

USE OF HUMIC ACID AND SOME BIOFERTILIZERS TO REDUCE NITROGEN RATES ON CUCUMBER (*Cucumis sativus* L.) IN RELATION TO VEGETATIVE GROWTH, YIELD AND CHEMICAL COMPOSITION.

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ABSTRACT

Two field experiments were carried out during the two summer seasons of 2007 and 2008 at Kaha Hort. Res. Station, Kaliobia Governorete. Humic acid and *Azotobacter* and *Azospirillum* as bacteria fixing nitrogen along with nitrogen levels 30, 60 and 90 kg N/fed. plus uninoculated plants were evaluated under recommended N dose 120 kg N/fed., in addition to their interaction on growth, yield and chemical composition of cucumber plants. A split plot design with three replicates was used. The results revealed that, humic acid at 0.5 % as soil application increased the vegetative growth expressed as number of leaves, plant height (cm), plant fresh and dry weight (gm) and leaf area (cm²) and increased significantly fruits/plant, plant yield (kg/fed.) Early and total yield (ton/fed.).

Also, soil application of humic acid at 0.5 % had significant effect on nitrogen, phosphorus and potassium. However, NO₃ in cucumber fruits was not significantly affected with humic acid.

Biofertilizers (*Azotobacter* and *Azospirillum* as bacteria fixing nitrogen had a significant on all studied character in both seasons, the highest values were obtained by inoculation cucumber plants with *Azotobacter* and 90 kg N/fed.

The interaction between humic acid and biofertilizers plus nitrogen levels had significant effect on all treats under study in both seasons.

Generally, it could be concluded that, soil application of humic acid at 0.5% twice after 36 and 50 day after sowing at rate 25 ml/plant and inoculation plants with *Azotobacter* after 30 day after sowing at rate 25 ml/plant along with 90 kg N/fed gave the highest values of yield and NO₃- concentration on cucumber fruits were within the safe levels from human

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the important cucurbitaceous crops grown in Egypt. The cultivated open field area reached 71932 feddan with average yield of 9.33 ton /fed. The excess use of nitrogen fertilizers in agriculture can lead to nitrate accumulation in plants and ground water pollution, nitrate accumulation in edible plants is a problem when eaten, part of ingested nitrate may be converted to nitrite causing methaemoglobinaemia or oveen to carcinogenic nitrosamines (Alexander, 1977).

Accordingly, active researches must be conducted to find ways of reducing nitrate accumulation in vegetable crops.

Humic acid had significant effect on vegetative growth and yield of potato plants (Awad and El-Ghamry 2007). Enhancement of plant growth using humic acid had been reported to be due to increasing nutrients uptake

such as N, Ca, P, K, Mg, Fe, Zn and Cu (Adani, *et al* 1998 and David, *et al* 1994), and enhancement of photosynthesis, chlorophyll density and plant root respiration has resulted in greater plant growth with humate application (Chen and Avid 1990 and Smidova 1960). The application of biofertilizers to avoid environmental pollution. *Azotobacter* and *Azospirillum* were found to have not only the ability to fix nitrogen but also to release certain phytohormones of gibberelline and indolic nature, which could stimulate plant growth, absorption of nutrients and photosynthesis process (Tien *et al.*, 1979; Rynders and Vlassak, 1982 and Fayez *et al.*, 1985).

Azotobacter is also known to produce an either soluble fungistatic substance which inhibits the growth of fungi like *Alternaria*, *Fusarium* and *Rhizoctonia solani* (Gupta *et al.*, 1995). Single inoculation of cucumber and tomato plants with *Azotobacter* caused an increase in nitrogen content by 44.3 and 50% in cucumber and tomato plants compared with uninoculated plants, respectively (Gomaa, 1995). Gharib (2001) found that inoculated cucumber plants with *Azotobacter* plus phosphate dissolving bacteria (PDB) led to significant increases in early and total yield at the half dose of the normal mineral nitrogen. Also, Hanna *et al.*, 2005 found that application of chicken manure with biofertilizers (*Azotobacter* + *Azospirillum*) significantly increased vegetative growth and early and total yield of cucumber.

The objective of the present work was to study the effect of humic acid and inoculation with *Azotobacter* and *Azospirillum* plus different doses of N-chemical fertilizer (30, 60 and 90 kg N/fed. Uninoculated plants received the recommended N-dose (120 kg N/fed) were also involved on vegetative growth, yield and its components and chemical composition on cucumber plants.

MATERIALS AND METHODS

Two field experiments were carried out during the two summer seasons of 2007 and 2008 at Kaha Hort. Res. Station, Kaliobia Governorete to study the effect of humic acid and *Azotobacter* and *Azospirillum* as bacteria fixing nitrogen and nitrogen N-levels 30, 60 and 90 kg N/fed. plus uninoculated plants received the recommended N dose 120 kg N/fed., on growth, yield and chemical composition of cucumber plants.

Physical properties for experimental soil (texture class) was clayey and pH 7.9 and 8.1 in the first and second year, respectively. The experimental was carried out in a split plots design with three replications. The main plots were included humic acid and without humic which subdivided to seven sub plots (recommended dose 120 kg N/fed, $\frac{3}{4}$ R-dose + *Azotobacter*, $\frac{3}{4}$ R-dose + *Azospirillum*, $\frac{1}{2}$ R-dose + *Azotobacter*, $\frac{1}{2}$ R-dose + *Azospirillum*, $\frac{1}{4}$ R-dose + *Azotobacter*, $\frac{1}{4}$ R-dose + *Azospirillum*) the experiment includes 14 treatments, which were the combination between two humic acid and 7 biofertilizer and nitrogen levels.

Cucumber seeds (Amira II hybrid were sown on the second week of March for both seasons of study. Seeds were sown in hills 30 cm apart on one side of ridge of 5 meters in length and one meter in width. Thinning took

place after complete germination, i.e., two weeks after seed sowing, leaving one plant per hill. The sub plot area was 15 m² and each one consisted of three rows. Three commercial fertilizers were used; ammonium nitrate (33% N), calcium super phosphate (15.5% P₂O₅) and potassium sulphate (48% K₂O). In applying the fertilizers, the quantity devoted for each plot was divided as follows:

- Nitrogen fertilizer treatments was divided into three equal parts; the first was added after three weeks from sowing, the second after two weeks from first part and third two weeks after second part latter.
- Phosphorus fertilizer was divided into two equal parts; the first was applied to soil before sowing and second after three weeks from sowing.
- Potassium fertilizer was divided into equal parts, the first was added after three weeks from sowing and the second after two weeks (36 days after sowing).

Humic acid at 0.5% was added beside cucumber plants after 36 and 50 days after sowing. An efficient of *Azotobacter chroococcum* and *Azospirillum lipoferum* were obtained from microbial collection of Dept. Agric. Microbiology, Fac. Agric. Ein Shams University, Cairo, Egypt.

The mother culture of *Azotobacter* strain was grown on modified a Shhy's medium of Abdel-Malek and Ishac (1968) while *Azospirillum* isolate was grown on nutrient broth medium oxoid Manual, (1965). Inoculum was prepared by subculturing the *Azotobacter* and *Azospirillum* mother culture on nutrient agar in kolle flasks for 72 hr, after which the heavy growth was then scratched and transferred sterile tap water and thoroughly mixed. The prepared inoculum was then used to inoculate plants at rate of 25ml/plant after one month from planting bacterial growth conditions: total bacteria counts was determined on nutrient agar medium (Difeo Manual, 1977 at 30°C for 48 to 72 hr. But *Azotobacter* and *Azospirillum* were calculated on Ashby's medium Abdel-Malek and Ishad, (1968) and Dobereiner, (1988) media at 30°C for 10 days, respectively.

A random sample of five plants from each sub plot were taken at the flowering stage for determination of vegetative growth, i.e., plant height, number of leaves, plant fresh and dry weight and leaf area (cm²) of the six leaf from the meristemic top of the main stem.

Number of fruits/plant, plant yield (kg), early yield (ton/fed) fruits of first six harvests from each treatment were weighted to calculate the early yield per fed. and total yield (ton/fed.). All fruits harvested from each treatment through harvesting period were weighted to calculate the total yield per fed.

Total nitrogen, phosphorus and potassium were determined in leaves at the beginning of flowering according to the method described by Pregl (1945), Murphy and Riley (1962) and Brown and Lilleland (1946). Nitrate was determined in fruits according to Singh (1988).

All obtained data were statistically analyzed for variance and the mean values were compared at 5% levels of LSD according to (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Vegetative growth parameters:

Effect of humic acid

Data recorded in table (1) explain that growth parameters of cucumber plants expressed as No. of leaves, plant height (cm), plant fresh and dry weight (gm) and leaf area (cm²) were significantly influenced by humic acid however, number of leaves and fresh weight were not significantly affected in the second season. The highest values of most these traits were obtained by soil application of humic acid. These results could be due to the role of humic acid which enhance photosynthetic process, stimulate root growth and development of chlorophyll and proliferation of desirable micro-organisms in soil (Liu *et al.*, 1998).

Table (1): Effect of humic acid and biofertilizers on plant vegetative growth of cucumber during 2007 and 2008 seasons.

Treatments	No. of leaves		Plant height (cm)		Plant fresh weight (gm)		Plant dry weight (gm)		Leaf area (cm ²)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Humic acid	24.38	22.71	104.38	107.95	339.05	340.90	44.07	44.54	182.81	159.95
Without	21.86	21.57	98.24	89.20	331.33	329.86	42.35	42.56	165.86	147.76
L.S.D at 5%	0.82	n.s	4.1	18.12	7.08	n.s	1.7	0.78	7.7	9.42
120 N/fed(R) kg	25.0	23.7	111.16	97.83	357.00	342.5	45.88	44.08	186.66	162.83
Azt. + ¼ R	29.0	26.5	115.66	101.50	364.33	365.00	48.25	48.02	197.83	169.66
Azs. + ¼ R	21.5	21.3	105.33	106.33	354.50	343.3	44.81	44.03	179.33	156.83
Azt. + ½ R	24.16	23.0	104.66	105.16	334.0	336.33	42.30	44.25	175.5	155.66
Azs. + ½ R	20.8	20.17	98.0	98.16	326.33	332.33	41.12	43.11	169.3	151.16
Azt. + ¼ R	22.16	21.0	89.3	94.00	313.33	317.5	41.04	41.78	159.2	142.83
Azs. + ¼ R	19.2	19.33	84.5	87.00	305.50	310.67	39.08	39.62	152.00	138.00
L.S.D at 5%	0.32	1.19	2.9	n.s	3.95	11.83	1.68	0.81	4.93	3.1

R: Recommended dose (120 kg N/fed.). Azt: Azotobacter Azs: Azospirillum

In addition, Atiyeh *et al.*, (2002) found that increasing humic acid from 50 to 500 mg/kg soil significantly increased growth of cucumber plants in terms of plant height, leaf area, shoot, root and dry weight. The beneficial effects of humic acid have been attributed to improvements in soil properties and structure (Kahsnitz 1992), to greater availability of mineral nutrients to plants (Gilot 1997) and increased microbial population and biologically active metabolites such as plant growth regulators (Tomati and Gali, 1995).

Effect of biofertilizers

Data in table (1) show that growth parameters of cucumber plants i.e., number of leaves and leaf area were reduced by nitrogen stress (30 kg/fed) in both season. Similar results were obtained by Byari and Mirdad (1996), they found that reducing nitrogen stress by increasing the level of nitrogen application of 10 to 300 kg N/ha increased plant growth.

Effect of interaction between humic acid and biofertilizers

The interaction between humic acid and Azotobacter and Azospirillum had a significant effects on plant height, plant fresh and dry weight and leaf area in both seasons (Table 2).

Table (2): Vegetative growth of cucumber plant as affected by interaction between humic acid and biofertilizers during 2007 and 2008 seasons.

Treatments		No. of leaves		Plant height (cm)		Plant fresh weight (gm)		Plant dry weight (gm)		Leaf area (cm ²)	
		2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Humic acid	120kgN/fed(R)	26.30	25.33	114.66	117.33	361.66	346.67	48.13	45.83	195.00	166.00
	Azt. + ¾ R	30.32	28.00	119.66	122.33	370.33	375.00	50.36	39.33	205.66	171.67
	Azs. + ¾ R	22.33	21.33	105.66	106.33	349.33	351.66	46.76	45.10	184.66	161.00
	Azt. + ½ R	25.67	22.70	107.33	115.33	337.33	339.33	41.76	44.63	180.00	160.67
	Azs. + ½ R	21.67	20.30	103.33	106.30	330.33	339.70	40.56	44.55	174.33	154.33
	Azt. + ¼ R	23.66	21.33	92.66	97.33	315.00	320.00	41.26	42.10	175.00	155.67
	Azs. + ¼ R	20.68	20.00	87.33	90.67	309.33	314.00	39.66	40.24	165.00	150.33
Without	120kgN/fed(R)	23.66	22.00	107.66	78.33	352.33	338.33	43.36	42.24	178.33	159.67
	Azt. + ¾ R	27.66	25.00	111.66	80.66	358.32	355.0	46.13	46.07	190.00	167.67
	Azs. + ¾ R	20.60	21.30	105.00	106.33	341.67	355.0	42.85	42.96	175.00	152.66
	Azt. + ½ R	22.66	23.30	102.00	95.00	330.66	333.30	42.83	43.86	171.00	150.67
	Azs. + ½ R	20.00	20.00	92.66	90.00	322.33	325.00	41.67	41.66	164.33	148.0
	Azt. + ¼ R	20.66	20.66	87.00	90.66	312.33	315.01	40.82	41.45	143.33	130.00
	Azs. + ¼ R	17.66	18.66	81.66	83.33	301.67	307.30	38.50	38.99	139.00	125.60
L.S.D at 5%		0.45	1.78	4.08	38.13	5.58	17.66	2.38	1.21	6.97	4.38

R: Recommended dose (120 kg N/fed.), Azt: Azotobacter Azs: Azospirillum

Data indicate that the highest values were recorded with soil application of humic acid along with 3/4 recommended dose of N-chemical fertilizer (90 kg N/fed) and inoculated cucumber plants with Azotobacter. The lowest values were obtained without soil application of humic acid along with inoculated cucumber plants with Azospirillum and 30 kg N/fed. Such results could be due to enhancement of plant growth after using humic acid. Increasing nutrients uptake such as N, P, Ca, K, Mg, Fe, Zn and Cu is illustrated in this respect (Adani *et al.*, 1998 and David *et al.*, 1994).

Also, such results may suggest that N-fixing bacteria (Azotobacter and Azospirillum) have the ability to supply the grown plants with some their nitrogen requirements, in addition to their ability to release plant promoting substances, mainly IAA, gibberillin and cytokinine-like substances which could stimulate plant growth absorption of nutrients and efficiency of nutrient and the metabolism of photosynthates (Dobereiner, 1988; Tien *et al.*, 1979; Reynders and Vlassak, 1982).

Yield and its components

Effect of humic acid

Results in table (3) show that soil application of humic acid significantly increased the number of fruits/plant, yield (kg/plant) and early and total yield of cucumber plants in both seasons. Similar results were obtained by Awad and El-Ghamry (2007). Results illustrated by Chen and Aviad (1990) demonstrated that humic materials led to increase the permeability of plant membranes, promote the uptake of nutrients, increase soil moisture holding capacity, improve soil, reduce impacts of disease and stimulate plant growth.

Effect of biofertilizers

Data in table (3) show that inoculated cucumber plants with (Azotobacter and Azospirillum) significantly increased number of fruits/plant, yield of plant (kg) and early and total yield. The highest values were obtained

by inoculated cucumber plants with Azotobacter along with 3/4 recommended dose of N-chemical fertilizer (90 kg N/fed.) and the lowest values were obtained by inoculated plants with Azospirillum and 1/4 recommended dose of N-chemical fertilizer (30 kg N/fed.) These results are in agreement with that reported by Gharib 2001 and Hanna, *et al.*, (2005) on cucumber.

Table (3): Effect of humic acid and biofertilizers on yield and its component of cucumber during 2007 and 2008 seasons.

Treatments	No. of fruits/plant		Plant yield (kg)		Early yield (ton/fed)		Total yield (ton/fed)	
	2007	2008	2007	2008	2007	2008	2007	2008
Humic acid	10.18	10.31	1.08	1.09	4.76	4.81	13.65	13.92
Without	8.75	7.93	0.84	0.91	4.07	3.72	11.67	10.68
L.S.D at 5%	0.26	0.7	0.07	0.04	0.28	0.45	0.78	1.16
120 kg N/fed.(R)	9.35	9.98	1.05	0.97	4.18	4.47	12.51	13.41
Azt. + ¼ R	10.41	10.74	1.12	1.09	4.9	5.13	13.69	14.38
Azs. + ¼ R	10.35	10.08	1.08	1.07	4.7	4.67	13.65	13.82
Azt. + ½ R	9.65	9.26	0.97	1.04	4.61	4.47	12.92	12.45
Azs. + ½ R	9.35	8.4	0.88	1.02	4.57	3.74	13.23	11.28
Azt. + ¼ R	8.61	7.74	0.82	0.92	4.20	3.73	11.73	10.45
Azs. + ¼ R	8.25	7.65	0.81	0.87	3.75	3.55	10.86	10.30
L.S.D at 5%	0.46	0.57	0.05	0.08	0.39	0.31	0.97	0.68

R: Recommended dose (120 kg N/fed.). Azt: Azotobacter Azs: Azospirillum

Effect of interaction between humic acid and biofertilizers

The interaction between humic acid and inoculated cucumber plant with Azotobacter and Azospirillum had a significant effects on yield of plant (kg) and early and total yield in both seasons (table 4).

Table (4): Yield and its components as affected by interaction between humic acid and biofertilizers on cucumber during 2007 and 2008 seasons.

Treatments	No. of fruits/plant		Plant yield (kg)		Early yield (ton/fed)		Total yield (ton/fed)		
	2007	2008	2007	2008	2007	2008	2007	2008	
Humic acid	120 kg N/fed(R)	9.5	11.13	1.16	0.99	4.17	4.98	12.53	14.95
	Azt. + ¼ R	11.4	12.35	1.27	1.22	5.51	5.88	15.37	16.84
	Azs. + ¼ R	11.30	11.16	1.20	1.18	5.20	5.36	15.03	15.52
	Azt. + ½ R	11.03	10.50	1.10	1.16	5.01	5.08	14.00	14.11
	Azs. + ½ R	10.10	9.60	1.00	1.15	5.08	4.13	14.76	12.87
	Azt. + ¼ R	9.13	8.80	0.93	1.00	4.46	4.24	12.48	11.89
	Azs. + ¼ R	8.80	8.63	0.90	0.93	3.90	3.99	11.36	11.63
Without	120 kg N/fed(R)	9.20	8.82	0.93	0.95	4.18	3.97	12.49	11.89
	Azt. + ¼ R	9.41	9.13	0.96	0.97	4.29	4.38	12.00	12.29
	Azs. + ¼ R	9.41	9.00	0.95	0.97	4.23	4.16	12.26	12.12
	Azt. + ½ R	8.86	8.03	0.84	0.92	4.22	3.85	11.83	10.82
	Azs. + ½ R	8.60	7.20	0.75	0.90	4.04	3.34	11.70	9.70
	Azt. + ¼ R	8.10	6.68	0.70	0.85	3.94	3.22	10.96	9.01
	Azs. + ¼ R	7.70	6.67	0.70	0.81	3.61	3.09	10.35	8.98
L.S.D at 5%	0.65	0.81	0.07	0.12	0.48	0.43	1.38	0.96	

R: Recommended dose (120 kg N/fed.). Azt: Azotobacter Azs: Azospirillum

Data indicate that the highest values were obtained by soil application of humic acid and inoculated cucumber plants with *Azotobacter* along with 3/4 recommended dose of N-chemical fertilizer (90 kg N/fed.) And, the lowest values were obtained without soil application of humic acid and inoculated cucumber plants with *Azospirillum* which gave the lowest values of plant growth. Similar results were obtained by Byari and Mirdad 1996. Also, Hammad and Abdel-Ati (1998) found that on potato fixed amount of nitrogen by *Azospirillum* was less than 20 kg N/fed. Subba Rao (1982) reported that *Azospirillum* on certain varieties of corn may fix a maximum of 20 kg N/fed/year.

Table (5): N%, P%, K% and NO₃⁻ (ppm) of cucumber as affected by humic acid and biofertilizers during 2007 and 2008 seasons.

Treatments	N %		P %		K %		NO ₃ ⁻ (ppm)	
	2007	2008	2007	2008	2007	2008	2007	2008
Humic acid	2.11	1.99	0.201	0.188	2.47	2.40	65.47	66.47
Without	1.77	1.87	0.157	0.170	2.04	2.22	58.41	60.47
L.S.D at 5%	0.09	0.09	n.s	0.011	0.28	0.16	n.s	n.s
120 kg N/fed.(R)	2.99	2.98	0.233	0.223	2.49	2.76	93.3	101.0
Azt. + ¼ R	1.92	2.15	0.184	0.210	2.40	2.73	63.00	71.00
Azs. + ¼ R	1.86	1.95	0.183	0.190	2.37	2.47	62.37	57.00
Azt. + ½ R	1.83	1.73	0.176	0.170	2.30	2.23	60.50	56.00
Azs. + ½ R	1.83	1.65	0.174	0.160	2.28	2.09	53.35	55.00
Azt. + ¾ R	1.63	1.53	0.155	0.150	1.99	1.93	50.95	51.50
Azs. + ¾ R	1.53	1.53	0.148	0.150	1.94	1.94	50.13	51.17
L.S.D at 5%	0.15	0.12	0.014	0.01	0.16	0.18	12.63	10.15

R: Recommended dose (120 kg N/fed.). Azt: *Azotobacter* Azs: *Azospirillum*

Chemical composition

Effect of humic acid

Data in table (5) show that nitrogen, phosphorus and potassium were significantly influenced by soil application of humic acid in both seasons. These results may suggest that humic acid stimulate root growth and enable better uptake of nutrients (Liu *et al.*, 1998; Zhang *et al.*, 2003; Awad and El-Ghamry 2007). Data in table (5) show also that nitrate concentration (ppm) was not significantly affected by soil application of humic acid in both seasons.

Effect of biofertilizers

Data in table (5) indicate that minerals content and NO₃ concentration were significantly affected by biofertilizers. The highest contents were obtained by fertilization cucumber plants with 120 kg N/fed. (uninoculated plants). Mahmoud *et al.*, 2009 on cucumber found that mineral nitrogen in cucumber led to a clear increase in nitrogen-% and nitrate concentration in cucumber leaves and fruits and higher than N% organic. Similar findings were obtained by Anga (2001) who found an in line in nitrate concentration on spinach leaves with mineral N-fertilizer. On the other hand, the lowest values were obtained by inoculated cucumber plant with *Azospirillum* along with 30 kg N/fed in both seasons.

Table (6): N%, P%, K% and NO₃⁻(ppm) of cucumber as affected by interaction effect between humic acid and biofertilizers on cucumber during 2007 and 2008 seasons.

Treatments		N %		P %		K %		NO ₃ ⁻ (ppm)	
		2007	2008	2007	2008	2007	2008	2007	2008
Humic acid	120 kg N/fed(R)	3.08	3.0	0.288	0.21	2.74	2.64	94.26	104.6
	Azt. + ¼ R	2.13	2.03	0.204	0.20	2.66	2.60	69.90	68.33
	Azs. + ¼ R	2.06	1.66	0.198	0.16	2.60	2.10	68.43	55.33
	Azt. + ½ R	2.03	1.70	0.195	0.17	2.60	2.23	67.06	58.26
	Azs. + ½ R	2.05	1.43	0.193	0.14	2.55	1.84	47.00	48.33
	Azt. + ¼ R	1.76	1.70	0.165	0.16	2.54	2.13	56.80	56.00
	Azs. + ¼ R	1.60	1.56	0.160	0.15	2.08	1.99	54.00	52.33
Without	120 kg N/fed(R)	2.9	2.96	0.176	0.23	2.23	2.88	92.33	98.00
	Azt. + ¼ R	1.7	2.26	0.164	0.22	2.13	2.85	56.10	75.33
	Azs. + ¼ R	1.66	2.23	0.166	0.22	2.14	2.84	56.30	74.60
	Azt. + ½ R	1.63	1.73	0.157	0.17	2.05	2.23	53.93	58.60
	Azs. + ½ R	1.60	1.63	0.155	0.16	2.02	2.08	53.26	54.60
	Azt. + ¼ R	1.50	1.60	0.144	0.16	1.90	2.05	49.87	54.00
	Azs. + ¼ R	1.40	1.50	0.136	0.14	1.80	1.90	47.10	50.00
L.S.D at 5%		0.22	0.17	0.02	0.018	0.23	0.25	17.87	14.35

R: Recommended dose (120 kg N/fed.). Azt: Azotobacter Azs: Azospirillum

Effect of interaction between humic acid and biofertilizers

The interaction effect between humic acid and inoculated cucumber plants with *Azotobacter* and *Azospirillum* had a significant effect on N,P,K and NO₃ in both seasons (table 6).

Results indicate that the highest values were obtained by soil application of humic acid and 120 kg N/fed. (recommended dose of N-chemical fertilizer). The lowest values were obtained without soil application of humic acid and inoculated plants with *Azospirillum*. Such results may indicate enhancement of plant growth using humic acid had been reported to be due to increasing nutrients uptake such as N, P, K, Ca, Mg, Fe, Zn, and Cu (Adani *et al.*, 1998 and David *et al.*, 1994). And N-chemical fertilizer led to increase N-uptake than N-organic which is low release (Mahmoud *et al.*, 2009), and inoculated with *Azospirillum* fixed nitrogen less than 20 kg N/fed (Hammad and Abdel-Ati 1998 and Abdel-Ati *et al.*, 1996).

Humic acid addition increase the total bacterial count in all treatments but it had a negative effect on both *Azotobacter* and *Azospirillum* counts (Table 7). Total bacteria, *Azotobacter* and *Azospirillum* counts were in the maximum being (160 x 10⁷ cfu, 6x 10⁶ cfu and 8 x 10⁶ cfu), but lowest value were 15 x 10⁷ cfu, 0.7 x 10⁶ cfu and 0.95 x 10⁶ cfu, respectively.

Generally, it could be concluded that, soil application of humic acid and inoculated cucumber plants with *Azotobacter* along with 3/4 recommended N-chemical fertilizer dose were the best treatment for maximizing the growth, yield and nutritional status of cucumber plants and low NO₃ content in fruits. So, it concluded as the best treatment on basis of yield and NO₃ safety for human nutrition.

Table (7): Effect of humic acid and nitrogen level on total bacteria count and biofertilizers in rhizosphere soil of cucumber plants during 2007 and 2008 seasons

Treatments		Total bacteria count x 10 ⁷		Azotobacter count x10 ⁶		Azospirillum count x 10 ⁶	
		2007	2008	2007	2008	2007	2008
Humic	120 kg N/fed.(R)	94	92	1.0	0.9	1.1	1.2
	Azt. + ¾ R	75	74	2.8	2.5	1.8	1.7
	Azs. + ¾ R	70	66	2.3	2.5	2.6	2.7
	Azt. + ½ R	104	102	3.3	3.1	2.3	2.2
	Azs. + ½ R	123	115	3.2	3.0	4.0	4.0
	Azt. + ¼ R	109	107	5.7	5.5	6.3	6.4
	Azs. + ¼ R	89	84	5.6	5.12	8.0	7.8
Without humic	120 kg N/fed.(R)	78	80	0.8	0.7	0.95	0.96
	Azt. + ¾ R	160	130	4.1	4.2	3.5	3.3
	Azs. + ¾ R	32	34	3.3	3.1	4.5	4.4
	Azt. + ½ R	92	87	5.4	5.3	5.0	4.8
	Azs. + ½ R	20	21	5.6	5.8	6.0	6.1
	Azt. + ¼ R	95	94	6.0	5.5	5.4	5.5
	Azs. + ¼ R	15	16	5.8	5.2	7.0	7.1

R: Recommended dose (120 kg N/fed.). Azt: *Azotobacter* Azs: *Azospirillum*

REFERENCES

- Abdel-Ati, Y.Y.; A.M.M. Hammad and M.Z.H. Ali (1996): Nitrogen fixing and phosphate solubilizing bacteria as biofertilizers for potato plants under Minia condition. Proc. 1st, Egyptian –Hungarian, Horticultural conf., Kafr El-Sheik; Egypt 15-17 Sept.
- Abd-El-Malek, Y. and Y. Z. Ishac (1968). Evaluation of methods used in counting *Azotobacter*. J. Appli. Bacteriol., 31: 267-275.
- Adani, F.; P.Genevini; P.Zaccheo and Zocchi, (1998): The effect of commercial humic acid on tomato plant growth and mineral nutrition. J. plant nutr. 21: 561-575.
- Alexander, M. (1977): Introduction to soil microbiology. 2nd Ed., John Wiley & Sans., Inc New York.
- Anga, M.A. (2001): Studies on the effect of mineral and biofertilization on yield and quality of spinach. M.Sc. Thesis Fac. of Agric., Alex. Univ. Vegetable crops Dept.
- Atiyeh, R.M.; S. Lee, C.A. Edwards, N.Q. Arancon, J.D. Metzger (2002): The influence of humic acids derived from earth worm – processed organic wastes on plant growth. Bioresource Technology 84: 7-14.
- Awad, El.M.M. and A.M. El-Ghamry (2007): Effect of humic acid effective microorganisms (EM) and magnesium on potato in clayey soil. J. Agric. Sci. Mansoura Univ., 32(9): 7629-7639.
- Brown, J.D. and O. Lilleland (1946): Rapid determination of potassium and sodium in plant material and soil extracts by flame photometry. Proc. Amer. Soc. Hort. Sci., 48: 341-346.
- Byari, S.H. and Z.M. Mirdad (1996): The response of greenhouse cucumber cultivars to nitrogen sources: 1-Vegetative growth. J.Agric. Sci. Mansoura Univ. 21(5): 1861- 1872.
- Chen, Y. and T. Aviad (1990): Effect of humic substances on plant growth. p. 161-186.

- In: Y.Chen and T. Aviad (eds). Humic substances in soil and crop sciences. Amer. Soc. Agron. Soil Sci. Amer., Madison. Wis.
- David, P.P.; P.V. Nelson and D.C. Sandres (1994): A humic acid improves growth of tomato seedling in solution culture. J.P. nutrition, 17:173-184.
- Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures (1977). Difco Laboratories Incorporated (9th ED), Detroit , Michigan . USA . 451.
- Dobereiner, J. (1988). Isolation and identification of root associated diazotrophs. Plant Soil. 110: 207.
- Eickhorst, T. and R. Tippkotter. (2008). Detection of microorganisms in undisturbed soil by combining fluorescence in situ hybridization (FISH) and micropedological methods. Soil Biology & Biochemistry. 40: 1284–1293.
- Fayez, M.; F.M. Emam and Makboul (1985): The possible use of nitrogen fixing *Azospirillum* as biofertilizer to wheat plants. Egypt. J. Microbial., 20(2), 199-2006.
- Gharib, M.G. (2001): Response of two cucumber cultivars to biofertilization under plastic house condition. M.Sc. Thesis, Fac. of Agric. Cairo Univ. 183 pp.
- Gilot, C. (1997) Effect of a tropical geophageous earthworm. *M. anomala* (Megascolecidae) on soil characteristics and production of yam crop in Ivory Coast. Soil Biology and Biochemistry 29: 353-359.
- Gomaa, A.M.H. (1995): Response of certain vegetable crops to biofertilization. Ph.D. Thesis, Fac. Agric., Cairo Unvi. Egypt.
- Gupta, S.; D.K. Arora and A.K. Srivastava (1995): Growth promotion of tomato plants by rhizobacteria and imposition of energy stress on *Rhizoctonia solani*. Soil Biology and Biochem., 27: 1051-1058.
- Hammad, A.M.M. and Y.Y. Abdel-Ati (1998): Reducing of nitrate and nitrite contents of potato tubers via biofertilization with *Azospirillum* and VA-mycorrhizal fungi. J. Agric. Sci. Mansoura Univ., 23(6): 2597-2610.
- Hanna, m.m.; S.A. Kabeel and F.M.A. Darwish (2005): Effect of organic and biofertilizers on growth yield and fruit quality of cucumber (*Cucumis sativus* L.) grown under clear polyethylene low tunnels. J. Agric. Sci., Mansoura Univ., 30(5): 2827-2841.
- Kahsnitz, H.G. (1992): Investigations on the influence of earthworms on soil and plant. Botanical Archives (I), 315-331.
- Liu, C.R.; J. Cooper and D.C. Bowman (1998): Humic acid application affect photosynthesis, root development and nutrient content of creeping bentgrass. Hort. Sci., 33: 1023-1025.
- Mahmoud, E.; N. Abdel-Kader; P.Robin; N. Akkal and Lamyaa Abdel-Rahman (2007): Effect of different organic and inorganic fertilizers on cucumber yield and some soil properties. World Journal of Agricultural Sciences 5(4): 408-414.
- Murphy, J. and J.P.R. Riley (1962): A modified single solution method for determination of phosphorus in natural waters. Anal. Chem. Acta., (27): 31-36.
- Oxoid Manual of Culture Media, ingredients and other laboratory services other laboratory services (1965), London.
- Pregl, E. (1945). Quantitative organic microanalysis. 4thEd. Chunndil, London.

- Reynders, L. and K. Vlassak (1982) Use of Azospirillum brasilense as biofertilizer in intensive wheat cropping. Plant and Soil, 66, 217.
- Singh, J.P. (1988): A rapid method for determination of nitrate in soil and plant extracts. Plant and Soil. 110: 137-139.
- Smidova, M. (1960): The influence of humic acid on the respiration of plant roots, Biol. Plant. 2: 152-164.
- Snedecor, G.W. and Cochran, W.G. (1980). Statistical methods 7th Ed. The Iowa State Univ. Press Ames, Iowa, U.S.A.
- Subba Rao, N.S. (1982): Advances in agricultural microbiology. Oxford & IBH publishing Co. New Delhi, India.
- Tien, T.M.; M.H. Gaskins and D.H. Hubble (1979): Plant growth substances produced by A. brasilense and their effect on the growth of pearl millet. Appl. Environ. Microbiol. 37, 1016.
- Tomati, U. and E. Galli (1995): Earthworms soil fertility and plant productivity. Acta Zoolgica Fennica 196, 11-14.
- Zhang, X.; E.H. Ervin and R.E. Schmidt (2003): Physiological effects of liquid applications of a seaweed extracts and humic acid on creeping. J.Amer. Soc. Hort. Sci. 128(4): 492-496.

استخدام حمض الهيوميك وبعض المخصبات الحيوية لخفض معدلات التسميد النيتروجيني للخيار وعلاقة ذلك بالنمو الخضري والمحصول والتحليل الكيماوي

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تم تنفيذ تجربتين حقليتين خلال الموسمين الصيفيين ٢٠٠٧ و ٢٠٠٨ على محصول الخيار هجين أميرة II في مزرعة محطة بحوث البساتين بقها محافظة القليوبية لدراسة استخدام حمض الهيوميك (إضافة أرضية - بدون إضافة) وكذلك تلقيح النباتات ببكتريا الأروتوباكتر والأزوسبيريلليم مع إضافة مستويات مختلفة من التسميد النيتروجيني الكيماوي (٣٠، ٦٠، ٩٠ كجم/ن/فدان بالإضافة إلى معاملة الكنترول (نباتات غير ملقحة ومسمدة بـ ١٢٠ كجم/ن/فدان) وتأثير ذلك على النمو الخضري والمحصول والتحليل الكيماوي.

ويمكن تلخيص النتائج المتحصل عليها فيما يلي:

- الإضافة الأرضية لحمض الهيوميك بتركيز ٠.٥ % بمعدل ٢٥ مل للنبات مرتين بعد ٣٦، ٥٠ يوم من الزراعة أدى إلى زيادة النمو الخضري والمحصول ومحتوى أوراق الخيار من النيتروجين والفوسفور والبوتاسيوم زيادة معنوية في كلا الموسمين ولم يتأثر محتوى الثمار من النترات معنويًا بإضافة حمض الهيوميك.
 - أوضحت النتائج أن تلقيح نباتات الخيار ببكتريا الأروتوباكتر بمعدل ٢٥ مل/النبات بعد شهر من الزراعة بالإضافة إلى التسميد بـ ٩٠ كجم/ن/ الفدان أعطى أعلى القيم لجميع الصفات المدروسة.
 - أثر التفاعل بين حمض الهيوميك وتلقيح النباتات ببكتريا الأروتوباكتر مع التسميد النيتروجيني معنويًا على جميع الصفات المدروسة.
- وعلى ذلك يمكن التوصية بإضافة حمض الهيوميك كإضافة أرضية بتركيز ٠.٥ % بمعدل ٢٥ مل للنبات بعد ٣٦، ٥٠ يوم من الزراعة بالإضافة إلى تلقيح النباتات بـ ٢٥ مل من بكتريا الأروتوباكتر مع تسميد نباتات الخيار بـ ٩٠ كجم/ن/الفدان وذلك للحصول على أعلى محصول وجوده مع تقليل التأثير الضار للأسمدة النيتروجينية حيث كان محتوى الثمار من النترات في الحدود الآمنة للإنسان.

قام بتحكيم البحث

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