates were collected from 400 seedlings and centrifuged at 5000 rpm for 10 min to remove any cellular debris; supernatants were passed through a syringe filter of pore size 0.45mm. The supernatant subject to refrigerator (-80 °C) and was concentrated. The residues were re-suspended in 50 ml distilled water with pH < 4, followed by a liquid-liquid partitioning step and phase separation by adding ethyl acetate three times Schwab *et al.* (1983). The extracts evaporated to dryness by aeration and dissolved in ethanol to prepare 0, 500, 1000, µg/mL then bioassayed against the causal agent of sorghum root rot.

### Extraction of sorghum root parts

Sorghum (*Sorghum bicolor* L.) roots were gathered from Baloza Research Station, Desert Research Center, North Sinai, Egypt during 2015. Then, plant roots were dried and one hundred grams of ground root tissues were extracted with 500mL methanol overnight. Then the extract put on shaker for 5hrs. The extract was filtered through two layers of sheath cloth to remove fibers, and followed by filter paper. The methanol evaporated and the aqueous phase partitioning was done with ethyl acetate, the extracts were concentrated under vacuum and stored at 4 °C for bioassay against sorghum root rot pathogen and analysis. The extracts were evaporated to dryness and stored at -20 °C until bioassay.

### Bioassay against fungus

#### Isolation of fungi from diseased root of sorghum plants

Plant roots were cut into small pieces and surface sterilized with sodium hypo-chloride (0.3% v/v) for 1-2min followed by alternate

washing with sterile distilled water and 70% ethyl alcohol, root pieces were air dried and then transferred to previously sterilized potato dextrose agar medium poured in sterilized petri plates incubated at 25 °C for 5 days, fungi are purified by hyphal tip method on potato dextrose agar medium, purified fungus was identified according to its morphological characteristics in Plant Protection Laboratory of Desert Research Center.

### Bioassay against pathogenic fungus

Crude extracts were evaporated and 50 mg dissolved with aqueous methanol out of this replaced with sterilized distilled water, while 1 mL from 500 and 1000 µg/mL concentration was added for each Petri dish (9 cm) containing PDA medium before its solidification and rotated gently to ensure equal distribution of crude extract, 1 cm plug of *Bipolaris sorokiniana* culture was transferred to the middle of the Petri dishes (Kumar and Kaushik, 2013). The dishes were put in growth chamber at 25 °C with four replications, data of fungal growth was recorded after four days of incubation.

### Identification of active metabolites

High-performance liquid chromatography (HPLC) with Mass detector (Agilent, USA, Triple Quad LC-MS/MS Model 6460 Mass Spectrometer) were used to analyze and identify the extracts. Whereas the HPLC system has a reversed phase C<sub>18</sub> column and Mobile phase consisted of distilled water (A) and methanol (B). The gradient elution started at 5% B for 5 min and continued with a percentage of B and time as follows: 95% B at 37 min, 95% B for 62 min at a flow rate of 0.33 ml/min and injection volume: 5 µl according to (Mabry et al., 1970).

FTIR spectra were scanned using a Perkin Elmer FTIR spectrometer model L1600300 Spectrum Two LiTa made in UK equipped (S. N: 94754) with DTGS detector with a resolution of 4 cm<sup>-1</sup>, number of scanning of 32 co-adding in the 400-4000 cm<sup>-1</sup> region. Spectra

were acquired using FTIR Spectrum 10™ software and the spectra were recorded as transmission values at each data point.

### Statistical analysis

Experiments were design in Randomized Complete Block Design and subjected to statistical analysis by ANOVA through the Least Significant Difference (L.S.D<sub>0.05</sub>) using the method described by (Snedecor and Cochran, 1990).

## Results and Discussion

### Effect of sorghum plants on bacterial counts in rhizosphere soil

The obtained results in Table (1) show that total bacterial counts in rhizosphere soil samples ranged from (1.1 x 10<sup>3</sup> to 1 x 10<sup>6</sup>, 1.2 x 10<sup>3</sup> to 2.6 x 10<sup>6</sup>, 1.07 x 10<sup>3</sup> to 1.2 x 10<sup>6</sup> and 9.9 x 10<sup>2</sup> to 1.3 x 10<sup>6</sup> CFU/mL) in rhizosphere soil samples 1, 2, 3 and 4 respectively, in non-rhizosphere soil samples the total bacterial counts ranged from (9 x 10<sup>2</sup> to 6.7 x 10<sup>5</sup>, 1.04 x 10<sup>3</sup> to 1.74 x 10<sup>5</sup>, 9 x 10<sup>2</sup> to 2 x 10<sup>5</sup> and 9.4 x 10<sup>2</sup> to 5 x 10<sup>5</sup> CFU/mL) for all samples 1, 2, 3 and 4 respectively, and the values of R/S (counts in the rhizosphere/counts in the non-rhizosphere soil) were more than one.

It is concluded that sorghum plants exhibited marked effects on bacterial growth in rhizosphere soil, this enhancement occurred due the effect of biochemical molecules secreted by sorghum plant roots in this region of soil.

### Effect of sorghum root exudates against fungal growth

Sorghum root exudates were collected after 28 days and extracted with equal volume of ethyl acetate by partitioning three times, then the crude extract were dried and sequence concentrations were prepared and bioassayed against fungal growth. The results showed that 500, 1000 µg/mL concentrations afforded reduction over the control by 17.53, 45.63%, respectively (Table 2) and (Figure 1) after four days of fungal growth, however after 7 days the reduction reached 17.28 and 36.4% over of the control.

**Table 1** Total microbial counts in rhizospheric and non -rhizospheric soil of sorghum.

R <sup>1</sup>				S <sup>2</sup>				R/S			
Total bacterial counts (CFU/ml)				Total bacterial counts (CFU/ml)							
1.1x10 <sup>3</sup>	1.2x10 <sup>3</sup>	1.07 x10 <sup>3</sup>	9.9 x10 <sup>2</sup>	9.9 x10 <sup>2</sup>	1.04 x10 <sup>3</sup>	9 x 10 <sup>2</sup>	9.4 10 <sup>2</sup>	1.1	1.2	1.2	1.05
8.2x10 <sup>3</sup>	8.8x10 <sup>3</sup>	5.1 x10 <sup>3</sup>	4.9 x10 <sup>3</sup>	2.5 x10 <sup>3</sup>	4.2 x10 <sup>3</sup>	4.7 x10 <sup>3</sup>	4.2 x10 <sup>3</sup>	3.3	2.1	1.07	1.15
5.6 x10 <sup>4</sup>	5.20 x10 <sup>4</sup>	3.5 x10 <sup>4</sup>	3.8 x 10 <sup>4</sup>	1.2 x 10 <sup>4</sup>	1.3 x 10 <sup>4</sup>	1.3 x 10 <sup>4</sup>	2.1 x 10 <sup>4</sup>	4.57	4.05	2.58	1.79
3 x10 <sup>5</sup>	4.3x10 <sup>5</sup>	2.5 x10 <sup>5</sup>	2.4 x10 <sup>5</sup>	9.3 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	8 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>	3.14	8.67	3.08	2.12
1x10 <sup>6</sup>	2.6x10 <sup>6</sup>	1.2 x10 <sup>6</sup>	1.3 x10 <sup>6</sup>	6.7 x10 <sup>5</sup>	1.74 x10 <sup>5</sup>	2 x10 <sup>5</sup>	5 x10 <sup>5</sup>	1.70	15.57	5.01	2.67
LSD (0.05) 5.52				2.06							

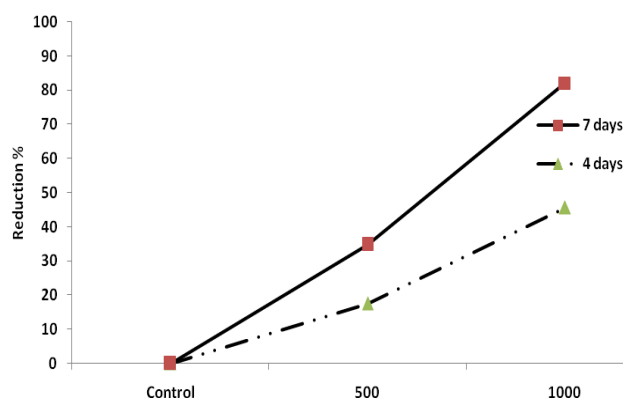
<sup>1</sup>R = Bacterial counts in the rhizospheric soil.<sup>2</sup>S = Bacterial counts in the non-rhizospheric soil.

R/S = Counts in the rhizosphere/counts in the non-rhizosphere.

**Table 2** Activity of sorghum root exudates (ethyl acetate extracts) on fungal growth at four and seven days-incubation period.

Concentration (ppm)	4 days		7 days	
	Mean of fungal growth (mm)	Reduction (%)	Mean of fungal growth (mm)	Reduction (%)
Control	3.01.46	0.00	5.40 ± 0.46	0.00
500	2.49 ± 0.35	17.53	4.47 ± 0.35	17.28
1000	1.64 ± 0.06	45.63	3.43 ± 0.40	36.40
LSD (0.05)	1.17			

± Standard deviation.

**Figure 1** Activity of sorghum root exudates (ethyl acetate extracts) on fungal growth.

### Effect of sorghum root parts extract against fungal growth

Table 3 shows the effect of different prepared concentrations from sorghum root parts ethyl-acetate extract on fungal growth. Sorghum root parts were extracted with orbital shaker, then the fibers removed by double layers of sheath clothes

after that the crude extracts were centrifuged and filtrated with filter paper Whatman number (1), then the extract evaporated and weighed, four sequence concentrations were prepared (200, 400, 800 and 1600ppm) in addition to control. The obtained data revealed that the reduction of fungal growth increased by increasing concentration, the

reduction ranged from 49.71 to 71.68%, the highest reduction was achieved from 1600 $\mu\text{g/mL}$ , while the lowest reduction was achieved from the lowest concentration 200 $\mu\text{g/mL}$ .

**Table 3** Activity of sorghum root parts extract with ethyl acetate.

Concentration (ppm)	Mean of fungal growth (mm)	Reduction (%)
Control	5.77 $\pm$ 0.201	0.00
200	2.90 $\pm$ 0.62	49.71
400	2.80 $\pm$ 0.40	51.445
800	2.53 $\pm$ 0.25	56.07
1600	1.63 $\pm$ 0.25	71.68
F value	5.54	
LSD (0.05)	0.81	

Statistical analysis by ANOVA described by L.S.D at  $P \leq 0.05$ .

### Qualitative identification of bacterial isolates and sorghum extracts

#### Metabolites analysis by Fourier Transform Infrared Spectroscopy (FTIR)

The ethyl acetate extract of sorghum root exudates FTIR presented in (Table 4) showed that, ten spectra could be identified as O-H stretching vibration (3443 $\text{cm}^{-1}$ ), N-H stretching vibration (2957 $\text{cm}^{-1}$ ), C-H stretching vibration-asym (2925 $\text{cm}^{-1}$ ), C-H stretching vibration- sym (2855 $\text{cm}^{-1}$ ), C = C stretching vibration (2079 $\text{cm}^{-1}$ ), C = O stretching vibration (1725), C = C stretching vibration (1638  $\text{cm}^{-1}$ ), C = N stretching vibration (1461 $\text{cm}^{-1}$ ), C-H vibration- sym (1384 $\text{cm}^{-1}$ ), CH<sub>2</sub> wagging vibration (1274 $\text{cm}^{-1}$ ), respectively.

**Table 4** FTIR spectra of sorghum root exudates, hexane and ethyl acetate root parts extracts (Transmission).

Root exudates (ethyl acetate)	Root part (ethyl acetate)	Root part (hexane)
3443	3423	3414
2957	2928	2927
2925	2072	2854
2855	1637	2040
2079	1514	1703
1725	1457	1648
1638	1384	1493
1461	1258	1453
1384	1169	1383
1274	1125	1270

#### FTIR analysis of sorghum root parts

Profile extracts presented in (Table 4) functional groups ranging from 1125 to 3423 $\text{cm}^{-1}$ , these spectra could be attributed to O-H stretching vibration (3423 $\text{cm}^{-1}$ ), asym. CH<sub>2</sub> stretching vibration (2928 $\text{cm}^{-1}$ ), asym. N = C = S stretching vibration (2072 $\text{cm}^{-1}$ ), C = O stretching vibration (1637 $\text{cm}^{-1}$ ), C = C stretching vibration (1514 $\text{cm}^{-1}$ ), C = N stretching vibration (1457 $\text{cm}^{-1}$ ), C-H sym. vibration (1384 $\text{cm}^{-1}$ ), C-C-C asym. stretching vibration (1258 $\text{cm}^{-1}$ ), C-O-C stretching vibration (1169 $\text{cm}^{-1}$ ) and C-C stretching vibration(1125 $\text{cm}^{-1}$ ), respectively.

The frequencies of sorghum hexane extracts presented (Table 4) ranging from 3414 to 1270  $\text{cm}^{-1}$  could be assigned to N-H stretching vibration (3414), O-H stretching vibration (2927  $\text{cm}^{-1}$ ), C-H stretching vibration- sym (2854 $\text{cm}^{-1}$ ), asym. N = C = S stretching (2040 $\text{cm}^{-1}$ ), C = C stretching vibration (1703 $\text{cm}^{-1}$ ), C = O stretching vibrations (1648), C = N stretching vibration (1493), C-H asym. vibration (1453 $\text{cm}^{-1}$ ), N-O stretching vibration (1383  $\text{cm}^{-1}$ ) and = C-H vibrations (1270 $\text{cm}^{-1}$ ), respectively.

#### Metabolites analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The bioactive constituents were responsible for the activity against the tested fungus from sorghum and its associated microbes were identified through LC-MS/MS after extraction and evaluation.

(A) The sorghum root exudates metabolites were extracted by partitioning with ethyl acetate three times with equal volume, then after dryness the sample dissolved in methanol for analysis through LC-MS/MS. The first signals deduced from  $m/z$  117 [M + 2H] which have the molecular weight 115 might be proline. The second compound corresponding to LC-MS/MS analysis deduced from  $m/z$  197 [M + CH<sub>3</sub>OH + H] which have molecular weight 164 could be coumaric acids. The third compounds present in the profile had formed at  $m/z$  at  $m/z$  163 [M + H] had molecular weight of

162 might be 3-hydroxycoumarin the fourth compound had formed fragment ion at  $m/z$  180 with molecular weight 123 might be benzoxazolone. The fifth one was deduced from  $m/z$  217 [M + Na] which have molecular weight 194 to be ferulic acids. The sixth compound appeared on LC profile deduced from  $m/z$  441 (M + K + Na) had molecular ions 358 which could be conclusively identified as sorgoleone the seventh compound deduced from  $m/z$  368 (2M + 3H<sub>2</sub>O + 2H) which have molecular weight 170, could be Gallic acid. The last compound gave protonated molecule ions  $m/z$  149 [M + H] with molecular weight 148 which might be identified as cinnamic acid. (Table 5 and Figure, 2).

**(B) The sorghum root part extracts** were extracted by shaking in methanol 70% and followed by partitioning with ethyl acetate, after dryness the samples and analyzed by LC-MS/MS. The first compound was deduced from 197 [M + CH<sub>3</sub>OH + H] which have molecular weight 164 and might be coumaric acid. The second compound corresponding to the analysis deduced from  $m/z$  245 [M + 2K + H] had molecular weight of 168 might be identified as vanillic acid.

The third compound had formed product ion at  $m/z$  328 [M + CAN + H] had molecular weight of 286 and might be identified as luteolin. The fourth one gave protonated molecule ions  $m/z$  149 [M + H] which have molecular weight 148 and might be identified as cinnamic acid. The fifth compound deduced from  $m/z$  163 (M + H) with a molecular weight (162) could be 3-Hydroxycoumarin. The sixth compound identified from  $m/z$  368 (2M + 3H<sub>2</sub>O + 2H) which have molecular weight 170 could be gallic acid. The seventh deduced from the protonated molecule ions at  $m/z$  217 (M + Na) and might be ferulic acid with molecular weight 194. The last compound appeared on LC profile deduced from  $m/z$  441 (M + K + Na), 419 (M + K + 2Na) had molecular ions

358, it could be conclusively identified as sorgoleone (Table 6 and Figure, 3).

This study deals with sorghum plant exudates and their activity against pathogenic fungus *Bipolaris sorokiniana* and identifying these signals which reflected root exudates activity. The results of this study are in agreement with those of Bonkowskia *et al.* (2000) who indicated that soil fauna has an important function in regulating rhizosphere microbial processes and therefore significantly affects plants growth. Plant growth can enhanced by beneficial bacteria and fungi (Saharan and Nehra, 2011) and induce against diseases (Ramamoorthy *et al.*, 2001). Bio-control traits of the Plant Growth Promoting Rhizobacterial isolates such as siderophore production and HCN production have also been studied (Apastambh *et al.*, 2016). Total counts of bacterial cell were investigated in both rhizosphere and non rhizosphere soil and the obtained results (Table 1) showed that the total bacterial counts in rhizosphere exceeded the total counts of bacteria in non-rhizosphere. These results are in agreement with Alpehi *et al.* (1996) who in their study separated the rhizosphere soil of wood-barley *Hordelymus europaeus* L. from the adjacent soil with 45- $\mu$ m gauze and found that the microbial biomass in the rhizosphere was almost doubled compared to the two adjacent 3mm wide compartments without roots.

**Table 5** Detected compounds from Sorghum root exudates through LC-MS/MS analysis.

Compounds	Molecular weight
Proline	115
Coumaric acid	164
3-Hydroxycoumarin	162
2-Benzoxazolone	123
Ferulic acid	194
Sorgoleone	358
Gallic acid	170
Cinnamic acid	148

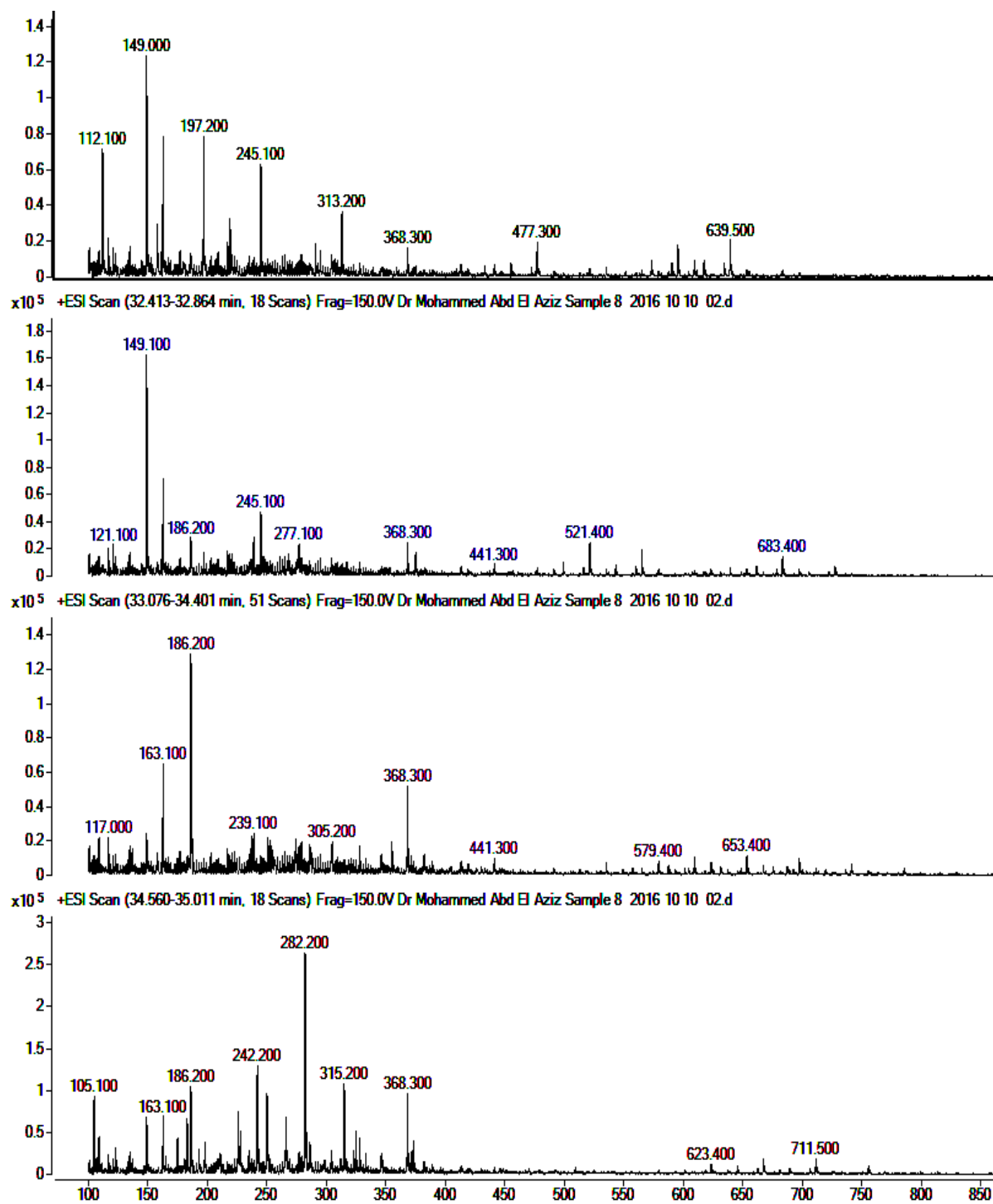


Figure 2 Sorghum root exudates extracted by ethyl acetate through LC-MS/MS.

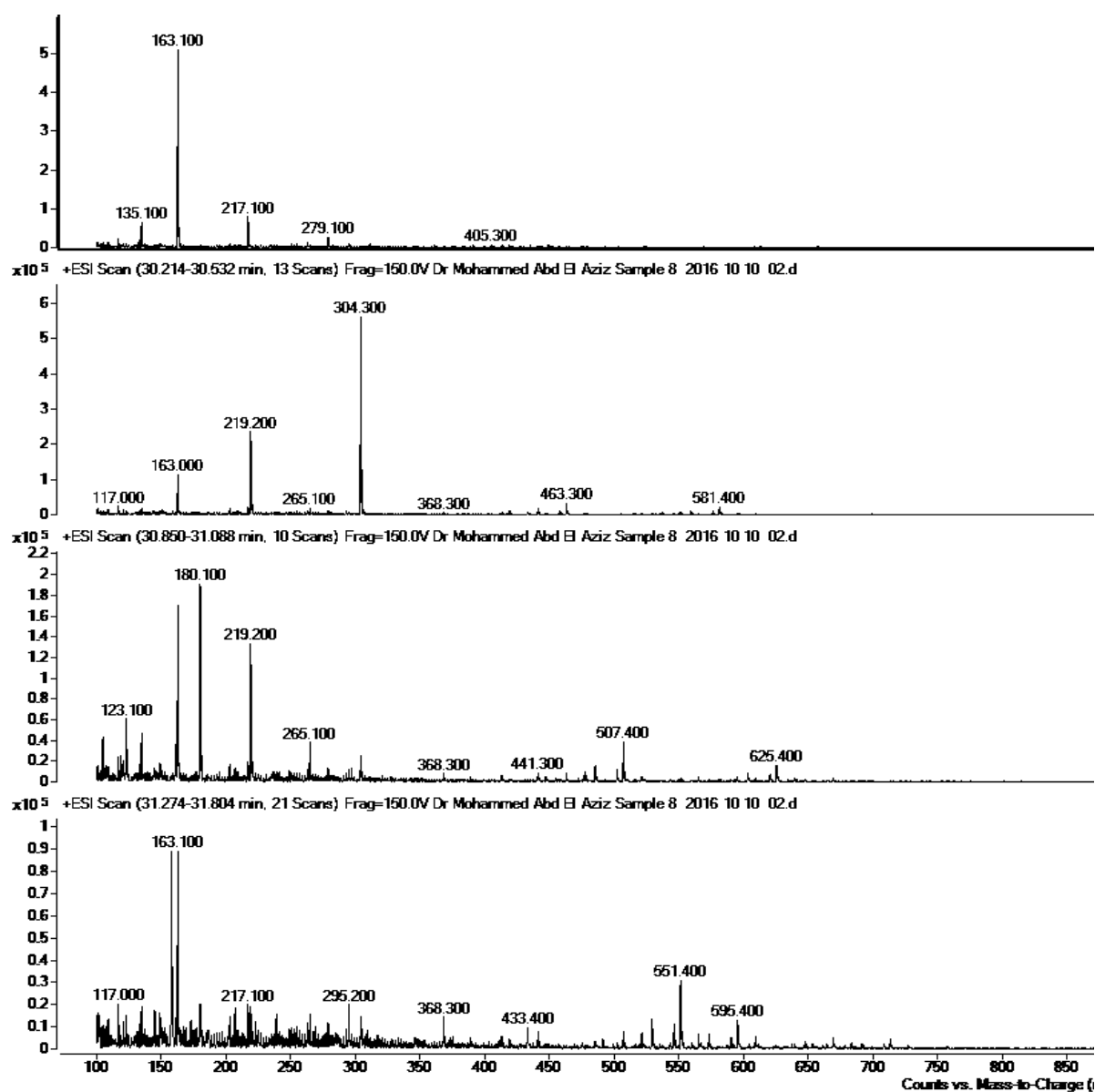


Figure 2 Sorghum root exudates extracted by ethyl acetate through LC-MS/MS (continued).

Table 6 Detected compounds from Sorghum root parts by LC-MS/MS.

Compounds	Molecular weight	Compounds	Molecular weight
Coumaric acid	164	3-Hydroxy Coumarin	162
Vanillic acid	168	Gallic acid	170
Luteolin	286	Ferulic acid	194
Cinnamic acid	148	Sorgoleone	358

In this part of study the antifungal activities of root exudates were bio-assayed against sorghum root rot pathogen isolated from diseased sorghum plants. After the pathogenicity test *in vitro*, the

obtained results indicated that sorghum roots and their exudates have varied allelopathic properties, attributed to many allelochemicals as indicated from the LCMS/MS analysis. Sorgoleone,



benzquinones and organic acids as well as flavonoids were detected (Tables, 5 and 6). These results are in agreement with Dayan *et al.* (2003) who reported that sorgoleone (2-hydroxy-5-methoxy-3-[(Z, Z)-8, 11, 14-pentadecatriene]-p-benzoquinone) is the main allelopathic component of the oily root exudates of sorghum *Sorghum*

*bicolor* (L.) Moench. Sorghum (*Sorghum bicolor*) root hairs release a substantial amount of phenolic lipids including sorgoleone, 3-pentadecatriene benzoquinone. Based on the spectral data, the active compounds were identified as diketopiperazine [cyclo(l-Pro-d-Leu)]. (Dayan *et al.* 2007).

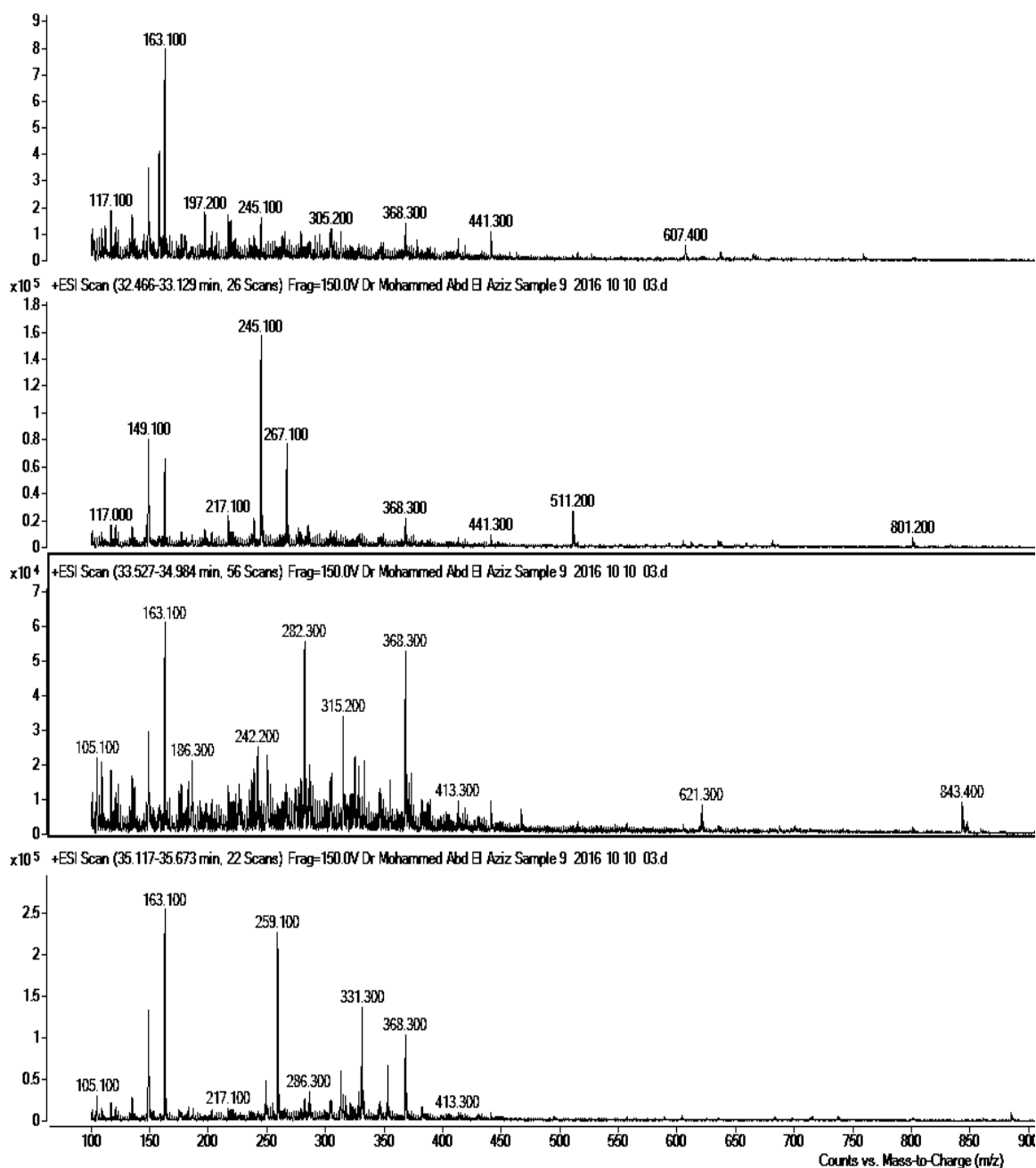
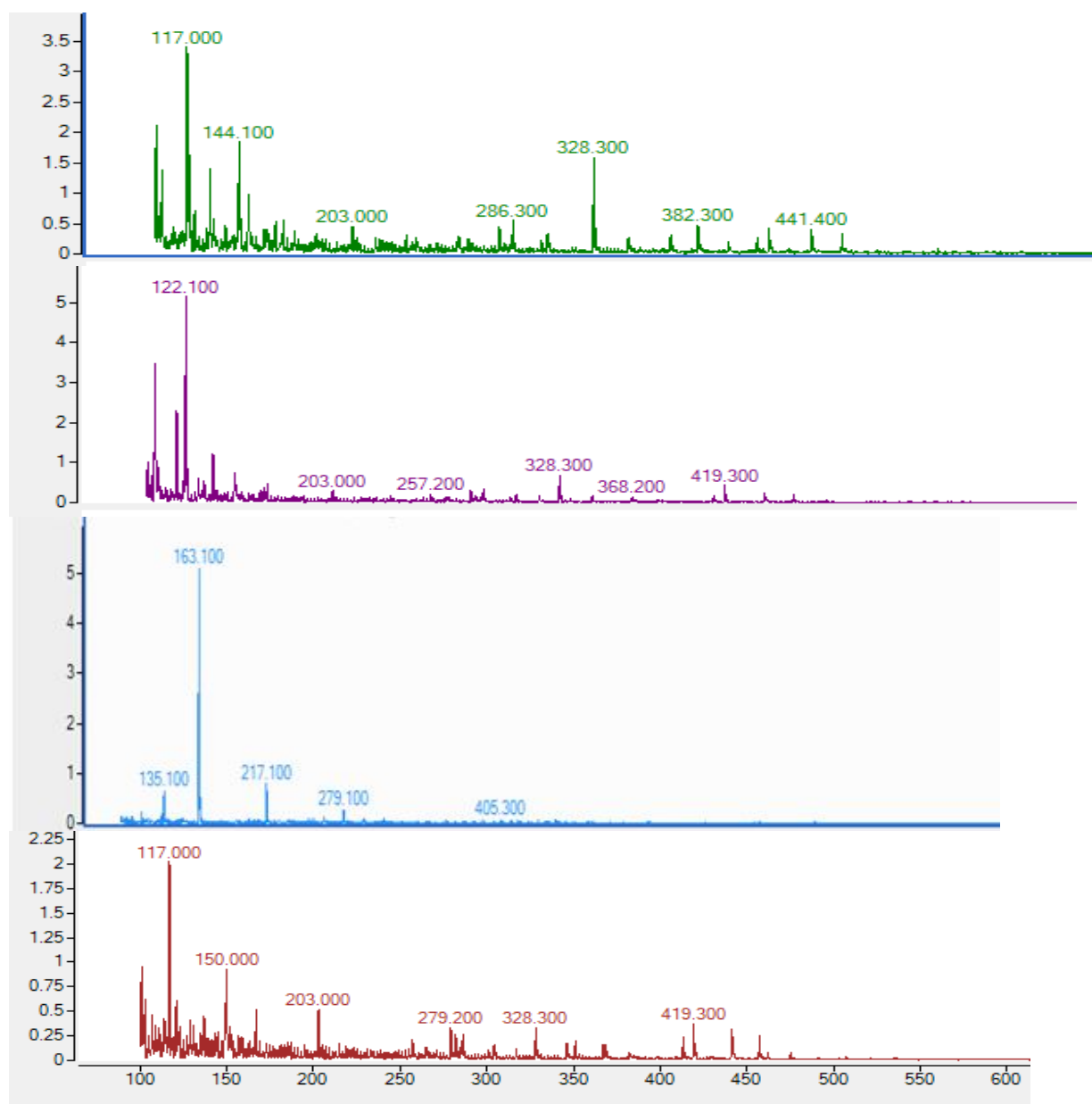


Figure 3 Sorghum root part extracts analyzed by LC-MS/MS.



**Figure 3** Sorghum root part extracts analyzed by LC-MS/MS (continued).

Rhizobacteria (*Bacillus* sp., *Enterobacter* sp, *Pseudomonas* sp.) isolated from native wild desert plants were found to have antagonistic activity against *F. oxysporum* and *Sclerotinia sclerotiorum* as well as unique PGP traits e.g., N<sub>2</sub>-fixation, indole acetic acid and siderophores production, mineral phosphate and zinc

solubilization, and antagonistic potentials (El-Sayed *et al.* (2014). *Pseudomonas fluorescens* was isolated from sorghum roots and surrounding soil with the goal of finding isolates that significantly inhibited sorghum fungal pathogens. *Pseudomonas fluorescens* was collected from seedlings of sorghum

cultivars RTx433 and Redlan and wheat cultivar Lewjain, grown in two soils (Harris *et al.* 2013). Plants attract specific microbes and therefore alter the composition and diversity of microbial communities in the rhizosphere in a plant-specific manner (Broeckling *et al.* 2008; Houlden *et al.* 2008).

This study is concerned with the idea that the sorghum plants could be well-matched with the associated bacteria in rhizospheric soil whereby the allelopathic abilities of sorghum is arranged through their interaction via the mediated allelochemicals as well as their growth and productivity. The role of rhizospheric bacteria is very important to sorghum growth, whereas the transduction signals activity as positive or negative as well as their concentrations should be undertaken and need further investigation especially under the stress conditions.

## References

- Alpei, J., Bonkowski, M. and Scheu, S. 1996. Protozoa, Nematoda and Lumbricidae in the rhizosphere of *Hordelymus europaeus* (Poaceae): Faunal interactions, response of microorganisms and effects on plant growth, *Oecologia*, 106: 111-126.
- Apastambh, A. R., Tanveer, K. and Baig, M. M. V. 2016. Isolation and Characterization of Plant Growth Promoting Rhizobacteria from Banana Rhizosphere. *Int. J. Curr. Microbiol. App. Sci.*, 5 (2): 59-65.
- Asiedu, A., E. Lied, Nilsen, R. and Sandne, K. 1993. Effect of processing (sprouting and/or fermentation) on sorghum and maize: II. Vitamins and amino acid composition. *Biological utilization of maize protein. Food Chem.*, 48: 201-204.
- Bais, H. P., Park, S. W., Weir, T. L., Callaway R. M. and Vivanco, J. M. 2004. How plants communicate using the underground information superhighway. *Trends in Plant Science*, 9: 26-32.
- Bonkowskia, M., Cheng, W., Griffithsc, B. S., Alpeid, J. and Scheu, S. 2000. Microbial-faunal interactions in the rhizosphere and effects on plant growth. *European Journal of Soil Biology*, 36: 135-147.
- Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K. and Vivanco, J. M. 2008. Root exudates regulate soil fungal community composition and diversity. *Applied Environmental Microbiology* 74 (3): 738-744.
- Curl, E. A. and Truelove, B. 1986. *The Rhizosphere (Advanced Series in Agricultural Sciences, Vol. 15)*. Springer Verlag, New York, USA.
- Dayan, F. E., Kagan, I. A. and Rimando, A. M. 2003. Elucidation of the biosynthetic pathway of the allelochemical sorgoleone using retrobiosynthetic NMR analysis. *The Journal of Biological Chemistry*, 278: 28607-28611.
- Dayan, F. E., Watson, S. B. and Nanayakkara, N. P. D. 2007. Biosynthesis of lipid resorcinols and benzoquinones in isolated secretory plant root hairs. *Journal of Experimental Botany*, 58 (12): 3263-3272.
- Dowling, D. N. and O'Gara, F. 1994. Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. *Trends in Biotechnology*, 12: 133-141.
- Dubrovskaya, E., Pozdnyakova, N., Golubev, S., Muratova, A., Grinevm, V., Bondarenkovam, A. and Turkovskaya, O. 2016. Peroxidases from root exudates of *Medicago sativa* and *Sorghum bicolor*: Catalytic properties and involvement in PAH degradation. *Chemosphere*, 169: 224-232.
- Duffy, B. 2003. Pathogen self-defense: Mechanisms to counteract microbial antagonism. *Annual Review of Phytopathology*, 41: 501-38.
- El-Sayed, W., Akhkha, A., El-Naggar M. Y. and Elbadr, M. 2014. In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. *Frontiers in Microbiology*, 5 (651): 1-11.
- Harris, F., Sattler, L. D., Scottand, E. and Pedersen, J. F. 2013. Characterization of fluorescent *Pseudomonas* spp. associated with roots and soil of two sorghum genotypes". *European Journal of Plant Pathology*, 136: 469-481.

- Houlden, A., Timms-Wilson, T. M., Day, M. J. and Bailey, M. J., 2008. Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. *FEMS Microbial Ecology*, 65 (2): 193-201.
- Hrynkiwicz, K. and Baum, C. 2012. The potential of rhizosphere microorganisms to promote the plant growth in disturbed soils. In: Malik, A. and Grohmann, E. (Eds.), *Environmental protection strategies for sustainable development, strategies for sustainability*, Springer, New York, pp. 35-64.
- Kumar, S. and Kaushik, N. 2013. Endophytic Fungi Isolated from Oil-Seed Crop *Jatropha curcas* Produces Oil and Exhibit Antifungal Activity. *PLoS ONE* 8 (2): e56202. doi:10.1371/journal.pone.0056202.
- Li, X. G., Zang, T. L., Wang, X. X., Hua, K., Zhao, L. and Han, Z. M. 2013. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-borne pathogen. *International Journal of Biological Science*, 9 (2): 164-173.
- Lugtenberg, B. and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, 63: 541-556.
- Mabry, T. J., Markham, K. R. and Thomas, M. B. 1970. *The Systematic Identification of Flavonoids*. Springer-Verlag, New York.
- Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. and Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection*, 20: 1-11.
- Saharan, B. S. and Nehra, V. 2011. Plant growth promoting rhizobacteria: A critical review. *Life Science Medical Research*, 21: 1-29.
- Schwab, S. M., Menge, J. A. and Leonard, R. T. 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of Sudangrass roots in relation to vesicular-arbuscular mycorrhiza Formation. *Plant Physiology*, 73: 761-765.
- Snedecor, G. W. and Cochran, W. G. 1990. *Statistical Methods*, 8<sup>th</sup> edition. Ames Iowa: Iowa State University Press.
- Walker, T. S., Bais, H. P., Grotewold, E. and Vivanco, J. M. 2003. Root Exudation and Rhizosphere Biology. *Plant Physiology*, 132: 44-51.
- Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. *Experimental Botany*, 52 (suppl 1): 487-511.
- Zhu, X., Weston, P., Norton, S., North, T., Godwin, I. and Weston, L. 2017. Comparison of Australian and African Sorghum root exudates: A case of long-term evolutionary adaptation? Abstract from 8<sup>th</sup> World Congress of Allelopathy, Marseille, France.

## فعالیت آللوپاتی عصاره و مواد مترشحه ریشه سورگوم *Sorghum bicolor* روی قارچ *Bipolaris sorokiniana*

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دریافت: ۱۰ آبان ۱۳۹۶؛ پذیرش: ۲۶ خرداد ۱۳۹۷

**چکیده:** گیاه سورگوم *Sorghum bicolor* L. از طریق ریشه مواد آللوکمیکیالی را به داخل خاک ترشح می کند که این مواد در ارتباط با میکروارگانسیم های خاک می تواند در حفاظت گیاه در برابر بیماری ها مؤثر باشد. نتایج به دست آمده نشان می دهد که عصاره و مواد مترشحه ریشه سورگوم به طور مؤثری روی جمعیت باکتری های موجود در ناحیه ریزوسفر خاک مؤثر است. در این پژوهش مواد مترشحه ریشه سورگوم استخراج گردید و در غلظت های ۵۰۰ و ۱۰۰۰ پی پی ام روی قارچ *Bipolaris sorokiniana* آزمایش گردید. درصد کاهش رشد قارچ ۴ و ۷ روز پس از تیمار مورد ارزیابی قرار گرفت. نتایج نشان داد که در غلظت های مورد استفاده بعد از ۴ روز رشد قارچ به ترتیب ۱۷/۵۳ و ۴۵/۶۳ درصد نسبت به شاهد کاهش یافته است. این در حالی است که بعد از ۷ روز رشد قارچ در غلظت های مورد استفاده به ترتیب ۱۷/۲۸ و ۳۶/۴۰ درصد کاهش یافته است. در مورد عصاره ریشه سورگوم کاهش رشد قارچ با افزایش غلظت بالا رفته است. به طوری که رشد قارچ در غلظت ۲۰۰ پی پی ام ۴۹/۷۱ درصد و در غلظت ۱۶۰۰ پی پی ام به ۷۱/۶۷ درصد کاهش داشته است. تجزیه مواد آللوکمیکیالی گیاه سورگوم با دستگاه LC-MS/MS و FTIR نشان داد که پرولین، کوماریک اسید، سینامیک اسید، هیدروکمارین، بنزوکسازولون، فروریک اسید و سورگولئون مواد اصلی ترکیبات مترشحه از ریشه را تشکیل می دهند. اما مواد موجود در عصاره ریشه سورگوم شامل کوماریک اسید، سینامیک اسید، وانیلیک اسید، لوتئولین ۳-هیدروکسی کمارین، گالیک اسید، فروریک اسید و سورگولئون می باشند. بنابراین مواد طبیعی مترشحه از ریشه و مواد استخراج شده از عصاره ریشه در بیوکنترل بیماری نقش مؤثری دارند و می توانند به عنوان مولکول های بیوشیمیایی مؤثر در جهت رسیدن به کشاورزی پایدار مورد استفاده قرار گیرند.

**واژگان کلیدی:** سورگوم، آللوکمیکیالی ها، مواد مترشحه ریشه، ریزوسفر، LC-MS/MS، FTIR