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EFFECT OF RHIZOBIUM BACTERIA ON NITROGEN FERTILIZER REQUEST OF COMMON VETCH (*Vicia sativa* L.)

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Abstract

Common vetch (Vicia sativa L.) is a single-year legume feed plant, grown in plant-animal production systems for grass and grain purposes in many parts of the world, and is widely used in the feeding of farm animals. Turkey is the genetic center of common vetch and 59 of the existing species are naturally present in the Turkish vegetation.
The aim of this study was to determine the effect of nitrogenous fertilizer on plant and the appropriate dose of nitrogenous fertilizer and to investigate the effect of plant growth, yield, amount of N uptake by the plant, which is Rhizobium bacterial inoculation. Experiment was carried in controlled greenhouse conditions and used 'Tamkoc 2000' variety of common vetch seeds and applied Rhizobium leguminosarum L. bacterial and 3 doses of nitrogen (0-3-6 kg/da nitrogen) fertilizer.
According to the results of the research it was observed that only the difference in the content of N in grain among the examined parameters. This value is 3.89% N in bacteria-overgrown plants and 3.39 % N in bacteria-free plants.

***Key words**: Common vetch, nitrogen fertilizer, Rhizobium leguminosarum* L.

INTRODUCTION

Common vetch (*Vicia sativa* L.) is an annual forage legume which is cultivated in numerous parts of the world in plant and animal production systems and commonly used in the feeding of the livestock (Cabellero et al., 2001; Ramos et al., 2000; Açıkgöz, 2001; Chowdhurry et al., 2001; Han, 2010). Roughage obtained from legumes is an important source of fodder and is richer than the other roughage in terms of minerals, vitamins and most importantly proteins (Ensminger et al., 1990; Karabulut et al., 2007). The chemical composition of the legume forages varies depending on the species, geographical regions and agricultural practices and their protein content can range between 20-45% (Dixon, Hosking, 1992; Abreu, Bruno-Soares, 1998; Filya et al., 2002). In areas where the production of oilseed residues is low or costly due to their high crude protein content, legume forages are used as an alternative source of protein. The amount of cultivated land in Turkey, including fallow lands, is 20.5 million hectares, and within this cultivated land, the area for the cultivation of fodder crops has reached to 1.8 million hectares due to the governmental incentives (TUIK, 2013). Again, with the governmental incentives, among all the forage cultivation lands, common vetch cultivation land has ranked the second with an area of 499000 hectares, following alfalfa cultivation land (629000 hectares).
Seed of the common vetch plant is green and has a good fodder. Common vetch is also a good alternation and green manure crop (Avcıoglu, Soya, 1977). Common vetch has 150 species (Tosun, 1974) and 59 of these species grow naturally in Turkish vegetation (Elci, Acıkgoz, 1993). In particular, *Vicia cracca* are found as natural plantations in certain regions of Anatolia, and as well as being a good source of nectar, these plantations are used as quality crude fodder after being reaped and dried during the fruit-set period (Tamkoc, 1999).
Use of legumes in the fallow lands of Europe and USA as a source of biological nitrogen fixation has been a revived focus of attention. The amount of nitrogen fixed by the legumes in fallow lands (BNF) can be affected and change due to a wide spectrum comprising factors such as the soil characteristics, environmental conditions and land use management (Ledgard, Steele, 1992). Common vetch, which is one of

these forage legumes, is the most frequently cultivated vetch species (Anonymous, 2011). Fodder of common vetch is very tasty and nutritious due to its high crude protein content. Extensive studies on the effect of *Rhizobium* inoculation on nitrogen use are inadequate for common vetch plant.

The aim of this study is to identify the effect of appropriate *Rhizobium* inoculation for common vetch on the vetch's requirement for nitrogenous fertilizer, to determine the appropriate fertilizer dose, and to investigate its effects on plant development, yield and the amount of nitrogen bound by the plant.

MATERIALS AND METHODS

This study was performed in the greenhouse of Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition under controlled conditions. In the study, common vetch seed of 'Tamkoc 2000' cultivar was used. Trial were established based on the pattern randomized block design and replicated 4 times. Suitable bacteria *(Rhizobium leguminosarum* L.) for common vetch were taken from Ankara Soil, Fertilizer and Water Resources Central Research Institute. Bacterial inoculation was carried out at a ratio of 1 gram of bacteria per 100 grams of seed. In order to ensure better adherence of the inoculation material to the seeds, 4% sugar solution prepared at a dimly lit location which does not receive sunlight and was added on the seeds. Than the seeds were dried out and planted and irrigation was performed with pure water. The trial plan is as follows:

- Inoculated with bacteria;
- Uninoculated;
- 3 nitrogen doses (0-30-60 kg ha^{-1} nitrogen) (using 46% urea) application.

The experiment was established on April 2016.

The soil samples used in the experiment were taken from a depth of 0-30 cm at Selcuk University of Faculty of Agriculture Sarıcalar Experimental area in Konya. The collected soil sample was passed through a 4 mm sieve. Than the experiment soil was sterilized for 2 hours at 121^0C. 0.8 kg soil has been put in each pot. The test plants were irrigated by using pure water and maintain field capacity during plant growth process. The experiment was terminated when the capsule formation was complete at the end of a development period of 4 months.

Measurements and analyzes performed on plants

Plant height (4 measurements were made at 10-days intervals after the first plant exit), bean length, number of grains per bean, leaf chlorophyll amount (with SPAD 502 chlorophyll meter), the grain yield values in the pot were observed. At the end of the trial, the plant was cut from the soil level and shoots and roots were weighed separately and then washed. After being dried at 65°C and being subjected to the required pretreatments.

They were solved by wet method using sulfuric acid and hydrogen peroxide (Bayraklı, 1987). The phosphorus in the plant extracts obtained after wet decomposition was read at the spectrophotometer based on the yellow color method and the potassium was read using atomic absorption (Kacar, Inal, 2010), and the % nitrogen content was calculated using Kjeldahl procedure. The obtained nitrogen values were multiplied by 6.25 to calculate crude protein ratios.

The amount of Nitrogen fixed from the atmosphere by the plants

The plant weight and nitrogen fixation capacity were determined during the development period of the plants.

The amount of nitrogen fixed from the atmosphere was calculated using the following formula (Yaman, Cinsoy, 1996; Öğütücü, 2000).

mg N / pot = [(root dry weight x root nitrogen content %)] + [SB (plant stem biomass dry weight or weight of aboveground parts of plant) x stem nitrogen content %] + (amount of dry weight of grain x grain nitrogen content %) / 100) x 1000

The amount nitrogen fixed nitrogen (kg N/da)

It was calculated using the equation: *Total nitrogen amount of the inoculated plant – total nitrogen amount of the uninoculated control* (Yaman, Cinsoy, 1996; Öğütücü, 2000).

SE (symbiotic efficiency) (%)

It was calculated using the equation: *Amount of nitrogen of the plant grain to which the isolate was inoculated / Amount of nitrogen in grain of nitrogen control × 100* (Beck et al., 1993; Materon et al., 1995).

Efficiency level

Equation: *Mean dry matter weight of test plant/ mean dry matter weight of nitrogen control plant) x 100*

The following limits have been taken into consideration when grouping the isolates based on their efficiency levels:

- 100: Highly efficient;
- between 100-75: Efficient;
- between 75-50: Moderately efficient;
- between 50-25: Slightly efficient;
- <25: Inefficient.

Soil analysis

Some of the physical and chemical properties of the test soil before the trial are given in Table 1. The soil used in the study is clay loam, mild alkaline (pH 7.75), limy (12.94%), less salty, little organic matter and its useful phosphorus concentration is very little. It was found that iron and zinc content is sufficient, and the copper and manganese content is high.

Table 1. Physical and chemical properties of the soil used in experiment

Parameters	Value	Parameters	Value
Texture class	Clay loam	P (mg kg^{-1})	5.15 (very little)
$CaCO_3$ (%)	12.94 (limy)	Fe (mg kg^{-1})	3.69 (enough)
pH (1/2.5 H_2O)	7.75 (mild alkaline)	Cu (mg kg^{-1})	1.85 (high)
EC (mS/cm)	249.0 (less salty)	Mn (mg kg^{-1})	14.73 (high)
Organic Matter (%)	1.98 (little)	Zn (mg kg^{-1})	0.74 (enough)
Volume weight(g/cm^3)	1.25	Field capacity (%)	27.34

Statistical analysis

The data were subjected to analysis of variance using the MINITAB 16 statistical package and means were separated according to the least significant differences (LSD) test.

RESULTS AND DISCUSSIONS

The effect of applying different doses of nitrogen fertilizer to the common vetch plant and the effect of *Rhizobium* bacteria inoculation on developmental parameters such as root weight, stem biomass, grain weight, bean length, number of seeds in the bean were found to be statistically insignificant (Tables 2 and 3). The highest root weight and stem biomass were observed in N30B + application. It was previously reported that inoculation with *Rhizobium leguminosarum* of common vetch (*Vicia sativa* L.) compared to non-inoculated cultivars were increased seed yield, biological yield, straw yield, pod length, number of seed per pod, number of pods, main stem length and thousand seed weight of common vetch cultivars (Albayrak et al., 2006).

Table 2. The effect of nitrogen fertilizer doses and bacterial inoculation on the mean values of various plant developmental parameters

N Doses (kg/ha)	Root weight (g/pot)	Stem Biomass (g/pot)	Grain weight (g/pot)	Bean length (cm)	Number of bean seeds (piece)
N0	1.04	14.16	1.48	2.84	2.01
N30	1.05	13.75	0.95	2.49	1.67
N60	0.70	13.05	1.05	2.52	1.85
Bacteria inoculation	Root weight (g/pot)	Stem Biomass (g/pot)	Grain weight (g/pot)	Bean length (cm)	Number of bean seeds (piece)
B+	0.89	13.65	1.06	2.62	1.72
B-	0.97	13.66	1.26	2.61	1.97

Table 3. The effect of nitrogen fertilizer doses and bacterial inoculation on plant developmental parameters

N Doses (kg/ha)	Bacteria inoculation	Root weight (g/pot)	Stem Biomass (g/pot)	Grain weight (g/pot)	Bean length (cm)	Number of bean seeds (piece)
N0	B+	1.25	14.39	1.46	2.89	1.83
N0	B-	0.84	13.93	1.50	2.78	2.19
N30	B+	0.91	13.93	0.74	2.42	1.62
N30	B-	1.20	13.58	1.17	2.57	1.71
N60	B+	0.52	12.64	0.99	2.55	1.99
N60	B-	0.88	13.46	1.10	2.49	1.99

The effect of nitrogenous manure application on and bacterial inoculation to the common vetch plant on the plant height values measured at 10 day-intervals was found to be statistically insignificant; the simultaneous effect of nitrogen application and bacterial inoculation was found to be significant only in the second measurement period ($P<0.05$). At the last measurement, the plant height varied between 32.8-37.4 cm (Table 4). The highest plant heights were measured at a field experiment in Gümüşhane, Turkey, as 41.20 cm, 38.90 cm, 38.70 cm and 38.60 cm (Yolcu, 2011).

It was found that nitrogen application and bacterial inoculation on the leaf chlorophyll content have a significant effect. The highest chlorophyll content (39.0) was observed upon N0B + application ($P<0.05$) (Table 4).

Table 4. The effect of nitrogen doses and bacterial inoculation on plant height and leaf chlorophyll (SPAD)

N doses (kg/ha)	Plant height (cm)				Chlorophyll
	1.	2.	3.	4.	
N0	27.4	32.3	32.4	33.9	36.54
N30	30.7	33.7	33.5	35.1	34.64
N60	30.5	33.7	35.8	35.1	35.35
Bacterial	1.	2.	3.	4.	Chlorophyll
B+	30.7	33.6	33.8	35.6	34.44
B-	28.4	32.8	34.0	33.8	36.58
N doses and Bacterial	1.	2.	3.	4.	Chlorophyll
N0 B+	29.3	31.8	32.4	34.4	39.0
N0 B-	25.5	32.8	32.4	33.5	34.0
N30 B+	33.3	38.5	36.1	37.4	31.8
N30 B-	28.0	29.0	30.9	32.8	37.5
N60 B+	29.4	30.7	32.8	34.9	32.5
N60 B-	31.5	36.7	38.8	35.2	38.2
LSD Value P<0.05	-	*	-	-	*

Statistically significant difference was observed in the K content of the grain and root of common vetch plant upon both bacterial inoculation and the interaction of nitrogen dose and bacterial inoculation. The nutrient concentration values obtained from the leaf, grain and root of plants are given in Table 5. There was no significant difference between the applications in terms of leaf, grain and root mean nitrogen contents. Bacterial inoculation increased the content of crude protein in grain ($P<0.01$). When the potassium contents of grain and root have been examined, it has been detected that nitrogen dose, bacteria inoculation, interaction of nitrogen dose and bacteria inoculation have statistically significant. The P contents of the grain and root were affected from nitrogen doses ($P<0.01$). The highest grain K content, root K and P contents were observed at the application of N30 dose (30 kg N/ha).

When the effect of nitrogenous fertilizer application and bacterial inoculation on the crude protein content of the grain was analyzed, it was found that bacterial inoculation increased crude protein ($P<0.05$), with the highest crude protein content being 24.75% upon N60B+ application (Figure 1). Phosphorus content of the grain was the highest with 0.62%, upon N30B+ application (Figure 2). Crude protein content was reported to be between of 15.15-20.69% under the ecological conditions of Iğdır (Temel et al., 2015). Crude protein content of 21 different vetch genotypes grown in Kahramanmaras was reported to vary between 23.47% and 18.80% (Yılmaz, Erol, 2012), and in studies carried out under different ecological conditions it was found that the crude protein contents of vetch varieties ranged between 9.08-22.30% (Yücel et al., 2012; Kaplan, 2013; Yücel et al., 2013; Yılmaz, Erol, 2015).

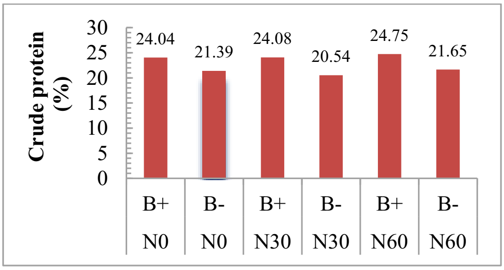

Figure 1. The effect of nitrogen doses and bacterial inoculation on the crude protein content of the grain (%)

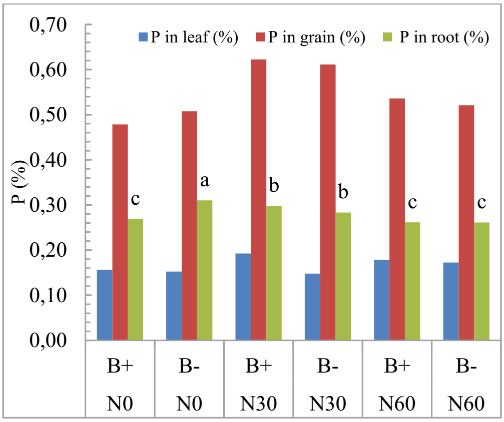

Figure 2. The effect of nitrogen doses and bacterial inoculation on the phosphorus content of leaf, grain and root (%)

Table 5. Effect of nitrogen doses and bacterial inoculation on N, P, and K concentrations of common vetch plant

N doses (kg/ha)	Leaf N	Grain crude protein	Root N	Leaf K	Grain K	Root K	Leaf P	Grain P	Root P
	(%)								
N0	1.26	22.71	2.14	2.50	1.25 b	1.79 a	0.15	0.49 b	0.29 a
N30	1.40	22.31	2.30	2.24	1.53 a	2.04 a	0.17	0.62 a	0.29 a
N60	1.45	23.2	2.59	2.34	1.29 b	1.17 b	0.18	0.53 ab	0.26 b
LSD Value P<0.01	-	-	-	-	0.174	0.305	-	0.09	0.009
Bacterial	Leaf N	Grain crude protein	Root N	Leaf K	Grain K	Root K	Leaf P	Grain P	Root P
	(%)								
B+	1.42	24.29	2.45	2.48	1.31	1.85 a	0.18	0.54	0.28 b
B-	1.32	21.2	2.24	2.24	1.40	1.49 b	0.16	0.54	0.28 a
LSD Value P<0.01	-	*	-	-	-	**	-	-	**

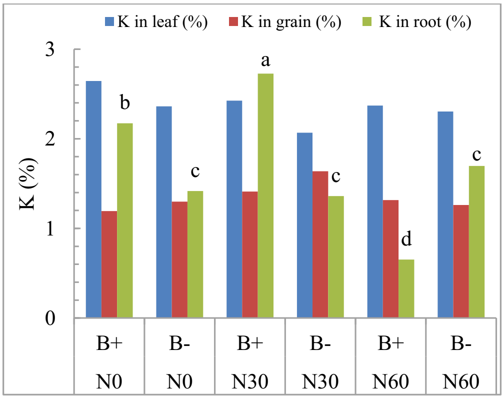

Figure 3. The effect of nitrogen doses and bacterial inoculation on the potassium content of leaf, grain and root (%)

The amount of nitrogen fixation at the application of N30 dose in the plant inoculated with *Rhizobium* bacteria was 34.42 mg N/pot, which was the highest (Table 6). The amount of nitrogen fixation varied between 6.99 and 10.74 kg N/da. In this case, it can be said that the amount of nitrogen fixed from the atmosphere by the vetch plant is significantly increased by bacterial inoculation. This increase is more pronounced when 30 kg N/ha nitrogenous fertilizer is applied. Symbiotic efficiency in the plant inoculated with the bacterial isolate varies between 102.9-108.3. This value is higher when 30 kg N/ha is applied, compared to the other values (Table 6). The isolate belongs to the "highly efficient" group in terms of its efficiency level (Holding, Kong, 1963).

Table 6. The effect of bacterial inoculation on the amount of nitrogen bound by the plant, symbiotic efficiency and the level of efficiency

N doses (kg/ha)	Nitrogen bounds by the plant (mg N/pot)	Nitrogen bounds by the plant (kg N/da)	Symbiotic efficiency	Level of efficiency
N0	24.91±2.60	7.81±0.77	107.60±4.81	118.58±4.89
N30	34.42±6.76	10.74±2.12	108.35±4.65	113.18±27.96
N60	22.46±7.99	6.99±2.52	102.90±1.01	99.95±18.88
LSD value P<0.05	-	-	-	-

CONCLUSIONS

The results of the study show that the common vetch plant should be inoculated with the appropriate *Rhizobium* bacteria prior to seeding and the seeding must be performed only afterwards, and 30 kg N/ha nitrogenous fertilizer application would be sufficient. In today's economic conditions, fertilizer prices are gradually increasing. Excessive use of nitrogenous fertilizer causes the problem of nitrate pollution in the soil and the water. Therefore, bacterial inoculation on vetch before being grown is an important practice. We must be performed these application for economic reasons and in order to eliminate the problem of environmental pollution.

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