

Effect of Different Rates of Bacteria (*Rhizobium leguminosarum*) Inoculated in Seed on Yield and Some Quality Parameters of Common Vetch (*Vicia sativa* L.)

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ABSTRACT

This research was conducted at Akdeniz University, Faculty of Agriculture's research greenhouse in 39 plastic pots. Different bacteria solutions (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 mL bacteria solution per 1 kg seed) were inoculated in the seeds of common vetch plant (*Vicia sativa* L.) with each replication. In addition, the most convenient bacteria dosage and differences between bacterial and artificial fertilizer applications were determined by applying (13 applications in total) sole fertilizer so that 1 kg of nitrogen (N) falls per decare in 1 pot and by applying control (non-inoculated seeds) for 1 pot. Analyses revealed that bacteria solution applications of 45 mL on plant height; 5 mL on plant nitrogen content; 0, 5, 10, 15 and 25 mL on plant phosphorus content; 25 mL on plant potassium content; 0, 5, 35, 40 and 45 mL on fresh weight; 55 mL on root weight; 30 mL on number of bacteria in soil; 30 mL on dry matter percentage and 5 mL on crude protein percentage have taken the first place. Fertilizer application did not sustain in any first place for any of the features, which indicate that bacteria applications can give better results.

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Adi Fiğ (*Vicia* L.) Tohumlarına Farklı Oranda Bakteri (*Rhizobium leguminosarum*) Aşılmasının Verim ve Bazı Kalite Özellikleri Üzerine Etkisi

ÖZET

Bu araştırma, Akdeniz Üniversitesi Ziraat Fakültesi deneme serasında 39 adet plastik saksılarda yürütülmüştür. Adi fiğ (*Vicia sativa* L.) bitkisinin tohumlarına her bir tekerrürde farklı bakteri solüsyonu (bir kilogram tohuma; 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 mL bakteri solüsyonu) uygulanmıştır. Ayrıca 1 saksıda dekara 1 kg azot düşecek şekilde sadece gübreleme ve 1 saksıda kontrol (bakteri uygulanmamış tohumlar kullanılmış) uygulanarak (toplam 13 uygulama) en uygun bakteri dozu aşılması ile bakteri ve suni gübre uygulamaları arasındaki farklılıklar da belirlenmiştir. Sonuçlara bakıldığında bitki boyunda 45; bitki azot içeriğinde 5; bitki fosfor içeriğinde 0, 5, 10, 15 ve 25; bitki potasyum içeriğinde 25; yaş ağırlıkta 0, 5, 35, 40 ve 45; kök ağırlığında 55; topraktaki bakteri sayısında 30; kuru madde oranında 30 ve ham protein oranında 5 mL bakteri solüsyonu uygulamalarının ilk sırada yer aldıkları anlaşılmaktadır. Gübre uygulamasının ele alınan hiçbir özelliğe ilk sırada yer almaması bakteriyel uygulamalarının daha iyi sonuçlar verebileceğinin göstergesidir.

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INTRODUCTION

Leguminous plants compose a major part of plants used for animal feeding purposes all over the world. They grow naturally in meadows and pastures and are

essential in supplying roughage for animals. Leguminous plants' nutritional value and their expediency on animals are significantly high (Anil et al., 1998). Leguminous plants are not only used in

animal feeding but also in human nutrition and biological nitrogen fixation (Anjum et al., 2006).

Vetches have bacteria called *Rhizobium*, which fix the free nitrogen in the air. In this symbiotic relationship, *Rhizobium* take the free nitrogen from the air and quickly convert it into ammonium and protein compounds. Thus, the plant obtains the nitrogen needs from *Rhizobium* (Cassida, 2004). Leguminous plant seedlings use the nitrogen in the soil to grow until their roots form nodules. If there isn't enough nitrogen in the soil, seedlings get damaged and their growth decelerate due to nitrogen deficiency. If these bacteria are not sufficient and effective in the soil, seeds should be inoculated with the appropriate type of *Rhizobium* according to their varieties (Önder and Akçin, 1991).

Nitrogen is a basic element for protein synthesis which enables organisms to survive (Canfield et al., 2010). Nitrogen (N₂) in the form of gas is present in the atmosphere, but most living organisms cannot metabolize nitrogen in this form (Cheng, 2008). This gas needs to be converted to ammonia (NH₃) to be useful (Jia and Quadrelli, 2014). This process is called nitrogen fixation (Cleveland et al., 1999) and is an important processing the nitrogen cycle (Gruber and Galloway, 2008).

It has been shown that the use of chemical fertilizers containing nitrogen, phosphorus and potassium are effective for increase of yield of meadows and pasture plants (Elliott and Abbott, 2003). However, chemical agricultural practices (including chemical fertilizers) create environmental problems (Socolow, 1999). For these reasons, alternative approaches that are environment-friendly (such as microbial fertilizers) are proposed to increase the yield in agricultural areas (Cebel, 2004).

In this research, the effect of *Rhizobium leguminosarum*, a nitrogen fixing bacteria, on yield and quality of common vetch was examined.

MATERIALS and METHODS

Materials

As plant material, Gülhan 2005, a variety of common vetch which is widely cultivated, was used in this research. As bacteria inoculant, *Rhizobium leguminosarum* bacteria strains, provided by NCIMB (National Collection of Industrial, Food and Marine Bacteria) were used.

Methods

Bacteria kept as dry dust at -20°C were prepared for inoculation in the microbiology laboratories of Antalya Wastewater Treatment Plant Water Quality Control Unit. Initially, inactive bacteria were dissolved in 0.5 mL Maximum Recovery Diluent (MRD), 0.9% sodium chloride, Tryptic Soy Broth (TSB), peptone water, sterile distilled water. This solution, then, was added

to 500 ml TSB media and incubated at 25°C for 3 days. At the end of the incubation period, a turbidity was formed in the medium indicating bacterial growth. Number of bacteria present in the medium was determined by using the dilution plate count method and expressed as colony forming unit (cfu) ml⁻¹. In this process, 1 mL of the medium with bacterial growth was transferred to a tube containing 9 mL of TSB. This tube constitutes the 10⁻¹ dilution. 1 mL from this dilution tube was then transferred to another tube to create 10⁻² dilution. This step was repeated several times to prepare serial dilution tubes. After that, 1 ml from each dilution tube was transferred to separate plates containing YMA (Yeast Mannitol Agar) nutrient media. The plates were incubated at 25 °C for 3 days and then colonies were counted. Following this enumeration, number of bacteria in the media were determined to be 3x10⁴cfuml⁻¹.

Seeds inoculated with bacteria were planted in pots, eight in each, on January 4th, 2016. On January 13th, seedlings were observed in pots. On January 27th, subtilization process was carried out in order to leave 4 plants in each pot. On February 1st, plants were disinfested of leaf miner (*Liriomyza trifolii*) and plant lice (*Aphidoidea*) and on April 15th plants were harvested.

Applications

Inoculation to seeds was done by taking 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 ml kg⁻¹ respectively from the bacteria produced in YMA nutrient media. Sucrose was used as an adhesive during inoculation. In addition to this, control (non-inoculated seeds) and also fertilizer in 1 pot were applied. Seeds to be planted in pots were randomly selected among the inoculated seeds. All 13 applications were planted in pots with a mixture of soil, perlite and turf and were covered with a thin layer of turf. In order to compare the effect of the bacteria with the plants grown with fertilizer, fertilizer with 1 kg of nitrogen per decare (Açıköz, 2001) was applied. Therefore, a total of 13 applications (11 bacteria, 1 control and 1 fertilizer) were carried out in each replication. They were then irrigated periodically.

Yield Factors

Heights of 4 plants in each experimental unit were measured and mean values were calculated. Right after the shaping process, their fresh weights were determined. Plant roots in each pot were washed with water to remove soils and their weights were measured. For each application, plants whose fresh weight (A) has been pre-determined were kept at 70°C for 48 hours in order to determine their dry weight (B) and their dry matter percentages were calculated with the formula (B/A)*100.

Quality Factors

Plants which were dried in incubators and whose dry weights were measured, were pulverized by grinding. The nitrogen contents of the samples were then determined with Kjeldahl method (AOAC, 1990). Crude protein percentages of the samples were determined by multiplying nitrogen content with the coefficient 6.25. Dried plant samples were wet digested in HNO₃+HClO₄ solution and analyzed with, ICP-AES (Varian Liberty Series II) to determine their phosphorus and potassium content.

Others

Total number of aerobic mesophilic bacteria was determined by using the dilution plate count method and expressed as cfu g⁻¹ dry weight soil (Parkinson et al., 1971). All resulting data were analyzed using the SAS 9.1 statistical software and the differences

between the averages were determined by the LSD test.

During the period in which the research was carried out, min.-max. temperature and min.-max. humidity were 8.4-32.3 °C and 21.5-69.3% in January; 14.3-40.2 °C and 15.4-60.4% in February; 12.6-41.4 °C and 15.0-56.7% in March, respectively.

According to the climate data obtained from the greenhouse every day, environmental conditions was appropriate for both bacterial growth and common vetch cultivation during the experiment.

RESULTS and DISCUSSIONS

Yield Factors

Averages of plant height, fresh weight, root weight and dry matter percentages obtained from this study are given in Table 1..

Table 1. Mean Values of Yield Parameters

Tablo1. Verim Özelliklerinin Ortalamaları

Dosages (ml kg ⁻¹) <i>Dozlar (ml kg⁻¹)</i>	Plant Height (cm) <i>Bitki Boyu (cm)</i>	Fresh Weight (g plant ⁻¹) <i>Taze Ağırlık (g bitki⁻¹)</i>	Root Weight (g plant ⁻¹) <i>Kök Ağırlığı (g bitki⁻¹)</i>	Dry Matter (%) <i>Kuru Madde (%)</i>
Control (<i>Kontrol</i>)	118.00	101.55	36.62	36.06
Fertilization (<i>Gübreleme</i>)	108.83	92.81	35.60	39.20
5	112.00	102.47	34.98	34.72
10	112.25	93.67	33.69	37.55
15	106.25	84.67	33.46	39.95
20	126.83	94.57	35.23	37.36
25	113.17	69.99	31.93	46.28
30	115.67	78.59	35.48	46.70
35	114.17	98.23	35.57	37.76
40	124.33	98.60	37.07	37.79
45	128.17	97.68	36.22	38.30
50	122.33	88.89	35.75	41.00
55	125.33	87.39	38.42	44.41
LSD	20.35	26.08	2.77	10.47

LSD: Least Significant Differences

When the averages of plant height were examined, it was seen that the values ranged from 106.25 to 128.17 cm. The highest plant height was achieved with 45 ml kg⁻¹dosage and the lowest plant height was obtained with 15 ml kg⁻¹dosage. This indicates that dose increase in the bacteria inoculation raises the plant height. Gök and Onaç (1995) stated that bacteria inoculation positively affects the vegetative development. Karadavut and Özdemir (2001) determined in a study carried out on chickpea under Hatay province conditions that bacteria inoculation increases the plant height. Söğüt (2005) conducted bacteria inoculation and nitrogen fertilizer application on some soybean varieties and found out that bacteria inoculation increased plant height more in comparison to nitrogen fertilizer application. Kaçar et al. (2005)

indicated based on a study carried out on some French bean's varieties under Bursa province conditions for 2 years that bacteria inoculation had no statistical effect on plant height increase. However, they reported differences with increasing nitrogen fertilization dosages. Özrenk et al. (2003) determined that bacteria inoculation increased plant height in proportion to control in chickpea. Meral et al. (1998) found in a study carried out on chickpea under Ankara province conditions that bacteria inoculation to seed increased plant height compared to control. Önder (1992) indicated that inoculation increases plant height more than control in dwarf French beans. In a study conducted with chickpea under Eskişehir province conditions, inoculation increased the plant height by 1.6-3.6% in varieties (Çakır, 2005).

When the mean values of fresh weight were evaluated, no linear relationship was observed between bacterial dose increase and fresh weight increase. In that, the lowest fresh weight was obtained with 25 ml kg⁻¹ dose and the highest fresh weight was observed at 10 ml kg⁻¹ dose. Meral et al. (1998) determined that bacteria inoculation increased plant height compared to control in chickpea.

In the mean value of root weight, while 55 ml kg⁻¹ dose gave higher values than other dosages did, 25 ml kg⁻¹ dose resulted in the lowest value. The highest root weight average at the highest dose, 55 ml kg⁻¹, suggests that bacteria inoculation positively affects the root development. Meral et al. (1998) determined that bacteria inoculation on chickpea plants increased root weight compared to control. Ünver et al. (1999) stated in a study conducted on lentil and Hungarian vetch that nitrogen and phosphorus-based fertilizers applied during plantation along with bacteria inoculation resulted in better development in aerial and

belowground plant parts.

While control dose and 5 ml kg⁻¹ dose gave the lowest dry matter values, other doses of bacteria inoculations increased dry matter percentages. This suggests that bacteria inoculations contribute positively to the increase in dry matter. In a study conducted under Ankara and Samsun province conditions, Albayrak and Sevimay (2005) determined that bacteria inoculations resulted in an increase in both fodder and seed yield compared to control in all varieties. It has been indicated that with *Rhizobium* inoculation dry matter content accumulation significantly increased in total N content and all plant parts including Nodules.

Quality Factors

The mean values of plant nitrogen content, phosphorus content, potassium content and crude protein content are given in Table 2.

Table 2. Mean Values of Quality Parameters

Tablo 1. Kalite Özelliklerinin Ortalamaları

Dosages (ml kg ⁻¹) Dozlar (ml kg ⁻¹)	Nitrogen Content (%) Azot İçeriği (%)	Phosphorus Content (%) Fosfor İçeriği (%)	Potassium Content (%) Potasyum İçeriği (%)	Crude Protein (%) Ham Protein (%)
Control (Kontrol)	2.55	0.25	2.16	15.91
Fertilization (Gübreleme)	2.75	0.22	2.29	17.21
5	3.42	0.26	2.06	21.34
10	2.81	0.27	2.37	17.53
15	3.03	0.25	2.08	18.94
20	2.56	0.17	1.87	16.00
25	2.95	0.25	2.60	18.41
30	2.70	0.22	2.21	16.85
35	2.60	0.21	2.07	16.25
40	2.85	0.23	2.11	17.79
45	2.59	0.20	2.07	16.21
50	2.89	0.21	2.03	18.04
55	2.99	0.24	1.98	18.97
LSD	0.52	0.08	0.30	3.23

LSD: Least Significant Differences

When considering the effect of these treatments on plant nitrogen content, it can be seen that the values vary between 2.55 and 3.42% and that while the highest value was achieved with 5 ml kg⁻¹ dose, the lowest one was obtained with the control treatment. Therefore, bacteria inoculation plays a positive role in increasing the content of nitrogen in the plant. Özrenk et al. (2003) reported that bacteria inoculation in chickpea plant positively affects plant nitrogen content. Similarly, Doğan et al. (2007) determined that inoculation increased nitrogen content in peanut.

It was observed that plant phosphorus content varied between 0.17 and 0.27% and that while the highest average was achieved with 10 ml kg⁻¹, the lowest value was observed with 20 ml kg⁻¹. Özrenk et al. (2003)

indicated that bacteria inoculation in chickpea positively affects plant phosphorus content. Şahin (2008) stated in a study carried out with chickpea in Tokat Region that in the wake of the inoculations, significant differences occurred in total N and available P contents of the soil.

Plant potassium content was found to be between 1.87% and 2.60%. The highest value was obtained with 25 ml kg⁻¹ dose while the lowest one was with 20 ml kg⁻¹ dose.

While the control treatment gave 15.91% crude protein percentage, values from other treatments varied between 16.00 and 21.34%. This indicates that bacteria inoculation increases crude protein percentage in plants at a certain rate. In addition, crude protein

content of the treatment receiving N fertilizer was found to be 17.21%. This indicates that nitrogen fertilizer also increases protein content compared to the control. In the 3-year doctoral dissertation with dwarf French bean varieties, Önder (1992) observed that bacteria inoculation increased the crude protein compared to control. However, Yağmur and Engin (2005) ascertained in a 2-year study on chickpea under Van province ecological conditions that bacteria inoculation did not influence crude protein percentage. At the beginning of the experiment, initial number of total aerobic mesophilic bacteria in the test soil was found to be 1.0×10^6 cfu g⁻¹dw. Results of enumeration of bacteria in soil at the end of the experiment are given in Table 3. Statistical analysis showed that effects of applications on bacterial number in soil were significant ($p < 0.05$). According to LSD Test, the highest bacterial number was obtained from the application rate of 30 ml. In general, bacterial numbers elevated with increasing application rate until 30 ml. With application rates above 30 ml, however, bacterial numbers slightly decreased and stabilized. This shows that rates above 30 ml may not provide extra benefits in terms of economics.

Table 3. Mean Values of Total Aerobic Mesophilic Bacterial Number in Soil

Tablo 3. Topraktaki Toplam Aerobik Mezofilik Bakteri Sayısı

Dosages (mL kg ⁻¹)- Dozlar (ml kg ⁻¹)	Number of Total Aerobic mesophilic Bacteria ($\times 10^6$ cfu g ⁻¹ dw soil) Toplam Aerobik Mezofilik Bakteri Sayısı ($\times 10^6$ cfu g ⁻¹ dw soil)
0	1.167 A B
5	1.800 A B
10	1.700 A B
15	1.800 A B
20	2.200 A B
25	2.233 A B
30	2.767 A
35	2.200 A B
40	2.300 A B
45	2.000 A B
50	2.000 A B
55	2.000 A B
Soil with fertilizer (Gübreli toprak)	1.200 A B
Initial Soil (Başlangıç toprağı)	1.000 B

CONCLUSIONS

Analyses revealed that application dosages of 45 ml on plant height; 5 ml on plant nitrogen content; 0, 5, 10, 15 and 25 ml on plant phosphorus content; 25 ml on plant potassium content; 0, 5, 35, 40 and 45 ml on fresh weight; 55 ml on root weight; 30 ml on number of bacteria in soil; 30 ml on dry matter percentage and 5 ml on crude protein percentage took the first place.

In general, higher plant height and root weight values

and dry matter contents were achieved with application dosage of 40 ml and above. On the other hand, lower application rates yielded higher values in fresh weight, plant nitrogen, phosphorus and potassium contents, and crude protein content.

Total aerobic mesophilic bacterial number of the soil prepared at the beginning of the experiment was determined as 1.0×10^6 cfu g⁻¹dw. Following the experiment, the fact that 2.767×10^6 cfu g⁻¹dw soil was achieved with 30 ml dose indicates that bacterial application increases the number of bacteria in the soil. In addition, the highest value of root weight that was seen with 55 ml dose suggests that bacteria inoculation positively contributes mostly to the development of root structure. The fact that chemical fertilizer application did not yield highest values in any of the features indicates that bacteria applications may be more effective and give better results.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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