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Effect of Humic Acid and Indole Butyric Acid on Vegetative Growth and Nutritional Status of *Jatropha curcas* Seedlings

[1]Dorria M.M. Ahmed, [2]Aml R.M. Yousef, [2]Enas A.M. Ali and S.M.A. Sarrwy[1]

[1]Pomology and [2]Horticultural Crops Technology Departments, National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt

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ABSTRACT

Jatropha (*Jatropha curcas* L.) as a commercially viable biofuel crop is recognized as a promising feedstock for biodiesel production. In addition, its high resistance to drought and salinity. The objective of this study was to investigate the effect of IBA and Humic acid at different concentrations on the vegetative growth characteristics and the mineral status of jatropha curcas plants. A pot experiment was carried out in the greenhouse of the National Research Centre, Dokki, Giza, Egypt. The seeds of Jatropha were sown and transplanted at pots and were received seven treatments at different concentrations and combinations of IBA and humic acid. These materials were used as 2 and 4 g /L for IBA, while, HA were applied at 100 and 200 ppm solely and in combinations. The plants were then grown in a partially shaded greenhouse for three months during two successive seasons of 2015 and 2016.

Results reveal that all treatments significantly promote the vegetative growth characters of Jatropha seedlings compared with untreated ones (control). These promotive effect was increased by decreasing the applied materials concentrations wither they were used individually or in combinations. The greatest values of seedling height, stem height and diameter, number of leaves/seedling and leaf area as well as fresh and dry weight of Jatropha seedlings were obtained by treatment No. 6 (2 g/L Hu + 100 ppm IBA). On the other hand, doubling the concentration of both humic acid and IBA substances (2 g/L HA + 100 ppm IBA) scored the greatest value of chlorophyll pigments content. These promotive effect of humic acid (HA) and Indole butyric acid (IBA) with their combination at low doses were reflexed positively on both macro and trace elements as well the developmental growth during the third month whereas, they scored the highest significant value in this respect .Finally, promotive effect can be achieved on the vegetative growth parameters and minerals of Jatropha seedlings with the applications of both humic and indole butyric acid in combination at 2 g/L Hu + 100 ppm IBA.

Key words: Jatropha seedlings, humic acid, Indole butyric acid, vegetative growth and developmental growth

Introduction

Jatropha curcas L., is a multipurpose plant that belongs to the family Euphorbiaceae and its value not only for the medicinal properties and resistance to various stresses but also for its use as an oilseed crop (Openshaw, 2000). Different parts of Jatropha are used for various purposes, such as animal feed, the production of various medicines and in the cosmetic industry, among other uses (Carels, 2009). Recently, the seeds have received tremendous attention because their oil can be converted to biodiesel. Moreover, Jatropha has been considered to be a potential bioenergy crop in arid and semiarid regions due to its high drought and salt resistance (Silva *et al*., 2010 and Maes *et al*., 2009). Seed and oil yield in Jatropha are affected by several factors, including genetics (Kaushik *et al*., 2007), age of the plant, field site characteristics, such as rainfall, soil type and fertility (Ouwens *et al*., 2007) and agronomic practices, such as plant spacing, pruning, irrigation and fertilization (Ghosh *et al*., 2011). To achieve a good yield, Jatropha needs certain good management practices, such as plant nutrition and minimizing loss of water through evaporation (Behera *et al*., 2010).

Corresponding Author: Dorria M.M. Ahmed, Pomology National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt. E-mail: Dorriaahmed@hotmail.com

In horticulture, seedling production is one of the production stages and is great importance system, since the performance of the crop in the field depends on the agronomic quality of the seedlings. Humic substances are composed of humic acids, fulvic acids and humin from biochemical transformations of compounds of soil organic matter such as lignin, cellulose, hemicellulose, sugars and amino acids (Primo *et al*., 2011). Humic acid and fulvic acid are a principal component of humic substances (65-70%), which are the major organic constituents of soil (Sani, 2014). Humic acid (HA) could be used as one of the main organic fertilizers, which is an important component of humic substances. Humic acid is an effective agent to use as a complement to synthetic or organic fertilisers (Khaled and Fawy, 2011). Moreover, humic acid might show anti-stress effects under abiotic stress conditions such as unfavorable temperature, salinity, pH, etc. The major functional groups of humic acid (HA) include carboxyl, phenolic hydroxyl, alcoholic hydroxyl and ketone (Cacco and Agnolla, 1984).

Plant growth hormones have also been reported to affect seed germination and dormancy by affecting different parts of the seed (Idu *et al*., 2007). Indole 3-Butyric Acid (IBA) is the leading plant hormone used to promote the formation of roots by breaking root apical dominance induced by cytokinin in plant (Cline, 2000). IBA is widely used because it is nontoxic for most plants over a wide range and promotes root growth in a large number of plant species (Hartmann *et al*., 1990). Recent investigations on IBA biosynthesis show that its concentrations in plants may be regulated by plant hormones and various stresses (Ludwig-Müller *et al*., 1995 and 2000).

El-Abd (1997) reported that the application of IBA with the lowest concentration increased root fresh weight. Moreover, all IBA treatments were effective in increasing the number, length and fresh weight of adventitious roots in cucumber cuttings as compared with control cuttings

The objective of this study was to evaluate the effect of different doses of humic acid (HA) and indole butyric acid (IBA) each individual or in combination treatments on vegetative growth and mineral contents of Jatropha seedlings.

Materials and Methods

A pot experiments was conducted in the greenhouse of the National Research Centre (NRC), Dokki, Giza governorate, Egypt during two successive seasons of 2015 and 2016. The objective of this study was to investigate the effect of IBA and humic acid at different concentration on the vegetative growth characteristics and the mineral status of jatropha curcas plants.

Seeds of Jatropha (*Jatropha curcas* L.) were sown at April 15th, 2015 and 2016 in seven treatments as different concentrations and combinations of Indole butyric acid (IBA) and humic acid (HA) for 24 hours. Pots of 15 cm in diameter and 20 cm deep were used. Each pot contained 15 kg of air dried clay sandy loam soil in equal proportion in ratio. Jatropha seeds were thinned twice, the 1stweek after one week of sowing and the 2nd two weeks later and leave three plants/pot. The plants were then grown in a partially shaded greenhouse for three months.

Soil: some and general physical and chemical characteristics of the soil used were: sand=30.8%, silt=30.2%, clay=34.0%, porosity=49%, bulk density=1.27g/cc, particle density=2.57g/cc, pH (1:2.5) = 7.15, EC dSm-1 (1:5) =1.3, $CaCO_3$% = 2.53 - OM% = 33.5 and Soluble cations (meq./100g): = 1.3.

Irrigation: was conducted using fresh water to refill the water shortage, the plants were irrigated every 2 days with 200 mL which was sufficient for leaching to occur from the bottom of all of the pots. A nutrient fertilizer solution (19:19:19) was weekly used. After transplantation. The treatments included seven solely treatments or in combinations with 4 replicates were applied as follows:

T1- Control (only water)
T2- 100 ppm IBA
T3- 200 ppm IBA
T4- 2 g/L Humic acid
T5- 4 g/L Humic acid
T6- 2 g/L Humic+100 ppm IBA
T7- 4 g/L Humic+200 ppm IBA

Foliar spray of previous applications was applied six times at two weeks intervals. Each treatment contained six pots, one for each sample and each pot contain three plants.

Vegetative growth: plant samples were taken at 15 days intervals for each treatment to determine the developmental growth parameters at the plant organs (leaves, stem, and root) as plant height (cm), stem length and diameter (cm), number of green leaves and their areas (cm^2).

Fresh, dry and moisture content:

Plant organ samples (leaves, stem and root) were collected, cleaned, weighed, and dried in oven at 70ºC, until weight constant and then grounded for determining moisture content percentage using the methods outline by Wilde *et al*. (1985).

Minerals Determination:

Plant samples were collected, cleaned, dried in oven at 70° C and ground in a stainless-steel mill. Digestion and determination of minerals was done using the methods described by Cottenie *et al*. (1982).

Chlorophyll a, b and total contents: were determined according to the methods described by Saric *et al*. (1967).

Statistical analysis:

The design for this experiment was a completely randomized design (CRD) with three replications. Data were analyzed with the analysis of variance (ANOVA) procedure of MSTATC program**.** When significant differences (P>0.05) were described by Snedecor and Cochran (1980).

Results

1-Seedling vegetative growth parameters:

1.1. Stem

IBA and humic substances have a pronounced influence on the vegetative growth of Jatropha plants, as an average of two seasons. Results in (Table 1) show a higher significant length and diameter of Jatropha plants with IBA and humic acid than untreated plants (control). The increment was observed with decreasing concentrations either used alone or in combinations. In this respect, the combination treatment / T6 (2 g/L humic+100 ppm IBA) had the highest shoot length (121.0 cm), while the control plants had the lowest length (63.67 cm). IBA applications significantly affected the Jatropha shoot diameter and recorded the highest stem thickness (1.83 cm) with low IBA application dose solely/T2, compared with the other treatments, followed by the same concentration combined with humic acid (2 g/L Humic+100 ppm IBA) /T6 that recorded 1.80 cm as shoot diameter. Concerning Jatropha seedlings height, it could be notice that the treatment No. 6 had the tallest Jatropha plants since it recorded 160.50 cm as an average of the two seasons, while the shortest value (82.33 cm) was obtained with untreated plants.

1.2. Leaves

Concerning the number of leaves per Jatropha seedlings, it seems that all treatments of IBA and HA showed higher number of leaves when compared with untreated one (control) which had the least leave number per seedling (15.0). Also, it is observed from Tables (2) that lower dose of both materials used gave higher values than higher dose. Meanwhile, humic acid treatments especially the combined applications recorded the highest significant leaves number per Jatropha seedlings (36.0 and 28.0) with T6 (2 g/L humic+100 ppm IBA) and T7 (4 g/L humic+200 ppm IBA) respectively, as an average of the two seasons of study.

As for leaves area, it was affected by different applications, since humic treatment solely or in combined with indole butyric acid affected this parameter. where, treatment No.6 (2g/L humic+100ppm IBA) showed the highest leave area (258.4 cm^2) followed by treatment No. 4 (213.8 cm^2), while, the treatments of IBA at 200 ppm /No. 3 and control one appeared lower leaves areas (166.20 and 172.70 cm^2), respectively in Jatropha seedlings.

1.3. Root

Regarding the root measurements per Jatropha seedlings, results in Table (3) clear that using humic and IBA treatments with low concentrations either alone or as combined applications, recorded the significant positive effect in both studied seasons (2015 and 2016) as compared with the control and the other treatments. Combined treatment No. 6 (2 g/L Humic+100 ppm IBA) gave the highest averages (39.0 and 1.75cm) for root length and diameter, respectively.

1.4. Fresh, dry weight and moisture content:

Results from Tables (1) showed that fresh and dry matter weights of shoot were significantly higher values of Jatropha plants treated with Indole butyric acid (IBA) and Humic acid (HA) substances. The highest fresh and dry matter weights (155.9g and 24.42g) were obtained from combined treatment of low dose of IBA and Humic applications (2g/L Humic acid+100 ppm IBA)/T6, compared with untreated plant (37.57 d , 15.67 b) as an average of the two successive seasons respectively. Meanwhile, the moisture content did not show any significant differences between all treatments.

As for Jatropha leaves, they had the highest fresh and dry weights content (48.40g and 10.34g) by the application of Humic acid at 2 g/L, respectively with comparison of control plants resulted (25.98g and 8.33g) in fresh and dry matter content respectively. In general, the percentages of moisture content were committable to the previous trend of fresh and dry matter weights of Jatropha plants in all applications of IBA and Humic acid (Table, 2).

From Tables (3) the fresh and dry matter weights of root Jatropha plants showed higher values with low doses of Indole butyric acid (IBA) and humic acid (HA) substances. The different trends between the two applications depend on solely or combined uses as an average of the two successive seasons. Moreover, the combined treatment of low dose of IBA and Humic applications (2g/L Humic acid+100 ppm IBA) /T6, had the highest fresh and dry matter weights (39.25 a, 11.14 a) with the highest moisture content (71.06%). Untreated Jatropha plants gained the higher result fresh weight (37.57g), but with lowest values of plant root (7.91bc), as calculated results of two seasons.

Table 1: Effect of humic acid and indole butyric acid at different concentrations on vegetative growth of stem jatropha plant as average of two seasons.

Treatment	Plant stem characters					
	Plant height (cm)	Length (cm)	Diameter (cm)	fresh weight (g)	dry weight (g)	Moisture content (%)
T1- Control	82.33 e	63.67 e	1.33 cd	37.57 d	15.67 b	84.32 a
T2- 100 ppm IBA	129.30 c	89.33 bcd	1.83 a	74.07 b	14.36 cd	80.81 a
T3- 200 ppm IBA	115 d	86.00 cd	1.37 bcd	47.76 cd	9.67 c	79.67 a
T4- 2 g/L Humic acid	129.70 c	99 b	1.50 abcd	83.52 b	14.03bcd	83.32 a
T5- 4 g/L Humic acid	114.80 d	84.67 d	1.13 d	76.85 b	11.18 c	79.42 a
T6- 2 g/L Hu+100 ppm IBA	160.50 a	121 a	1.80 ab	155.9 a	24.42 a	79.25 a
T7- 4 g/L Hu+200 ppm IBA	143.00 b	94.50 bc	1.70 abc	54.18 c	16.80 b	56.08 b

1.5. Chlorophyll content

The results in Table (2) show a positive and significant response of chlorophyll pigment contents (chlorophyll a, b and total chlorophyll) under the applications of indole butyric acid (IBA) and humic acid (HA) compared with the control of the leaves of Jatropha seedling plants as the average of the two seasons of the study. Generally, Jatropha seedlings had higher chlorophyll a content than chlorophyll b in all treated seedlings in this respect. The obtained results, reflected the increase of chlorophyll pigment contents (a, b and total) with the increment of solely IBA concentration (200g/L) /T3 application, which recorded the highest values (22.13, 11.37 and 33.50 mg/ 100g FW) of both. a, b and total chlorophyll, respectively. Meanwhile, the lowest chlorophyll contents were noticed in the untreated plants (16.69, 7.44 and 24.13 mg/100g FW). On the other hand, low dose of the combined HA and IBA substances (2g/L HA+100 ppm IBA) /T6 gave higher chlorophyll pigments content (22.47, 6.88 and 29.35 mg/100g FW) than the higher combined dose (4g/L HA+200 ppm IBA of both. a, b and total chlorophyll, respectively.

Table 2: Effect of humic acid and indole butyric acid at different concentrations on vegetative growth of leaf jatropha plant as average of two seasons.

Treatment	Plant leaf characters							
	leaves number	Leaf area (cm^2)	Chll. a mg/100 gm FW	Chll. b mg/100 gm FW	Total Chll. mg/100 gm FW	fresh weight / (g)	Dry weight / (g)	Moisture content (%)
T_1- Control	15.0 c	172.70 bc	16.69 b	7.44 bc	24.13 c	25.98 c	8.33 bc	67.52 c
T_2- 100 ppm IBA	27.67 b	191.60 bc	17.11 b	8.40 b	25.51 c	44.05 ab	8.9 bc	79.79 a
T_3- 200 ppm IBA	24.33 b	166.20 c	22.13 a	11.37 a	33.50 a	34.33 bc	8.06 bc	76.51 ab
T_4- 2 g/L Humic acid	26.0 b	213.80 b	23.38 a	6.93 c	30.31 ab	48.40 a	10.34 ab	78.54 a
T_5- 4 g/L Humic acid	22.33 b	182.50 bc	21.20 a	6.29 c	27.49 bc	24.30 c	7.71 c	68.57 c
T_6- 2 g/L Hu+100ppm IBA	36.0 a	258.40 a	22.47 a	7.52 b	29.35 b	32.58 bc	12.48 a	74.05 abc
T_7-4g/L Hu+200ppm IBA	28.33 b	188.10 bc	21.21 a	6.88 c	28.73 b	43.48 ab	8.35 bc	69.66 bc

Table 3: Effect of humic acid and indole butyric acid at different concentrations on vegetative growth of roots jatropha plant as average of two seasons

Treatment	Plant root characters				
	Length (cm)	Diameter (cm)	fresh weight (g)	dry weight (g)	Moisture content (%)
T_1- Control	20.0c	1.03 b	34.37 a	7.91 bc	71.15 a
T_2- 100 ppm IBA	32.33 ab	1.73 a	28.59 b	9.57 ab	69.81 ab
T_3- 200 ppm IBA	27.67 b	1.33 ab	18.72 c	8.70 abc	48.49 c
T_4- 2 g/L Humic acid	24 bc	1.43 ab	25.61 b	8.28 bc	67.40 ab
T_5- 4 g/L Humic acid	24.33 bc	1.30 ab	19.45 c	6.72c	65.45 b
T_6- 2 g/L Hu+100 ppm IBA	39.0 a	1.75 a	39.25 a	11.14 a	71.06 ab
T_7- 4 g/L Hu+200 ppm IBA	35.0 a	1.43 ab	15.98 c	7.20 bc	54.77 c

2-Seedling mineral contents:

Variance analyses results for leaves macro and microelements of jatropha seedling are given in Table (4). Macroelement (N, P, K, Ca, and Mg) and microelements (Fe, Mn, Cu, and Zn) were significantly influenced by the humic acid (HA) and Indole butyric acid (IBA) treatments. All these minerals were also affected by the HA x IBA interaction significantly as compared with control treatment.

2-1. Macro elements

As for IBA, treading jatropha seedlings with 100ppm IBA recorded the highest content of macro elements such as N, P, K, Ca and Mg (2.54, 0.44, 2.50, 0.93 and 0.54 % respectively) followed by 200 ppm IBA (2.31, 0.27, 2.49, 0.92 and 0.45 % respectively). Regarding humic acid, the maximum N, P, K, Ca and Mg content in leaves was obtained by application of HA at 2g/L (2.12, 0.36, 2.11, 1.14 and 0.55 %) followed by 4g/L (2.18, 0.33, 1.66, 0.98 and 0.50 %). The interactions treatment between humic acid and Indole butyric acid (2g/L Hu+100 ppm IBA) gave the highest values of N (2.67%), P (0.45%), K (2.55%), Ca (1.31%) and Mg (0.85%) compared with the other treatments and control.

2-2. Micro elements

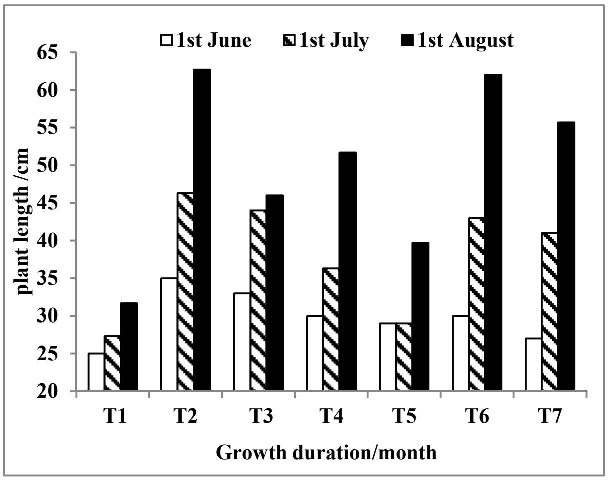
Humic acid (Hu) and Indole butyric acid (IBA) applications and their interactions increased microelements content of jatropha seedlings as compared with untreated plant (control).The highest content of microelements such as Fe, Mn, Cu and Zn (156.33, 115.33, 36.33 and 19.67 ppm) were obtained from IBA treatment at100ppm (165, 125.33, 37.33 and 25 mg respectively), followed by 200 ppm IBA. In addition, humic acid (Hu) treatments showed the same trend with different concentrations. The most effective treatment was the interactions between humic acid and indole butyric acid (2g/L Hu+100 ppm IBA)/T6 followed by T7(4g/L Hu+200 ppm IBA) compared with the other treatments and untreated plant.

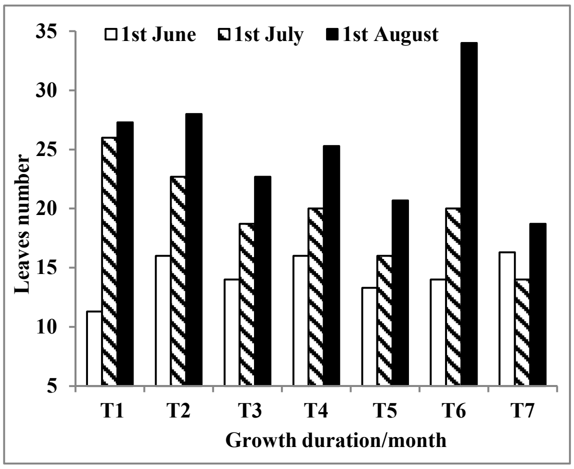
Table 4: Effect of humic acid and indole butyric acid each as a single or in different combinations on macro and microelements content of leaves jatropha plant as average of two seasons.

Treatments	Macroelements (%)					Microelements (ppm)			
	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
T_1- Control	1.60 g	0.183 f	1.78 e	0.85 e	0.44 g	165 d	130.67 c	20.42 f	28.67 c
T_2- 100 ppm IBA	2.54 c	0.44 b	2.50 b	0.93 d	0.54 d	51.02 ef	115.33 e	37.33 d	25 e
T_3- 200 ppm IBA	2.31 d	0.27 e	2.49 b	0.92 d	0.45 f	156.33 e	125.33 d	36.333 d	19.67 g
T_4- 2 g/L Humic acid	2.116 e	0.36 c	2.11 c	1.14 b	0.55 c	148 f	108 f	40.67 c	26.33 d
T_5- 4 g/L Humic acid	2.18 e	0.33 d	1.66 f	0.98 c	0.50 e	173.33 c	106.67 f	32.33 e	23.67 f
T_6- 2 g/L Hu+100 ppm IBA	2.67 a	0.45 b	2.55 a	1.31 a	0.85 a	201.67 a	140.67 a	51.67 a	34.67 a
T_7- 4 g/L Hu+200 ppm IBA	2.66 b	0.50 a	1.98 d	1.14 b	0.63 b	183.33 ab	134 b	44 b	32 b

3. Developmental growth

The effect of different doses of humic acid (HA), indole butyric acid (IBA) and their interactions on the developmental growth of jatropha seedlings are illustrated in Figures (1 and 2). Results show that jatropha seedling height and leaves number had significantly and positive increase during the growth duration (monthly), respectively. In general, the lesser dose of 2 g/L (Hu) or100 ppm (IBA) gave the tallest plants and great leaves number of jatropha seedling. Meanwhile, the higher dose was recorded the lower plant height and leaves number. Moreover, the interaction between humic acid and indole butyric acid (2 g/L Hu+100 ppm IBA)/ T6 was the most effective treatment for developmental growth at the third month of jatropha seedling characters (plant height and leaves number) compared with the untreated plant.





Figs. 1 and 2: Effect of humic acid and indole butyric acid at different concentrations on growth duration of plant height and leaves number of jatropha seedlings as average of two seasons.

Discussion

The obtained results, regarding the effect of humic substances on jatropha seedlings are in agreement with those obtained by Silva *et al*. (2010) who reported that the humic substances have a very pronounced influence on the growth of plant roots and enhanced root initiation and increased root growth which known root stimulator. Furthermore, Yildirim (2007) reported that the humic substances are increase yield and quality in a number of plant species. Also, promote plant growth enormously due to increasing cell membrane permeability, respiration, photosynthesis, oxygen and phosphorus uptake, and supplied root cell growth (Russo and Berlyn, 1990), and increased the chlorophyll content (Baldotto *et al*., 2009), increased the capacity of moisture retention in the soil or substrate (Khaled and Fawy, 2011).The lowest doses of humic substances application increased the nutrient uptake of wheat as reported by Asik *et al*. (2009).

From the previous results, it can be concluded that humic acid is technically not a fertilizer, although in some walks people do consider it. Humic acid is an effective agent to use as a complement to synthetic or organic fertilizers. In many instances, regular humic acids use will reduce the need for fertilization due to the soil and plant ability to make better use of it. In some instances, fertilization can be eliminated entirely if sufficient organic material is present and the soil can become self-sustaining through microbial processes and humus production. Hussein (2014). Moreover, Defline *et al.* (2005) specified that the foliar application of humic acids caused a transitional production of plant dry mass with respect to the unfertilized control. The application of humic reduces the requirement of other the fertilizers. It also increases crop yield, soil aeration, and drainage can also be improved by humic, the establishment of desirable environment for the development of microorganisms. Increase in the protein and mineral contents of most crops is possible by the application of humic substances Abd El-Kader *et al.* (2012). However, the obtained results are in harmony with those obtained by Chunhua *et al.* (1998) who reported that humic acid has many effects due to their increase of cation exchange capacity which affects the retention and availability of nutrients, or due to a hormonal effect, or the combination of both. Also, increasing nutrient uptake Russo and Berlyn, (1990). Abd El-Razek, *et al.* (2012) found that foliar and/or soil application of humic acid had a positive effect on growth of Florida Prince peach tree.

On the other hand, indole-3-butyric acid significantly increased vegetative growth, yield and their components and chemical constituents of maize grains (Amin *et al*., 2006). Treating different plant cuttings by dipping in IBA at 500 to1000 ppm for few seconds promote adventitious roots formation, root number and root length (Robbins *et al.,* 1983).

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