ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi*

DOI: 10.5152/AUAF.2023.23084

Effects of Different Phosphorus Sources on the Yield and Yield Components of Forage Pea

Çeşitli Fosfor Kaynaklarının Yem Bezelyesinde Kuru Ot Verimi ve Kalite Özellikleri Üzerine Etkileri

Dilara KAYNAR[1]
Binali ÇOMAKLI[2]

[1]Kastamonu Üniversitesi, Devrekani TOBB MYO, Veterinerlik Bölümü, Kastamonu, Türkiye
[2]Atatürk Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Erzurum, Türkiye

ABSTRACT

This experiment was conducted to evaluate the effects of two different doses (B0 or B1) of phosp horus-solubilizing bacteria (*Bacillus megaterium* M-3) inoculation, two different doses (0 or 3 t ha^{-1}) of poultry manure, and three different doses (0, 50, and 100 kg P_2O_5 ha^{-1}) of commercially available phosphorus fertilizer on the dry matter yield, plant height, crude protein, neutral detergent fiber, and acid detergent fiber contents of forage pea in the irrigated condition of Erzurum between 2009 and 2010. While the effects of bacteria inoculation and poultry manure applications on dry matter yield varied over the years, an increase was observed in dry matter yield with increasing doses of phosphorus fertilizer. In addition, considering the 2-year averages, the highest dry matter yield considering the 2-year averages was obtained with the application of bacteria, poultry manure, and phosphorus fertilization together. Therefore, in order to obtain the highest dry hay yield in Erzurum and similar ecological conditions with low and/or medium phosphorus content in the soil and irrigated, *Bacillus megaterium* inoculation and 3 t ha^{-1} poultry manure together with 100 kg ha^{-1} P_2O_5 application can be recommended.

Keywords: Dry matter yield, phosphorus fertilization, phosphorus-solubilizing bacteria, poultry manure

ÖZ

Bu deneme, iki farklı dozda (B0 veya B1) fosfor çözücü bakteri (*Bacillus megaterium* M-3) aşılaması, iki farklı dozda (0 veya 3 t ha^{-1}) tavuk gübresi ve 3 farklı dozda (0, 50 ve 100 kg P_2O_5 ha^{-1}) ticari fosforlu gübre uygulamasının yem bezelyesinin kuru madde verimi, bitki boyu, ham protein, NDF ve ADF içerikleri üzerine etkilerini değerlendirmek amacıyla 2009–2010 yılları arasında Erzurum'da sulu koşullarda yürütülmüştür. Bakteri aşılaması ve tavuk gübresi uygulamalarının kuru madde verimi üzerine etkileri yıllara göre değişirken, artan dozlarda fosforlu gübre uygulaması ile kuru madde veriminde artış gözlenmiştir. Ayrıca iki yıllık ortalamalar dikkate alındığında en yüksek kuru madde verimi bakteri, tavuk gübresi ve fosforlu gübrelemenin birlikte uygulanması ile elde edilmiştir. Bu nedenle, toprakta düşük ve/veya orta düzeyde fosfor içeriğine sahip, sulanan Erzurum ve benzer ekolojik koşullarda en yüksek kuru madde verimini elde etmek için *Bacillus megaterium* aşılaması ile beraber 3 t ha^{-1} tavuk gübresi ve 100 kg ha^{-1} P_2O_5 uygulaması önerilebilir.

Anahtar Kelimeler: Kuru ot verimi, fosforlu gübreleme, fosfor çözücü bakteri, tavuk gübresi

Received/Geliş Tarihi: 22.02.2023
Accepted/Kabul Tarihi: 25.04.2023
Publication Date/Yayın Tarihi: 18.05.2023

Corresponding Author/Sorumlu Yazar: Dilara KAYNAR
E-mail: dkaynar@kastamonu.edu.tr

Cite this article as: Kaynar, D., & Çomaklı, B. (2023). Effects of different phosphorus sources on the yield and yield components of forage pea. *Research in Agricultural Sciences*, 54(2), 81-88.

Introduction

Forage pea (*Pisum sativum* spp. *arvense* L.) is an annual cold-season legume plant with high forage yield and hay quality. Besides yielding high amounts of quality hay, this plant is of importance since it can be involved in short-term cropping systems and increase the amount of nitrogen that is present in the soil. Phosphorus plays an important role in increasing hay production and improving root and nodule development, nutrient intake, and plant growth in forage peas, as with the other forage legumes (Mitran et al., 2018). The use of inorganic, organic, and biological fertilizers are the major applications utilized in order to replenish the plant nutrients that are depleted in agricultural soils such as phosphorus (Masarirambi et al., 2012). However, the phosphate anions in chemical fertilizers form unsoluble salt complexes by interacting with Ca^{2+}, Fe^{3+}, and Al^{3+} ions in the soil and, as a consequence of this interaction, the amount of phosphorus available in the soil for plants decreases to 5–25% (Lambers & Plaxton, 2018; Schnug & Haneklaus, 2016). Moreover, the intense use of inorganic fertilizers causes soil degradation, which has a negative effect on the crop yield. This might be avoided by limiting the amount of inorganic fertilizers used (Debele, 2021). In addition, the practice of sustainable agriculture requires the use of organic fertilizers in combination with inorganic fertilizers because of increasing fertilizer prices and in order to decrease their negative impacts on the environment such as the accumulation of cadmium (Chukwu et al., 2014; Kaynar, 2014; Roba, 2018). Microorganisms that are used as biological fertilizers are known as plant growth-promoting rhizobacteria (PGPR). These microorganisms promote plant growth by increasing the amount of phosphorus that plants can use in soil. Plant growth-promoting rhizobacteria enable the dissolution of phosphorus that is actually available in the soil but has formed a complex by altering the enzyme and hormone secretion, which in turn reduces the need for chemical fertilizers (Chen & Liu, 2019; Meena et al., 2017). Thus, in studies employing biological fertilizers and organic fertilizers such as cattle manure and poultry manure, it was determined that the physical and chemical properties of the soil improved, and the yield increased in many plants (Azmi et al., 2019; Eleduma et al., 2020; Türkkan & Kibar, 2022).

The objective of this experiment was to determine the effects of applications of phosphorus-solubilizing bacteria inoculation, poultry manure, and mineral phosphorus on the dry matter yield and quality characteristics of forage peas.

Methods

The field experiment was carried out in the experimental field of the Agriculture Faculty of Agriculture of Atatürk University in Erzurum. The experiment used a randomized complete block with three replications with a factorial arrangement. Two levels of bacteria inoculation (B0 or B1), two doses of poultry manure (0 or 3 t ha^{-1}), and three doses of phosphorus (0, 50, and 100 kg P_2O_5 ha^{-1}) were applied. Each plot was 5 × 3 m in size, with a 0.5 m buffer inside each edge and a 2 m buffer outside. Six lines of plants were sown with a 30 cm distance between the rows (Tan, 2018).

Annual total precipitation was 410.2 mm, whereas it was 437.8 mm in the first year and 475.9 mm in the second year, and the values were higher than the long-term average. June in the first year and May in the second year received more precipitation (Table 1). The long-term (1929–2009) average temperature was 5.3°C, and the annual mean temperature values were higher (5.8°C for the first year and 7.9°C for the second year) than the long-term average. The highest temperature was 17.2°C in July in the first year and was 19.5°C in July in the second year.

The seeds to be sown in the experiments were inoculated with *Rhizobium leguminosarum* bacteria obtained from Ankara Soil and Fertilizer Research Institute. Phosphorous fertilizer (triple superphosphate) and poultry manure were mixed into the soil using a harrow. *Bacillus megaterium* was prepared at 10^8 CFU mL^{-1} density after the incubation and was inoculated to the seeds to be sowed in the plots. Kirazlı variety of forage peas (12 kg da^{-1}) (*Pisum sativum* ssp. *arvense* L.) were planted in preprepared seed beds at 4–6 cm depth (Tan, 2018) on the first year April 20 and on April 29 in the second year by using a hand drill. Seeds inoculated with phosphorus-solubilizing bacteria (*Bacillus megaterium* M-3) were sowed using different planting drills in order to prevent contamination. In 2 years, they were irrigated three times when the plants turned dark green due to moisture deficiency in the soil in the growing season.

Using the Bouyoucus hydrometer method (Gee & Hortage, 1986), the soil texture was found to be loamy in both years. The pH levels of soils were potentiometrically determined to be neutral (7.45 in the first year and 7.65 in the second year) by utilizing a pH meter (McLean, 1983). The lime content of the soil was found to be at the medium level (0.82% in the first year and 0.85% in the second year) using a Scheibler calcimeter volumetrically (Nelson, 1982). The organic matter content of the soil was determined in the "low-level class" (1.40% in the first year and 1.80% in the second year) by using the Smith-Weldon method (Nelson & Sommers, 1982). Using the flame photometry method (Thomas, 1982), the

Table 1.
Climatic Data of Experimental Area in 2009, 2010, and Long-Term Average at Erzurum

	Total Precipitation (mm)			Average Temperature (°C)			Average Relative Humidity (%)		
	2009	2010	LYA	2009	2010	LYA	2009	2010	LYA
J	2.30	52.20	19.80	−12.10	−4.30	−9.70	82.40	84.00	77.00
F	18.80	14.80	24.80	−3.10	−1.80	−8.60	84.70	82.30	77.00
M	51.10	82.20	31.00	−0.70	3.10	−2.80	73.80	69.10	75.00
A	42.70	54.20	58.40	4.30	5.60	5.40	64.60	71.30	66.00
M	43.20	63.60	70.00	10.00	10.40	10.50	61.00	69.60	63.00
J	76.20	50.50	41.60	14.70	15.90	14.90	65.00	60.10	58.00
J	29.20	55.50	26.20	17.20	19.50	19.30	60.70	56.00	52.00
A	22.80	9.00	15.10	17.10	20.30	19.40	50.60	44.80	49.00
S	43.70	8.80	20.00	12.40	17.00	14.30	53.10	48.10	52.00
O	51.00	72.20	47.90	8.70	9.20	7.60	62.40	70.20	65.00
N	41.40	0.00	32.90	1.80	1.80	−0.10	75.70	66.10	73.00
D	15.40	12.90	22.50	−1.10	−1.90	−6.60	84.70	76.60	78.00
Total/average	437.80	475.90	410.20	5.80	7.90	5.30	68.20	66.50	65.40

Note: LYA = long year average.

potassium content was high (118 kg ha^{-1} in the first year and 158 kg ha^{-1} in the second year). Soil's phosphorus content was found insufficient (27.5 kg ha^{-1} in the first year and 62 kg ha^{-1} in the second year) according to the molybdophosphoric acid method (Olsen & Summers, 1982).

After removing one row from each side of the plots and a 0.5 m area from the start or end of each plot, the harvesting process was carried out. The plants were harvested when they formed (Tan, 2018) by using a scythe. Before the plants were harvested, ten plants were selected from each parcel, and plant heights were measured. Harvested plants were oven-dried at 68°C to a constant weight and they were weighed in order to determine the dry matter yield (Jones, 1991).

After weighing, the oven-dried plant samples were grounded and passed through a 2 mm sieve for chemical analysis. The total nitrogen content of plants was determined using the Kjeldhal method, and the crude protein content was calculated by multiplying by 6.25 (Jones, 1991). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) ratios were determined by using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY) according to the procedure described by Van Soest et al. (1991).

The data were analyzed using variance analysis in JMP package software (SAS Institute, 2002). Mean values were compared using the LSD test (Yıldız & Bircan, 1994).

Results and Discussion

Plant growth-promoting rhizobacteria inoculation resulted in a significant increase in the plant height. The plant height, which was 88.33 cm on average, increased to 89.88 cm with PGPR inoculation. However, the application of poultry manure had no significant effect on the plant height (Table 2). On the other hand, applications of phosphorus fertilizer caused an increase in plant height in parallel with the phosphorus doses and reached the highest value of 90.79 cm in 100 kg ha^{-1} P_2O_5. In a similar study, it was reported that the increasing phosphorus doses yielded a higher level of increase in the plant height compared to the other yield factors (Yılmaz, 2008). There was no statistically significant difference found between plant height values by the years. Besides, PGPR inoculation yielded a higher level of increase in the

Table 2.
Effects of Phosphorus-Solubilizing Bacteria, Poultry Manure, and Phosphorus Fertilizer Applications on Plant Height (cm), Dry Matter Yield (t ha^{-1}), Crude Protein Ratio (%), ADF (%), and NDF (%) Ratio in Forage Pea

		PH	DHY	CPR	ADF	NDF
B	B_0	86.77^B	3.73^B	17.65	24.20	38.84^A
	B_1	89.88^A	3.94^A	17.88	23.89	37.49^B
	Av.	88.33	3.83	17.76	24.04	38.17
P	P_0	85.16^C	3.60^C	16.85^B	24.05	38.01
	P_{50}	89.04^B	3.81^B	17.92^A	24.09	38.42
	P_{100}	90.79^A	4.10^A	18.51^A	24.00	38.08
	Av.	88.33	3.83	17.76	24.04	38.17
PM	PM_0	88.08	3.77^B	17.61	24.14	37.64^B
	PM_1	88.58	3.90^A	17.92	23.95	38.70^A
	Av.	88.33	3.83	17.76	24.04	38.17
Years	2009	87.97	4.01^A	18.97^A	23.55^b	36.26^B
	2010	88.69	3.66^B	16.55^B	24.54^a	40.08^A
	Av.	88.33	3.83	17.76	24.04	38.17
	B	**	**	NS	NS	*
	P	**	**	**	NS	NS
	PM	NS	**	NS	NS	*
	Y	NS	**	**	*	**
	B × P	*	**	NS	NS	NS
	B × PM	**	**	*	NS	NS
	B × Y	*	**	NS	*	*
	P × Y	*	**	NS	*	*
	PM × P	*	NS	NS	*	NS
	PM × Y	NS	**	**	NS	NS
	B × P × PM	NS	NS	NS	NS	NS
	B × P × Y	*	**	NS	NS	NS
	B × PM × Y	*	**	NS	*	NS
	P × PM × Y	NS	NS	NS	NS	**

Note: *Values shown in capital letters are significant at 1% ($p < .01$), and small letters are significant at 5% ($p < .05$).
ADF = acid detergent fiber; B = bacteria; CPR = crude protein rate; DHY = dry hay yield; NDF = neutral detergent fiber; NS = none significant; P = phosphorus; PH = plant height; PM = poultry manure; Y = year.

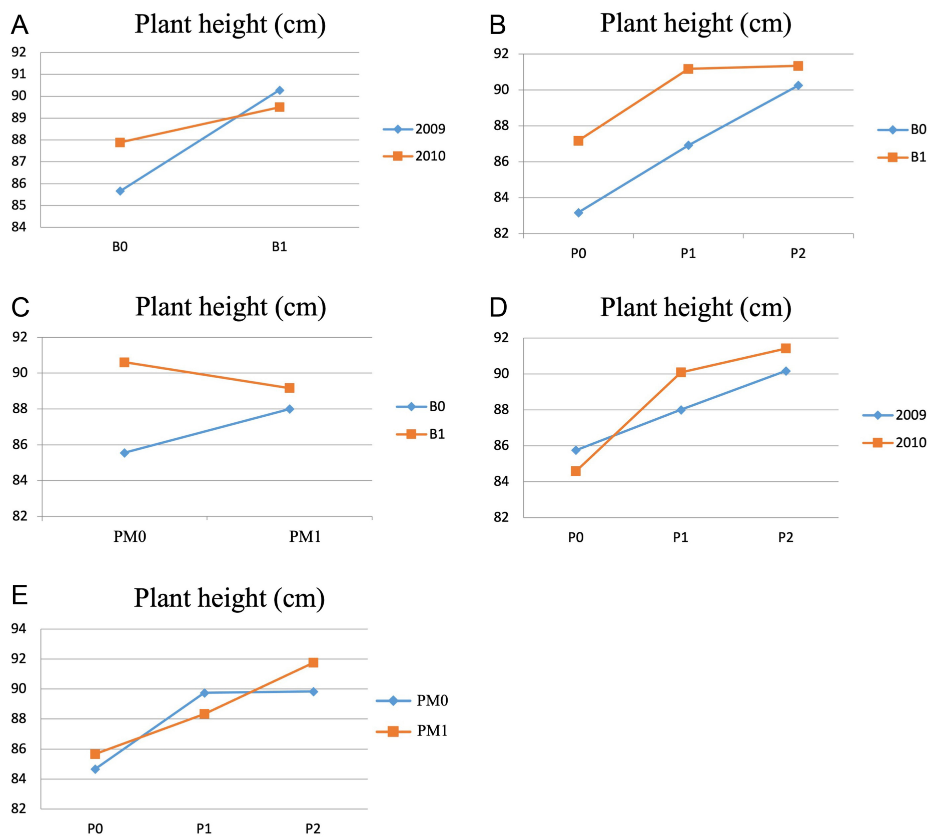

Figure 1.
A–E, Effects of Phosphorus-Solubulizing Bacteria, Poultry Manure, and Phosphorus Fertilizer Applications on Plant Height (cm) in Forage Pea. B × Y, B × P, B × PM, P ×Y, PM × P.

plant height in the first year in comparison to the second year, and as a result of different effects, B × Y interaction was found statistically significant ($p < .05$) (Figure 1A).

Plant height increased linearly depending on increased P doses under B0 conditions, whereas plant height increases ceased after P1 doses under B1 conditions. It caused the B × P interaction to be statistically significant ($p < .05$) (Figure 1B). Poultry manure application increased the plant height under the B0 condition, whereas it caused a decrease under the B1 condition. This result showed that the B × PM interaction was statistically significant ($p < .01$) (Figure 1C). This result might be because the phosphorus arising from the bacteria inoculation and the phosphorus content of poultry manure might have reached the potentially toxic level for nitrogen-fixing bacteria (Amba et al., 2011). While the plant height linearly increased in parallel with phosphorus doses in the first year, a remarkable increase was observed after the P1 dose in the second year. This different reaction caused a significant P × Y interaction ($p < .05$) (Figure 1D). The plant height increase ceased after the P1 dose without poultry manure application, whereas plant heights increased after the P1 dose together with the poultry manure application. It resulted in a significant PM × P interaction ($p < .05$) (Figure 1E). Therefore, it was determined that the use of inorganic fertilizer and poultry manure resulted in a higher increase in yield when compared to the sole use (Almaz et al., 2017).

Plant growth-promoting rhizobacteria inoculation statistically significantly increased the dry matter yield ($p < .01$). Dry matter yield was found to be 3.73 t ha^{-1} without PGPR inoculation, whereas it increased to 3.94 t ha^{-1} in PGPR inoculation plots (Table 3). The effect of poultry manure application on dry matter yield was statistically significant ($p < .01$). Dry matter yield, which was 3.77 t ha^{-1} without poultry manure application, increased to 3.90 t ha^{-1} with poultry manure application. Besides, dry matter yield statistically significantly increased with increasing phosphorus doses ($p < .01$), and the highest level (4.10 t ha^{-1}) was obtained with P2 dose. Dry matter yield decreased from 4.01 t ha^{-1} in the first year to 3.66 t ha^{-1} in the second year, and a statistically significant difference was found between dry matter yield values by the years ($p < .01$). It is known that the reaction of legumes to phosphorus is higher than other plants (Mitran et al., 2018) and phosphorus fertilizers increase the yield in cases of phosphorus deficiency (Sümer & Erten, 2022). However, in some cases, the efficiency of phosphorus for plants might be low due to the activity of soil microorganisms, even if the amount of phosphorus is sufficient. Because these bacteria increase the yield by transforming the phosphorus into a form that is available for plants (Chauhan et al., 2022; Matse et al., 2020; Öksel et al., 2022). Poultry manure application had a positive effect on the dry matter yield. In previous studies, it was reported that poultry manure application increased hay yield (Hoover et al., 2019; Lin et al., 2018).

Bacteria inoculation and poultry manure application increased the dry matter yield in the first year but caused a decrease in the second year. As a result of these different effects of bacteria and poultry manure on dry matter yield in different years, B × Y and PM × Y interactions were found to be significant ($p < .01$) (Figure 2A and B). With increasing phosphorus doses, there was a linear increase in dry matter yield in the first year, whereas

Table 3.
Chemical Composition of Poultry Manure

Parameter	Value
pH (1:5.0)	6.50
Organic matter (%)	22.00
Al (mg/kg)	315.26
B (mg/kg)	20.36
Ca (mg/kg)	18546.00
Cd (mg/kg)	0.092
Cr (mg/kg)	1.23
Cu (mg/kg)	36.12
Fe (mg/kg)	352.00
K (mg/kg)	12312.00
Mg (mg/kg)	3451.00
Mn (mg/kg)	172.35
Na (mg/kg)	956.00
Ni (mg/kg)	2136.00
P (mg/kg)	4845.00
Pb (mg/kg)	0.049
S (mg/kg)	452.13
Zn (mg/kg)	185.62

a higher level of increase was observed after the P1 dose in the second year. This different reaction suggests that the P × Y interaction was significant ($p < .01$) (Figure 2C). These results might be due to the changes in temperature and precipitation values between the years. While dry matter yield increased linearly in parallel with phosphorus doses under the B1 condition, a higher level of increase was observed after the P1 dose under the B0 condition. As a result of this different reaction, the B × P interaction was found to be significant ($p < .01$) (Figure 2D). It is known that plants benefit from fertilizers more effectively when using PGPR together with inorganic fertilizers (Abbas et al., 2013; Celik et al., 2020). While poultry manure application increased the dry matter yield under the B0 condition, it had no effect under the B1 condition. As a result of these different effects, B × PM interaction was found to be significant ($p < .01$) (Figure 2E).

The effects of PGPR inoculation and poultry manure applications on the crude protein ratio of the plant were insignificant. Besides that, phosphorus fertilizer application also significantly increased the crude protein ratio ($p < .01$).

In general, the crude protein ratio and mineral content increase with the use of phosphorus fertilizer (Belete et al., 2019), and it was reported in previous studies that phosphorous fertilizers increased the crude protein ratio in common vetch and forage pea (Kaynar, 2014; Yüksel & Türk, 2019). A statistically significant difference was also found between crude protein ratios over the years ($p < .01$). Application of poultry manure decreased the crude protein ratio under the B0 condition, whereas it increased the crude protein ratio when applied under the B1 condition. This reaction caused a significant B × PM interaction ($p < .05$) (Figure 3A). This different reaction might be because the bacteria showed a different activity in relation to different phosphorus concentrations in the soil (Gupta et al., 2015). Poultry manure application decreased the crude protein ratio in the first year but increased it in the

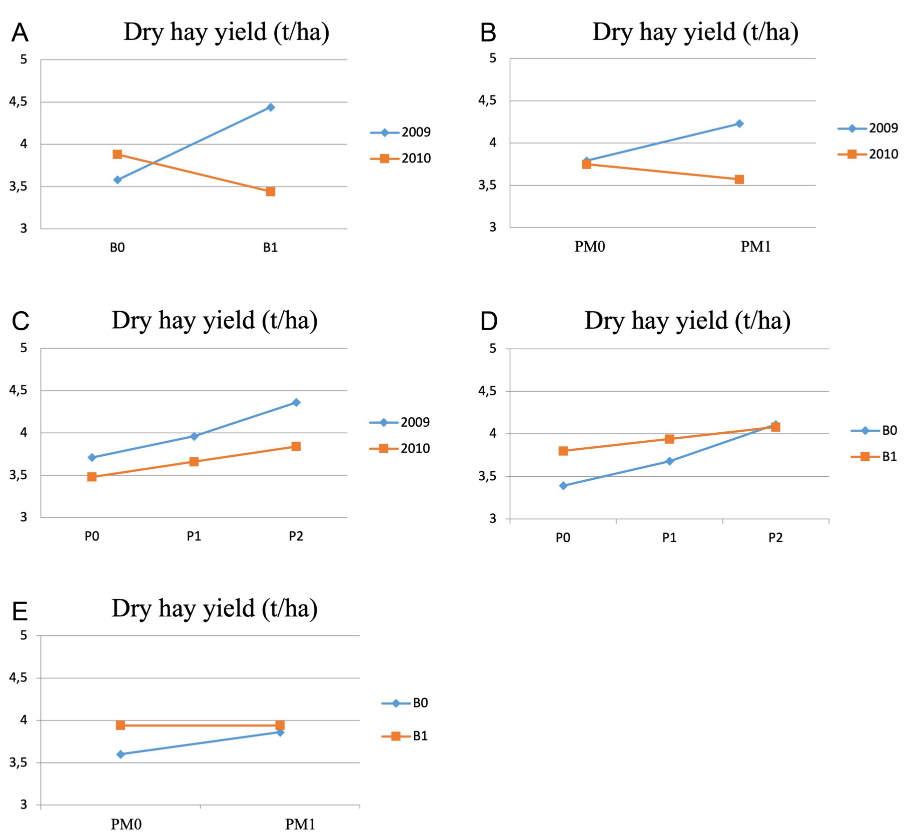

Figure 2.
A–E, Effects of Phosphorus-Solubulizing Bacteria, Poultry Manure, and Phosphorus Fertilizer Applications on Dry Matter Yield (t ha⁻¹) in Forage Pea; B × Y, PM × Y, P × Y, B × P, B × PM.

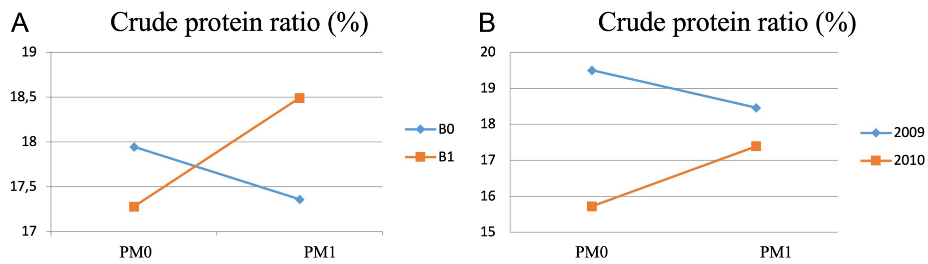

Figure 3.
A, B, Effects of Phosphorus-Solubulizing Bacteria, Poultry Manure, and Phosphorus Fertilizer Applications on Crude Protein Ratio (%) in Forage Pea; B × PM, PM × Y.

second year. As a result of this different reaction, the PM × Y interaction was found to be significant ($p < .01$) (Figure 3B).

Plant growth-promoting rhizobacteria inoculation caused a decrease in the NDF ratio of plants ($p < .05$), whereas poultry manure application increased it ($p < .05$). Moreover, the effect of phosphorus application on the NDF ratio was found to be statistically nonsignificant. A statistically significant difference was found between the years ($p < .01$). The results showed that the NDF ratio was lower than 41%, which is the upper limit for quality forage (Yavuz et al., 2009). Compared to the second year, the bacteria inoculation caused a more remarkable decrease in the NDF ratio in the first year. It resulted in a significant B × Y interaction (Figure 4A). The NDF ratio decreased after the P1 dose in the first year, whereas it increased in parallel with increasing phosphorus doses in the second year. As a result of this different reaction, the P × Y interaction was found to be significant ($p < .05$) (Figure 4B). High NDF and ADF ratios indicated increased lignification and consequently decreased the digestibility of hay (Budak & Budak, 2014).

According to the results, the effects of phosphorus, poultry manure applications, and PGPR inoculation on ADF ratio were found not to be statistically significant, and a statistically significant difference was found between the years ($p < .01$). Acid detergent fiber content of forage peas was found to be lower than 31%, which is the upper limit for quality roughage (Yavuz et al., 2009). However, bacteria inoculation and phosphorus application decreased the ADF content in the first year but increased it in the second year. This finding caused significant B × Y and P × Y interactions ($p < .05$) (Figure 5A and B). In addition,

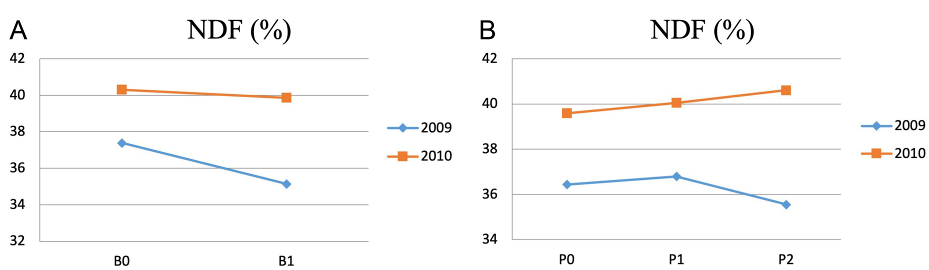

Figure 4.
A, B, Effects of Phosphorus-Solubulizing Bacteria, Poultry Manure, and Phosphorus Fertilizer Applications on NDF Ratio (%) in Forage Pea; B × Y, P × Y.

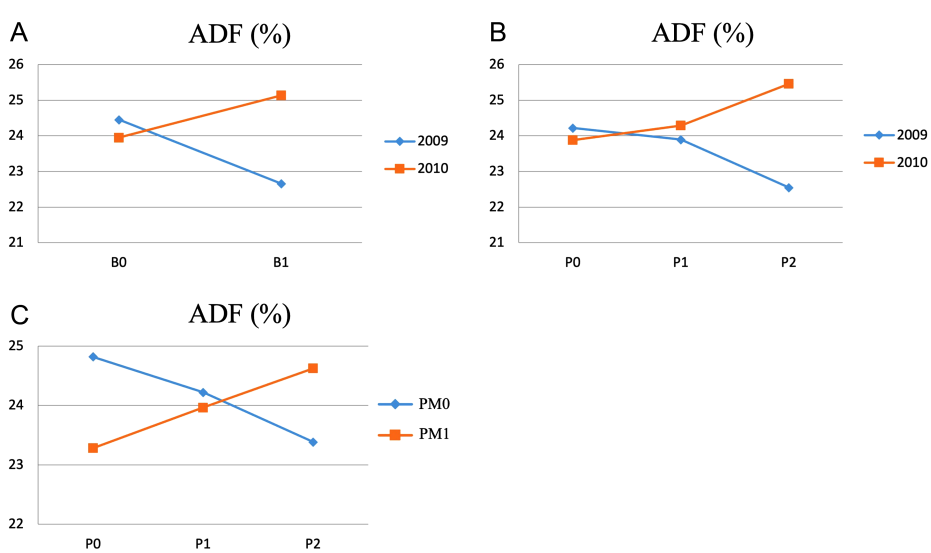

Figure 5.
A–C, Effects of Phosphorus-Solubulizing Bacteria, Poultry Manure, and Phosphorus Fertilizer Applications on ADF (%) in Forage Pea; B × Y, P × Y, PM × P.

the ADF ratio linearly increased in parallel with increasing phosphorus doses with poultry manure application, whereas it decreased after the P1 dose without poultry manure application. This different reaction caused a significant PM × P interaction ($p < .05$) (Figure 5C).

It is known that ADF and NDF ratios of forage peas, like other forage legumes, are generally lower in comparison to other plants (Osman et al., 2010). The reason for finding the NDF and ADF ratios of hay higher in the second year was probably because the temperature was higher in the second year than in the first year.

Conclusion and Recommendations

The combined application of organic and inorganic fertilizers increases soil productivity and yield and reduces the harmful effects of inorganic fertilizers on the environment. Thus, the combined use gains importance as an alternative way for sustainable soil productivity and sustainable agriculture. Hence, in the present study examining the effects of bacteria inoculation, poultry manure, and phosphorus fertilizer on the dry matter yield and yield components of forage peas, it was determined that the use of bacteria inoculation and poultry manure application in combination with phosphorus fertilizer resulted in a higher level of dry matter yield in comparison to using them solely.

Furthermore, the change in the results of bacteria inoculation and poultry manure over the years suggests that further studies, including different doses of poultry manure or PGPR inoculation together with different organic phosphorus sources, are needed.

Thus, to obtain the highest forage pea dry hay yield in Erzurum and similar ecological conditions with low and/or medium phosphorus content in the soil, it is recommended to the application of 100 kg ha^{-1} P_2O_5 in addition to 3 ton ha^{-1} poultry manure together with PGPR inoculation.

Peer-review: Externally peer-reviewed.

Author Contributions: D.K., B.Ç; Design – D.K., B.Ç.; Supervising – D.K., B.Ç.; Funding – D.K., B.Ç.; Materials – D.K., B.Ç.; Data Collection and/or Processing – D.K., B.Ç.; Analysis and/or Interpretation – D.K., B.Ç.; Literature Review – D.K., B.Ç.; Writing – D.K., B.Ç; Critical Review – D.K., B.Ç.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declare that this study has received no financial support. Ataturk University, Department of Scientific Research Project Coordination provided the fund support.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – D.K., B.Ç.; Tasarım – D.K., B.Ç.; Denetleme – D.K., B.Ç.; Kaynaklar – D.K., B.Ç.; Malzemeler – D.K., B.Ç.; Veri Toplanması ve/veya İşlemesi – D.K., B.Ç.; Analiz ve/veya Yorum – D.K., B.Ç.; Literatür Taraması – D.K., B.Ç.; Yazıyı Yazan – D.K., B.Ç.; Eleştirel İnceleme – D.K., B.Ç.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Atatürk Üniversitesi Bilimsel Araştırma Proje Koordinasyon Daire Başkanlığı fon desteği sağlamıştır.

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