E G E U N İ V E R S İ T Y

F A C U L T Y O F A G R İ C U L T U R E

Department of Soil Science and Plant Nutrition

Number: **32807152/347**
Bornova

Subject:
24.04.2014

To BAGBANLAR FERTİLİZER AND AGRİCULTURAL PRODUCTS SAN.LTD. STİ

On the microbial fertilizer commercial preparation ESBİOFUL prepared by your company is attached “Biological Activity Research Final Report” prepared by Hakan Cakıcı, assistant professor of our department.

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Biological Activity Research Result Report of ESBIOFUL (Pseudomonas fluorescens) Commercial Preparation

Researcher

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Ege University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition

Porpose

- Determination of the effect of PGPR *Pseudomonas fluorescens* (ESBİOFUL) on seedling development.
- Determination of the effect of PGPR *Pseudomonas fluorescens* (ESBIOFUL) on plant growth parameters that directly affect the yield and suitability of soils of our country.

Introduction

Pseudomonas fluorescens ranking in the group of plant growth-inducing root bacteria (PGPR) is a bacterium that contributes to the increase of yield in plant groups and other economically important plants by establishing a relationship with plant roots. It is known that PGPR bacteria are effective on plant growth by making nutrients necessary for plant in soil to be converted into useful form.

Material and Method

In this study was used "ESBIOFUL" preparation produced and delivered us by BAGBANLAR FERTİLİZER AND AGRİCULTURAL PRODUCTS SAN.LTD. STİ.

"Albeni" tomato sort was used in the experiments which were established to determine the effect of the preparation on seedling and plant growth parameters. *In the seedling development experiment*, the tomato seeds were inoculated with the suspension prepared from ESBİOFUL (1/100) for 2 hours. The Seedling Experiment was established with 2 applications and 4 replicates, impending inoculated and unvaccinated (control) seeds. In the trial established according to the pattern of random plots, each viola (104 seedlings) was evaluated as a parcel.

The seedling experiment conducted in the greenhouse environment, under the temperature of 15-25 C^0 night-day and humidity-controlled conditions of 60-70% was completed at the end of the 42-day (six weeks) cultivation period and the length, root and green components moisture and dry weight and dry matter measurements were carried out in the seedlings.

The potting experiment, which was established to determine the effect of the preparation on plant growth parameters, also used "Albeni" type tomato seedlings. In the trial; form of four different applications, control, seed grafted seedling, seed vaccination + vaccination with irrigation, were performed. Potting experiment was established with total (4 × 4 × 3) 48 pieces of pots with 4 applied 4 reparations and 3 reparations in each reparation according to the experimental pattern of random parcels 1.5 kg soil area. Trial soil; slightly alkaline reaction (pH: 7.4), unsalted (<0.03%), calcareous (4%), low organic matter (1.8%), loamy textured, K, Ca, Mg sufficient, N, P, Fe, Zn, Cu and Mn are medium-level.
The preparation used in the experiment was implemented in the manner ruffling to irrigation water in the ratio of 1/500, after transferring seedlings inoculated to seeds in the ratio of 1/100 and tomato seedlings. In the control pots, no application expect standard irrigation has been performed.

Seedlings grown in pots were allowed to develop in greenhouse in 15-25 C^0 day and night temperature and 60-70% humidity controlled conditions for 42 days (6 weeks). The pots are irrigated as standard in order to maintain 20% moisture by taking into consideration the soil capacity and useful water. No pruning was made to the plants.

Plant growth parameters (plant height, green part, root and total moisture weight) were completed and tested at the end of the six week development period after the seedlings were harvested. The parameters related to fresh vegetable material (green part, root and total dry weight) were recorded. In addition, the samples were dried in 105^0 C and dry matter (DM) was determined.

The experiments were carried out in randomized plots with 4 replications consisting of 3 tomato plants. SPSS program package version 15.0 and p = 0.05 level LSD test were applied to the obtained group averages.

Research Results

Plant height was measured at the end of the six-week development period in the seedling development trial (Table 1). Plant height in the measurements obtained; ESBİOFUL applications showed a statistically significant increase compared to control.

Table 1. Effect of ESBIOFUL preparation on the length and root length of tomato seedlings.

Application / Dose	Seedling length (Cm)	Average (Cm)	Root length (cm)	Average (Cm)
	12,05		9,64	

Control	13,71	13,36 b	10,90	10,40 b
	13,43		10,66	
	14,25		10,40	
ESBİOFUL 1/100	14,89	15,04 a	11,85	12,04 a
	15,83		12,77	
	15,32		12,16	
	14,13		11,40	
LSD		***1,117****		***1,515****

As can be seen in Tables 2, 3 and 4, the preparation significantly increased the plant growth (root and green component and dry weights and dry matter amounts) of the plant according to the untreated Control. This effect was statistically significant.

Table 2. Effect of ESBIOFUL preparation on the moisture weight of tomato seedlings (g / seedlings)

Application/Dose	**Green part (g)**	**Average (g)**	**Root (g)**	**Average (g)**	**Total (g)**	**Average (g)**
Control	16,06	17,66 b	11,63		27,69	30,25 b
	18,28		13,40		31,68	
	17,91		12,06		29,97	
	18,39		13,30		31,69	
ESBİOFUL 1/100	18,84	19,73 a	13,94		32,78	34,00 a
	21,10		15,03		36,13	
	20,42		14,07		34,49	
	18,58		14,04		32,62	
LSD		***2,012****		***1,084****		***3,023****

Table 3. Effect of ESBIOFUL preparation on dry weight (g/seedling) of tomato seedlings (65^0 C).

Application/Dose	**Green part (g)**	**Average (g)**	**Root (g)**	**Average (g)**	**Total (g)**	**Average (g)**
Control	1,98	2,21 b	1,32	1,44 b	3,30	3,65 b
	2,27		1,51		3,78	
	2,23		1,43		3,66	
	2,36		1,52		3,88	
ESBİOFUL 1/100	2,36	2,59 a	1,53	1,63 a	3,89	4,22 a
	2,88		1,80		4,68	
	2,68		1,67		4,35	
	2,44		1,54		3,98	

LSD	***0,353****		***0,188****		

Table 4. Effect of ESBIOFUL preparation on dry matter (%) ratio of tomato seedlings (105^0C).

Application/ Dose	**Green part Dry Matter Rate (%)**	**Average (%)**	**Root Dry Matter Rate (%)**	**Average (%)**	**Total Dry Matter Rate (%)**	**Average (%)**
Control	6,64	7,02 b	5,80	6,10 b	5,90	6,27 b
	7,18		6,25		6,41	
	6,97		6,08		6,21	
	7,32		6,28		6,59	
ESBİOFUL 1/100	7,62	7,76 a	6,47	6,55 a	7,04	7,20 a
	8,14		6,87		7,63	
	7,82		6,50		7,29	
	7,46		6,37		6,86	
LSD		***0,635****		***0,419****		***0,703****

The important positive effect of the applications on the growth parameters of seedlings shows that ESBIOFUL will benefit in the cultivation of healthy and mature seedlings.

In the pot experiment, the measurements of the plant growth parameters were made at the end of the six-week development period after the growth of the seedlings. As can be seen in Tables 5.6 and 7, ESBIOFUL applications showed significant effect on plant height, green parts, root moisture and dry weights compared to control. Similar effects were observed in dry matter amounts of plant parts.

Table 5. ESBIOFUL applied tomato plants after 6 weeks of application of the length.

	Application	**Plant height (cm)**	**Average**
1	Control	40	39,25 b
		38	
		43	
		36	
2	Seed Vaccination	46	48,00 a
		52	
		52	
		42	
3	Vaccination with	44	44,00 ab
		42	

	irrigation	48	
		42	
4	Seed Vaccination + Vaccination with irrigation	46	48,75 a
		52	
		54	
		43	
LSD			***5,361****

As shown in Table 5, the size of tomato plants increased with ESBIOFUL applications. This effect was statistically significant and Seed vaccination and Seed vaccination + vaccination with irrigation were the most effective applications.

Green parts, root and total moisture weights increased with ESBİOFUL applications according to the control statistically (Table 6). These data recorded as a result of the applications, water and nutrients from the soil ESBİOFUL `s taking the soil and so on. It shows that it will contribute positively to physiological abilities. Accordingly, it is seen that plant parameters for yield will be supported by ESBIOFUL application.

Table 6. Effect of ESBIOFUL applications on moisture weights of tomato plants (g / plant).

	Application	**Green part Moisture weight (g)**	**Average (g)**	**Root moisture weight (g)**	**Average (g)**	**Total Moisture weight (g)**	**Average (g)**
1	**Control**	235	230,00 c	44	43,00 c	279	273,00 c
		224		42		266	
		247		46		293	
		214		40		254	
2	**Seed Vaccination**	293	306,00 c	57	57,50 a	350	363,50 a
		339		61		400	
		307		58		365	
		285		54		339	
3	**Vaccination with irrigation**	275	271,50 b	51	50,50 b	326	322,00 b
		265		49		314	
		282		53		335	
		264		49		313	
4	**Seed vaccination+ Vaccination with**	295	311,25 a	56	58,25 b	351	369,50 a
		325		60		385	
		332		62		394	
		293		55		348	

	irrigation						
LSD			***32,059*****		***4,269*****		***36,213*****

The dry weight of tomato plants applied to ESBIOFUL increased statistically as compared to control plants (Table 7). All ESBIOFUL applications on total dry weight were found to have an important effect on control. However, there was no difference in effectiveness between the applications.

Table 7. Effect of ESBIOFUL applications on dry weight (g / plant) of tomato plants (65^0C).

	Application	**Green part Dry weight (g)**	**Average (g)**	**Root dry weight (g)**	**Average (g)**	**Total dry weight (g)**	**Average (g)**
1	**Control**	31,35	30,69 c	5,17	5,06 c	36,52	35,75 c
		29,95		4,96		34,91	
		32,93		5,41		38,34	
		28,54		4,70		33,24	
2	**Seed Vaccination**	39,16	40,82 a	6,70	6,68 a	42,86	46,75 ab
		45,10		7,07		52,17	
		40,83		6,72		47,55	
		38,20		6,24		44,44	
3	**Vaccination with irrigation**	36,56	36,09 b	5,90	5,94 b	42,46	42,03 b
		35,23		5,86		41,09	
		37,50		6,24		43,74	
		35,10		5,76		40,86	
4	**Seed Vaccination + Vaccination with irrigation**	39,23	40,89 a	6,49	6,71 a	45,72	47,61 a
		42,16		6,95		49,11	
		43,23		7,09		50,32	
		38,96		6,34		45,30	
LSD			***4,061*****		***0,448*****		***5,093*****

In green part and root part, there was a statistically increase in dry matter by ESBIOFUL application. With this result, it has been observed that the application of ESBIOFUL in the appropriate dosage provides benefits in terms of dry matter accumulation in the plant. The increase in the dry matter content of the plant material contributes to the plant's overall development table and also affects the yield by making agricultural products more valuable in terms of the extent to which it should contain (figure 1.2.3).

Table 8. Effect of ESBIOFUL applications on dry matter (%) ratio of tomato plants (105^0 C).

	Application	Green part dry matter (g)	Average (g)	Root dry matter (g)	Average (g)	Total dry matter (g)	Average (g)
1	**Control**	6,71	6,45 b	4,79	4,64 c	5,90	
		6,30		4,50		5,55	
		6,90		5,07		6,13	
		5,90		4,22		5,11	
2	**Seed vaccination**	6,80	7,00 a	5,65	5,82 a	6,46	
		7,60		6,30		7,22	
		7,20		6,00		6,84	
		6,40		5,33		6,08	
3	**Vaccination with irrigation**	6,90	6,76 ab	5,30	5,19 b	6,55	
		6,60		5,07		6,27	
		7,30		5,61		6,94	
		6,25		4,84		5,93	
4	**Seed vaccination + Vaccination with irrigation**	6,95	7,06 a	5,79	5,78 a	6,60	
		7,20		6,00		6,84	
		7,60		6,33		7,22	
		6,50		5,00		6,18	
LSD			***0,413****		***0,539****		***0555****

Figure 1. Tomato plants applied Control and ESBIOFUL.

Figure 2. Examples of seedling trials (Week 6).

Figure 3. Examples of pot experiment (Week 6).

Conclusion and Opinion

The results of the studies we conducted and the above-mentioned results indicate that the commercial preparation of ESBIOFUL (Pseudomonas fluorescens) has a positive and significant effect on plant growth and other growth and yield parameters.

The storage time of *Pseudomonas fluorescens*, the organism in the content, can maintain the number of living organisms unchanged approximately 1 (one) month between +2 and $+8^0$ C. This bacterium can work best in the soil pH: 6,0-7,5; temperature 20-25^0C; humidity 20-25%.

Considering the slightly alkaline (pH: 7,4) reaction of the soil in which the experiment is carried out, average 20^0C temperature and 20% humidity conditions, it is concluded that the preparation will be useful in fulfilling the functions envisaged in the soil and growing conditions of our country.

As a result of this data, our observations on plant growth, and statistically splitting up of numerical values, according to **ESBİOFUL commercial preparation**

published in the Official Gazette No. 29.03.2014 and No 28956 **“Organic, Organomineral, Fertilizers and Soil regulators used in agriculture, with Microbial, Enzyme explicit and Production of Other Products”**, it was concluded that “Our country is in compliance with this and has a positive effect on production” (24.04.2014).

Best regards,

Asst. Prof. Hakan CAKICI

Ege University, Faculty of Agriculture

Department of Soil Science and Plant Nutrition

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Biological Activity Research Result Report of Foliar Application of ESBIOFUL (Pseudomonas fluorescens) Commercial Preparation

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Ege University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition

Porpose

Is the determination of the effect of foliar application of PGPR *Pseudomonas fluorescens* (ESBİOFUL) on the plant growth parameters that directly affect the yield.

Introduction

It is known that *Pseudomonas fluorescens* in the soil growth stimulating root bacteria (PGPR) group is effective in plant application as well as in foliar applications.

Material and Method

In this study was used “ESBIOFUL” preparation produced and delivered us by BAGBANLAR FERTİLİZER AND AGRİCULTURAL PRODUCTS SAN.LTD. STİ.

In order to determine the effect of foliar application on plant growth parameters, “Albeni" type tomato seedlings were used in the pot experiment.

This experiment; was carried out with the report results of study dated 24.04.2014 and numbered 32807152/347 and introduced by E.U., Faculty of Agriculture, Department of Soil Science and Plant Nutrition. Potting experiment was established with 2 applied 4 replications and 3 pots in each of the samples according to the experimental pattern of the random parcels with a total of 1.5 kg of soil area (2 × 4 × 3) with 24 pots. Trial soil; slightly alkaline reaction (pH: 7.4), unsalted (<0.03%), calcareous (4%), low organic matter (1.8%), loamy textured, K, Ca, Mg enough N, P, Fe, Zn, Cu and Mn is moderate.

The results of the pathogenic test of “ESBIOFUL” microbiological fertilizer commercial preparation, carried out by Gazi University Center for Life Sciences application and Research Center were investigated. Accordingly, it has been observed that the application of this preparation from the Leaf will not pose any problems in terms of human and environmental health.

The preparation used in the experiment was applied three times 10 days after spraying tomato seedlings at 1/1000 ratio. In the control pots, no application other than standard irrigation has been performed. Seedlings placed in pots were allowed to develop in greenhouse in 15-25^0 C night and day temperature and 60-70% humidity controlled conditions for 42 days (6 weeks). The pots are irrigated as standard in order to maintain 20% moisture by taking into consideration the soil capacity and useful water. No pruning was made to the plants.

At the end of the six-week development period, the measurements of plant growth parameters (plant height, green parts, root and total wet weight) were completed and the trial was evaluated. The fresh vegetable material was stored in the drying cabinet working at 65^0 C for 4 days and the parameters of the dried plant material (green parts, root and total dry weight) were recorded. In addition, samples were dried in 105^{0}C and dry matter (km) was determined.

The experiments were carried out in randomized plots with 4 replications consisting of 3 tomato plants. The SPSS program package version 15.0 and LSD test were applied to the obtained group averages.

Research Results

At the end of the six-week development period, the plant growth parameters were measured. As shown in Table 1,2,3, ESBİOFUL foliar applications showed statistically significant effect on plant height, green parts, root wet and dry weights compared to control.

Table 1. ESBIOFUL applied tomato plants after 6 weeks in length (cm)

Application	Plant heigth (cm)	Average
Control	40	39,25 b
	38	
	43	
	36	
Foliar application	46	42,75 a
	40	
	42	
	43	
***LSD** (%5)*		*3,455**

As shown in Table 1, the length of tomato plants increased by ESBIOFUL applications. This effect was statistically significant.

It was determined that tomato plants applied ESBIOFUL from Leaf showed statistically significant increases over total wet weight compared to control plants (Table 2). While ESBIOFUL applications on total wet weight were significantly

more effective than control, no effect on green parts and root wet weights was determined.

Table 2. Effect of ESBIOFUL applications on the wet weights of tomato plants (g / plant)

	Application	Green part Wet weight (g)	Average (g)	Root green weight (g)	Average (g)	Total green weight (g)	Average (g)
1	**Control**	235	230,00	44	43,00	279	273,00 b
		224		42		266	
		247		46		293	
		214		40		254	
2	**Foliar application**	241	242,25	46	46,25	287	288,50a
		235		44		279	
		238		46		284	
		255		49		304	
LSD(%5)			***Unimportant***		***Unimportant***		*14,252**

Dry weight of tomato plants applied to ESBİOFUL was found to increase with statistical significance compared to control plants (Table 3). The effect of ESBIOFUL applications on the green parts and total dry weight was found to be significant compared to the control.

Table 3. Effect of ESBIOFUL applications on the dry weight (g / plant) of tomato plants (65^0C).

	Application	Green part dry weight (g)	Average (g)	Root dry weight (g)	Average (g)	Root dry weight (g)	Average (g)
1	**Control**	**Control**	30,69 b	5,17	5,06	36,52	35,75 b
		Control		4,96		34,91	
		32,93		5,41		38,34	
		28,54		4,70		33,24	
2	**Foliar application**	32,44	35,04 a	5,98	6,07	38,42	41,11 a
		38,28		6,75		45,03	
		36,12		6,42		42,54	
		33,32		5,14		38,46	

LSD(%5)	***4,005****		***Unimportant***		***5,112****

The rate of dry matter in green parts and root parts was increased with ESBIOFUL foliar application (Table 4). As a result, it was observed that ESBIOFUL foliar application benefited from the dry matter accumulation vaccine in the plant. The increase in the rate of dry matter of the plant material contributes to the overall growth table of the plant and also affects the yield by making the agricultural products more valuable in terms of the scope that it should contain.

Table 4. Effect of ESBIOFUL applications on dry matter (%) ratio of tomato plants (105^0C).

	Application	**Green part dry weight (g)**	**Average (g)**		**Root dry weight (g)**	**Average (g)**	**Root dry weight (g)**	**Average (g)**
1	**Control**	6,71	6,45 b		4,79	4,64 b	5,90	5,67 b
		6,30			4,50		5,55	
		6,90			5,07		6,13	
		5,90			4,22		5,11	
2	**Foliar application**	32,44	6,88 a		5,45	5,43 a	6,25	6,42 a
		38,28			5,05		6,00	
		36,12			5,44		6,49	
		33,32			5,78		6,36	
LSD(%5)			***0,612****			***0,486****		***0,720****

Conclusion and Opinion

The results of the research we conducted and the results of the above-mentioned ESBIOFUL (*Pseudomonas fluorescens*) microbial fertilizer applied to the plant development, growth and yield parameters were determined to affect positively and significantly.

The storage time of *Pseudomonas fluorescens*, the organism in the content, can maintain the number of living organisms unchanged approximately 30 (thirty) days between +2 and $+8^0$ C. This bacterium can work best in the soil pH: 6,0-7,5; temperature 20-25^0C; humidity 20-25%.

The experiment was conducted under 15-25^0C night-day temperature and 60-70% humidity controlled conditions in sera environment and potted soil was mild alkaline (PH: 7.4) reaction, ambient temperature averaged 20^0C temperature and

20% humidity. In addition, according to the results of pathogen test conducted by Gazi University Department of Life Sciences Application and Research Center of microbial fertilizer commercial preparation named ESBİOFUL, it has been observed that foliar application will not create any problems in terms of human and environmental health.

As a result of this data, our observations on plant growth, and statistically splitting up of numerical values, according to **ESBİOFUL commercial preparation** published in the Official Gazette No. 29.03.2014 and No 28956 **"Organic, Organomineral Fertilizers and Soil regulators used in agriculture, with Microbial, Enzyme explicit and Production of Other Products"**, it was concluded that **"'Our Country Will Be Useful In Fulfilling The Requirements For Breeding Conditions"** (04.07.2014).

Best regards,

Asst. Prof. Hakan CAKICI

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Department of Soil Science and Plant Nutrition