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EFFECT OF SOIL AND FOLIAR APPLICATION OF HUMIC ACID ON GROWTH AND PRODUCTIVITY OF SOYBEAN PLANTS GROWN ON A CALCAREOUS SOIL UNDER DIFFERENT LEVELS OF MINERAL FERTILIZERS

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ABSTRACT

A field experiment was carried out on a calcareous soil at Abou massou village (48 km south-west to Alexandria) to determine the effect of humic acid (HA) at the rates of 0 , soil application of (15 kg HA / fed and 30 kg HA/fed) , foliar spray of 0.1% HA and mixture of (15 kg HA/fed as soil application + 0.1% HA as foliar spray along with mineral fertilizers (MF) at the rates of 0 , 75 and 100% of the recommended dose of N, P and K (RDF) on some physical and chemical properties of the studied soil as well as soybean yield and its components.

The obtained results indicated that soil application of HA significantly increased soil organic matter content and positively affected bulk density and total porosity (decrease bulk density and increase total porosity). Available N, P and K in soil showed pronounced increases due to the soil application of HA and/ or MF , with a superiority for the treatment of 30 Kg HA + 100% RDF over the other treatments. Growth traits , N, P and K content in seeds as well as seed yield and yield components of Soybean considerably increased as a result of soil or foliar application of HA and/ or MF and the increase progressed with increasing the rate of HA (From 15 kg HA to 30 kg HA/Fed) or MF (From 75 to 100% RDF). The combined application of HA and MF was more prominent in enhancing the aforementioned parameters compared to the treatments received solely application of MF. In this respect, the highest values of these parameters were produced by the combined application of 30 kg HA/fed with 100% RDF.

Keywords: Humic acid, mineral fertilizers, soil properties, plant growth, Soybean.

INTRODUCTION

Humic substances (humic and fulvic acids) are the major component of soil organic matter and the term of "humus" is widely accepted as synonymous for the soil organic matter (Chen. and Aviad, 1990). The humic substances in the soil have multiple affects that can greatly benefit plants growth (Lobartini *et al*., 1997 ; Nardi *et al*., 2002 and Sangeetha *et al*., 2006). Many investigators classified the beneficial effects of humic acids on plant growth into direct and indirect effects. Indirect effects involve improvement of the soil properties such as aggregation, aeration, permeability, water holding capacity, micronutrients transport and availability (Tan, 2003). Direct effects are those which require uptake of humic substances into the plant tissue resulting in various biochemical effects (Chen and Aviad, 1990 and Nardi *et al*., 2002). Humic substances affect the solubility of many nutrient elements by building complex forms or chelating agents of humic matter with metallic cations (Lobartini *et al*.,1997).

In many studies, humic acids have been found to affect biological activity and soil properties (Soong,1980 and Tajuddin, 1992), decrease the loss of moisture and enhance the water retention (Cheng *et al*.,1998), decrease soil bulk density and increases total porosity and soil organic matter content (Salib *et al*., 2003 and zaky *et al*.,2006).

Humic acids plays a major role in plant nutrients uptake and growth parameters in plant in both vegetative and genetative stages (Ulukan, 2008) on wheat and Shehata and EL-Helaly (2010) on snap bean .

The increment of growth parameters and crop yields due to HA application may be attributed to that HA is an important component since it constitutes a stable fraction of carbon, thus regulating the carbon cycle and release of nutrients, including nitrogen, phosphorus, and sulfur, which decreasing the need for inorganic fertilizer for plant growth. HAs stimulate plant growth by the assimilation of major and minor elements, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production (Ulukan, 2008). Moreover, Russo and Berlyn (1990) reported that, humates (granular and liquid forms) can reduce plant stress that involved plant diseases as well as enhance plant nutrient uptake. In addition, HA can be used as a growth regulator by regulate endogenous hormone levels (Frgbenro and Agboola, 1993 and Piccolo *et al*., 1992).

El-Ghamry *et al*. (2009) reported that all morphological characteristics , yield components, macronutrients content as well as chlorophyll content of faba bean significantly increased by foliar application of humic acid. Humic acid applied to wheat in a calcareous soil as soil application (1 and 2 g/Kg soil) and foliar spray (0.1 and 0.2%) had a significant positive effect on dry weight and NPK uptake of wheat (Katkat el at., 2009).

The enhancing effect of humic acids was also observed on the dry matter yield of corn and oat seedling (Albuzio *et al*., 1994 and Khaled and Fawy, 2011). Moreover, the effect of humic acids on numerous plants such as tomato (Padem an Ocal, 1999), strawberry (Neri *et al*., 2002), spinach (Ayas and Gulser, 2005) and bean (Zaky *et al*., 2006) have been well documented.

The mechanism of humic acids in promoting plant growth is not completely known, but several explanations proposed. Nardi *et al*. (2002) attributed the beneficial effect of humic acid on plant growth to the increasing cell membrane, oxygen uptake, respiration and photosynthesis, nutrients uptake, root and cell elongation and ion transport. Moreover, Nardi *et al*. (1999) mentioned that the positive effect of humic acids on plant growth may be due to its acting as plant growth hormones.

The purpose of this work aimed to determine the effect of soil and foliar application of humic acid along with different levels of mineral fertilizers on macronutrients content, Soybean yield and its component as well as macronutrients availability in soil.

MATERIALS AND METHODS

A field experiment was carried out during the summer season of 2009 on a calcareous soil at Abou Masooud farm (48 Km south-west to Alexandria) Alexandria Governorate, Egypt. Some physical and chemical properties of the studied soil are presented in Table (1).

Table (1): Some physical and chemical properties of the soil under investigation.

Practicle size distribution in presence of $CaCO_3$				
Clay	**(%)**	**15.2**	**EC_e (dS/m)**	**2.36**
Silt	**(%)**	**20.7**	**Cations meq /L :**	
Fine sand	**(%)**	**43.2**	**Ca^{2+}**	**7.88**
Coarse sand	**(%)**	**20.9**	**Mg^{2+}**	**4.85**
Textural class :	**Sandy clay loam**		**Na^+**	**10.4**
Bulk density (g/cm^3)		**1.40**	**K^+**	**0.73**
Total porosity	**(%)**	**47.2**	**Anions meq /L :**	
$CaCO_3$	**(%)**	**33.4**	**CO_3^{2-}**	**0.00**
O.M.	**(%)**	**1.09**	**HCO_3^-**	**5.82**
pH(1-2.5 susp.)		**8.15**	**Cl^-**	**7.24**
Available macronutrients			**SO_4^{2-}**	**10.8**
Available N	**mg /kg soil**	**64.0**		
Available P	**mg/kg soil**	**10.7**		
Available K	**mg/kg soil**	**315**		

Soybean grains variety Giza 35 were sown in plots with 10.5 m^2 in area (3 x 3.5 m). The experiment was designed in a split plot design with three replicates. The treatments included three levels of mineral fertilizers, i.e. 0 , 75% and 100% of the recommended dose of N, P and K in the form of ammonium sulphate (20.5 % N), superphosphate (15.5 % P_2O_5) and potassium sulphate (48 % K_2O) respectively. While humic acid (HA) was applied in five levels, i.e., 0 (no humic acid), soil application of (15 kg HA/fed and 30 kg HA/fed), foliar spray of 0.1% HA and mixture of 15 kg HA/fed as soil application + 0.1% HA as foliar application.

Soil application of humic acid was applied in one dose before sowing and foliar spray was done on 30, 45 and 60 days after sowing. All the agricultural recommended practices were followed as usual including the irrigation processes.

Yield and its components:

At harvest (120 days after sowing), number of pods/plant, 100 seeds weight (g) and seed yield (kg/fed) were recorded. Samples of soybean seeds were digested using H_2SO_4 and H_2O_2. Total nitrogen was determined using the standard procedure of micro-kjeldahl as described by black (1965). Total phosphorus and potassium were determined according to Jackson (1973).

Soil analysis:

Soil samples were collected from all experimental plots after 70 days from sowing. Organic matter content was determined by the Walkey and

Black method (Black, 1965). Available N, P and K in soil were determined according to Jackson (1973).

Statistical analysis:

All obtained data were statistically analyzed and compared by using least significant differences (L.S.D) according to the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Organic matter content, bulk density and total porosity:

Organic matter content as affected by the application of humic acid (HA) and mineral fertilizers is presented in Table (2), data showed that organic matter was significantly increased upon the soil application of HA and progressed with increasing its rate from 15 to 30 kg HA/fed. Otherwise, no significant changes were observed in organic matter content due to the foliar application of HA and / or mineral fertilizers alone. These results are in agreement with those of Zaky *et al*. (2006)**.**

Table (2): Some soil properties as affected by humic acid and NPK fertilizers.

Treatments		(B) Fertilizer levels (% recommended dose of NPK)											
(A)		**Organic matter (%)**				**Bulk density (g/cm^3)**				**Total porosity (%)**			
Humic acid		**0**	**75**	**100**	**Mean**	**0**	**75**	**100**	**Mean**	**0**	**75**	**100**	**Mean**
H_0		0.98	1.01	1.02	1.00	1.38	1.35	1.35	1.36	47..9	49.1	49.1	48.7
H_1		1.10	1.13	1.13	1.12	1.30	1.27	1.26	1.28	50..9	52.1	52.5	51.8
H_2		1.17	1.20	1.21	1.19	1.23	1.21	1.21	1.22	53.6	54.3	54.3	54.1
H_3		1.01	1.04	1.03	1.03	1.35	1.33	1.33	1.34	49.1	49.8	49.8	49.6
H_4		1.12	1.15	1.15	1.14	1.28	1.25	1.24	1.26	51.7	52.8	53.2	52.6
Mean		1.08	1.11	1.11		1.31	1.28	1.28		50.6	51.6	51.8	
$LSD_{0.05}$	A	0.08				0.06				2.25			
	B	N.S				N.S				N.S			
	AxB	N.S				N.S				N.S			

H_0: No humic acid (HA)
H_1:Soil application of HA (15 kg/Fed)
H_2:Soil application of HA (30 kg/Fed)
H_3:Foliar spray of 0.1% HA
H_4:Soil application of HA (15 kg/Fed) + Foliar spray of 0.1% HA

Concerning the effect of applied HA and mineral fertilizers on bulk density and total porosity (Table 2), results indicated that bulk density and total porosity were positively affected by the soil application of HA. A marked decrease in bulk density was occurred at the two rates of soil application of HA and the decrease was more pronounced at the rate of 30 kg HA/Fed. Aggregation resulting from HA must have been the main cause for such a reduction in the bulk density. Similar results were obtained by salib (2003) who found that treating clay soil with HA resulted in a decrease in bulk density. Bulk density is a function of total porosity, therefore, decrease bulk density means increased total porosity . Thus in view of the fact that total porosity is directly deduced from bulk density , any trend of change concerning total porosity would be exactly similar to that of bulk density with a reverse direction .

Available N, P and K in soil:

Regarding the affect of applied HA and mineral fertilizers on the available N , P and K , data in Table (3) indicated that available N , P and K increased considerably due to the application of mineral fertilizers and increasing the rate of mineral fertilizers from 75 to 100% of the recommended dose of fertilizers (RDF) was accompanied by pronounced increases in available N , P and K in soil .

Table (3): Effect of humic acid and NPK fertilizers on available macronutrients (mg/kg soil).

Treatments		(B) Fertilizer levels (% recommended dose of NPK)											
(A)		Available N				Available P				Available K			
Humic acid		0	75	100	Mean	0	75	100	Mean	0	75	100	Mean
H_0		58.1	72.5	77.3	69.3	10.1	13.0	14.2	12.4	267	290	304	287
H_1		65.4	80.2	85.7	77.1	11.5	14.3	15.0	15.0	287	308	326	307
H_2		70.6	86.1	92.0	82.9	12.7	15.4	16.3	16.3	309	334	350	331
H_3		58.6	72.4	77.5	69.5	10.3	12.9	14.1	14.1	265	293	308	289
H_4		65.9	81.0	86.2	77.7	11.7	14.4	15.4	15.4	290	315	330	312
Mean		63.7	78.4	83.7		11.3	14.0	15.0		284	308	324	
$LSD_{0.05}$	A	4.76				0.74				17.4			
	B	4.82				0.85				18.1			
	AxB	8.15				N.S.				N.S.			

See footnotes of Table 2 for treatment designations.

Soil application of HA was associated with significant increases in available N, P and K. The combined application of HA and mineral fertilizers recorded higher values in respect to N, P and K as compared to the treatments received solely application of mineral fertilizers, and the highest values of available N, P and K (92.0, 16.3 and 350 mg/kg soil, respectively) were obtained under the treatment of 30 kg HA/ Fed along with 100% RDF. Such increase in N , P and K as a result of HA addition may be attributed to the improving in soil nutrients retention and enhancing soil microbial activity which works to convert the organic from of nutrients to mineral form (Stevenson,1994). Similar observations were also obtained by Zaky *et al*.(2006) who mentioned that treated soil with HA through the irrigation water caused marked increases in available N , P and K in soil .

Growth traits:

Data in Table (4) showed that the single application of mineral fertilizers significantly augmented plant hight, number of branches and dry weight of shoot. the more the rate of mineral fertilizers was the more the effect was. The relative increase of plant height, number of branches and dry weight of shoot (regardless of HA application) were 7.49, 15.0 and 8.99%, respectively with increasing the rate of mineral fertilizers from 75 to 100 RDF.

Regarding HA application, results showed that the treatments receiving HA in both soil or foliar application caused pronounced increases in plant hight, number of branches and dry weight of shoot compared to the untreated ones. This occurred with the two rates of mineral fertilizers. In this concern, the highest values of plant hight (86.5cm), number of branches (4.00) and dry weight of shoot (108 g/plant) were recorded at the rate of 30 kg HA/Fed along with 100% RDF . Humic acid stimulate plant growth by the assimilation of major and minor elements, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production (Ulukan, 2008) .

In addition, HA can be used as a growth regulator by regulate endogenous hormone levels (Piccolo *et al*., 1992). These findings are in accordance with those obtained by El-Ghamry *et al*. (2009) on Faba bean, El-Bassiony *et al*. (2010) on Snap bean and Shehata and El-Helaly (2010) on Snap bean.

Table (4): Effect of humic acid and NPK fertilizers on growth traits of soybean at 90 days age.

Treatments		(B) Fertilizer levels (% recommended dose of NPK)											
(A)		Plant hight (cm)				No. of branches				Dry weight of shoot (g/plant)			
Humic acid		0	75	100	Mean	0	75	100	Mean	0	75	100	Mean
H_0		51.7	67.0	72.8	63.8	2.00	2.67	3.00	2.56	54.7	82.8	90.2	75.9
H_1		60.7	74.8	80.1	71.9	2.33	3.00	3.67	3.00	66.1	92.7	100	86.3
H_2		65.3	80.4	86.5	77.4	2.67	3.33	4.00	3.33	71.0	98.1	108	92.4
H_3		59.1	71.9	76.0	69.0	2.33	3.00	3.33	2.89	63.9	86.2	94.6	81.6
H_4		64.0	77.4	83.2	74.9	2.67	3.33	3.67	3.22	69.2	96.0	104	89.7
Mean		60.2	74.3	79.8		2.40	3.07	3.53		65.0	91.2	99.4	
$LSD_{0.05}$	A	3.77				0.28				5.63			
	B	4.31				0.30				6.15			
	AxB	N.S.				0.52				10.9			

See footnotes of Table 2 for treatment designations.

Nitrogen, P and K contents in soybean seeds:

Date presented in Table (5) showed marked increases in N, P and K concentrations in soybean seeds due to the application of mineral fertilizers and progressed with increasing its rates from 75 to 100% RFD. Nitrogen, P and K concentrations increases by 3.68, 13.2 and 5.39% (irrespective of HA application) respectively with increasing mineral fertilizers rates from 75% to 100% RDF.

Humic acid in both soil or foliar application had a significant positive effect on N, P and K concentrations in soybean seeds and treatments received HA recorded higher values as compared to those treatments with no HA addition and that occurred under the two applied rates of mineral fertilizers. In this respect, the highest values of N, P and K concentrations (5.62, 0.65 and 1.84%, respectively) were produced by the combined application of 30 kg HA/fed with 100% RDF. The enhancing affect of HA on N,P and K concentrations may be due to better development root systems (David *et al*.1994),increased the permeability of plant membranes (Ulukan,2008). Furthermore, humic substances may interact with the phospholipids structures of cell membranes and react as carriers of nutrients through them. These results are in agreement with those obtained by El-Ghamry *et al*.(2009) who mentioned that foliar application of humic acid (0.1

and 0.2%) caused considerable increases in N,P and K content in faba bean seeds. Also, Khaled and fawy (2011) reported that applied humic acid in soil application (2 and 4 g/kg soil) and foliar spray (0.1 and 0.2%) significantly increased N, P and K uptake by corn plants.

Table (5): Effect of humic acid and NPK fertilizers on macronutrients concentration in soybean seeds.

Treatments		(B) Fertilizer levels (% recommended dose of NPK)											
(A)		N %				P %				K %			
Humic acid		0	75	100	Mean	0	75	100	Mean	0	75	100	Mean
H_0		4.23	4.91	5.10	4.75	0.35	0.47	0.54	0.45	1.32	1.56	1.66	1.51
H_1		4.58	5.18	5.31	5.02	0.41	0.53	0.60	0.51	1.45	1.67	1.76	1.63
H_2		4.81	5.33	5.62	5.25	0.44	0.58	0.65	0.56	1.55	1.77	1.84	1.72
H_3		4.54	5.09	5.24	4.96	0.39	0.50	0.57	0.49	1.40	1.61	1.73	1.58
H_4		4.70	5.27	5.49	5.15	0.43	0.58	0.63	0.55	1.52	1.72	1.80	1.68
Mean		4.57	5.16	5.35		0.41	0.53	0.60		1.45	1.67	1.76	
$LSD_{0.05}$	A	0.18				0.03				0.28			
	B	0.21				0.04				N.S.			
	AxB	0.35				0.06				N.S.			

See footnotes of Table 2 for treatment designations.

Seed yield and yield components:

Results in Table (6) revealed that the addition of mineral fertilizers significantly increased number of pods plant^{-1}, weight of 100 seeds and seed yield. The higher the rate of mineral fertilizers the more pronounced the effect was in increasing all parameters of soybean yield and its components. Values of the relative increase of number of pods plant^{-1}, weight of 100 seeds and seed yield (irrespective of HA application) were 8.56, 3.78 and 6.57 %, respectively with increasing mineral fertilizers rate from 75 to 100% RDF.

Table (6): Effect of humic acid and NPK fertilizers on yield and yield components of soybean .

Treatments		(B) Fertilizer levels (% recommended dose of NPK)											
(A)		No. of pods plant^{-1}				Weight of 100 seeds (g)				Seed yield (kg/fed)			
Humic acid		0	75	100	Mean	0	75	100	Mean	0	75	100	Mean
H_0		35.7	46.2	50.4	44.1	16.0	17.8	18.6	17.5	794	1342	1472	1203
H_1		41.3	52.3	57.0	50.2	16.9	18.5	19.2	18.2	1053	1504	1590	1382
H_2		45.9	58.0	63.2	55.7	17.5	19.1	19.7	18.8	1294	1597	1694	1528
H_3		40.4	50.5	54.1	48.3	16.6	18.3	19.0	18.0	1010	1462	1545	1339
H_4		44.0	56.2	60.8	53.7	17.2	18.8	19.5	18.5	1232	1553	1651	1479
Mean		41.5	52.6	57.1		16.8	18.5	19.2		1077	1492	1590	
$LSD_{0.05}$	A	3.74				0.47				70.2			
	B	3.91				0.50				76.8			
	AxB	6.56				N.S.				N.S.			

See footnotes of Table 2 for treatment designations.

With regard to HA application , results showed that HA in both soil or foliar application significantly enhanced number of pods plant^{-1}, weight of 100 seeds and seed yield and such position effect holds true under the two applied rates of mineral fertilizers. The combined application of HA and mineral fertilizers gave higher values in respect to the aforementioned parameters compared to the treatments received single application of mineral fertilizers.

In this concern , the greatest values of number of pods plant^{-1} (63.2),weight of 100 seeds (19.7 g) and seed yield (1694 kg /fed) were scored at the rate of 30 kg HA/fed along with 100% RDF. El-bassiony *et al*. (2010) found that pod yield of snap bean improved by increasing rates of mineral fertilizers and humic acid as foliar spray and the highest values of pod yield were recorded under the rate of 0.2% HA along with 100% RDF. Similar results were obtained by Zaky *et al*. (2006) on bean and El-Ghamry *et al*. (2009) on faba bean.

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تأثير الاضافة الارضية والرش لحامض الهيوميك على نمو وإنتاجية نباتات فول الصويا الناميه فى أرض جيرية تحت مستويات مختلفة من التسميد المعدنى.

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إجريت تجربة حقلية فى أرض جيرية بقرية أبو مسعود جنوب الاسكندرية ب ٤٨ كم وذلك لتقييم التأثير المشترك لحامض الهيوميك بمعدلات : صفر و إضافة أرضية لحامض الهيوميك (١٥ كجم/فدان و ٣٠كجم/فدان) ، ٠,١% اضافة بالرش و خليط من الإضافة الأرضية لحامض الهيوميك بمعدل ١٥ كجم/فدان + ٠,١% إضافة بالرش ، وكذلك التسميد المعدنى تحت معدلات صفر، ٧٥%, ١٠٠% من المعدلات المقترحة لكل من النتروجين والفوسفور والبوتاسيوم ، وتأثير ذلك على محتوى التربة من المادة العضوية والعناصر الكبرى والكثافة الظاهرية والمسامية الكلية للتربة وكذلك على صفات النمو الظاهرية للنبات ومحتوى البذور من العناصر الكبرى وإنتاجية محصول فول الصويا .

وقد اظهرت النتائج المتحصل عليها أن الإضافة الأرضية لحامض الهيوميك أدت إلى زيادة مرنوية فى محتوى التربة من المادة العضوية وكذلك أثرت بصورة إيجابية على الكثافة الظاهرية والمسامية الكلية (حيث نقصت الكثافة الظاهرية وزادت المسامية الكلية) كما أدت الإضافة الأرضية لحامض الهيوميك وكذلك التسميد المعدنى إلى زيادة ملحوظة فى كل من النيتروجين والفوسفور والبوتاسيوم الميسر فى التربة وكانت الأفضلية للمعاملة ٣٠كجم حامض هيوميك / فدان + ١٠٠% من المعدلات الموصى بها للتسميد .

أدت الإضافة الأرضية والرش لحامض الهيوميك وكذلك التسميد المعدنى إلى زيادة ملحوظة لكل من صفات النمو الظاهرية ومحتوى البذور لكل من النيتروجين والفوسفور والبوتاسيوم وكذلك محصول البذور ومكونات المحصول لفول الصويا ، وكانت الزيادة أكبر وضوحاً عند زيادة كل من معدل إضافة حامض الهيوميك (من ١٥ كجم/ فدان الى ٣٠كجم/ فدان) ومعدل إضافة التسميد المعدنى (من ٧٥%الى١٠٠%من المعدلات الموصى بها). أدت الإضافة المزدوجة لكل من حامض الهيوميك والتسميد المعدنى إلى زيادة أكثر وضوحاً فى كل من هذه الصفات المشار إليها سابقاً عنها فى حالة الإضافة المنفردة للتسميد المعدنى.

وعموماً فقد تحققت أعلى القيم لكل من المتغيرات السابق ذكرها تحت الإضافة المزدوجة لكل من حامض الهيوميك بمعدل ٣٠كجم/ فدان +١٠٠% من المعدلات الموصى بها للتسميد.

قام بتحكيم البحث

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