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Significance of Foliar Spray with Some Growth Promoting Rhizobacteria and Some Natural Biostimulants on Yield and Quality of Cucumber Plant

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TWO field experiments were conducted at El-Kafr El-Gedid Village, Kafr El-Sheikh Governorate, Egypt, during summer seasons of 2012-2013 and 2013-2014. The study is an attempt to improve vegetative growth, yield and quality of cucumber "hybrid Prince" using some growth promoting rhizobacteria and/or biostimulants. Cucumber plants were sprayed with seaweed extract (2.5 g/L), fishmeal extract (10 g/L), *Azospirillum* liquid culture (15 ml/L), *Trichoderma* liquid culture (15 ml/L), compost tea (65 ml/L) and their mixture compared to control (tap water).The spray with the different materials were carried out after 15 days of transplanting, then two times later at 15 days intervals. The application with the different spray materials alone or mixed attained significant increases in vegetative growth (leaf area (cm^2), leaves dry matter percentage and number of female flowers/plant), fruit length (cm), fruit diameter (cm), early and total fruit yield (t/fed.) over water sprayed control plants. Furthermore, the spray materials positively increased total soluble solids, N, P and K percentages in leaves as well as chlorophyll content. Also, the mixture treatment gave the highest net return L.E/fed. (6925).

The mixture was the best in this context, so that, spraying cucumber plants with the mixture treatment three times is recommended to improve yield and quality of the plant.

Keywords: Cucumber, Seaweed, Fishmeal, *Azospirillum*, *Trichoderma*, Compost tea.

Cucumber (*Cucumis sativus* L) is an important vegetable and one of the most popular members of the cucurbitaceae family (Thoa, 1998). It is thought to be one of the oldest vegetables cultivated by man with historical records dating back 5,000 years (Wehner and Guner, 2004).

In recent years, the world focused his attention to minimize environmental pollution and human health impacts, by reducing the use of synthetic fertilizers and chemicals in crops production. Especially, vegetables which eat freshly eaten (IFAOM/SOEL, 2000 and FAO/TTC, 2001).

Compost tea extract has been defined simply as a liquid extract from composted material that may contain organic and inorganic soluble nutrients, and a large number of microorganisms including bacteria and fungi (ROU, 2003b). Compost tea is manufactured and used on a wide range of crops such as vegetables, fruit, vines, cotton, cereals, and trees. The production and application of compost tea is primarily focused on disease suppression, supplementing plant nutrients and increasing soil microbiology to improve soil structure, water percolation/retention, rooting depth and consequently improved plant growth.

Seaweed extract application for different crops is consider a great importance for its high contents from organic matter, micro elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins and amino acids and also, rich in growth regulators such as auxins, cytokinin and gibberellins (Khan *et al.*, 2009). The beneficial effect of seaweed extract is a result of many components that may work synergistically at different concentrations (Fornes *et al*., 2002).

Fishmeal fertilizers, also, are excellent sources of nutrition for soils and plants whereas fishs contain the full spectrum of nutrients. Plants rapidly respond to it and grow vigorously when regularly fertilized with fish fertilizers.

Azospirillum brasilense is one of the best bacterial biostimulants which release plant phytohormones, amino acids, antioxidants, siderofores and antibiotic in addition to fixing atmospheric nitrogen in the rhizosphere or phyllosphere (Martinez-Viveros *et al*., 2010). The effect of *Trichoderma viride* on growth of plants can increase percent of germination and produce some metabolites of the antagonistic microbes which stimulate plant growth. Such metabolites may include antibiotics, siderophores, hydrogen cyanide, enzymes, or growth stimulating hormones such as auxins and gibberellins and some of the metabolites facilitate transformation of unavailable mineral and organic compounds into available forms to the plant (Haikal, 2008).

Hence, the goal of the current research was to improve vegetative growth and yield as well as fruits quality of cucumber plants using some natural extracts which nontoxic, environmentally friendly, organic and costless.

Materials and Methods

Two field experiments were conducted at El-Kafr El-Gedid Village, Kafr El-Sheikh Governorate, Egypt, during summer seasons of 2012-2013 and 2013-2014. This work is an attempt to study the response of cucumber plants (*Cucumis sativus* cv. Prince Hybrid), to foliar spray application with some biostimulants. Also, to study the possibility of using such costless and safety methods for maximizing growth, productivity and fruit quality. Plants were sprayed three times at 20 L/feddan with diluted 1:5. The first one was at 15 days after transplanting and repeated each 15 days by intervals. All cultural practices for growing cucumber plants were performed as recommended by Egyptian Ministry of Agriculture for mineral fertilizers. Treatments were as follows:

• Compost tea extract at 65 ml/L.

• Seaweed extract at 2.5 g /L.

• Fishmeal extract at 10 g/L.

• *Azospirillum brasilense* liquid culture at 15 ml/L. (1×10^9 cfu/ml^{-1}).

• *Trichoderma viride* liquid culture at 15 ml/L. (1×10^6 spores/ ml^{-1}).

• Mixture**.** (Which contains, *Azospirillum, Trichoderma*,seaweed extract and fishmeal extract were added with the above mentioned rates to the filtrated final compost tea).

• Control (Tap water).

The previous treatments were arranged in four replicates using complete randomized block design and the plot area was 20 square meter, the spacing between plants was 40 cm. ($1m^2$ included 2.5 plant).The experimental soil analysis is shown in Table 1.

TABLE 1. Some physical, chemical and biological characteristics of the experimental soil.

Clay %	Silt %	Sand %	Texture	pH	EC ($ds.m^{-1}$)	O.M (%)
18.15	36.50	46.35	Loam	7.82	0.190	1.66

Cations (mg/L)				Anions (mg/L)				Macro-nutrient (ppm)		
Na+	Ca++	Mg^{++}	K^+	CO3–	HCO3-	Cl-	SO4–	N	P	K
0.12	0.83	0.47	0.57	-	1.00	0.81	0.18	7.10	6.16	371

Total count of bacteria (cfu/g dry weight)	Total count of fungi (cfu/g dry weight)	Total count of actinomycetes (cfu/g dry weight)
$185X10^7$	$77X10^5$	$68X\ 10^5$

Physical, chemical and biological analyses of soil were determined by Department of Soil Chemistry and Department of Agriculture Microbiology, Soil, Water and Environment Research Institute, Agric. Res. Center.

Compost tea extract

The stock solution of compost tea was prepared in Bacteriology Lab., Soil, Water and Environment Research Institute, Sakha Agricultural Research Station by soaking 5 Kg of matured compost with 0.5 L molass in 50 L of dechlorated water for three days in a polyethelen compost tea machine (100 L/capacity) with continuous areation during the period of soaking by an air pump with using PVC pipe that was dipped in it (Ingham, 2005). After soaking time, the liquid mixture was filtrated by plastic net.

Seaweed extract

Seaweed extract product imported by Techno Green Comp. Group, Cairo, Egypt. The stock solution of seaweed extract was prepared in Bacteriology Lab., Soil, Water and Environment Research Institute, Sakha Agricultural Research

Station by dissolving 2.5 g/L of dechlorated water for two days in a glass stock (20 liter) with continuous areation using air pump. Its constituent was as follows:

PH	9-11	**K_2O (%)**	≥18.0
Organic Matter (%)	40.0-50.0	**Alginic Acid (%)**	≥10
Total Nitrogen (%)	≥1.0	**Cytokinin & Gebberellin**	600 ppm
P_2O_5 (%)	≥6.0	**Dry Matter (%)**	≥95.0
Other Ingredients: S, Mg, Ca, Na, Alginic acid, Amino acid, Mannitol, Auxin and Natural Plant Hormones.			

Fishmeal extract

Fishmeal extract product was prepared in Bacteriology Lab., Soil, Water and Environment Research Institute, Sakha Agricultural Research Station by dissolving 10 g fish powder /L of dechlorated water for two days in a glass stock (20 liter) with continuous areation using air pump.

Azospirillum brasilense liquid culture

Azospirillum semi solid Döbereiner medium (Döbereiner *et al*., 1976) contained (g/L of distilled water): Malic acid, 5.0, KH_2PO_4, 0.4, K_2HPO_4, 0.1, $MgSO_4.7H_2O$, 0.2, NaCl, 0.1, $CaCl_2.7H_20$. 0.02, $FeCl_3.6H_2O$, 0.01, $NaMoO_4.2H_2O$, 0.002, Agar, 1.75. *Azospirillum brasilense* was grown up for 3 days at 30˚C.

Trichoderma viride liquid culture

Potato dextrose agar medium (Tsao, 1970) contained (g/L of distilled water): Extract of 200 g potatoes, dextrose, 20, Yeast extract, 0.5, Distilled water up to final volume of 1000 ml, Agar, 15-20.

200 g potatoes were peeled, sliced and boiled water in 500 ml distilled water until soften and then filtered. The filtrate (extract) was used for medium preparation.

Trichoderma viride was grown up for 5 days at 30˚C.

Experimental parameters

Vegetative growth

• Leaf area (cm^2) of the sixth leaf from the meristemic top of the main stem. Ten plants of each treatment and the area was determined by using L1-3000-Portable Area Meter (PAM).

• Leaf dry matter (%).

• Number of female flowers / plant.

Fruit yield

• Early yield (t/fed.): Fruits of first eight harvests from each treatment were weighted to calculate the early yield per feddan.

• Total yield (t/fed.): All fruits harvested from each treatment along the harvesting period were weighted to calculate the total yield per feddan.

• Fruit characteristics: Ten fruits from each treatment through 8 picking were taken randomly for determining average fruit characters as follows:

- Fruit weight (g). - Number of fruits/ feddan.
- Fruit length (cm). - Fruit diameter (cm).
- Total soluble solids in fruits (TSS) %.

Chemical constituents

• Total nitrogen, phosphorus and potassium were determined in the dry matter of leaves according to the methods described by Pregl (1945), Trough and Mager (1939) and Browns and Lilliland (1946) respectively.

• Total chlorophyll: Total chlorophyll was determined by Minolta chlorophyll metter SPAD- 502 in the felid.

Fruit quality

Included, average fruit length (cm), and fruit diameter (cm). Total soluble solids (TSS %) was determined in fruit juice by a hand refractometer according to A.O.A.C. (1965).

Fruit characteristics were determined in fruits picked in the same day (which its flowers were previously labeled at the same opening day).

Microbial estimations

It was estimated by counting total count of bacteria, fungi and actinomycetes according to Allen (1959).

Statistical analysis

All data obtained during both seasons of study were subjected to analysis of variance and significant differences among means were determined at 5% level of significance according to (Snedecor and Corchran, 1972).

Results and Discussion

Vegetative growth

Data of Table 2 indicated that foliar spray cucumber plants with some biostimulants (compost tea, fishmeal extract, and seaweed extract) and/or microorganisms *i.e. Azospirillum brasilense* and *Trichoderma viride* led to improvement for leaf area, number of female flowers and leaves dry matter percentage. The foliar spray with compost tea attained significant increaments for these characteristics through both growing seasons, followed by spray with liquid culture of *Azospirillum*, then fishmeal extract and finally liquid culture of *Trichoderma*. However, spray with the mixture of compost tea with the other studied materials showed the highest increases at all, and the differences were significant over control plants, which gave 93.00, 37.50 and 24.23 at the first

season (2013) and 95.25, 38.50 and 25.78 at the second (2014) respectively, compared to 61.50, 17.00 and 15.81 at season 2013 and 63.25, 20.00 and 17.44 at season 2014 for control treatment. These are in agreement with those results obtained by Chamangasht *et al*., (2012) they indicated that biostimulants (the mixture of *Azotobacter*, *Azospirillum*, *Pseudomonas* and compost tea) can improve plant growth and yield of lettuce. Also these are in agreement with Abou-El-Hassan & Desoky (2013) they reported that using compost tea of compost increased the vegetative properties of lettuce plants. Also, In this regard, (Fathy *et al*., 2000 and El-Desouky *et al*., 2011) found that spraying tomato plants with biostimulants significantly increased plant length , leaves No./plant, branching and leaf area / plant.

TABLE 2. Effect of foliar spray with plant growth promoting rhizobacteria and/or biostimulants on vegetative growth characters of cucumber plants.

Treatments	Leaf area (cm2)		Leaves dry matter (%)		Number of female flowers/plant	
	Seasons					
	2013	2014	2013	2014	2013	2014
Compost tea extract	82.50	85.00	21.89	23.32	34.25	33.75
Seaweed extract	81.00	81.25	19.25	21.22	22.00	24.50
Fishmeal extract	76.00	77.75	18.36	19.19	20.75	23.75
Azospirillum liquid	80.75	81.25	21.00	21.94	27.25	28.50
Trichoderma liquid	65.00	70.25	17.24	18.13	19.50	22.00
Mixture	93.00	95.25	24.23	25.78	37.50	38.50
Control	61.50	63.25	15.81	17.44	17.00	20.00
F. test	**	**	**	**	**	**
L.S.D 5%	3.22	2.37	1.13	1.08	1.29	2.04

Fruit yield

Data presented in Table 3 showed that the foliar spray with biostimulants, microorganisms and/or their mixture significantly increased fruit weight (g), early fruits yield (t/fed.) and total fruits yield (t/fed.) of cucumber plants. The results revealed that foliar spray with the mixture gave the highest increases over control (tap water spray), which were highly significant. These values recorded 156.00, 4.87 and 14.75 at season 2013 and 160.25, 4.85 and 15.44 at 2014, then the spray with compost tea which, also, gave highly significant increases over control. It attained 151.25, 3.80 and 12.90 at season 2013 and 154.75, 3.90 and 13.13 at season of 2014 compared to 95.00, 2.18 and 9.49 at season 2013 and 104.25, 2.28 and 9.96 respectively. These results could be explained as a reflection of the beneficial effects of some growth promoting rhizobacteria and compost tea that contains many macro and micro nutrients in available form, natural hormones, vitamins and antioxidants which be available for plant and so reflect on plant growth and its composition (Abbasi *et al*., 2002, Biocycle, 2004 and Meshref *et al*., 2010) that led to improve number of fruits and total yield of cucumber plants.

TABLE 3. Effect of foliar spray with plant growth promoting rhizobacteria and/or biostimulants on yield of cucumber plants.

Treatments	Average fruit weight (g)		Early yield (t/feddan)		Total yield (t/feddan)	
	Seasons					
	2013	2014	2013	2014	2013	2014
Compost tea extract	151.25	154.75	3.80	3.90	12.9	13.13
Seaweed extract	141.50	145.25	2.65	2.79	10.6	10.98
Fishmeal extract	121.75	131.00	2.54	2.59	10.15	10.80
Azospirillum liquid	149.00	152.25	3.28	3.26	12.15	12.25
Trichoderma liquid	107.50	114.25	2.45	2.54	9.80	10.64
Mixture	156.00	160.25	4.87	4.85	14.75	15.44
Control	95.00	104.25	2.18	2.28	9.49	9.96
F. test	**	**	**	**	**	**
L.S.D 5%	4.001	3.78	0.23	0.17	0.65	0.56

Chemical constituents

The foliar spray with microorganisms, biostimulants and their mixture led to consistent increases in chlorophyll values (SPAD units), N, P and K % over the treatment of tap water spray (control), through the two studying seasons (Table 4). The mixture treatment gave the highest increases over control, which exhibited 4.35, 4.77, 0.70 and 5.37 at season 2013 compared to treatment of control that gave 31.15, 2.80, 0.30 and 2.55 respectively. Most differences than control were significant. Results of season 2014 gave a similar trend. Similarly, Masarirambi *et al*. (2010) reported that there was relatively higher macro and micronutrients content in lettuce plants produced by foliar spray with biostimulants than those grown with conventional fertilizers. These effects might be due to the beneficial effects of compost tea and PGPR that increase supply of macro and micro nutrients in available form for plants. Also, Fish fertilizers contain significant quantities of protein nitrogen as well as a healthy balance of all 18 nutrients known to be significant for crop growth. All of these mineral nutrients are in protein chelated forms which are usable by the crops and additionally are resistant to loss from leaching. Fish also contains more than 60 other trace minerals which have positive effects on soil biology and crop health. As were suggested by Meshref *et al*. (2010) and Saharan & Nehra (2011).

(Fathy *et al*., 2000 and El-Desouky *et al*., 2011) reported that spraying tomato plants with biostimulants significantly increased mineral content (P, K, Ca and Mg), carbohydrate, sugar contents and total chlorophyll.

Shehata *et al*. (2011) mentioned that spraying growth promoting rhizobacteria significantly increased N, P, and K and content of leaves of celeriac plant.

TABLE 4. Effect of foliar spray with plant growth promoting rhizobacteria and/or biostimulants on some chemical content for leaves of cucumber plants.

Treatments	Chlorophyll content (SPAD unit)		N (%)		P (%)		K (%)	
	Seasons							
	2013	2014	2013	2014	2013	2014	2013	2014
Compost tea extract	36.00	38.47	4.35	4.42	0.68	0.70	4.90	4.97
Seaweed extract	35.80	36.82	4.07	4.10	0.58	0.60	4.37	4.57
Fishmeal extract	35.30	35.20	3.65	3.75	0.47	0.51	4.12	4.40
Azospirillum liquid	31.67	31.37	4.47	4.30	0.59	0.56	4.97	4.90
Trichoderma liquid	31.97	32.50	3.87	3.95	0.47	0.48	4.02	4.02
Mixture	40.35	40.00	4.77	4.85	0.70	0.71	5.37	5.55
Control	31.15	30.87	2.80	3.02	0.30	0.36	2.55	3.42
F. test	**	**	**	**	**	**	**	**
L.S.D 5%	2.3	1.47	0.31	0.25	0.04	0.03	0.29	0.31

Fruit quality

Data of Table 5 exhibited some of quality characteristics of cucumber fruits *i.e.* T.S.S %, fruit length (cm) and fruit diameter (cm). All the studied foliar application treatments gave increaments over tap water spray treatment (control), through the two studying seasons. The treatment which attained the highest effect was the spray with the mixture followed by compost tea then *Azospirillum*, while the spray with the diluted liquid culture of *Trichoderma* gave the lowest positive effects. Mostly, variation between revealed significances. Results of season 2014 had the same trend of those of 2013. (Abou-El-Hassan *et al.*, 2014) showed that addition of plant growth promoting rhizobacteria (PGPR) or compost tea to produce good yield and quality of cucumber plants under sandy soil. Similarly, spray of fruit trees with componds containing amino acids, plant phytohormones, humate, N, P, K and some microelements contribute in improving tree growth and quality of yield (Eissa, 2003). Likewise, foliar application with some PGPR types gave results similar to the action of these treatments (Nour El-Din, 2006).

Economic estimation

Data of Table 6 included costs of cultivation of one feddan of cucumber (fixed and changed), price of the produced fruits (L.E) and the net of feddan at each foliar spray treatment. The control treatment (tap water spray) gave the lowest net return (238 L.E. /fed.), while the treatment of spray with the mixture of biostimulants and microorganisms attained the highest net return (6925 L.E. /fed.) followed by the treatment of compost tea (4375 L.E./fed.) then the treatment of *Azospirillum* which gave 3200 L.E./fed.

TABLE 5. Effect of foliar spray with plant growth promoting rhizobacteria and biostimulants on fruit quality of cucumber plants.

Treatments	T.S.S in fruits (%)		Average fruit length (cm)		Average fruit diameter (cm)	
	Seasons					
	2013	2014	2013	2014	2013	2014
Compost tea extract	4.87	4.92	13.72	13.82	3.50	3.47
Seaweed extract	4.57	4.75	12.52	12.92	3.07	3.17
Fishmeal extract	4.50	4.52	11.32	12.10	2.95	3.02
Azospirillum liquid	4.22	4.30	13.25	13.32	3.25	3.22
Trichoderma liquid	3.97	4.12	11.32	11.60	2.77	3.52
Mixture	5.25	5.47	14.12	14.87	3.80	3.85
Control	4.15	4.22	10.67	11.12	2.5	2.87
F. test	**	**	**	**	**	**
L.S.D 5%	0.37	0.22	0.42	0.34	0.13	0.12

TABLE 6. Economic estimation of cucumber foliar spray with some biostimulants, microorganisms and their mixture.

Treatments	Fruit yield (t/fed.)	Price (L.E.)	Fixed costs (L.E.)	Changed costs (L.E.)	Total costs (L.E.)	Net return (L.E.)
Compost tea extract	13.02	16275	11750	150	11900	4375
Seaweed extract	10.79	13488	11750	200	11950	1538
Fishmeal extract	10.48	13100	11750	50	11800	1300
Azospirillum liquid	12.20	15250	11750	300	12050	3200
Trichoderma liquid	10.22	12775	11750	300	12050	725
Mixture	15.10	18875	11750	200	11950	6925
Control	9.59	11988	11750	-	11750	238

- Fixed costs (L.E.) includes, rent of field (3000), Ploughing (150), nurses (4000), pesticides (500), organic fertilizers (1500), mineral fertilizers (800), irrigation (300), harvesting (1500).
- Price of cucumber (1250 L.E. / ton).

Conclusion

It can be recommended that all applied of biostimulants and/or some growth promoting rhizobacteria had positive and promoting effects on cucumber plants growth. The results of the present investigation indicated that, foliar application of mixture, compost tea extract, seaweed extract, fishmeal extract and effective bacteria were superior effect on vegetative growth characters and some chemical compounds of fruits of cucumber plants. Also, foliar application led to producing higher total yield with best quality of cucumber fruits. It could be concluded that, mixture more effective treatment than other treatments for yield and quality of cucumber under the experimental conditions.

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معنوية الرش الورقي ببعض الكائنات الدقيقة المنتجة لمنظمات النمو وبعض المنشطات الحيوية علي محصول وجودة نبات الخيار

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أجريت دراسة حقلية لمدة عامين بقرية الكفر الجديد – محافظة كفرالشيخ – مصر. خلال المواسم الصيفية ٢٠١٢/٢٠١٣ ، ٢٠١٣/٢٠١٤ بهدف زيادة النمو الخضري، المحصول والجودة لنبات الخيار "هجين برنس" باستخدام بعض الكائنات الدقيقة المنظمة للنمو و/ أو المنشطات الحيوية حيث تم رش نباتات الخيار بمستخلص الطحالب ٢,٥ جرام/لتر، مستخلص السمك ١٠ جرام/لتر، مزرعة سائلة من الأزوسبيريلم ١٥ مل/ لتر، مزرعة سائلة من الترايكوديرما من ١٥ مل/لتر، شاي الكمبوست ٦٥ مل/لتر ومخلوط منهم علاوة علي الكنترول (ماء) كمعاملة مقارنة.

وأجري الرش بهذه المعاملات المختلفة بعد ١٥ يوم من الشتل ثم الرش مرتين كل ١٥ يوم علي فترات متقطعة. وأظهرت النتائج زيادة معنوية سواء في صورة منفردة أو مخلوطة في النمو الخضري (مساحة الورقة/نبات، عدد الأزهار المؤنثة/نبات والوزن الجاف/نبات) بالاضافة الي قياس المحيط الطولي والعرضي للثمرة والمحصول المبكر والكلي من الثمار مقارنة بالكنترول. كما أظهرت النتائج زيادة ايجابية في تقدير المواد الكلية الذائبة والنسبة المئوية للنيتروجين، الفوسفور والبوتاسيوم بالاضافة الي محتوي النبات من الكلوروفيل. كذلك اعطت معاملة المخلوط أعلي عائد صافي وصل الي ٦٩٢٥ جنيها مصريا للفدان.

وتوصي الدراسة باستخدام معاملة المخلوط من الكائنات الدقيقة المنتجة لمنظمات النمو و المنشطات الحيوية لمدة ثلاث مرات علي فترات متقطعة لزيادة المحصول وصفات الجودة لنبات الخيار.