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Determination of the Effects of Bacterial Fertilizer on Yield and Growth Parameters of Tomato

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ABSTRACT

Use of fertilizers is important in agricultural production. In particular, it is more focused on organic fertilizer that increases yield and quality of crops and is not harmful to environment at the same time. In this study, the effect of bacterial biofertilizers on yield and some quality parameters were analyzed in two tomato cultivars (*Solanum lycopersicum L.*, cv. Cevahir F1 and Pala F1) in greenhouse conditions. Three different concentrations (1, 3, and 5 gL^{-1} and control i.e. un-inoculated) and two different bacterial fertilizers including A: Azotobacter spp. (1×10^{9} CFU), and B: mixture of *Bacillus subtilis* and *Bacillus megatarium* (1×10^{9} UFC) were evaluated in a completely randomized design with 3 replications. The treatments were applied one week after planting of the seedlings. The solutions were given to the root zone of the plant and repeated 3 times at intervals of 10 days. Average fruit weight, fruit number per plant, fruit weight per plant, plant height, fruit width and length, total soluble solid, pH, ascorbic acid, chlorophyll content, dry matter yield and mineral content were evaluated on tomato. The effects of treatments were found significant on plant growth parameters. Bacterial fertilization increased yield and other parameters in all treatments. Besides, the effects of treatments were significant on mineral content of tomato fruits.

Keywords: Biofertilizer, PGPR, Plant development, *Solanum lycopersicum* L.

INTRODUCTION

In the production of horticultural crops, the producers and consumers having high education and income levels, especially in the developed countries, have attempted to produce clean products that do not harm the natural balance, do not pollute the environment, and are not toxic to humans and other living things. This production system is called biological, ecological or organic agriculture (Er and Başalma, 2008; Ozyazıcı *et al*., 2010; Kodaş, 2011).

Organic fertilizer and plant wastes are used in organic farming to improve the physical, biological, and chemical properties of the soil; in this way, the continuity of soil fertility is provided. In this agricultural system, crop rotations, organic wastes, animal fertilizers, green fertilizers and mineral rocks are used for feeding the soil and provide the plant nutrients. In addition, bacterial fertilizers are also used as soil improvers. Bacterial fertilizers refer to containing active strains of microorganisms mainly Plant Growth-Promoting Rhizobacteria (PGPR) in sufficient numbers. They are used to either fix atmospheric nitrogen or solubilize plant nutrients like phosphates, and stimulate plant growth through synthesis of growth-promoting substances (Tilak, 1991; García-Fraile, *et al*. 2017). Moreover, bacterial fertilizers use in agricultural fields is reasonable due to their

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plant nutrients content and high humic acid and low toxic element content.

Plant roots take up essential plant nutrients from the soil (Mills and Jones, 1996; Fageria, 2016), therefore, enhanced plant development depends on good root growth. Promotion of root growth by PGPR is one of the important potential and organic tools for increasing nutrient uptake (Lucy *et al*., 2004; Biswas *et al*., 2000; Adesemoye *et al*., 2009). In previous studies, it was found that PGPR could promote and increase plant growth and yield in mulberry (Sudhakar *et al*., 2000), apricot (Esitken *et al*., 2002, 2003), sugar beet and barley (Cakmakci *et al*., 2001), tomato and pepper (Sahin *et al*., 2000), bean (Diéz-Méndez *et al*., 2015) and spinach (Jiménez-Gómez *et al*., 2018).

Almost all of the vegetables, due to substances they contain, as well as to protect and improve human health, to increase in their effects, are also depend on grown by organic means (Ozer, 2017).

Tomato (*Solanum lycopersicum L.*) is one of the most produced and economically important vegetables grown worldwide (FAOSTAT, 2017). The aim of this study was to determine the effects of two different certifiable organic bacterial fertilizer on growth and yield of tomato under greenhouse conditions.

MATERIALS AND METHODS

Growth Conditions and Plant Materials

This study was conducted under greenhouse conditions at Atatürk University, Turkey, in 2016. Used greenhouse was 12.5 m wide and 20 m long (250 m^2). The sidewalls were 70 cm high, with glass cover and natural ventilation from the roof. Two tomato cultivars (*Solanum lycopersicum L*., cv. Cevahir F1 and Pala F1) were cultivated under natural light conditions. The seedlings of tomato cultivars were purchased from a commercial company in Erzincan, Turkey. Tomato seedlings were planted with 50 cm row spacing and 50 cm on-row plant spacing in the second week of May, in 2016. The experiment ended on 15 October in the same year.

Bacterial Fertilizers

Three different concentrations (1, 3, and 5 gL^{-1} and control (un-inoculated) and two different and certifiable bacterial fertilizers including A: Azotobacter spp. ((1×10^9 UFC) and B: mixture of *Bacillus subtilis* and *Bacillus megatarium* (1×10^9 UFC) were used in this study. The bacterial fertilizers were obtained from Professor Dr. Metin Turan (Yeditepe University, Department of Genetics and Bioengineering, İstanbul, Turkey). These bacterial fertilizers contained also 15% organic matter, 6% organic C, 13% humic+fulvic acid and enzyme, acid phosphatase, urease, denitrogenase, protease, (30 U mL^{-1} from each) besides the PGPR used in the present study.

Application Procedure

The treatments included three different dosses of bacterial fertilizers (1, 3, and 5 gL^{-1}) and control (uninoculated). This experiment consisted of a completely randomized design with 3 replications and each replication had 10 plants. The treatments were applied one week after planting of the seedlings. The solutions were given to the root zone of the plants and repeated 3 times at intervals of 10 days.

Data Collection and Statistical Analysis

The measurements taken included average fruit weight (g) (Biçer, 2011), fruit number per plant, fruit weight per plant, plant length (cm), fruit width and length (cm), total soluble solid (^{o}Brix) (using refractometer, with the refractive index accuracy of ±0.0002 and the °Brix range of 0–95%, with temperature correction.) (Bao *et al*., 2014), pH (using a pH meter, with an accuracy of

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0.001.) (Bao *et al*., 2014), ascorbic acid (mg 100 g^{-1}) (with a Merck reflectometer set (Merck RQflex) (Ozer, 2017), chlorophyll content [A chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) was used] (Sahin *et al*. 2018), dry matter yield (%) and mineral content in tomato fruit. Macro and micro nutrients (N, K, P, Mg, S, Ca, Na, Fe, Mn, Zn, Cu, Pb, B and Cd) contents of fruits were also determined. Plant samples were oven-dried at 68°C for 48 hours and were then ground. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Sahin *et al*. 2018). Potassium (K), Ca and Mg were determined after wet digestion of dried and ground sub-samples in a H_2SO_4 -Se-Salicylic acid mixture. Phosphorus (P) was determined spectrophotometrically by the vanadomolybdophosphoric-yellow method (Lott *et al*., 1956). Potassium (K) and Ca were determined by flame photometry, and Mg, Cu, Fe, Mn, Na, Zn, Pb, B and Cd were determined by atomic absorption spectrometry using the methods of AOAC, (1990). Boron was determined, after dry-ashing of plant samples, spectrophotometrically at 550 nm by the curcumin method (Odom, 1992). All data in the present study were processed by SPSS and the means were separated by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Plant Growth

Significant differences were observed between Pala F1 and Cevahir F1 cultivars in terms of the evaluated parameters, except for plant height, fruit length, and chlorophyll content. Application of B-5 significantly increased fruit number per plant (6.83), fruit weight per plant (2.16 kg), total soluble solid (6.23 (^{o}Brix)), ascorbic acid (173.50 mg 100 g^{-1}), chlorophyll content and dry matter yield (5.87 %) when compared to the control plants (Table 1). Similar results have been reported by Jackson *et al*. (1964) and Tilak, (1991) in tomato. They found that inoculation with Azotobacter quickened the stem and leaf growth of tomato. In addition, De Silva *et al*. (2000) reported that the leaf area and stem diameter of high bush blueberry was increased by applying PGPR. Pirlak *et al*. (2007) determined that inoculation of PGPR increased yield and growth in apple.

Both bacterial fertilizers were found statistically significant in terms of ascorbic acid and chlorophyll content between cultivars and applications. The higher ascorbic acid and chlorophyll content were found in B-5. Bacterial fertilizers were found as increasing in ascorbic acid (Aiyer *et al*., 1964; Bangerth, 1976) and chlorophyll content (Zodape *et al*., 2011) in tomatoes.

Fruit Nutrient Contents

The effects of bacterial fertilizer applications on mineral (N, P, Mg, Ca, Na, K, Cu, Mn, Fe and Zn) contents of tomato were significant at P< 0.05, 0.01 and 0.001 (Table 2). All bacterial fertilizer applications affected the plants by increasing N, P, K, Mn, Zn, Pb, B and Cd contents of the fruits (Table 2).

The highest average K (23767.50 mg kg^{-1}) and B (26.01 mg kg^{-1}) contents were obtained from A-5 application in both tomato cultivars. B-3 and A-3 applications increased N content of the fruit in Pala F1 cultivar. In addition, *Azotobacter* spp. (1×10^9) (A) treatment increased Pb, B and Cd contents of the fruit in Cevahir F1 cultivar. As the concentration of organic bacterial fertilizer increase N, P, K, Ca and Mn contents of fruits increased in the present study (Table 2).

Kalantari *et al*. (2010) reported that application of compost and vermicompost increased the concentrations of P, K, Ca, and Mg in the shoot. However, Mg, Pb, and Zn declined with increase in the concentration of organic bacterial fertilizer in present study (Table 2). Yıldırım *et al*. (2010) reported

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Table 1. Effect of different bacterial fertilizers on plant growth, yield and quality of fruit. [a]

Treatments/Cultivars	Fruit number per plant			Fruit weight per plant (kg)			Average weight (kg) per fruit		
	Pala F1	Cevahir F1	Mean	Pala F1	Cevahir F1	Mean	Pala F1	Cevahir F1	Mean
Control	4.24 c*	19.57 ^{ns}	11.90 ^{NS}	1.23 e**	3.50 ^{ns}	2.37 ^{NS}	0.29 ^{ns}	0.18 ^{ns}	0.23 ^{NS}
A-1	5.89 ab	19.72	12.8	1.96 abc	3.4	2.68	0.33	0.17	0.25
B-1	4.49 bc	20	12.24	1.36 de	3.37	2.36	0.3	0.17	0.24
A-3	5.67 abc	19.81	12.74	1.73 bcd	3.1	2.41	0.31	0.16	0.23
B-3	5.50 abc	20.52	13.01	1.67 cd	3.28	2.48	0.3	0.16	0.23
A-5	5.76 abc	18.05	11.9	2.12 ab	3.21	2.66	0.38	0.18	0.28
B-5	6.83 a	20.79	13.81	2.16 a	3.19	2.68	0.32	0.15	0.24
Mean	5.48 ***	19.78	12.63	1.75 ***	3.29	2.52	0.32 ***	0.17	0.24

Treatments/Cultivars	Fruit length (mm)			Fruit width (mm)			Plant length (cm)		
	Pala F1	Cevahir F1	Mean	Pala F1	Cevahir F1	Mean	Pala F1	Cevahir F1	Mean
Control	57.57 ^{ns}	64.58 ^{ns}	61.07 ^{NS}	87.71 ^{ns}	77.88 ^{ns}	82.79 ^{NS}	316.33 ^{ns}	307.56 ^{ns}	311.95 ^{NS}
A-1	66.07	61.47	63.77	85.89	74.82	80.36	342	320.67	331.33
B-1	62.07	59.49	60.78	88.55	74.3	81.43	315	316.67	315.83
A-3	57.12	61.16	59.14	86.45	78.92	82.69	337.67	314.22	325.95
B-3	60.98	61.42	61.2	83.75	75.81	79.78	296	318.67	307.33
A-5	63.84	61.49	62.67	85.39	76.19	80.79	321.17	328.22	324.7
B-5	60.14	59.02	59.58	84.74	73.64	79.19	289.67	321.67	305.67
Mean	61.11 ^{NS}	61.23		86.07 ***	75.94		316.83 ^{NS}	318.24	

Treatments/Cultivars	Ascorbic acid (mg 100 g^{-1})			pH			Total soluble solid (^{o}Brix)		
	Pala F1	Cevahir F1	Mean	Pala F1	Cevahir F1	Mean	Pala F1	Cevahir F1	Mean
Control	152.00 ab***	89.00 b***	120.50 BC***	4.46 ^{ns}	5.71 ^{ns}	5.08 ^{NS}	5.93 a**	4.45 ^{ns}	5.19 A**
A-1	83.50 c	165.00 a	124.25 BC	4.43	4.69	4.56	4.47 c	4.55	4.51 C
B-1	46.00 d	89.50 b	67.75 D	4.41	5.05	4.73	5.40 ab	4.48	4.94 ABC
A-3	94.65 c	47.50 c	71.08 D	4.52	4.31	4.42	4.90 bc	4.44	4.67 BC
B-3	131.50 b	85.50 b	108.50 C	4.44	4.69	4.56	4.83 bc	4.4	4.62 BC
A-5	172.00 a	170.00 a	171.00 A	4.49	4.48	4.48	5.67 ab	4.44	5.06 AB
B-5	173.50 a	99.00 b	136.25 B	4.52	4.91	4.72	6.23 a	4.43	5.33 A
Mean	121.88 **	106.5		4.46 *	4.83		5.35 ***	4.45	

Treatments/Cultivars	Dry matter yield (%)			Chlorophyll content		
	Pala F1	Cevahir F1	Mean	Pala F1	Cevahir F1	Mean
Control	4.43 b*	5.23 ^{ns}	4.83 ^{NS}	55.93 a*	57.00 a**	56.47 AB***
A-1	4.14 b	5.33	4.74	53.37 a	54.27 a	53.82 B
B-1	4.24 b	5.15	4.7	47.95 b	47.95 b	47.95 C
A-3	4.09 b	5	4.55	56.60 a	56.63 a	56.62 AB
B-3	4.75 b	5.37	5.06	58.00 a	57.50 a	57.75 A
A-5	4.03 b	4.93	4.48	56.70 a	59.87 a	58.28 A
B-5	5.87 a	5.03	5.45	56.23 a	57.93 a	57.08 AB
Mean	4.51 *	5.15		54.97 ^{NS}	55.88	

[a] A: Bacterial fertilizer of Azotobacter spp.((1×10^9); B: Bacterial fertilizer mixture of *Bacillus subtilis* and *Bacillus megatarium* (1×10^9); Control: 0; A/B-1: 1 g L^{-1};A/B-3: 3 g L^{-1}; A/B-5: 5 g L^{-1}. * *P*< 0.05, ** *P*< 0.01, *** *P*< 0.001. Means sharing similar letter are statistically not different.

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Table 2. Effect of different bacterial fertilizers on fruit chemical contents of tomato cultivars.

Treatments /Cultivars	N (%)			P ($mg\ kg^{-1}$)			K ($mg\ kg^{-1}$)		
	Pala F1	Cevahir F1	**Mean**	Pala F1	Cevahir F1	**Mean**	Pala F1	Cevahir F1	**Mean**
Control	2.36 b***	3.35 ^{ns}	2.85 C**	3444.00 c**	3800.33 ^{ns}	3622.17 C**	22303.33 ^{ns}	19646.67 ^{ns}	20975.00 C*
A-1	2.95 a	3.53	3.24 AB	3518.33 c	4249	3883.67 BC	20645	22258	21451.50 BC
B-1	2.85 a	3.46	3.16 B	3522.33 c	4089.67	3806.00 BC	21435.33	22343	21889.17 ABC
A-3	3.13 a	3.7	3.42 AB	3899.67 ab	4311.67	4105.67 AB	23749.67	22953.67	23351.67 AB
B-3	3.13 a	3.84	3.49 A	4014.67 a	4446	4230.33 A	23439.33	23266	23352.67 AB
A-5	3.09 a	3.8	3.45 AB	3996.67 a	4434.33	4215.50 A	23548.33	23986.67	23767.50 A
B-5	3.07 a	3.64	3.36 AB	3688.00 bc	4392	4040.00 AB	22541.67	23621	23081.33 AB
Mean	2.94 ***	3.62		3726.24 ***	4246.14		22523.24 ^{NS}	22582.14	

Treatments /Cultivars	Ca ($mg\ kg^{-1}$)			Mg ($mg\ kg^{-1}$)			Fe ($mg\ kg^{-1}$)		
	Pala F1	Cevahir F1	**Mean**	Pala F1	Cevahir F1	**Mean**	Pala F1	Cevahir F1	**Mean**
Control	8561.33 ^{ns}	8185.33 ^{ns}	8373.33 ^{NS}	4444.67 a**	4919.33 ^{ns}	4682.00 ^{NS}	172.33 ^{ns}	223.00 ^{ns}	197.67 ^{NS}
A-1	7793.67	9149	8471.33	3714.00 b	4617.33	4165.67	128	214	171
B-1	7880	8851	8365.5	3692.33 b	5055.67	4374	144	226	185
A-3	8629	9256.67	8942.83	3748.67 b	4792	4270.33	157.67	230	193.83
B-3	8465.67	9131.33	8798.5	3853.33 b	4966	4409.67	152.67	239.33	196
A-5	8378.33	9002.33	8690.33	3581.00 b	4874	4227.5	151.33	237	194.17
B-5	8181.33	8943	8562.17	3846.67 b	4902.67	4374.67	162	251.33	206.67
Mean	8269.90 ***	8931.24		3840.10 ***	4875.29		152.57 ***	231.52	

Treatments /Cultivars	Mn ($mg\ kg^{-1}$)			Zn ($mg\ kg^{-1}$)			Cu ($mg\ kg^{-1}$)		
	Pala F1	Cevahir F1	**Mean**	Pala F1	Cevahir F1	**Mean**	Pala F1	Cevahir F1	**Mean**
Control	26.33 b*	25.20 ^{ns}	25.76 B**	48.40 a***	40.16 ^{ns}	44.28 A***	7.26 ^{ns}	7.58 ^{ns}	7.42 ^{NS}
A-1	31.78 a	28.37	30.07 A	39.26 b	43.38	41.32 AB	7.96	8.01	7.98
B-1	34.12 a	26.83	30.48 A	34.89 d	36.71	35.80 D	7.71	7.09	7.4
A-3	35.83 a	30.33	33.08 A	38.25 bc	38.07	38.16 BCD	8.13	8.75	8.44
B-3	33.83 a	29.04	31.44 A	39.40 b	43.19	41.29 AB	7.43	8.02	7.72
A-5	34.45 a	28.55	31.50 A	36.15 cd	37.91	37.03 CD	7.95	7.22	7.59
B-5	36.79 a	29.32	33.06 A	36.85 bcd	41.22	39.04 BC	8.06	7.58	7.82
Mean	33.30 ***	28.23		39.03 ^{NS}	40.09		7.78 ^{NS}	7.75	

Treatments /Cultivars	Pb ($mg\ kg^{-1}$)			B ($mg\ kg^{-1}$)			Cd ($mg\ kg^{-1}$)		
	Pala F1	Cevahir F1	**Mean**	Pala F1	Cevahir F1	**Mean**	Pala F1	Cevahir F1	**Mean**
Control	0.30 b***	0.30 ab*	0.30 AB***	21.80 ab*	21.29 d**	21.54 D***	1.10 bc***	1.08 c***	1.09 C***
A-1	0.35 a	0.28 abc	0.32 A	20.13 b	24.57 bc	22.35 CD	1.69 a	1.78 a	1.74 A
B-1	0.30 b	0.25 bc	0.27 B	20.08 b	23.49 cd	21.79 D	0.85 d	0.77 d	0.81 D
A-3	0.33 ab	0.32 a	0.33 A	22.75 ab	25.17 abc	23.96 BC	1.17 b	1.19 c	1.18 C
B-3	0.22 c	0.23 c	0.23 C	22.75 ab	26.44 ab	24.60 AB	0.84 d	0.91 d	0.88 D
A-5	0.29 b	0.26 abc	0.28 B	24.28 a	27.73 a	26.01 A	1.31 b	1.38 b	1.34 B
B-5	0.23 c	0.23 c	0.23 C	20.22 b	22.74 cd	21.48 D	0.94 cd	0.90 d	0.92 D
Mean	0.29 *	0.27		21.72 ***	24.49		1.13 ^{NS}	1.15	

A: Bacterial fertilizer of Azotobacter spp. ((1×10^9); B: Bacterial fertilizer mixture of *Bacillus subtilis* and *Bacillus megatarium* (1×10^9); Control: 0; A/B-1: 1 g L^{-1};A/B-3: 3 g L^{-1}; A/B-5: 5 g L^{-1}. * P< 0.05, ** P< 0.01, *** P< 0.001. Means sharing similar letter are statistically not different.

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that the yield, growth, and nutrition of broccoli plant under organic growing conditions significantly increased (Turan *et al*., 2004).

Also, Pirlak *et al*. (2007) found that inoculation of PGPR increased N, K, and Mn contents in apple. It is commonly known that increased N content in the plant results in higher uptake of nutrients elements such as K, Ca, Mg and P from the soil (Marschner, 1995).

The data presented in this study are in agreement with previous studies in that the amount of P, K, Ca, and Mg was significantly increased, probably, by N_2-fixing activity of the bacterial population.

CONCLUSIONS

In conclusion, bacterial fertilizers containing strains of microorganisms mainly plant growth-promoting rhizobacteria in sufficient numbers can be environmentally safe means of converting into an acceptable source of organic matter. Bacterial biofertilizers are excellent material for use in intensive vegetable production, as it improves yield and nutrition of the tomato. The results of our experiment may lead to increase in organic and high quality tomato production grown in the greenhouse conditions.

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تعیین اثر کود باکتریایی بر عملکرد و پارامترهای رشد گوجه فرنگی

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چکیده

در تولید محصولات کشاورزی، مصرف کود از اهمیت برخوردار است. تاکید این امر به ویژه بر کود آلی است که میزان عملکرد و کیفیت محصول را افزایش داده و در عین حال به محیط زیست صدمه نمی زند. در این پژوهش، اثرمصرف کود باکتریایی روی عملکرد و برخی پارامترهای کیفیت محصول دو کولتیوار گوجه فرنگی (*Solanum lycopersicum L.*)به نام هایPala F1 و Cevahir F1 در شرایط گلخانه بررسی شد. به این منظور، سه سطح غلظت (۱، ۳، و ۵ میلی گرم در لیتر و یک شاهد بدون تلقیح) از دو کود باکتریایی متفاوت شامل کود A : Azotobacter spp. (1x10^9 UFC)، و کود B: مخلوطی از *Bacillus subtilis* و*Bacillus megatarium* (1x10^9 UFC) در یک طرح آماری کاملا تصادفی در سه تکرار ارزیابی شد. تیمار ها یک هفته بعد از کاشت نشای بوته ها اعمال شد. محلول های آزمایش سه بار با فواصل ۱۰ روزه به ریشه ها افزوده شد. اندازه گیری ها شامل میانگین وزن میوه ها، تعداد میوه در هر بوته، وزن میوه در هر بوته گیاه، طول و عرض میوه، کل مواد جامد محلول، pH، اسید اسکربیک، محتوای کلروفیل، عملکرد ماده خشک، و عناصر معدنی بود. نتایج حاکی از معنادار بودن اثر تیمارها روی پارامترهای رشد گیاه بود. درهمه تیمارها، کوددهی باکتریایی منجر به افزایش عملکرد و دیگر پارامترها شد. افزون بر آن، اثرات تیمارها روی محتوای عناصرمعدنی در میوه های گوجه فرنگی معنادار بود.

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