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SHORT COMMUNICATION

Effects of Phosphorus Fertilizer, Poultry Manure Applications with *Bacillus megaterium* M-3 Inoculation on Yield and Yield Components of Common Vetch (*Vicia sativa*)

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ABSTRACT

Phosphorus is considered one of the essential elements for legumes as it increases nitrogen fixation due to nodulation. Hence, for high productivity in legumes, it should be added as inorganic or organic fertilizer to soils containing insufficient phosphorus. Especially in recent years, using inorganic and organic fertilizers together has gained importance in terms of sustainable agriculture, considering plant growth and soil and environmental health together. The effects of three different doses of phosphorus fertilizer (0, 50, 100 kg P_2O_5 ha^{-1}), and two different doses of poultry manure (0, 3 t ha^{-1}) applications with two different doses (B0 or B1) of phosphorus solubilizing bacteria (*Bacillus megaterium* M-3) inoculation on hay yield and yield components of common vetch were examined in this study. In fact, while the highest hay yield and crude protein rate were obtained by bacterial inoculation together with phosphorus fertilizer application, the effect of poultry manure application on yield and yield parameters was variable. Instead, the applications had no significant effect on the number of main branches, ADF and NDF rates. Consequently, the application of 100 kg P_2O_5 ha^{-1} together with *Bacillus megaterium* inoculation can be recommended for high hay yield in common vetch under irrigated conditions in areas with poor or medium soils in terms of phosphorus.

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1. Introduction

Common vetch is one of the critical leguminous forage crops in crop rotation systems due to its high dry matter yield, nutritional value, production of grain forage, and being an annual plant. Like other leguminous forage crops, common vetch requires additional phosphorus fertilization to achieve optimal yield (Mitran et al., 2018; Tan, 2018). Although inorganic fertilizers, among factors affecting agricultural productivity, are vital, their unconscious use can deteriorate the soils physical, chemical, and biological characteristics (TEMA, 2018). Furthermore, they are toxic element source for human, animal, and environmental health since the cadmium they contain accumulates in plants to which they are applied (Chukwu et al., 2014; Debele, 2021; Roba, 2018).

In recent years, a prominent approach in terms of sustainable agriculture is to alleviate the adverse effects of

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chemical fertilizers by incorporating natural sources such as organic fertilizers like poultry and cattle manures, along with microbial fertilizers to enhance the effectiveness of phosphorus (P) utilization for plant growth (Chauhan et al., 2022; Meraklı & Memon, 2020; Solmaz et al., 2022). Organic fertilizers increase organic matter, nutrient content, and aggregate stability in the soil and improve the quality and yield of crops without causing environmental pollution (Kocagöz et al., 2022). Poultry manure is a significant source of organic plant nutrients since it increases the soil organic matter, nutrient element content, and aggregate stability (Adeyemo et al., 2019; Masocha & Dikinya, 2022). On the other hand, the inoculation of soils with rhizobacteria, called as Plant Growth-Promoting Rhizobacteria (PGPR), including phosphorus-solubilizing bacteria, promotes plant growth by converting phosphorus in the soil into a form available to plants, and that this process promotes plant growth by making phosphorus available for plants by altering the activity of metabolic enzymes, synthesis of certain hormones and suppressing the pathogen development (Chen & Liu, 2019; Meena et al., 2017). Since it is not feasible to give up the use of inorganic fertilizers completely, which play a crucial role in agricultural production today, supporting fertilizer use with organic fertilizers is essential to alleviate their adverse effects (Cüre, 2022). Numerous studies investigated the effects of inorganic fertilizers on common vetch cultivation (Rafaat, 2022; Türk & Yıldız, 2016). Nevertheless, the number of studies studying the combined use of organic fertilizers and the increasingly popular bacterial inoculation with inorganic fertilizers is very limited. It is essential to investigate the impacts of these applications on the sustainability of agriculture and the health of soil. The principal objective of this study is to evaluate the individual and combined effects of phosphorus fertilizer, poultry manure treatments, and the phosphorus solubilizing bacteria (*Bacillus megaterium* M-3) inoculation on the achievement of maximum yields in common vetch.

2. Materials and Methods

The research was conducted in the experimental field of the Faculty of Agriculture, Atatürk University in Erzurum. The study was established with three replications according to the factorial arrangement in the randomized complete blocks trial design. The experiment included three different doses (0, 50 and 100 kg P_2O_5 ha^{-1}) of chemical phosphorus fertilizer (Triple superphosphate), two different doses (0 and 3 t ha^{-1}) of poultry manure (composition of poultry manure was given in Table 1) and two different doses (B0 and B1) of phosphorus solubilizing bacteria inoculation.

Table 1. Chemical composition of poultry manure.

Parameter	Value
pH (1:5.0)	6.5
Organic matter (%)	22.0
Al (mg kg^{-1})	315.3
B (mg kg^{-1})	20.4
Ca (mg kg^{-1})	18546.0
Cd (mg kg^{-1})	0.09
Cr (mg kg^{-1})	1.23
Cu (mg kg^{-1})	36.1
Fe (mg kg^{-1})	352.0
K (mg kg^{-1})	12312.0
Mg (mg kg^{-1})	3451.0
Mn (mg kg^{-1})	172.4
Na (mg kg^{-1})	956.0
Ni (mg kg^{-1})	2136.0
P (mg kg^{-1})	4845.0
Pb (mg kg^{-1})	0.049
S (mg kg^{-1})	452.1
Zn (mg kg^{-1})	185.6

The seeds used in the experiments were subjected to inoculation with *Rhizobium leguminosarum* bacteria, which were obtained from the Ankara Soil and Fertilizer Research Institute. A combination of phosphorous fertilizer and poultry manure was added into the soil using a harrow. A suspension of cultures of *Bacillus megaterium* was prepared at a density of 10^8 CFU mL^{-1} and his suspension was then used to inoculate the seeds intended for sowing in the plots. The Karaelçi variety of common vetch (*Vicia sativa*) (12 kg da^{-1}) were planted in pre-prepared seed beds at a depth of 4-6 cm (Tan, 2018).

Planting was conducted manually using a hand drill, with the first year planting took place on April 20 and the second year on April 29. To prevent contamination, different drills were used to sow seeds. The crops were irrigated three times over a two-year period when they turned dark green due to deficiency of moisture in the soil during the growing season.

Table 2. Total precipitation, temperature average and relative humidity average values of 2009, 2010 and long-term averages in Erzurum province.

	Total Precipitation (mm)			**Average Temperature (°C)**			**Average Relative Humidity (%)**		
	2009	2010	LYA	2009	2010	LYA	2009	2010	LYA
J	2.3	52.2	19.8	-12.1	-4.3	-9.7	82.4	84.0	77.0
F	18.8	14.8	24.8	-3.1	-1.8	-8.6	84.7	82.3	77.0
M	51.1	82.2	31.0	-0.7	3.1	-2.8	73.8	69.1	75.0
A	42.7	54.2	58.4	4.3	5.6	5.4	64.6	71.3	66.0
M	43.2	63.6	70.0	10.0	10.4	10.5	61.0	69.6	63.0
J	76.2	50.5	41.6	14.7	15.9	14.9	65.0	60.1	58.0
J	29.2	55.5	26.2	17.2	19.5	19.3	60.7	56.0	52.0
A	22.8	9.0	15.1	17.1	20.3	19.4	50.6	44.8	49.0
S	43.7	8.8	20.0	12.4	17.0	14.3	53.1	48.1	52.0
O	51.0	72.2	47.9	8.7	9.2	7.6	62.4	70.2	65.0
N	41.4	0.0	32.9	1.8	1.8	-0.1	75.7	66.1	73.0
D	15.4	12.9	22.5	-1.1	-1.9	-6.6	84.7	76.6	78.0
Total/Average	437.8	475.9	410.2	5.8	7.9	5.3	68.2	66.5	65.4

LYA: Long year average.

The dimensions of each plot were 5 × 3 m, with a distance of 0.5 m along each edge and a buffer zone of 2 m around the perimeter of the plot. A total of six rows of plants were sowed, with a spacing of 30 cm between each row (Tan, 2018).

The long year annual precipitation was recorded 410.2 mm, which is lower than the values observed in the first year (437.8 mm) and the second year (475.9 mm). It is worth noting that all three values above the long-term average precipitation. Table 2 indicates that a higher amount of precipitation was recorded in June of the first year and May of the second year.

The average temperature throughout the period from 1929 to 2009 was recorded as 5.3 °C. Furthermore, the annual mean temperature for the first year was 5.8 °C, while for the second year it was 7.9 °C, both of which were above the long-term average. In the first year, the maximum temperature recorded in July was 17.2 °C, but in the second year, the highest temperature recorded in July was 19.5 °C.

The plants were harvested by using a scythe when the lower pods started to fill with seeds (Tan, 2018). Harvesting was carried out after removal of one row from each side of the plots and a 0.5m area from the beginning or end of each plot.

Before harvest, the plant height of 10 randomly selected plants from each plot was measured, and the average number of main branches per plant was determined by counting the number of primary branches (Sümerli et al., 2002). Harvested plants were oven dried at 68 °C to constant weight and weighed to determine dry matter yield. Plant samples were then ground, passed through a 2 mm sieve and prepared for chemical analysis. The total nitrogen content of the plants was determined by the Kjeldhal method and the crude protein ratio was calculated by multiplying by 6.25 (Jones, 1991). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) ratios were determined using the Van Soest analysis method (Goering & Van Soest, 1970) (ANKOM Technology, Fairport, NY). The organic matter contents of the soils were determined by the Smith-Weldon method (D. W. Nelson & Sommers, 1982) and were evaluated in the low-level class (1.40% in the first year and 1.80% in the second year).

The textures of the soils were determined by the Bouyoucus hydrometer method (Gee & Bauder, 1986) and were in the loam class in both years. The lime content of the soil was determined volumetrically by Scheibler calcimeter (R. E. Nelson, 1982) as medium level (0.82% in the first year and 0.85% in the second year). In addition, the pH levels of soils were potentiometrically determined to be neutral by utilizing a pH meter (McLean, 1982) (7.45 in the first year and 7.65 in the second year).

Soil phosphorus contents were determined according to the phosphomolybdic acid method (Olsen & Summers, 1982), indicating insufficient (27.5 kg ha^{-1} in the first year and 62 kg ha^{-1} in the second year), and potassium content was determined by the flame photometry method (Thomas, 1982) were determined high (118 kg ha^{-1} in the first year and 158 kg ha^{-1}) in the second year).

Analysis of variance in the JMP package software (SAS, 2002) was used to analyze the data. Means were compared using the LSD test (Yıldız & Bircan, 1994).

3. Results and Discussion

The application of phosphorus fertilizer resulted in a significant increase in plant height ($p<0.01$), as given in Table 3. Plant height showed a positive correlation with increasing phosphorus levels, reaching the highest value of 63.8 cm with

100 kg ha^{-1} P_2O_5 application. Legumes demonstrate a greater sensitivity to phosphorus in comparison to other plant species (Mitran et al., 2018). In a previous study, Cömert (2014) reported that increased phosphorus fertilizer application increased the height of the common vetch. Similarly, poultry manure application also significantly increased plant height ($p<0.01$). Poultry manure is a valuable source of nitrogen and readily available phosphorus for soil enrichment. Moreover, its exceptional fertilizer efficiency has been found to significantly boost yields of crops in plant production (Kurt, 2019).

PGPR inoculation statistically significantly increased the plant height ($p<0.05$). Previous researches have indicated that bacteria inoculation increased plant growth (İmriz et al., 2014; Kaynar & Çomaklı, 2023). In addition, the values for plant height in the second year were higher than in the first year and a statistically significant difference was also found between plant height over the years ($p<0.01$). This situation might be linked to the more favorable precipitation and temperature conditions in the second year. PGPR inoculation increased the plant height under the PM0 conditions, but it decreased the plant height when inoculated under the PM1 condition. This response resulted in a significant B X PM interaction ($p<0.01$) (Figure 1a).

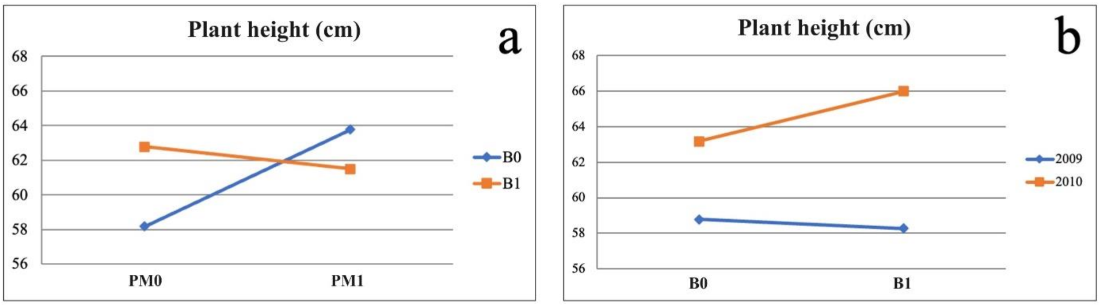

Figure 1. Effects of phosphorus solubilizing bacteria and poultry manure applications on plant height (cm) in common vetch. **(a)** Bacteria X Poultry manure interaction, **(b)** Bacteria X Year interaction.

This result could be attributed to the phosphorus concentration in the poultry manure, and the phosphorus resulting from the inoculation of the bacteria reached the maximum level that could be potentially toxic for the nitrogen-fixing bacteria (Amba et al., 2011; Truu et al., 2017) In addition, PGPR inoculation reduced plant height in the first year but caused a significant increase in the second year. It resulted in a significant B X Y ($p<0.01$) (Figure 1b).

The effects of applications on the number of main branches of the plant were insignificant and no significant interactions were found between the treatments or between the years. Similarly, it was reported by Sawires (2011), number of main branches in plants was not influenced by varying dosages of phosphorus.

On the other hand, as the phosphorus doses were raised, there was a statistically significant increase ($p<0.01$) in the dry matter yield of common vetch. This increase was reached the highest level (6.30 t ha^{-1}) with P2 dose (Table 3). Phosphorus plays a significant role in the energy conversion process and nitrogen fixation within the nodules of leguminous forage crops, making it an essential element for their growth and development (Öncan Sümer & Erten, 2022; Uyanık et al., 2011). Consequently, it is essential to ensure an adequate supply of phosphorus in order to achieve optimal productivity in forage legumes (Mitran et al., 2018; Udvardi & Poole, 2013). Previous studies have reported that phosphorus fertilizer increases the yield of common vetch and forage pea (Erkovan et al., 2014; Rafaat, 2022; Yüksel & Türk, 2019). Poultry manure application significantly increased the dry matter yield ($p<0.01$). The application of organic fertilizers, such as poultry manure, has been found to positively impact soil structure, aeration, and water infiltration, hence leading to an elevation in crop yields (Azmi et al., 2019; Türkkan & Kibar, 2022). Inoculation of plant with growth-promoting rhizobacteria resulted in a statistically significant increase in dry matter yield ($p<0.01$). Dry matter yield, which was 5.54 t ha^{-1} without PGPR inoculation, increased to 5.75 t ha^{-1} with PGPR inoculation. Phosphorus-solubilizing bacteria can convert bound phosphorus into a form that is readily accessible to plants through the production of diverse organic acids within the soil. This process ultimately leads to an improvement in plant productivity, as demonstrated by Meena et al. (2017) and Chen and Liu (2019). The difference in dry matter yield over the years was statistically significant ($p<0.01$), with an increase in the dry matter yield from 5.28 t ha^{-1} in the first year to 6.01 t ha^{-1} in the second year. Considering the two-year average results, the application of poultry manure in common vetch under B0 conditions increased dry matter yield, whereas it decreased the yield under B1 conditions. This difference resulted in a significant ($p<0.01$) B X PM interaction (Figure 2a). The difference might be related to the chemical composition of poultry manure. Despite providing additional nutrient elements, poultry manure may negatively affect microbial activity in the soil and cause to a decrease in plant yield (Li et al., 2011). This reduction in microbial activity is significant as soil microorganisms play a crucial role in facilitating the uptake of phosphorus and other essential nutrients by plants (Chauhan et al., 2022; Matse et al., 2020; Öksel et al., 2022). Dry matter yield increased linearly with increasing P doses under PM0 conditions, whereas the increase in dry matter yield stopped after P1 doses under PM1 conditions. This reaction caused a significant P × PM interaction ($p<0.01$) (Figure 2b).

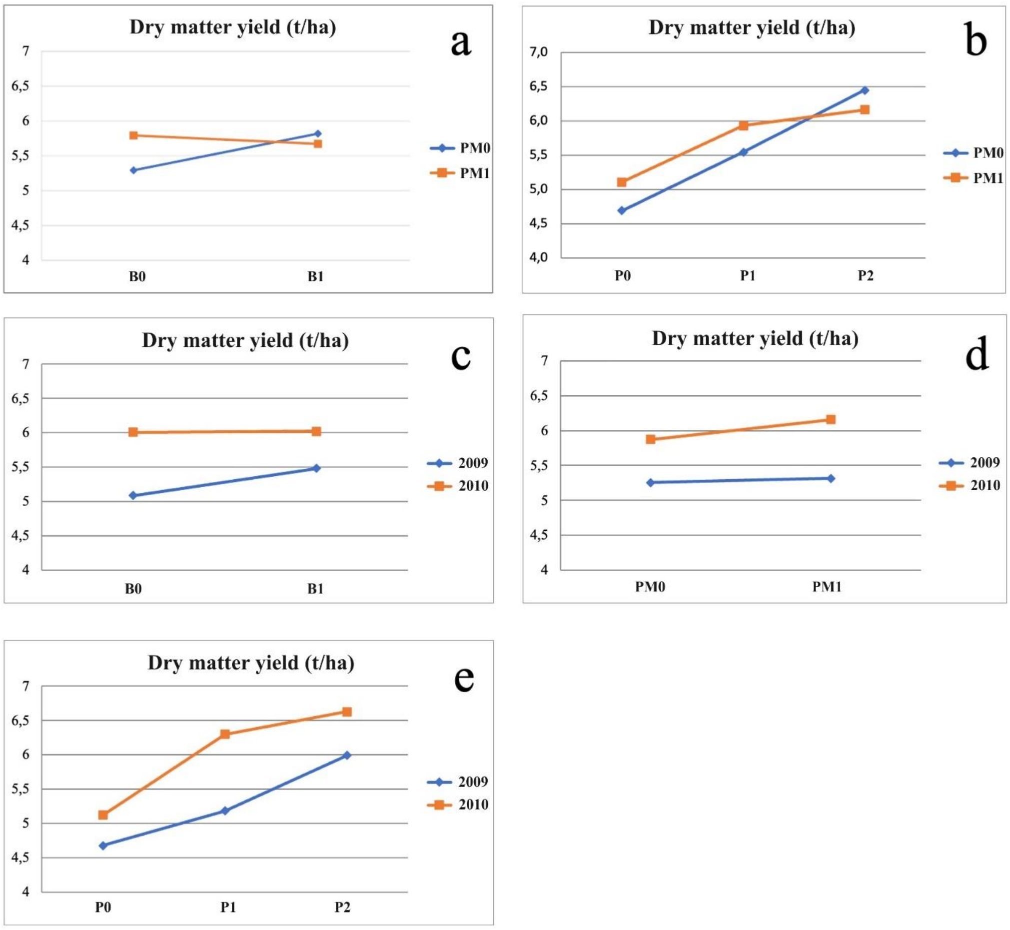

Figure 2. Effects of phosphorus solubilizing bacteria, poultry manure, and phosphorus fertilizer applications on dry matter yield (t/ha) in common vetch. **(a)** Bacteria X Poultry manure interaction, **(b)** Phosphorus X Poultry manure interaction, **(c)** Bacteria X Year interaction, **(d)** Poultry manure X Year interaction, **(e)** Phosphorus X Year interaction.

This suggests that the application of 50 kg P_2O_5 phosphorus, along with phosphorus provided by poultry manure, reached phosphorus saturation for common vetch. Indeed, poultry manure is known to be particularly rich in phosphorus for plants (Kurt, 2019). While dry matter yield increased with PGPR inoculation in the first year, it was ineffective in the second year. Because of these different effects, Y X B interaction was found to be significant ($p<0.01$) (Figure 2c). Previous studies indicated that factors such as irrigation, temperature, soil type, plant species, bacterial strain, and application method play a crucial role in the activity of bacteria (Gerçekçioğlu et al., 2018; Gupta et al., 2015). The application of poultry manure resulted in an increase in dry matter yield during the second year in comparison to the first year. This reaction caused a significant Y X PM interaction ($p<0.05$) (Figure 2d). The dry matter yield, which increased with the P1 dose in both years, further increased with the P2 dose in the first year but decreased with the P2 dose in the second year. This different response led to a significant ($p<0.01$) Y X P interaction (Figure 2e). These differences might be attributed to variations in temperature and precipitation values between the years. The application of poultry manure increased dry matter yield more in the second year when compared to the first year. This resulted in a significant ($p<0.05$) Y X PM interaction (Figure 2d). The dry matter yield, which increased with the P1 dose in both years, further increased with the P2 dose in the first year but decreased with the P2 dose in the second year. As a result of these different effects, Y X P interaction was found to be significant ($p<0.01$) (Figure 2e). These differences might be attributed to variations in temperature and precipitation values between the years.

The effects of phosphorus application on the crude protein ratio of the resulted in a significant increase in the crude protein content ($p<0.01$). Phosphorus has an influence on root development and the activity of *Rhizobium* bacteria, resulting in significant effect on nitrogen fixation (Yüksel & Türk, 2019). Moreover, the presence of phosphorus has been seen to enhance the crude protein content in legumes (Belete et al., 2019; Kaynar & Çomaklı, 2023).

Table 3. Effects of phosphorus fertilizer, poultry manure applications and PGPR (*Bacillus megaterium* M3) inoculation in common vetch (*Vicia sativa*) plant height (cm), number of main branches (pieces), hay yield (t ha^{-1}), crude protein ratio (%), NDF (%) and ADF (%) rates.

		PH	NMB	DHY	CPR	NDF	ADF
P	P$_0$	59.8^{C}±1.17	1.91±0.10	4.90^{C}±0.15	17.07^{B}±0.29	40.5±0.17	30.2±0.18
	P$_{50}$	61.1^{B}±0.99	2.00±0.10	5.74^{B}±0.14	17.87^{A}±0.25	40.5±0.15	30.0±0.16
	P$_{100}$	63.8^{A}±0.85	1.91±0.08	6.30^{A}±0.10	18.33^{A}±0.23	40.6±0.15	30.2±0.17
	Av.	61.5±0.52	1.95±0.10	5.64±4.78	17.75±0.19	40.5±0.15	30.1±0.16
PM	PM$_0$	60.4^{B}±0.90	1.94±0.07	5.56^{B}±0.15	18.22^{A}±0.20	40.4±0.14	29.9±0.13
	PM$_1$	62.6^{A}±0.78	1.97±0.08	5.73^{A}±0.13	17.29^{B}±0.22	40.6±0.11	30.3±0.14
	Av.	61.5±0.42	1.95±0.85	5.64±3.90	17.75±0.15	40.5±0.12	30.1±0.13
B	B$_0$	60.9^{b}±0,90	1.94±0.08	5.54^{B}±0.16	17.53^{b}±0.13	40.6±0.11	30.2±0.15
	B$_1$	62.1^{a}±0.82	1.94±0.08	5.75^{A}±0.13	17.98^{a}±0.21	40.5±0.14	30.1±0.13
	Av.	61.5±0.42	1.94±0.85	5.64±3.90	17.75±0.15	40.5±0.12	30.1±0.13
Years	2009	58.5^{A}±0.43	2.00±0.04	5.28^{B}±0.11	17.38^{B}±0.22	40.3^{b}±0.13	30.2±0.15
	2010	64.5^{B}±0.89	2.02±0.10	6.01^{A}±0.15	18.13^{A}±0.22	40.7^{a}±0.11	30.0±0.13
	Av.	61.5±0.42	2.01±0.85	5.65±3.90	17.75±0.15	40.5±0.12	30.1±0.13
	P	**	ns	**	**	ns	ns
	PM	**	ns	**	**	ns	ns
	B	*	ns	**	*	ns	ns
	Y	**	ns	**	**	*	ns
	BxP	ns	ns	ns	ns	ns	ns
	BxPM	**	ns	**	ns	ns	ns
	BxY	**	ns	**	ns	ns	ns
	PMx P	ns	ns	**	ns	ns	ns
	PMxY	ns	ns	*	ns	ns	ns
	PxY	ns	ns	**	ns	ns	ns
	BxPxPM	ns	ns	**	ns	ns	ns
	BxPxY	ns	ns	**	*	ns	ns
	BxPMxY	**	ns	**	**	ns	ns
	PxPMxY	ns	ns	ns	*	ns	ns

#Values shown in capital letters and ** sign are significant at 1% ($P<0.01$), small letters and * sign are significant at 5% ($p<0.05$).
PH: Plant height; NMB: Number of main branches; DMY: Dry matter yield; CPR: Crude protein rate; ADF: Acid detergent fiber; NDF: Neutral detergent fiber, B: Bacteria; P: Phosphorus; PM: Poultry manure; Y: Year; Ns: None significant.

The application of poultry manure resulted in a significant decrease in crude protein content ($p<0.01$). This effect could be attributed to the high nitrogen content present in poultry manure. The application of nitrogen fertilizers has been widely recognized as a factor that induces stress in legume plants, leading to a decrease in protein content (Bayram et al., 2009). The PGPR inoculation resulted in a statistically significant increase in the crude protein content ($p<0.05$). The observed results can be attributed to the positive impacts of bacterial inoculation on the growth of root systems, the metabolic activity of microorganisms involved in nitrogen fixation, and the development of complex root systems, hence facilitating the establishment of a mutually advantageous symbiotic association (Bhat et al., 2013). Previous research has also reported that the utilization of Plant Growth-Promoting Rhizobacteria (PGPR) has a positive impact on the chemical composition of plants, leading to elevated levels of crude protein and mineral content (Erkovan et al., 2014; Yüksel & Türk, 2019).

The applications were not affected the ADF and NDF rates. The ADF and NDF values obtained after analysis in common vetch were found to be below the recommended thresholds of

31% for ADF and 41% for NDF, which are considered indicative of acceptable fodder quality (Yavuz et al., 2009). A statistically significant change ($p<0.05$) was observed in the NDF ratio between the two years, with an increase in NDF from 40.3% in the first year to 40.7% in the second year. This difference could potentially be attributed to the variability in temperature experienced over the duration of plant growth, as the second year of the experiment exhibited higher temperatures compared to the first year. According to Osman et al. (2010), it has been observed that the cellulose content in forage tends to rise in response to warm weather conditions.

4. Conclusion

The use of inorganic fertilizers in combination with organic fertilizers as an alternative approach to reducing the use of inorganic fertilizers has become increasingly important in the context of sustainable agriculture. For this reason, this study investigated the effects of phosphorus fertilizer and poultry manure applications with bacterial inoculation on yield and yield components of common vetch. Based on the two-year average results, it was found that the plots treated with 100 kg P_2O_5 per hectare together with PGPR inoculation yielded the highest dry matter and crude protein yields. Therefore, it can be suggested to apply phosphorus fertilizer of 100 kg ha^{-1} P_2O_5 with PGPR inoculation to optimize forage production in common vetch grown in Erzurum and comparable environmental circumstances with low to moderate phosphorus levels. Additionally, given the results, poultry manure appears promising as an important organic fertilizer source for sustainable agriculture. For this reason, new studies are needed to investigate issues such as different doses and application methods of poultry manure.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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