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### **Research Article** (Araștırma Makalesi)

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# The effects of some biofertilizers on yield, chlorophyll index and sugar content in sugar beet (*Beta vulgaris var. saccharifera* L.)

Bazı biyogübrelerin şeker pancarında (*Beta vulgaris var. saccharifera* L.) verim, klorofil indeksi ve şeker içeriğine etkisi

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#### ABSTRACT

**Objective:** The study was conducted to investigate the effects of commercial biofertilizers such as BM-Root-Pan, BM-Megaflu and BM-Coton-Plus on the yield, quality and chlorophyll content of sugar beet.

**Material and Methods:** The experiment was carried out in Karapınar-Konya in the 2017 sugar beet growing season. The experimental design was a randomized complete block design (RCBD) with three replications. Bio-fertilizers were applied to soil twice at the stage of BBCH 16-19 and BBCH 35.

**Results:** The differences among control, BM-Root-Pan, BM-Megaflu and BM-Coton-Plus for root weight, root length, root diameter, per plant sugar yield and root yield were significant. The highest root weight, root length, root diameter and per plant sugar yield recorded in BM-Megaflu with *Bacillus megaterium*, *Pantoea agglomerans* and *Pseudomonas fluorescens*.

**Conclusion:** Bio-fertilizers containing plant growth-promoting rhizobacteria had capable of increasing sugar beet yield.

### ÖΖ

**Amaç:** Bu çalışma, BM-Root-Pan, BM-Megaflu ve BM-Coton-Plus gibi bazı ticari biyogübrelerin, şeker pancarının verim, kalite ve klorofil içeriğine etkisini araştırmak amacıyla yapılmıştır.

**Materyal ve Yöntem:** Deneme 2017 yılı şeker pancarı yetiştirme sezonunda Konya İli Karapınar İlçesinde yürütülmüştür. Deneme tesadüf blokları deneme desenine göre 3 tekerrürlü olarak düzenlenmiştir. Biyogübreler, şeker pancarının BBCH 16-19 ve BBCH 35 büyüme dönemlerinde iki kez olmak üzere topraktan uygulanmıştır.

**Araştırma Bulguları:** Yumru ağırlığı, yumru uzunluğu, yumru çapı, bitki başına şeker verimi ve kök yumru verimi yönünden kontrol ile birlikte BM-Root-Pan, BM-Megaflu ve BM-Coton-Plus uygulamaları arasında önemli farklılıklar saptanmıştır. En yüksek yumru ağırlığı, yumru uzunluğu, yumru çapı ve bitki başına şeker verimi *Bacillus megaterium, Pantoea agglomerans* ve *Pseudomonas fluorescens* bakterilerini içeren BM-Megaflu uygulamasından elde edilmiştir.

**Sonuç:** Bitki büyüme teşvik edici bakteriler içeren biyogübrelerin şeker pancarı verimini artırabileceği kanısına varılmıştır.

#### INTRODUCTION

Sugar beet (*Beta vulgaris* var. *saccharifera* L., 2n = 18) is one of the most important sugar crops worldwide and accounts for approximately 25% of global sugar production. Root yield and sugar content are two major economic traits of the sugar beet (Wang et al., 2019).

In the world, sugar beet planting area, root yield and production during 2018/19 were 4.82 million hectares, 57 tonnes ha<sup>-1</sup> and 275.5 million tonnes, respectively (FAO, 2020). The sugar beet planting area was 310 thousand hectares and produced root was 18.1 million tonnes and the mean root yield per area was 58 tonnes ha<sup>-1</sup> in Turkey (TUIK, 2020). Also, granulated sugar was produced from the sugar beet about 2.7 million tonnes in the 2016/17 growing season. According to the granulated sugar data, digestion rate (sugar content) has been estimated as 14% countrywide (Anonymous, 2017).

Fertilizer management is one of the most factor affecting sugar beet productivity because of the very nutrient responsibility of high yielding cultivars (Zengin et al., 2009; Hergert, 2010). In recent years, the application of bio-fertilizers gained importance to alleviate for reducing natural deterioration as fertilizer used in crop production are mostly chemicals that cause environmental problems (Döbereiner, 1997; Parlakova and Dursun, 2019). The bio-fertilizers has identified as products based on beneficial microorganisms of proven efficacy that live in association or symbiosis with plants and naturally help their nutrition and growth. These preparations contained living cells of efficient nitrogen-fixing, P-solubilizing microorganisms (Chesti et al., 2013). The bio-fertilizers containing bacteria used for these purposes are species mainly belonging to genera Rhizobium, Azospirillum, Bacillus, Pseudomonas, Azotobacter, Burkholderia, Herbaspirillum. (Rosenblueth and Martinez, 2006).

The bio-fertilizer such as BM-Coton-Plus, BM-Root-Pan and BM-Megaflu are mixed microbial fertilizer containing *Bacillus megaterium*, *Paenibacillus polymyxa*, *Pantoea agglomerans*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Paenibacillus azotofixans* with functions such as nitrogen fixation, phytohormones production, solubilization of phosphorus, and increase in micronutrient uptake (Abdulla, 2018). Moreover, many researchers revealed their roles both directly and indirectly on plant growth promotion (Kloepper, 1992; Höflich et al., 1994; Lazarovits and Nowak, 1997; Glick, 2012).

Bacillus spp. inoculants positively affected root and shoot growth, biomass and yield in wheat (Rana et al. 2012), cluster bean (Yadav and Tarafdar, 2012), dry beans (Tozlu et al., 2012), rice (Beneduzia et al, 2008). Also, it was addressed that Pseudomanas had P-solubilization and IAA production roles in pearl millet (Misra et al., 2012) and wheat (Hussain and Hasnain, 2011). Bioformulations with different bacteria ingredients achieved promising results such as root and sugar yield in field condition of sugar beet (Cakmakci et al., 2001; Jorjani et al., 2011).

Our research aimed to study the effects of commercial products which include different bacteria on yield, chlorophyll and sugar content of sugar beet in field conditions.

#### **MATERIAL and METHODS**

The experiment was carried out under the condition of a farmer in Karapınar-Konya (37°44'20.4"N longitude and 33°25'32.1"E latitude) in 2017. Central Anatolia type of continental climate with semi-arid summer is dominant in the region. The mean temperatures of the growing season exhibited that July, August and September exceeds the long term mean temperatures while precipitations of the month viz., April, May, June, September and October were higher than that of the long term mean precipitations. There were no precipitations in July and September (Figure 1).

The soil characteristics of the experiment were loamy sand having pH 8.0 (slightly alkali), organic matter 2.06% (medium), available  $P_2O_5$  95.4 kg ha<sup>-1</sup> (high) and available  $K_2O$  1441 kg ha<sup>-1</sup> (very high). Also, the experiment area has lime content 48.5 (very high), medium Fe (4.21 ppm) and high Zn (1.93 ppm).

The sugar beet hybrid cultivar, Aranka (from KWS Company), was used in the study. The general characteristics of a cultivar are genetic monogerm, tolerant to *Cercospora beticola*, high yielding, ample sugar content and NZ type. Bio-fertilizers used in the study were commercial products such as BM-Coton Plus, BM-Root-Pan and BM-Megaflu (from BIOMARKET Company of Turkey). The list of bacteria contents belong to products is given in Table 1.



**Figure 1.** Precipitation (mm) and temperature (°C) values in Karapınar in 2017 and the long-term. **Sekil 1.** Karapınar'a ait 2017 yılı ve uzun dönem sıcaklık ortalamaları ve yağış miktarı.

Table 1. The list of bacteria content of bio-fertilizers	
Çizelge 1. Biyogübrelerin bakteri içerikleri	

Commercial Name		Number of Live Organisms (number of organisms/ml)			
BM-Root-Pan	Bacillus megaterium RCK-869	Paenibacillus polymyxa	Pantoea	<i>Bacillus subtilis</i> RCK-561	1,7x10 <sup>7</sup>
		RCK-540	ayyiomerans RR-120		
BM-Megaflu	Bacillus megaterium	Pantoea agglomerans	Pseudomonas fluorescens		2,1x10 <sup>8</sup>
BM-Coton-Plus	Bacillus subtilis PA1		Paenibacillus azotofixa	2,2x10 <sup>7</sup>	

The experiment was arranged in a "Randomized Complete Blocks Design" with 3 replications. Seeds were sown on April 17, 2017. Plots were 12 rows of 6.0 m x 5.4 m ( $32.4 \text{ m}^2$ ). The distance between rows and within rows were 0.45 m and 0.20 m, respectively. Plant density was approximately 110 thousand plants per hectare.

Two hundred kg of N, one hundred kg of P and 80 kg of K were applied per hectare before planting time. An additional 50 kg ha<sup>-1</sup> of N was given with the first irrigation. Two hoeing and two herbicide (Betanal maxxPro from BAYER; 47 g/L Desmedipham+75 g/L Ethofumesate+27 g/L Lenacil+60 g/L Phenmedipham) were applied for weed control. The recommended application dose of biofertilizers was 100 ml da<sup>-1</sup>. They were sprayed twice with the tractor pulverization at the BBCH 16-19 (6-8 leaves unfolded) and BBCH 35 (leaves cover 50% of the ground). Experimental plots were irrigated 7 times by sprinkler irrigation system.

After technologically maturity, sugar beet plants were harvested by hand on 16th October 2017. Root weight (kg), root length (cm), root diameter (cm), number of forking (number plant<sup>-1</sup>), dry matter (%) and sugar rate (%), were determined from randomly ten plants of each parcel. Also, the whole of parcels which leaving the outside rows as borders were harvested for yield calculate. The dry matter and sugar rate was determined using HANNA HI 96801 Refractometer. The chlorophyll content index (CCI) was determined by using APOGEE CCM-200 plus. CCI data were collected weekly from June and October in 10 randomly selected plants of each parcel.

All data were analyzed using TARIST statistical Package Program (Acikgoz et al., 1994). The differences between the means were compared by the least significant difference (LSD) at the 5% level (Steel and Torrie, 1980). The curve fitting for chlorophyll content was computed by the Microsoft Office Excel program and the most meaningful regression equation was obtained by the highest R<sup>2</sup>.

## **RESULT and DISCUSSION**

The variance analysis revealed the significant differences among the biofertilizers for all observed characteristics except the number of forking features (Table 2). Root weight, one of the most important characteristics in sugar beet, was between 0.81 and 1.08 kg while the highest values were recorded in BM-Megaflu (1.08 kg) and Control (0.95 kg). The root weight of BM-Coton-Plus (0.89 kg) and BM-Root-Pan (0.81 kg) had the lowest value in the same statistical group. Unlike other biofertilizers, BM-Megaflu contains Pseudomanas. In parallel with our findings, some researchers reported that *Bacillus megaterium*, *Pantoea agglomerans* and *Pseudomonas fluorescens* of within BM-Megaflu have increased the root weight (Jorjani et al., 2011; Kumar et al., 2014).

Root length ranged from 20.8 cm (Control) to 25.8 cm (BM-Megaflu). As for that other fertilizers, BM-Root-Pan 21.1 cm and BM-Coton-Plus 22.8 cm had root length. It can be seen clearly that all biofertilizers applications positively affected root length compared with control. Loper and Schroth (1986) stated that root length was shortened by species of Pseudomonas. But, Dal Cortivo et al (2017) reported that PGPR which applying as spraying to the leaf were increased the root length.

Bio-fertilizers	Root Weight (kg)	Root Length (cm)	Root Diameter (cm)	No. of Forking (No. of plant)	Sugar Yield Per Plant (g)	Root Yield (t ha <sup>-1</sup> )
BM-Root-Pan	0.81 b	21.1 c	10.97 b	1.50	0.126 b	75.11 b
BM-Megaflu	1.08 a	25.3 a	12.35 a	1.53	0.182 a	89.14 a
BM-Coton-Plus	0.89 b	22.8 b	11.43 ab	1.97	0.153 ab	86.48 a
Control	0.95 ab	20.8 c	12.35 a	1.50	0.158 a	83.56 a
LSD (0.05)	0.16	1.45	1.02		0.031	7.13

**Table 2.** Mean values regarding root and sugar yield with yield components.**Çizelge 2.** Verim bileşenleri ile kök ve şeker verimine ait ortalama değerler.

The same letters in a column are not significantly different at the 0.05 probability levels.

Aynı harfle gösterilen ortalamalar arasında önemli fark ( $P \le 0,05$ ) yoktur.

In terms of root diameter, BM-Megaflu (12.35 cm) and Control (12.35 cm) treatment had the highest values while BM-Coton-Plus (11.43 cm) and BM-Root-Pan (10.97 cm) treatment had the lowest in the root diameter. Under the conditions of Bursa, Canigenis (2012) was recorded root diameter 13.2 - 19.2 cm; Arslan (1994) found the root diameter 6.3 - 7.1 cm under Van conditions. The results in our study were different determined to be those found by the researchers. Unlike, it is similar to by Akcin et al (1992). According to these different results, it is thought that root length and root diameter may show high variation depending on the material and climatic conditions. The effect of bio-fertilizers on the number of forking per m<sup>2</sup> was non-significant. The higher values were recorded in BM-Coton-Plus (1.97) and BM-Megaflu (1.53). BM-Root-Pan (1.50 no. of fork) and Control (1.50 no. of fork) were the lowest treatments.

The root yield in our study was ranged between 75.11 t ha<sup>-1</sup> (BM-Root-Pan) and 89.14 t ha<sup>-1</sup> (BM-Megaflu). Root yield of BM-Megaflu (89.14 t ha<sup>-1</sup>), BM-Coton-Plus (86.48 t ha<sup>-1</sup>) and control (83.56 t ha<sup>-1</sup>) were statistically higher than that of BM-Root-Pan (75.11 t ha<sup>-1</sup>) (Fig. 2). Sahin et al (2004) and Karagoz et al (2018) reported the increasing the root yield by plant growth-promoting bacteria as similar to our study results. Also, Cakmakci et al (2011) stated that PGPR has increased the yield approximately at a rate 18%. As for our study, PGPR was increased the sugar beet yield in rate 7% as maximum (BM-Megaflu). According to these results, it is concluded that the positive effects of PGPR were changed depending on ecological conditions, soil characteristics and bacteria species.

The highest dry matter content in our study was obtained as 21.42% from BM-Coton-Plus application while the lowest dry matter content was determined from 20.52% BM-Root-Pan, 20.49% BM-Megaflu and 19.52% control applications, respectively (Figure 2). The dry matter mentioned are in parallel with Celikel (1989) and Kurtcebe (1999) but higher than 16.4 – 17.6% found by Turgut (2012). Also, Mrkovacki et al (1997) reported that N<sub>2</sub>-fixing bacteria were increased the dry matter and these bacteria efficacy changed depending on different factors.



Figure 2. Yield and technological features. *Şekil 2. Verim ve teknolojik özellikler.* 

No significant differences among biofertilizers were determined for sugar content. The highest value was found in BM-Coton-Plus (17.14%). This result was followed by BM-Root-Pan (16.39%), BM-Megaflu (16.39%) and Control (15.62%) (Figure 2). The sugar contents determined in our study were similar to those found by Johari et al (2008) and Rahimi et al (2016). On the contrary of our results, Canigenis (2012) and Erciyes et al (2016), recorded the sugar content as an average of 18%. But, the sugar content determined in our study higher than found from 14.21 – 15.63% values reported by Kulan et al (2016). These different results showed that the effect of the cultivar and soil characteristics, climatic differences and harvest time is important in determining sugar content.

Sugar yield per plant was between 0.13 and 0.18 g. The highest sugar yield per plant was recorded from BM-Megaflu (0.18 g) and Control (0.16 g). The sugar yield per plant was increased at the rate of 13% compared to the control. These results were followed by BM-Coton-Plus (0.15 g) and BM-Root-Pan (0.13 g) in the same statistical group (Table 2). Some researcher declared that *Bacillus subtilis, Bacillus megaterium, Paenibacillus polymyxa, Pantoea agglomerans and Pseudomonas fluorescens* of within BM-Megaflu were increased the root weight (Sahin et al 2004; Cakmakci et al 2005). The sugar yield per plant mentioned is in parallel with these researchers but lower than 18.7 – 22.8% found by Cakmakci et al (2011). 'Carter et al (1985) and Saglam et al (1996) emphasized that it is the crucial importance of the cultivar and soil characteristics, sowing time, use of nitrogen, precipitation amount and harvest stage for sugar yield per plant. Sugar yield ranged from 10.75 tonness ha<sup>-1</sup> (BM-Megaflu) to 12.44 tonnes ha<sup>-1</sup> (BM-Coton-Plus). These values were followed by BM-Root-Pan (12.34 tonnes ha<sup>-1</sup>) and Control (12.31 tonnes ha<sup>-1</sup>). The sugar yield referred are in parallel with Johari et al (2008) and Canigenis (2012) but lower than 12.63 – 15.50 tonnes ha<sup>-1</sup> found by Kulan et al (2016).

As can be seen in Figure 3, chlorophyll content index (CCI) increased from day 60 to day 113, whereas it decreased as the sugar beet aged (technological maturity). Although the effects of biofertilizers on CCI were not remarkable, the quadratic response was observed in CCI across the growing stages. These results were parallel to Yang and Lee (2001) and Liu et al. (2019). This change meant that assimilates produced in leaves more allocation to root. Also, Soler Rovira et al (2009) reported that not meant the excessive more increasing chlorophyll content the more yield.



Figure 3. Chlorophyll Content Index. Sekil 3. Klorofil İçerik İndeksi.

# CONCLUSION

The plants in BM-Megaflu applied parcels can be defined as taller and larger root, lower forking rate, high root and sugar yielding. The difference of BM-Megaflu from others is that it contains *Pseudomonas fluorescens* with a high number of live organisms. It can be speculated that the high performance of BM-Megaflu can come from these characteristics.

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