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Effect of different Humic Acids Sources on the Plant Growth, Calcium and Iron Utilization by Sorghum

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Humic acids are widely used in agriculture system for high quality plant production, but are these different source acids equally efficient. So, a pot experiment was carried out during two successive summer seasons (2016 and 2017) to study the effect of both individual and combined applications of different three sources of humic acids and either of Ca or Fe on growth and Ca and Fe utilization by sorghum plants, variety Giza 15, under sandy culture condition. Three humic acids isolated from different sources, *i.e.,* clayey soil (HAS); podrite (HAP) and compost (HAC) were used. Each HA was applied at rates of 0, 25, 50, 75 and 100 mg kg^{-1} refined sand. Either of Ca or Fe was added as acetate form (CH_3COO) at rates of 0, 25, 50 and 100 mg kg^{-1} refined sand. The experimental design was a split-split plot design with three replicates. Results showed that, applications of each three HA individually and in combination with Ca or Fe were associated by a significant increases of dry weights of shoots and roots of sorghum plants. The highest dry weights were found in the plants treated by HAC followed by those associated the treatments of HAP. So according the calculated RI (%) values for the mean treatments of different added Ca rates, the estimated dry matter yields of shoots and roots may be arranged as follows: HAC (137.0 and 179.1 %) > HAP (98.84 and 143.0 %) > HAS (98.04 and 84.31 %) respectively. While, the corresponding order with different addition of Fe rates were HAC (114.4 and 217.9 %) > HAP (108.8 and 148.2 %) > HAS (101.1 and 76.99 %) for shoots and roots dry matter yields, respectively. In addition, increasing application rates of HA and Ca or Fe were associated with increases of Ca and Fe uptake by both shoots and roots. The found order of the used HA on the increase of Ca and Fe uptake by shoots and roots of sorghum plants was: HAC > HAP > HAS. Moreover, the uptake of Ca and Fe by shoots was higher than that uptake by roots. On the other hand, increasing application rates of Ca and Fe decreased their utilization rate by sorghum plants. While, increasing application rates of HA were associated by an increase in the utilization rate of Ca and Fe by sorghum plants. Based on the obtained data, may be concluded that the fertilization program of sorghum plants must be include applications of HA especially HAC with Ca and Fe to obtained high quantity and quality of sorghum plants. Also, organic amendments must be used to increase Ca and Fe fertilizers efficiency.

Key words: Humic acids, Calcium, Iron, Nutrient uptake, Nutrient utilization rate, Sorghum plants

Introduction

Humic acids are a commercial product contains many nutrients which improve the soil fertility and increasing the phyto-availability of nutrients and consequently affected plant growth and yield. Humic acids particularly is used to remove or decrease the negative effects of mineral fertilizers and some chemicals forms in the soil. So, humic substances have many beneficial effects on soil and consequently on plant growth and are shown highly hormonal activity. These materials not only increase macronutrients contents and ions uptake but also enhance micronutrients of the plant organs (Brunetti et al. 2005). In addition, Montaser et al. (2011) reported that the humic acids (HA) may increase the permeability of plant membranes and enhance the uptake of

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nutrients. Moreover, HA may also improve soil nitrogen uptake and facilitate the uptake of potassium, calcium, magnesium and phosphorus, making these nutrients more mobile and available to plant root systems. Also, humic substances are organic substances of high molecular weights, and they are naturally widespread in aquatic and terrestrial environments. Iron-reducing bacteria can substitute humic substances for Fe(III) as the terminal electron acceptor. The ecological significance of humic acid reduction in natural environments is not well understood (Kappler et al. 2004). A further understanding of the relation between humic substance reduction and iron is important and requires knowledge about the diversity of microbes that might be responsible for this reduction in sedimentary environments.

Calcium is one of an essential macronutrients; however, its role is elusive. When examining total Ca in plants, the concentration is quite large (mM), but its requirement is that of a micronutrient (μM). Calcium is not usually limiting in field conditions, still there are several defects that can be associated with low levels of this ion, including poor root development, leaf necrosis and curling, blossom end rot, bitter pit, fruit cracking, poor fruit storage, and water soaking. The underlying causes for these effects are not entirely clear; nevertheless, two areas within the cell have been recognized as being important targets. First is the cell wall, where Ca plays a key role in cross-linking acidic pectin residues. The second is the cellular membrane system, where low Ca increases the permeability of the plasma membrane (Hepler, 2005).

Also, iron (Fe) is one of an essential micronutrients for plants. In this concern, Marschner (2012) reported that Fe is most important for the respiration and photosynthesis processes. Iron is also implied in many enzymatic systems like chlorophyll synthesis. The mobility of iron in the plant is very low. Therefore, the species of Fe in the soil environment could be summarized in the following: (1) Fe^{II} in primary minerals; (2) Fe^{III} in secondary minerals, as Fe crystalline minerals and poorly ordered crystalline (hydro) oxides; (3) soluble and exchangeable Fe; and (4) Fe bound to organic matter in soluble or insoluble forms. In addition, iron mobilization was influenced by the complexation of iron to soluble inorganic ligands through iron-binding molecules of low molecular weight called siderophores, which are released from microbes (Lemanceau et al. 2009) or phytosiderophores which are released from graminaceous plant species (Awad et al., 1988 and Awad et al. 1994). This process also causes iron to be removed not only from iron minerals but also from organic complexes such as organic acids, phenols, and soil humic substance ligands (Colombo et al. 2014).

Sorghum Vulgare var saccharatum, of family Gramineae, commonly called sorghum and also known as great millet, durra, jowari, or milo, is a grass species cultivated for its grain, which is used for food, both for animals and humans, and for ethanol production. Sorghum originated in northern Africa, and is now cultivated widely in tropical and subtropical regions. Sorghum is the world's fifth most important cereal crop after wheat, rice, maize and barley. Sorghum has a variety of uses including food for human consumption, feed grain for livestock and industrial applications such as ethanol production (Delserone, 2008). Also, FAO (2012) showed that, the area planted to sorghum worldwide has increased by 66 percent over the past 50 years while yield has increased by 244 percent. The leading producers of sorghum bicolor in 2011 were Nigeria (12.6%), India (11.2%), Mexico (11.2%) and the United States (10.0%). Sorghum grows in a wide range of temperature, high altitudes, toxic soils and can recover growth after some drought. It has four features that make it one of the most drought-resistant crops:

- It has a very large root-to-leaf surface area ratio.
- In times of drought, it will roll its leaves to lessen water loss by transpiration.
- If drought continues, it will go into dormancy rather than dying.
- Its leaves are protected by a waxy cuticle.

Thereafter, this pot experiment was carried out using sandy culture to study the effect of both individual and combined applications of different sources and rates of humic acids and either of Ca or Fe on sorghum plants growth and its chemical composition. Also, the role of humic acids on Ca and Fe utilization by sorghum plants was assessment.

Materials and Methods

This study was carried out using sandy culture as a pot experiment in greenhouse at the Experimental Farm of Soils, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt with sorghum plants (Sorghum Vulgare var saccharatum), variety Giza 15 as a test plant during two successive growth summer seasons of 2016 and 2017 to study the effect of both individual and combined applications of different three sources of humic acids and either of Ca or Fe at different application rates on sorghum plants growth and its some chemical composition. Also, the role of humic acids on Ca and Fe utilization by sorghum plants was assessment. Each humic acid was applied at rates of 0, 25, 50, 75 and 100 mg kg^{-1} sand. Either of Ca or Fe was added as acetate form (CH_3COO) and at rates of 0, 25, 50 and 100 mg kg^{-1} sand.

Humic acids preparation

In this study, three humic acids isolated from different sources were used. The first humic acid (HAS) used in this study was extracted from the clayey soil of Experimental Farm of the Soils, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt; the second humic acid (HAP) was isolated from podrite and the third one (HAC) was extracted from compost of clover straw, where each acid was applied at rates of 0, 25, 50, 75 and 100 mg kg^{-1} sand. These humic acids were extracted, fractionated and purified according to the methods described by Kononova (1966); Posner(1966); Chen et al. (1978) and Schnitzer and Khan (1978). The purified humic acids content of C, N, P and H was determined according to Mann and Sounders (1966) ; Bremmes and Mulvaney (1982) and Olsen and Sommers (1982). Humic acids content of oxygen (O) was calculated by subtracting the content (g kg^{-1}) of C, N, P, H, Ca and Fe from the total of 1000. Ash content (%) of these humic acids was estimated by burning the oven dry humic acid at 750 ^{o}C for 24 hr (Holder and Griffith, 1983). The obtained results of the total nutrients and the calculated atomic ratios for the three humic acids arre recorded in Table 1. Also, the studied humic acids contents of total acidity and some functional groups, *i.e.* carboxyl (COOH), total-OH, phenolic-OH and alcoholic –OH were determined according to the methods described by Dragunova (1958) ; Kukhareko (1937) and Brooks et al. (1958) and the obtained data are recorded in Table 2.

Sandy culture preparation

Sand used in this study was taken from desert part of Quessna region, Minufia Governorate. Sand was sieved through a 2 mm sieve, washed by tap water, treated with diluted HCl (6%) and H_2O_2 (30%) to remove the carbonate and oxidize the organic matter, respectively. The treated sand was washed several times with tap water followed by distilled water up to the sand become free from Cl. The refined sand was air-dried kept for using.

Nutrient solution

The pots were irrigated every two days in the using nutrient solution alternated with tap water at the moisture content of 60 % of water holding capacity. The composition of the complete essential nutrient (normal) solution was: 4 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 100 µM Fe-EDTA, 10 µM $MnSO_4$, 30 µM H_3BO_3, 1 µM $ZnSO_4$, 0.2 µM Na_2MoO_4, 0.1 µM $CoSO_4$, 0.1 µM $NiSO_4$ and 0.1 mM NaCl (Agarwala and Chatterjee 1996).

Pot experiment

A 360 plastic pots with 25 cm inter diameter and 20 cm depth were used in this study. Each pot was filled by 3 kg of refined and air-dried sand (sandy culture). Each pot was cultivated by 8 grains of sorghum, the rates of seeds cultivated were taken from Egyptian Ministry of Agriculture recommendations. In 17 May 2016 and 2017, the cultivated pots were irrigated using tap water at 60 % of water holding capacity. The layout of the experiment was a split – split - plot design, with the main plots arranged in a randomize complete block design, with three replicates. After 10 days of planting, the plants of each pot were thinned at 5 plants. After thinning directly, the pots were divided into three main groups (120 pot /main group) representing the main factor or humic acids (HAS ; HAP and HAC) treatments. The pots of each main group were divided into equal five sub groups (24 pot for each sub group) which treated by one application rate of humic acid (0, 25, 50, 75 and 100 mg kg^{-1} refined sand). At the same time, the pots of each subgroup were divided into two sub sub groups (12 pot / sub sub group) representing the treatment of Ca and Fe. The pots of each sub sub group were divided into equal four groups (3 pot for each group), where the pots of each final group were treated by one concentration of Ca or Fe. Both Ca and Fe was added as acetate form (CH_3COO) and at rates of 0, 25, 50 and 100 mg kg^{-1} refined sand with irrigation water.

TABLE 1. Chemical analysis of the studied three humic acids

Humic acids	Total nutrients (g kg^{-1})							Atomic ratios				Ash (%)
	C	H	N	P	O	Ca	Fe (mg kg^{-1})	C/H	C/O	C/N	C/P	
HAS	465.4	61.5	22.5	8.5	430.4	11.4	298	7.57	1.08	20.7	54.8	1.85
HAP	451.8	55.0	29.8	6.8	441.8	14.5	335	8.21	1.02	15.2	66.4	2.25
HAC	438.5	52.8	27.0	6.3	462.8	12.2	421	8.30	0.95	16.2	69.6	1.70

TABLE 2. Total acidity and some functional groups (meq $100g^{-1}$ HA) of the three studied humic acids

Humic acids	Total acidity	COOH	Total - OH	Phenolic - OH	Alcoholic -OH
HAS	580.4	270.1	445.8	310.3	135.5
HAP	630.8	305.4	505.6	350.4	150.2
HAC	710.50	330.4	527.6	380.1	182.5

Preparation and analyses of plant samples

At the end of the two seasons (after 65 days of planting), the plants of each treatment were harvested separately from each pot as a whole and separated into shoots and roots. Both shoots and roots of each sample were washed with tap-water and then 2 times with distilled water, air- dried, oven-dried at 70 ^{o}C for 48 hours until weight becomes constant and weighted to record the dry weights (g pot^{-1}). The plant samples were ground separately to a fine powder in a stainless grinder and stored in plastic bags until analysis. Half g portions of each dried plant sample was digested by a concentrated mixture of H_2SO_4 + $HClO_4$ at (5 : 0.5) ratio according to Chapman and Pratt (1961). The content of both Ca and Fe was determined in the find plant digests. The calcium was determined by titration method with EDTA standard solution and ammonium perpurity as indicator according to Cottenie *et al.* (1982). Also, iron content was measured using Perkin Elmer atomic absorption, spectrophotometer model 2830.

The assessment of the studied parameters

Relative increase percentage (RI) of the obtained dry matter yields of sorghum plants (shoots and roots) as affected by the studied treatments was calculated by the following equation:-

RI= [(dry matter yield of treated plants - dry matter yield of untreated plants)/ dry matter yield of untreated plants] ×100

The utilization rate for Ca and Fe application by shoots and roots of sorghum plants were fertilized by different three sources of humic acids may be calculated using the following equation:

$SU = [(PS_T - PS_O) / S_T] \times 100$ (Fouda *et al.* 2013).

where : SU (%) = Ca or Fe utilization rate.

PS_T = Ca or Fe uptake (mg pot^{-1}) by treated plants at different application rates.

PS_O = Ca or Fe uptake (mg pot^{-1}) by untreated plants with Ca or Fe.

S_T = Application rate (mg kg^{-1}) of Ca or Fe.

The data were exposed to statistical analysis according to Gomez and Gomez (1984). The significant differences among means were tested using the least significant differences (L.S.D.) at 5 % level of significant error.

Results and Discussion

Effect of different sources and rates of humic acids and four application rates of Ca and Fe on:

Dry matter yield of sorghum plants

Data in Tables 3 to 6 and Fig. 1 to 4 show, the dry matter yield (g pot-1) of sorghum plants (shoots and roots) and their relative increases (RI, %). Increasing rates of added humic acids were associated by a significant increases of both roots and shoots dry matter yield under different application rates of Ca and Fe. The found increases in dry matter yield of sorghum as a result of humic acids applications widely varied according to the added humic acid resource. According to the found dry matter yield and their relative increase (RI ,%) in the plants treated only with humic acids, the used humic acids takes the order: HAC = HAP > HAS for shoots and was HAC > HAP > HAS for roots (based on the mean values of the used humic acids applications in the two growing seasons). This order was cleared from the calculated values of relative increases (RI) of dry matter yields of sorghum (shoots and roots) plants as a results of humic acids application, where the highest positive values of RI (%) were found in the plants treated with HAC followed by these found in the plants treated with HAP. These increase of sorghum (shoots and roots) dry matter yield mainly resulted from many essential macro- and micronutrients presented in the used humic acids (Tables, 1 and 2) and also to their effect on improving growth media. The same significant increase effect of humic acid applications on the dry matter yield was reported by many investigators such as Morard et al. (2011) on various plant species; Mohamed (2012) on maize; Nada and Tantawy (2012) on tomato and Abd El-Kader (2016) on wheat. With all individual treatments of humic acids, the dry matter yields (g pot-1) for shoots were higher than those of roots (Tables 3 to 6).

Data in Tables 3 & 4 and Fig. 1 & 2 show, the effect of individual applications of Ca on dry matter yield of sorghum (shoots and roots) plants and their relative increase (RI, %). The dry matter yields of both shoots and roots increased significantly with the increase rate of added Ca, so RI (%) values were increased and become more positive at high rates of added Ca. For example RI increased from 37.68 % at rate of 25 mg Ca kg-1 to 105.71 % at rate of 100 mg Ca kg-1 for shoots dry matter yield and increased from10.30 % to 68.91 % with the increase of added Ca from 25 to 100 mg kg-1 for roots dry matter yield. Also, data in the same table showed that, at the same application rate of Ca, the dry matter yield of shoots was higher than that of roots. The significant increase effect of Ca on plant growth resulted from cross-linking acidic pectin residues. Also, Ca is the cellular membrane system, where low it increases the permeability of the plasma membrane (Hepler, 2005 and Marschner, 2012). In this respect Tuna et al. (2007) and Mohamed (2012) obtained on similar results.

In addition, combined applications of both humic acids (HAS, HAP and HAC) and Ca at different rates resulted in a significant increases of sorghum (shoots and roots) plants dry matter yield (g pot-1) compared with their individual applications (Tables, 3 and 4). These findings may be cleared and supported by calculated RI (%) values, where these values were increased with the increase rate of added humic acids and Ca. The found increases of dry matter yields of sorghum plants as a result of humic acids and Ca application together resulted from their effect on plant growth by increasing enzymes activity and metabolic processes and also to improve growth media conditions as premeditation by many investigators (Nardi et al., 2002; Marschner, 2012 and Mohamed, 2012). In all combined treatments of humic acids and calcium, dry matter yields of shoots were higher than those roots. At the same treatment of humic acid and Ca, RI (%) of shoots dry matter yield was higher than that of roots. This trend means that, shoots of sorghum plants appeared high response to different applications of humic acids and Ca higher than that found with the roots. Within the combined treatments of humic acids and Ca there are clear variations in the obtained dry matter yields of both shoots and roots affected by the source of added humic acid, where the highest yields and relative increase were found in the plants treated by HAC and the lowest values were found in the plants treated by HAS. This trend showed the clear effect of humic acid chemical composition on plant growth (Thah et al., 2006; Hussein & Hassan, 2011 and Turan et al., 2011).

TABLE 3 . Dry matter yield of shoots and roots (g pot^{-1}) of sorghum as affected by different sources and rates of humic acids under four application rates of Ca (mean values two seasons)

Humic acids treat-ments		Shoots					Roots				
		Added Ca rate (mg kg^{-1})					Added Ca rate (mg kg^{-1})				
Source	Rate (mg kg^{-1})	0	25	50	100	Mean	0	25	50	100	Mean
HAS	0	1.733	2.386	2.919	3.565	2.651	1.039	1.146	1.286	1.755	1.307
	25	1.930	2.690	3.235	4.154	3.002	1.068	1.460	1.470	1.972	1.493
	50	2.038	2.900	3.590	4.654	3.296	1.148	1.746	1.972	2.105	1.743
	75	2.183	3.283	4.669	5.632	3.942	1.181	2.143	3.094	3.137	2.389
	100	2.332	3.541	4.940	6.266	4.270	1.193	2.245	3.308	3.832	2.645
	Mean	2.043	2.960	3.871	4.854	3.432	1.126	1.748	2.226	2.561	1.915
L.S.D. at (0.05)		0.274	0.643	1.277	1.643		0.211	0.417	1.002	1.085	
HAP	0	1.733	2.386	2.919	3.565	2.651	1.039	1.146	1.286	1.755	1.307
	25	1.935	2.453	3.022	3.658	2.767	1.251	1.620	1.970	2.402	1.811
	50	2.010	2.718	3.438	4.472	3.160	1.512	2.427	2.760	2.837	2.384
	75	2.290	2.989	4.776	5.845	3.975	1.746	2.543	3.511	4.550	3.088
	100	2.451	3.469	5.696	7.093	4.677	2.749	2.967	4.737	5.691	4.036
	Mean	2.084	2.803	3.970	4.927	3.446	1.659	2.141	2.853	3.447	2.525
L.S.D. at (0.05)		0.281	0.675	1.288	1.721		0.536	0.518	1.023	1.283	
HAC	0	1.733	2.386	2.919	3.565	2.651	1.039	1.146	1.286	1.755	1.307
	25	1.946	2.964	3.764	4.902	3.394	1.553	1.931	2.104	2.693	2.070
	50	1.984	3.592	4.367	5.455	3.850	1.572	3.116	3.168	3.702	2.890
	75	2.295	4.258	5.843	7.450	4.962	2.559	3.134	4.313	5.610	3.904
	100	2.460	4.859	6.588	8.820	5.682	3.014	3.553	4.748	5.997	4.328
	Mean	2.084	3.612	4.696	6.038	4.108	1.947	2.576	3.124	3.952	2.900
L.S.D. at (0.05)		0.384	0.747	1.461	1.984		0.561	0.549	1.045	1.327	
Mean	0	1.733	2.386	2.919	3.565	2.651	1.039	1.146	1.286	1.755	1.307
	25	1.937	2.702	3.340	4.238	3.054	1.291	1.670	1.848	2.356	1.791
	50	2.011	3.070	3.798	4.860	3.435	1.411	2.430	2.633	2.881	2.339
	75	2.256	3.510	5.096	6.309	4.293	1.829	2.607	3.639	4.431	3.127
	100	2.414	3.956	5.741	7.393	4.876	2.319	2.922	4.264	5.173	3.670
General means		2.070	3.125	4.179	5.273	3.662	1.578	2.155	2.734	3.319	2.447
L.S.D. at (0.05)		0.301	0.700	1.375	1.759		0.529	0.545	1.031	1.289	

TABLE 4 . Relative increase (RI,%) of shoots and roots dry weight of sorghum as affected by different sources and rates of humic acids under four application rates of Ca (mean values of two seasons)

Humic acids treatments		Shoots					Roots				
		Added Ca rate (mg kg^{-1})					Added Ca rate (mg kg^{-1})				
Source	Rate (mg kg^{-1})	0	25	50	100	Mean	0	25	50	100	Mean
HAS	0	-	37.68	68.44	105.71	52.96	-	10.30	23.77	68.91	25.75
	25	11.37	55.22	86.67	139.70	73.24	2.79	40.52	41.48	89.80	43.65
	50	17.60	67.34	107.16	168.55	90.16	10.49	68.05	89.80	102.60	67.74
	75	25.97	89.44	169.42	224.99	127.46	13.67	106.26	197.79	201.92	129.91
	100	34.56	104.33	185.05	261.57	146.38	14.82	116.07	218.38	268.82	154.52
	Mean	17.90	70.80	123.35	180.10	98.04	8.35	68.24	114.24	146.41	84.31
HAP	0	-	37.68	68.44	105.71	52.96	-	10.30	23.77	68.91	25.75
	25	11.66	41.55	74.38	111.08	59.67	20.40	55.92	89.61	131.18	74.28
	50	15.98	56.84	98.38	158.05	82.31	45.52	133.59	165.64	173.05	129.45
	75	32.14	72.48	175.59	237.28	129.37	68.05	144.75	237.92	337.92	197.16
	100	41.43	100.17	228.68	309.29	169.89	164.58	185.56	355.92	447.74	288.45
	Mean	20.24	61.74	129.09	184.28	98.84	59.71	106.02	174.57	231.76	143.02
HAC	0	-	37.68	68.44	105.71	52.96	-	10.30	23.77	68.91	25.75
	25	12.29	71.03	117.20	182.86	95.85	49.47	85.85	102.50	159.19	99.25
	50	14.48	107.27	151.99	214.77	122.13	51.30	199.90	204.91	256.30	178.10
	75	32.43	145.70	237.16	329.89	186.30	146.29	201.64	315.11	439.94	275.75
	100	41.95	180.38	280.15	408.94	227.86	190.09	241.96	356.98	477.19	316.56
	Mean	20.23	108.41	170.99	248.43	137.02	87.43	147.93	200.65	280.31	179.08

Individual applications of Fe effect on sorghum plants (shoots and roots) calculated as g pot-1 have a significant increases as shown in Table 5 and Fig. 3 & 4. These increases were more clear at high rates of added Fe as shown from the calculated RI (%) values for both shoots and roots (Table 6). At the same rate of Fe applications, dry matter yields of shoots and its RI were higher than those of roots. This trend means that, Fe application have a greater effect on growth compared with that observed with that of roots. The beneficial effect of Fe on yield may be attributed to the enhancement effect of Fe on respiration and photosynthesis processes and many enzymes activities like chlorophyll synthesis. These results are comparable to those reported by Mohammad et al. (2009) on wheat ; Eisa and Taha (2010) on wheat and Helmy (2015) on maize.

Regarding to the specific effect of different individual applications of both Ca and Fe on the dry matter yield (g pot-1) of sorghum plants (shoots and roots) as listed in Tables (3 and 5) may be observed that, at the same rates of Ca or Fe , the found dry matter yields of both shoots and roots of sorghum plants treated by Fe were higher than those found in the plants treated by Ca. So, RI values (%) of dry matter yields of sorghum plants treated by Fe were high positive compared with these calculated for sorghum plants treated by Ca. This means that, Fe application to sorghum plants is very important to obtain on high yield of this plant. So, fertilization program of sorghum plants must be include Fe application.

Data in Tables 5 & 6 and Fig. 3 & 4 showed that, the combined effect of three humic acids varied in their resources and Fe at different application rates on both dry matter yield (g pot-1) of sorghum plants (shoots and roots) and their relative increase (RI, %). These data showed a significant increase of the found dry matter yield of sorghum plants and its relative increase as a result of humic acids and Fe application together. The high yields of dry matter of sorghum plants were found at high application rates of humic acid and Fe, where the highest values were found in the plants treated by HAC with Fe, but the lowest values were found in the plants treated by HAS with Fe. In this respect, Taha et al. (2006); Alhendawi et al. (2008); Eisa and Taha (2010) and Colombo et al. (2014) obtained on similar results.

TABLE 5 . Dry matter yield of shoots and roots (g pot⁻¹) of sorghum as affected by different sources and rates of humic acids under four application rates of Fe (mean values two seasons)

Humic acids treat-ments		Shoots					Roots				
		Added Fe rate (mg kg^{-1})					Added Fe rate (mg kg^{-1})				
Source	Rate (mg kg^{-1})	0	25	50	100	Mean	0	25	50	100	Mean
HAS	0	1.733	2.694	3.186	4.613	3.057	1.039	1.460	1.570	2.245	1.578
	25	1.930	2.915	3.309	4.711	3.217	1.068	1.492	1.790	2.386	1.684
	50	2.038	3.149	3.444	4.895	3.383	1.148	1.696	1.900	2.810	1.888
	75	2.183	3.260	3.862	5.523	3.709	1.181	1.806	1.947	2.826	1.939
	100	2.332	3.530	4.034	6.347	4.062	1.193	1.868	2.104	3.250	2.104
	Mean	2.043	3.110	3.567	5.218	3.486	1.126	1.664	1.862	2.703	1.839
L.S.D. at (0.05)		0.274	0.274	0.699	1.235		0.211	0.211	0.737	1.220	
HAP	0	1.733	2.694	3.186	4.613	3.057	1.039	1.460	1.570	2.245	1.578
	25	1.935	2.817	3.321	4.748	3.204	1.251	1.837	1.994	2.638	1.931
	50	2.010	2.866	3.592	4.982	3.361	1.512	2.104	2.229	3.140	2.245
	75	2.290	3.198	4.071	5.929	3.872	1.746	2.591	2.857	4.004	2.799
	100	2.451	3.690	4.797	7.454	4.597	2.749	3.988	4.192	6.437	4.341
	Mean	2.084	3.053	3.793	5.545	3.604	1.659	2.396	2.568	3.693	2.579
L.S.D. at (0.05)		0.281	0.281	0.687	1.254		0.536	0.536	0.764	1.310	
HAC	0	1.733	2.694	3.186	4.613	3.057	1.039	1.460	1.570	2.245	1.578
	25	1.946	3.087	3.272	4.895	3.299	1.553	2.167	2.543	3.470	2.434
	50	1.984	3.235	3.739	5.166	3.530	1.572	2.481	3.894	5.260	3.301
	75	2.295	3.309	4.416	6.236	4.065	2.559	4.066	4.490	6.359	4.369
	100	2.460	3.727	4.846	7.454	4.622	3.014	4.255	4.710	7.348	4.832
	Mean	2.084	3.210	3.892	5.673	3.715	1.947	2.886	3.441	4.936	3.303
L.S.D. at (0.05)		0.348	0.384	0.734	1.307		0.561	0.561	0.783	1.340	
Mean	0	1.733	2.694	3.186	4.613	3.057	1.039	1.460	1.570	2.245	1.578
	25	1.937	2.940	3.301	4.785	3.240	1.291	1.832	2.109	2.831	2.016
	50	2.011	3.083	3.592	5.014	3.425	1.411	2.094	2.674	3.737	2.478
	75	2.256	3.256	4.116	5.896	3.882	1.829	2.821	3.098	4.396	3.036
	100	2.414	3.649	4.559	7.085	4.427	2.319	3.370	3.669	5.678	3.759
General mean		2.070	3.124	3.751	5.479	3.606	1.578	2.315	2.624	3.777	2.573
L.S.D. at (0.05)		0.301	0.403	0.704	1.284		0.529	0.809	0.774	1.332	

TABLE 6 . Relative increase (RI,%) of shoots and roots dry weight of sorghum as affected by different sources and rates of humic acids under four application rates of Fe (mean values of two seasons)

Humic acids treat-ments		Shoots					Roots				
		Added Fe rate (mg kg^{-1})					Added Fe rate (mg kg^{-1})				
Source	Rate (mg kg^{-1})	0	25	50	100	Mean	0	25	50	100	Mean
HAS	0	-	55.45	83.84	166.19	76.37	-	40.52	51.11	116.07	51.93
	25	11.37	68.21	90.94	171.84	85.59	2.79	43.60	72.28	129.64	62.08
	50	17.60	81.71	98.73	182.46	95.13	10.49	63.23	82.87	170.45	81.76
	75	25.97	88.11	122.85	218.70	113.91	13.67	73.82	87.39	171.99	86.72
	100	34.56	103.69	132.78	266.24	134.32	14.82	79.79	102.50	212.80	102.48
	Mean	17.90	79.43	105.83	201.09	101.06	8.35	60.19	79.23	160.19	76.99
HAP	0	-	55.45	83.84	166.19	76.37	-	40.52	51.11	116.07	51.93
	25	11.66	62.55	91.63	173.98	84.96	20.40	76.80	91.92	153.90	85.76
	50	15.98	65.38	107.27	187.48	94.03	45.52	102.50	114.53	202.21	116.19
	75	32.14	84.54	134.91	242.12	123.43	68.05	149.37	174.98	285.37	169.44
	100	41.43	112.93	176.80	330.12	165.32	164.58	283.83	303.46	519.54	317.85
	Mean	20.24	76.17	118.89	219.98	108.82	59.71	130.60	147.20	255.42	148.23
HAC	0	-	55.45	83.84	166.19	76.37	-	40.52	51.11	116.07	51.93
	25	12.29	78.13	88.81	182.46	90.42	49.47	108.57	144.75	233.97	134.19
	50	14.48	86.67	115.75	198.10	103.75	51.30	138.79	274.78	406.26	217.78
	75	32.43	90.94	154.82	259.84	134.51	146.29	291.34	332.15	512.03	320.45
	100	41.95	115.06	179.63	330.12	166.69	190.09	309.53	353.32	607.22	365.04
	Mean	20.23	85.25	124.57	227.34	114.35	87.43	177.75	231.22	375.11	217.88

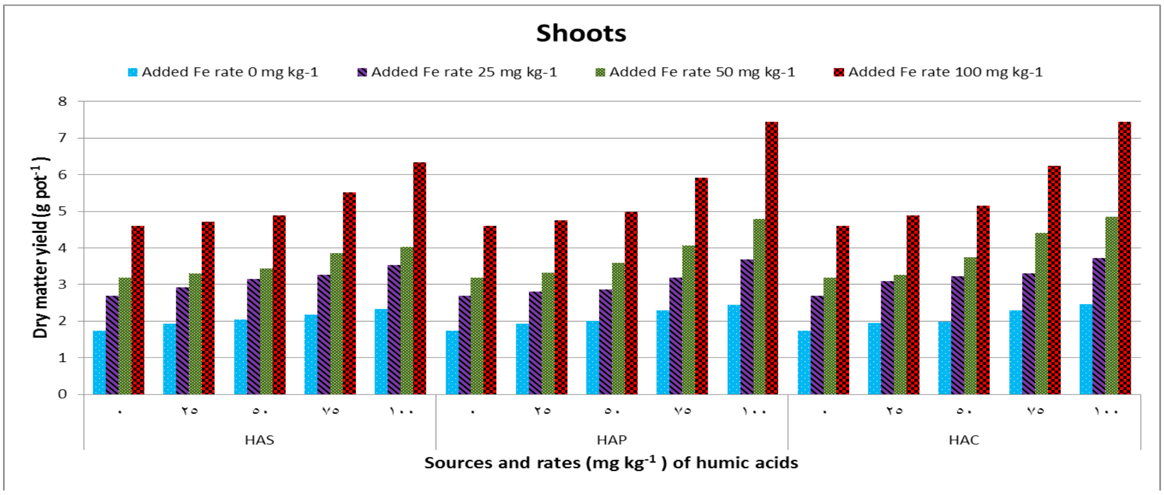

Fig. 1. Effect of different sources and rates of humic acids on shoots dry matter yield of sorghum plants under different application rates of Ca

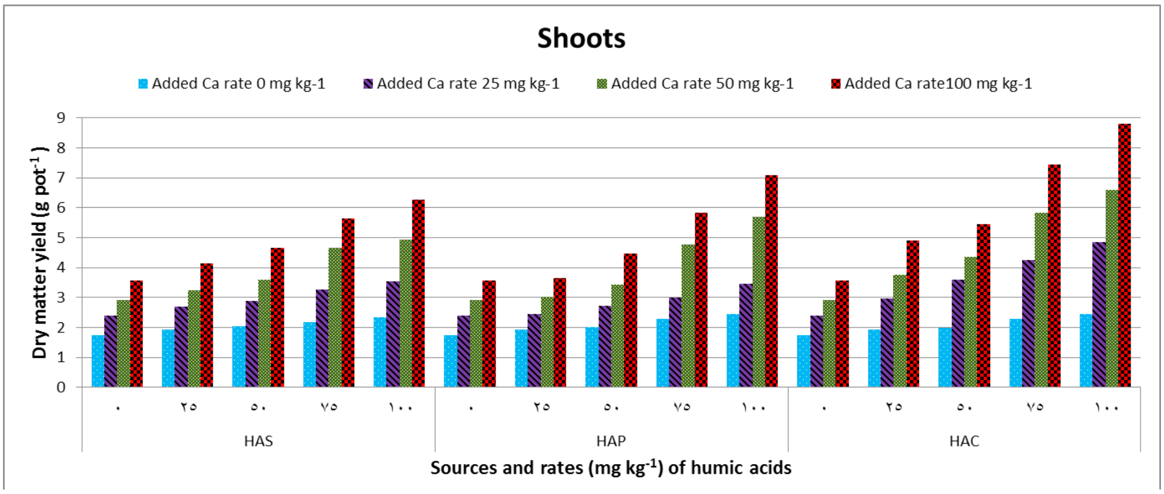

Fig. 2. Effect of different sources and rates of humic acids on roots dry matter yield of sorghum plants under different application rates of Ca

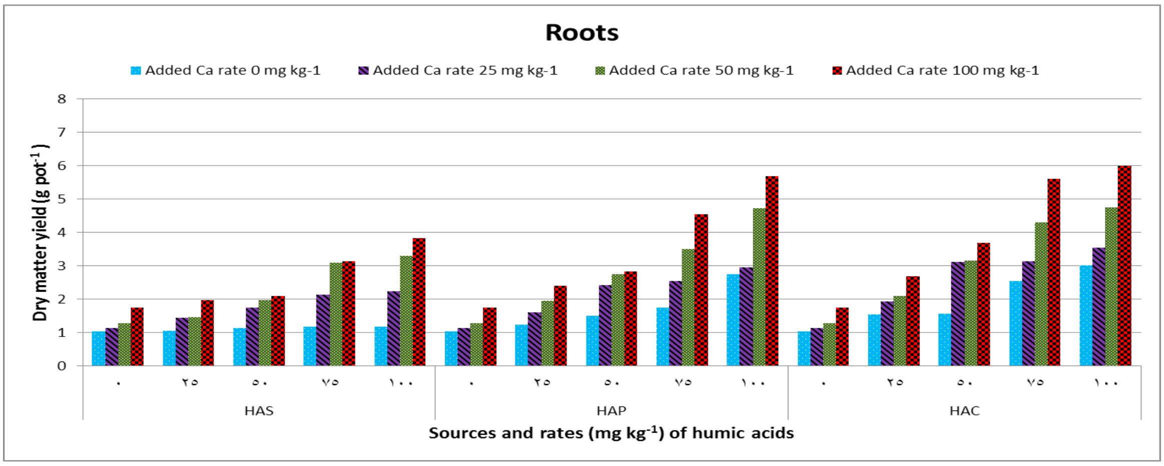

Fig. 3. Effect of different sources and rates of humic acids on shoots dry matter yield of sorghum plants under different application rates of Fe

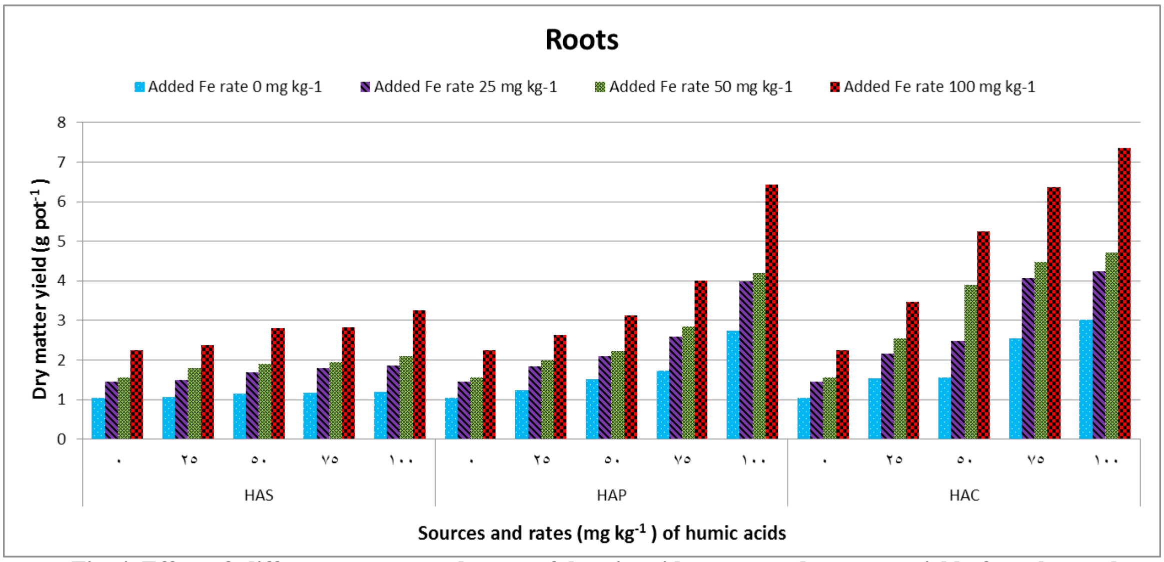

Fig. 4. Effect of different sources and rates of humic acids on roots dry matter yield of sorghum plants under different application rates of Fe

Calcium uptake

Data in Table 7 showed that , Ca uptake (mg pot-1) affected by individual and combined applications of the used three humic acids and Ca. These data showed, individual applications of humic acids resulted in a slight increase of Ca uptake by both shoots and roots. On the other hand, significant increase of Ca uptake was resulted from the individual Ca applications. More increases of Ca uptake by shoots and roots of sorghum plants were found in the plants treated by the combined applications of humic acids and Ca. These increase are in harmony with the effect of the studied treatments (humic acids and Ca) on the dry matter yield of sorghum plants either shoots and roots. At the same application rate of humic acid and Ca in combined applications, the highest Ca uptake by shoots and roots was found in the plants treated by HAC followed by that found with HAP application. This order is harmony with the chemical composition of the used humic acids and their content of Ca (Tables, 1 and 2). This finding in the current study is supported by Asik et al. (2009); Katkat et al. (2009); Hussein and Hassan (2011) and Mohamed (2012).

Iron uptake

Iron uptake (mg pot-1) by sorghum plants (shoots and roots) affected by individual and combined applications of the used three humic acids and Fe was studied and the obtained data recorded in Table (8). These data showed that, a slight increase of Fe uptake by both shoots and roots as a result of individual applications of humic acids, where the added three humic acids may be arranged according to their effect on Fe uptake by sorghum plants in the following order: HAC > HAP > HAS. This order are in harmony with both the chemical composition of the used humic acids especially their content of Fe (Tables, 1 and 2) and their effect on the dry matter yields of sorghum plants (Table, 5). Individual applications of Fe resulted in a significant increase of Fe uptake (mg/pot) by shoots and roots of sorghum plants. These results attributed to high content of soluble Fe in the growth media and its effect on the increase of sorghum dry matter yield. Before that, Lemanceau et al. (2009); Mohammad et al. (2009); Eisa and Taha (2010) and Colombo et al. (2014) obtained on similar results. More increases of Fe uptake by shoots and roots of sorghum plants were observed in the plants treated by humic acids and Fe together. These results are in agreement with those obtained by Mackowiak et al. (2001); Salib (2002); Sanchez et al. (2006); Alhendawi et al. (2008) and Morard et al. (2011).

Calcium and iron utilization

The presented data in Tables 9 and 10 showed that, the Ca and Fe utilization by shoots and roots of sorghum plants treated by three humic acids at different application rate when its added as alone or in combination with Ca OAC or Fe OAC. Increasing rate of added humic acids resulted in an increases of both Ca and Fe utilization by both shoots and roots of sorghum plants. Such increases attributed to enhanced effect of added humic acids on plant growth and the yields of dry matter yield as mentioned before that by Morard et al. (2011) ; Mohamed (2012) ; Nada and Tantawy (2012) and Abd El-Kader (2016).

At the same application rate of the used three humic acids, the obtained data pointed the following points: 1) Utilization rates of Ca and Fe with shoots were higher than those found for roots which are in harmony with the found dry matter yields for both shoots and roots, 2) With both shoots and roots, utilization rates of Ca were higher than those obtained for Fe, which may be attributed to the high dry matter yields of sorghum plants (shoots and roots) resulted from Ca applications compared with those resulted from Fe application and 3) According to the found utilization rate of Ca and Fe by both shoots and roots, the used three humic acids takes the order: HAC > HAP > HAS, where this order attributed two main resource's. The first is the chemical composition of these acids and their content of both functional groups and essential plant nutrients (Tables, 1 and 2), and the second is the effect of the added humic acids on the obtained dry matter yields of sorghum plants. In addition, the obtained data also showed that, utilization rates (%) of both Ca and Fe clearly decreased with the increase rate of added Ca and Fe. This trend was found with both shoots and roots at all application rates of the tested three humic acids. These findings means that, low application rates of Ca and Fe have a high efficiency on their uptake compared with those resulted from the high application rates. Such increments were obtained by Hepler (2005); Taha et al. (2006); Katkat et al. (2009) and Eisa and Taha (2010).

Conclusion

This study emphasized the great importance of the appropriate role of humic acids especially HAC in enhancing growth yield and promotes the uptake of Ca and Fe nutrients by sorghum plant under the grown on sandy culture. The interaction effect between humic acids and either of Ca or Fe application increased both sorghum yield quantity and quality. The best treatment is applying HAC at rate 100 mg kg-1 + Ca rate 100 mg kg-1 followed by HAC at rate 100 mg kg-1 + Fe rate 100 mg kg-1.

TABLE 7. Calcium uptake (mg pot^{-1}) in shoots and roots of sorghum as affected by different sources and rates of humic acids under four application rates of Ca (mean values of two seasons)

Humic acids treatments		Shoots Added Ca rate (mg kg^{-1})					Roots Added Ca rate (mg kg^{-1})				
Source	Rate (mg kg^{-1})	0	25	50	100	Mean	0	25	50	100	Mean
HAS	0	0.734	14.711	22.002	29.247	16.674	0.275	4.861	6.815	9.608	5.390
	25	0.787	14.945	22.962	33.542	18.059	0.344	5.212	6.892	9.930	5.595
	50	0.822	14.945	28.014	35.823	19.901	0.349	5.935	8.279	10.177	6.185
	75	1.134	16.642	28.654	37.992	21.106	0.377	6.613	9.478	11.375	6.961
	100	1.391	21.611	31.884	42.553	24.360	0.446	6.674	10.894	11.383	7.349
	Mean	0.974	16.571	26.703	35.831	20.020	0.358	5.859	8.472	10.495	6.296
HAP	0	0.734	14.711	22.002	29.247	16.674	0.275	4.861	6.815	9.608	5.390
	25	0.801	22.976	30.717	41.535	24.007	0.446	6.570	7.715	10.527	6.315
	50	0.904	23.013	32.300	41.867	24.521	0.447	7.720	9.654	11.241	7.266
	75	0.967	23.933	34.570	46.981	26.613	0.454	8.305	13.565	15.056	9.345
	100	1.481	24.206	37.353	47.800	27.710	0.458	8.572	14.232	17.245	10.127
	Mean	0.977	21.768	31.388	41.486	23.905	0.416	7.206	10.396	12.735	7.688
HAC	0	0.734	14.711	22.002	29.247	16.674	0.275	4.861	6.815	9.608	5.390
	25	1.166	25.190	33.867	46.560	26.696	0.611	8.690	11.572	16.158	9.258
	50	1.310	26.113	37.656	48.531	28.403	0.612	10.302	17.082	20.732	12.182
	75	1.426	26.605	40.886	55.859	31.194	0.636	11.907	17.565	26.985	14.273
	100	1.640	26.937	42.821	63.940	33.835	0.754	13.089	19.837	28.048	15.432
	Mean	1.255	23.911	35.446	48.827	27.360	0.578	9.770	14.574	20.306	11.307

TABLE 8. Iron uptake (mg pot-1) in shoots and roots of sorghum as affected by different sources and rates of humic acids under four application rates of Fe (mean values of two seasons)

Humic acids treatments		Shoots Added Fe rate (mg kg^{-1})					Roots Added Fe rate (mg kg^{-1})				
Source	Rate (mg kg^{-1})	0	25	50	100	Mean	0	25	50	100	Mean
HAS	0	1.914	14.317	18.930	21.965	14.282	0.918	7.161	9.577	13.424	7.770
	25	1.922	15.596	19.545	22.360	14.856	0.934	7.165	10.269	14.006	8.094
	50	2.006	15.670	19.963	24.841	15.620	0.939	7.426	12.551	15.213	9.032
	75	2.060	15.867	21.611	27.423	16.740	0.950	7.448	12.803	15.961	9.291
	100	2.137	17.306	21.783	32.288	18.379	0.970	7.492	17.615	16.598	10.669
	Mean	2.008	15.751	20.366	25.775	15.975	0.942	7.338	12.563	15.040	8.971
HAP	0	1.914	14.317	18.930	21.965	14.282	0.918	7.161	9.577	13.424	7.770
	25	1.986	16.544	19.754	28.708	16.748	1.000	8.266	14.012	14.756	9.509
	50	2.075	16.802	20.713	29.280	17.218	1.179	8.629	14.369	16.579	10.189
	75	2.111	17.761	21.587	32.546	18.501	1.291	10.108	14.565	20.558	11.631
	100	2.127	18.462	23.985	36.180	20.189	1.897	14.983	15.676	31.180	15.934
	Mean	2.043	16.777	20.994	29.736	17.388	1.257	9.829	13.640	19.300	11.007
HAC	0	1.914	14.317	18.930	21.965	14.282	0.918	7.161	9.577	13.424	7.770
	25	1.993	17.023	19.151	27.915	16.521	1.272	10.079	12.590	19.733	10.919
	50	2.101	17.269	20.947	27.915	17.058	1.319	10.638	18.667	29.131	14.939
	75	2.157	17.306	23.837	32.583	18.971	2.021	16.171	20.206	33.700	18.025
	100	2.165	17.983	25.165	39.483	21.199	2.229	16.469	20.394	35.961	18.763
	Mean	2.066	16.780	21.606	29.972	17.606	1.552	12.104	16.287	26.390	14.083

TABLE 9. Calcium utilization (%) by shoots and roots of sorghum as affected by different sources and rates of humic acids at four application rates of Ca (mean values of two seasons)

Humic acids treatments		Shoots				Roots			
Source	Rate (mg kg^{-1})	Added Ca rate (mg kg^{-1})				Added Ca rate (mg kg^{-1})			
		25	50	100	Mean	25	50	100	Mean
Control		55.91	42.54	28.51	42.32	18.34	13.08	9.33	13.58
HAS	25	56.63	44.35	32.76	44.58	19.47	13.10	9.59	14.05
	50	56.49	54.38	35.00	48.62	22.34	15.86	9.83	16.01
	75	62.03	55.04	36.86	51.31	24.91	18.20	11.00	18.04
	100	80.88	60.99	41.16	61.01	24.94	20.90	10.94	18.93
	Mean	64.01	53.69	36.45	51.38	22.92	17.02	10.34	16.76
HAP	25	88.70	59.83	40.73	63.09	24.50	14.54	10.08	16.37
	50	88.44	62.79	40.96	64.06	29.09	18.41	10.79	19.43
	75	91.86	67.21	46.01	68.36	31.40	26.22	14.60	24.07
	100	90.90	71.74	46.32	69.65	32.46	27.55	16.79	25.60
	Mean	89.98	65.39	43.51	66.29	29.36	21.68	13.07	21.37
HAC	25	96.10	65.40	45.39	68.96	32.32	21.92	15.55	23.26
	50	99.21	72.69	47.23	73.04	38.76	32.94	20.12	30.61
	75	100.72	78.92	54.43	78.02	45.08	33.86	26.35	35.10
	100	101.19	82.36	62.30	81.95	49.34	38.17	27.29	38.27
	Mean	99.13	74.84	52.34	75.49	41.38	31.72	22.33	31.81

TABLE 10. Iron utilization (%) by shoots and roots of sorghum as affected by different sources and rates of humic acids at four application rates of Fe (mean values of two seasons)

Humic acids treatments		Shoots				Roots			
Source	Rate (mg kg^{-1})	Added Fe rate (mg kg^{-1})				Added Fe rate (mg kg^{-1})			
		25	50	100	Mean	25	50	100	Mean
Control		49.61	34.03	20.05	28.56	24.97	17.32	12.51	18.27
HAS	25	54.70	35.25	20.44	36.80	24.92	18.67	13.07	18.89
	50	54.66	35.91	22.84	37.80	25.95	23.22	14.27	21.15
	75	55.23	39.10	25.36	39.90	25.99	23.71	15.01	21.57
	100	60.68	39.29	30.15	43.37	26.09	33.29	15.63	25.00
	Mean	56.32	37.39	24.70	39.47	25.74	24.72	14.50	21.65
HAP	25	58.23	35.54	26.72	40.16	29.06	26.02	13.76	22.95
	50	58.91	37.28	27.21	41.13	29.80	26.38	15.40	23.86
	75	62.80	38.95	30.44	44.06	35.27	26.55	19.27	27.03
	100	65.34	43.72	34.05	47.70	52.34	27.56	29.28	36.39
	Mean	61.32	38.87	29.61	43.26	36.62	26.63	19.43	27.56
HAC	25	60.12	34.32	25.92	40.12	35.23	22.64	18.46	25.44
	50	60.67	37.69	25.81	41.39	37.28	34.70	27.81	33.26
	75	60.60	43.36	30.43	44.80	56.60	36.37	31.68	41.55
	100	63.27	46.00	37.32	48.86	56.96	36.33	33.73	42.34
	Mean	61.17	40.34	29.87	43.79	46.52	32.51	27.92	35.65

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تأثير مصادر مختلفة من أحماض الهيوميك علي نمو و إستفادة نباتات السورجم من الكالسيوم و الحديد

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لقد إنتشر إستخدام أحماض الهيوميك في الزراعة من أجل إنتاج نباتي عالي الجودة ، ولكن هل هذه الأحماض المختلفة المصدر متساوية في الكفاءة؟ من أجل ذلك تم إجراء تجربة أصص ، وذلك بزراعة نباتات السورجم – صنف جيزة 51 علي مزرعة رملية خلال موسمي نمو صيف متتاليين 6102 – 7102 م ، وذلك لدراسة كلٍ من التأثير الفردي و المشترك لإضافة ثلاثة أحماض هيوميك ذات مصادر مختلفة مع إضافات مختلفة لكلٍ من الكالسيوم أو الحديد علي كلٍ من النمو و إستفادة نباتات السورجم من الكالسيوم و الحديد. وأحماض الهيوميك الثلاثة المستخدمة قد تم فصلها من مصادر مختلفة كما يلي: الأول حامض هيوميك قد تم فصله من الأرض الطينية ، و الثاني حامض هيوميك قد تم فصله من الحمأة والثالث حامض هيوميك قد تم إستخلاصه من كمبوست قش البرسيم. وكل حامض هيوميك منهم قد تم إضافته بمعدل صفر ، 52 ، 05 ، 57 و 001 ملليجرام / كيلوجرام رمل منقي . كما أن كلٍ من الكالسيوم و الحديد تم إضافتهما في صورة خلات و بمعدلات إضافة صفر ، 52 ، 05 و 001 ملليجرام / كيلوجرام رمل منقي. وأجريت التجربة في تصميم قطع منشقة مرتين بثلاثة مكررات.

وقد أوضحت النتائج أن إضافات أي حامض من أحماض الهيوميك الثلاثة المختلفة المصادر سواءً كانت منفردة أو متحدة مع الكالسيوم أو الحديد كان يُصاحبها زيادة معنوية في المادة الجافة للمجموع الخضري و الجذري لنباتات السورجم. ووجدت أكبر زيادة في الوزن الجاف في النباتات المعاملة بحامض الهيوميك المستخلص من كمبوست قش البرسيم ثم تلتها النباتات المعاملة بحامض الهيوميك المستخلص من الحمأة. وطبقاً لحسابات متوسط الزيادة النسبية (٪) في المادة الجافة للنباتات المعاملة بأحماض الهيوميك عند مختلف إضافات الكالسيوم ، فقد وجد إنه في المجموع الخضري و الجذري كانت كالتالي: حامض هيوميك البرسيم (731,0 و 971,1 ٪) > حامض هيوميك الحمأة (89,48 و 341,0 ٪) > حامض هيوميك الأرض (89,40 و 48,13 ٪) علي التوالي. بينما كان متوسط الزيادة النسبية (٪) في المادة الجافة للنباتات المعاملة بأحماض الهيوميك عند مختلف إضافات الحديد كالتالي: حامض هيوميك البرسيم (411,4 و 712,9 ٪) > حامض هيوميك الحمأة (801,8 و841,2 ٪) > حامض هيوميك الأرض (101,1 و 67,99 ٪) و ذلك لكلٍ من المجموع الخضري و المجموع الجذري علي التوالي. كما وجد أن زيادة معدلات الإضافة من كلٍ من أحماض الهيوميك و الكالسيوم أو الحديد يُصاحبها زيادة في إمتصاص الكالسيوم والحديد في كلٍ من المجموع الخضري و الجذري لنباتات السورجم، وقد أخذت أحماض الهيوميك نفس الإتجاه السابق حيثُ كان ترتيب إمتصاص الكالسيوم و الحديد في نباتات السورجم المعاملة بحامض هيوميك البرسيم > المعاملة بحامض هيوميك الحمأة > المعاملة بحامض هيوميك الأرض ، بالإضافة إلي أن الإمتصاص سواء للكالسيوم أو الحديد كان في المجموع الخضري > المجموع الجذري. وعلي الجانب الآخر، فقد وجد أن زيادة معدلات الإضافة لكلٍ من الكالسيوم و الحديد يُصاحبها نقص في إستفادة النبات بهما. في حين زيادة معدلات الإضافة لأحماض الهيوميك يُصاحبها زيادة في إستفادة النبات للكالسيوم والحديد.

وعموماً وطبقاً للنتائج المتحصل عليها، فإن الدراسة تُوصي بإنه يجب أن يحتوي برنامج التسميد في زراعة نباتات السورجم علي إضافة أحماض الهيوميك و خاصة المستخلصة من كمبوست قش البرسيم مع إضافة الكالسيوم والحديد وذلك لتحسين إنتاجية محصول السورجم سواءً من ناحية الكم أو الجودة. وأيضاً فإنه من الضروري إستخدام المحسنات العضوية حيثُ أنها تزيد من كفاءة التسميد بالكالسيوم و الحديد.