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ORIGINAL PAPER

Effects of phosphorus fertilizer applications on element concentrations, forage yield and quality in berseem clover (*Trifolium alexandrinum* L.)*

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Abstract

This study aimed to determine the effect of different levels of phosphorus doses on the nutrient concentrations, forage yield, and quality of a berseem clover (*Trifolium alexandrinum* L.) cultivar under semi-arid conditions. The research was conducted during the 2023-2024 growing season at the Research and Application Field of the Faculty of Agriculture, Siirt University, Siirt. The Derya cultivar of berseem clover was used as the plant material. Field trials were designed according to a randomized block design with four replications focusing on six different phosphorus doses (P_0=0.0 kg P ha^{-1}, P_3=0.3 kg P ha^{-1}, P_6=0.6 kg P ha^{-1}, P_9=0.9 kg P ha^{-1}, P_{12}=1.2 kg P ha^{-1}, P_{15}=1.5 kg P ha^{-1}). The parameters examined included plant height, main stem thickness, green forage yield, dry matter yield, crude protein (CP), acid detergent fiber (ADF) ratio, neutral detergent fiber (NDF) ratio, relative feed value (RFV), as well as mineral concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Ca/P ratio, and K/(Ca+Mg) ratios. The findings indicated that with increasing applications of phosphorus fertilizer, both plant height and main stem thickness, along with green and dry forage yields, demonstrated significant enhancement. The highest results were obtained by applying 0.9 kg of pure P ha^{-1} for forage production. It was concluded that phosphorus fertilizer applications did not affect the quality of green forage in berseem clover. Regarding the mineral content of berseem clover, it was found that phosphorus fertilization did not cause any differences in nutrient concentrations, such as P, Ca, and Mg, according to the doses. However, for potassium (K), negative effects were observed beginning from the application of 1.5 kg of P ha^{-1}.

Keywords: *Trifolium alexandrinum* L., plant height, forage yield, crude protein, nutrient content

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INTRODUCTION

Clover species are classified within the *Fabaceae* family and play a significant role as forage crops cultivated in temperate climates (Williams et al. 2019). Berseem (*Trifolium alexandrinum* L.), a member of the clover family, is widely cultivated in Egypt, India, and Pakistan due to its high nutritional value. Its fresh, succulent leaves and stems, along with its rapid regrowth ability, allow for an extended green forage season and supports multiple cuttings, making it an essential feed source for ruminant animals (Açıkbaş, Özyazıcı 2022). The production of safe and healthy animal-derived food products is closely linked to the quality of feed consumed by livestock (Bıçakçı and Açıkbaş 2018). Berseem clover is an important plant due to its high nutritional value and lack of adverse effects on animal health. With its rich mineral content, high digestibility, excellent palatability, high protein content, and potential to improve soil fertility, berseem is one of the most important and popular forages for ruminants (Garg et al. 2016, Roy et al. 2019, Khanduri et al. 2021). Owing to its strong nitrogen fixation capability, it also plays an important role in improving soil structure and contributing to the better growth of the subsequent crop (Akram et al. 2022). Depending on the variety and season, berseem can fix between 115 and 400 kg N ha^{-1} in the soil during the growing season (Graves et al. 1990).

Fertilization is one of the most important agricultural practices, affecting the yield and quality of the product obtained per unit area. Achieving the desired benefits from the applied fertilizer is only possible through conscious and technically appropriate fertilization. The forage yield increased through the application of chemical fertilizers even in small amounts can be sufficient for crop growth because of the high nutrient content of these fertilizers (Nand et al. 2018). Berseem is less dependent on nitrogen from fertilizer sources due to its symbiotic relationship with *Rhizobium leguminosarum* bv. *trifolii*, which provides a nitrogen source for itself and for the subsequent crops (Graves et al. 1990, Cosentino et al. 2014). As a leguminous plant, berseem clover requires a sufficient amount of phosphorus in its free form for better nodulation (Akram et al. 2022). In general, leguminous plants require more potassium and phosphorus than cereals because they can meet their nitrogen needs through biological nitrogen-fixing bacteria, which support plant growth (Ayub et al. 2012). The optimal supply of essential nutrients plays a crucial role in agricultural production in terms of both yield and quality characteristics (Ceritoglu et al. 2024). In this context, determining the optimal fertilizer doses for plants and applying fertilization programs based on these amounts are of great importance. The optimal fertilizer dose varies depending on factors such as the plant genus, species, and varieties, as well as climatic and soil characteristics. In determining appropriate fertilizer amounts, soil analysis and the soil-plant nutrition relationship play a crucial role. Research conducted on leguminous forage crops has reported that phosphorus fertilizer applications have a positive effect on yield and yield components (Albayrak et al. 2009, Fayetörbay et al. 2014, Yıldız, Türk 2015, Özyazıcı, Açıkbaş 2019).

In general, phosphorus is the most commonly deficient plant nutrient in Turkish soils, both in terms of spatial distribution and proportion. However, excessive and unregulated phosphorus fertilization practices have also led to significant challenges, rendering phosphorus management one of the major issues in agricultural soils. In this context, the rotation of forage crops used to meet roughage needs is limited. Although not widely cultivated, it is necessary to include berseem in the cropping pattern for the sake of sustainability, to understand its importance, and to determine the productivity benefits it provides to the soil. This study aims to determine the effects of different phosphorus doses on forage yield, quality, and nutrient concentrations in berseem clover (*T. alexandrinum* L.).

MATERIALS AND METHODS

Materials

The research was conducted under field conditions in Siirt province in the Southeastern Anatolia Region of Türkiye (37°58′13.20″N – 41°50′43.80″E), during the 2023-2024 growing season. The plant material used in the research was the Derya cultivar of berseem clover.

The study area is characterized by a continental climate, where all four seasons are distinctly observed. Summers are hot and dry, while winters are harsh. Rainfall is scarce between June and October. Most of the precipitation occurs in the spring, autumn, and winter months. According to the long-term (1927-2023) meteorological data (Anonymous 2024) for Siirt province, the current climate in the region is semi-arid. Figure 1 shows the average temperature and total precipitation of the years examined and the long-term averages. The average temperature values of the 2023-2024 growing year and the long-term averages were found to be close to each other. The total annual precipitation values of the trial year and the long-term averages showed differences between months (Figure 1).

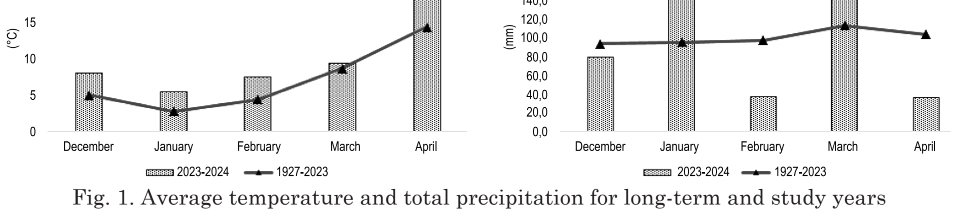

Fig. 1. Average temperature and total precipitation for long-term and study years

Soil samples were collected from the study area from a depth of 0-20 cm, by combining samples from five different locations into a single composite sample to determine some physical and chemical properties. Soil samples were analyzed for texture, pH, electrical conductivity (EC), lime, organic matter, available phosphorus, and potassium contents at the Science and Technology Application and Research Center of Siirt University. The pH and EC were determined using the 1:2.5 (w/v) soil-water mixture method (Horneck et al. 1989). Lime was detected using the calcimeter method. The Bouyoucus hydrometer method was used for texture analysis (Bouyoucos 1951). Soil organic matter was determined using the Walkley Black wet combustion method (Nelson, Sommers 1982). Available phosphorus was calculated by the sodium bicarbonate method using a spectrophotometer (Olsen et al. 1954), and available potassium was determined using the ammonium acetate method with a flame photometer (Ferrando et al. 2020). Berseem clover is grown in clay-textured soils that are non-saline, mildly alkaline, and moderately calcareous, with low amounts of organic matter and accessible phosphorus, but mediun available potassium. The extractable calcium concentration is sufficient and the magnesium content is moderate (Table 1).

Table 1

Some physical and chemical properties of the research area soils (0-20 cm)

Texture (%)			pH	EC (dS m^{-1})	Lime (%) ($CaCO_3$)	OM (%)	Available			
Sand	Clay	Silt					P (kg ha^{-1})	K (kg ha^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
36.2	36.3	27.5	7.80	0.05	1.98	0.60	54.5	443.8	1110.4	259.31

OM – organic matter, EC – electrical conductivity

Research subject and growth conditions

The field experiment was conducted using a randomized block design with four replications. Six different phosphorus doses (P_o=0.0 kg P ha^{-1}, P_3=0.3 kg P ha^{-1}, P_6=0.6 kg P ha^{-1}, P_9=0.9 kg P ha^{-1}, P_{12}=1.2 kg P ha^{-1}, P_{15}=1.5 kg P ha^{-1}) were the focus of the research. The phosphorus fertilizers, which form the focus of the research, were applied in the form of triple superphosphate (% 43-44 P_2O_5) at the determined doses along with the planting.

Berseem, which is grown as a winter crop, is best sown between September and December under Turkish conditions, while mid-October has been reported as the most suitable sowing time in regions where it is extensively cultivated, such as Pakistan and India (Jabbar et al. 2022, Praveen et al. 2022). Taking into account that the optimal temperature range for sowing is 20-25°C, which plays an important role in achieving good emergence (Praveen et al. 2022), the sowing was carried out accordingly. Berseem was sown as a winter crop, with the plants being sown in the first half of December (05.12.2023). Harvest was carried out on May 1, 2024, when 50% of the plants were in bloom (Anonymous 2019), and the results were obtained based on a single year.

In the study, parameters such as plant height, stem thickness, and green and dry matter yields were determined according to the guidelines reported by Anonymous (2019). In the study, from each plot consisting of six rows, one border row on each side and 50 cm from both ends were excluded as border effect. The remaining area was harvested, and the green herbage was weighed. The obtained values were then converted to a per-hectare basis to calculate green herbage yield. Subsequently, 0.5 kg of herbage samples were taken from the harvested forage of each plot and dried in an oven at 70°C for 48 hours. After drying, the samples were kept at room temperature for 24 hours and then weighed using a precision balance with 0.001 g sensitivity to determine the dry matter weight. The obtained dry herbage data were also converted to yield per hectare. The dried samples were ground and prepared for analysis. In the ground dry matter samples, the CP, ADF, NDF, total P, K, Ca, and Mg contents were determined using a NIRS (Near Infrared Reflectance Spectroscopy) device with the #IC-0904FE calibration set (Brogna et al. 2009). Additionally, the Ca/P and K/(Ca+Mg) ratios of the samples were determined in the study. The relative feed value (RFV) of berseem dry forage has been determined according to the Equations reported by Van Dyke and Anderson (2000).

Using Equation 1, digestible dry matter (DDM, %); with Equation 2, dry matter intake (DMI, %); and with Equation 3, relative feed value (RFV) were determined.

$$\text{DDM (\%)} = 88.9 - (0.779 \times \%\ \text{ADF}) \quad (1)$$

$$\text{DMI (\%)} = 120\ /\ \%\ \text{NDF} \quad (2)$$

$$\text{RFV (\%)} = \%\ \text{DDM} \times \%\ \text{DMI} \times 0.775 \quad (3)$$

Statistical analysis

In the study, percentage (%) values were subjected to angular (arcsine) transformation prior to statistical analysis. The experimental data were analyzed using analysis of variance (ANOVA) based on the randomized block design. Differences among treatment means were evaluated using the Tukey multiple comparison test, following a significant *F*-test result (Açıkgöz, Açıkgöz 2001).

RESULTS AND DISCUSSION

The average values regarding the effect of phosphorus fertilization on plant height, stem thickness, and green forage and dry matter yield in berseem are given in Table 2. According to the average data, it was determined that the yield increased in parallel with the increase in phosphorus fertilizer dose, up to the P_9 dose, after which there was a statistically significant decrease in yield. The highest plant height values were determined to be 78.7 cm and 80.3 cm at P_6 and P_9 fertilizer doses, respectively. The lowest plant height was obtained from the control (P_0) treatment, where no phosphorus fertilizer was applied (74.0 cm). This difference between doses was found to be statistically significant at the p<0.01 level (Table 2).

Table 2

Changes in yield and yield components of berseem according to different phosphorus fertilizer doses*

P doses (kg P ha^{-1})	Plant height (cm)	Stem thickness (mm)	Green forage yield (kg ha^{-1})	Dry matter yield (kg ha^{-1})
$_P0$	74.0 *c*	3.80 *c*	135.4 *c*	36.6 e
P_3	75.0 *bc*	4.03 *bc*	140.7 *c*	39.1 *de*
P_6	78.7 *a*	4.13 *bc*	162.4 *b*	45.0 *cd*
P_9	80.3 *a*	4.82 *a*	200.7 *a*	55.9 *a*
P_{12}	78.0 *ab*	4.50 *ab*	190.0 *a*	53.3 *ab*
P_{15}	77.9 *ab*	4.43 *ab*	170.7 *b*	49.2 *bc*
Significance level				
Tukey value	2.97	0.60	164.12	59.55
F probability	**	**	**	**

P_0= 0.0 kg P ha^{-1}, P_3= 0.3 kg P ha^{-1}, P_6= 0.6 kg P ha^{-1}, P_9= 0.9 kg P ha^{-1}, P_{12}= 1.2 kg P ha^{-1}, P_{15}= 1.5 kg P ha^{-1}, * The difference between the means shown with the same letter in the same group and in the same column is not statistically significant, ** $p<0.01$ significance level

Studies have emphasized that legumes have a high phosphorus requirement, and when there is a deficiency, phosphorus should be provided to the plants for adequate biomass production (Turk, Tawaha 2001, Turk et al. 2003, Yılmaz 2008). On the other hand, in the case of phosphorus deficiency, it is stated that young plants exhibit stunted growth and delayed maturation (Akdoğan, Kır 2020, Xie et al. 2021). Similar to the findings of this study, research conducted with different plant species has reported an increase in plant height depending on the phosphorus doses, with statistically significant differences compared to the control (Arslan et al. 2014, Türk et al. 2018, Akdoğan, Kır 2020, Eken, Türk 2021).

According to the average data, it was determined that the stem thickness increased in parallel with the increase in phosphorus fertilizer dose up to the P_9 dose, after which there were insignificant decreases in yield. The highest stem thickness value was determined to be 4.82 mm at the P_9 fertilizer dose. The lowest stem thickness was obtained from the control (P_0) treatment, where no phosphorus fertilizer was applied (3.80 mm). This difference between doses was found to be statistically significant at the $p<0.01$ level (Table 2). It was determined that stem thickness, which is a yield component, increased with the increase in phosphorus doses, and similar results were obtained in a phosphorus fertilization study conducted with sainfoin plants (Altıparmak 2016).

When examining the effect of phosphorus fertilization on green forage yield, the highest values were determined to be 200.7 kg ha^{-1} and 190.0 kg ha^{-1} at the P_9 and P_{12} fertilizer doses, respectively. The lowest green forage yield was obtained from the control (P_0) and P_3 fertilizer doses, where no phosphorus fertilizer was applied. This difference between doses was found to be statistically significant at the $p<0.01$ level (Table 2).

When evaluating the effect of phosphorus fertilization on dry matter yield, the highest value was determined to be 55.9 kg ha^{-1} at the P_9 fertilizer dose. The difference between the P_9 and P_{12} fertilizer doses for dry matter yield was found to be statistically insignificant. The lowest dry matter yield was determined to be 36.6 kg ha^{-1} in the control (P_0) group, where no phosphorus fertilizer was applied. This difference between doses was found to be statistically significant at the $p<0.01$ level (Table 2).

It has been observed that phosphorus fertilization has a positive effect on both green and dry matter yields. The observed increase in yield with rising phosphorus application doses suggests that phosphorus enhances photosynthetic activity in plants, thereby contributing to greater dry matter accumulation (Colomb et al. 2000, Kacar, Katkat 2009). Numerous studies have reported similar yield-enhancing effects of phosphorus fertilization in various legume plant species, including white clover (Acar, Önal Aşçı 2006), hairy vetch (Türk et al. 2009), narbon vetch (Bell et al. 2001, Türk et al. 2007, Özyazıcı, Açıkbaş 2019), and common vetch (Sürmen et al. 2011, Yıldız, Türk 2015).

The average values regarding the effect of phosphorus fertilization on CP, ADF, NDF, and RFV in berseem clover are given in Table 3. In berseem,

Table 3

Effects of different phosphorus fertilizer doses on forage quality in Berseem

P doses (kg P ha^{-1})	CP ratio (%)	ADF (%)	NDF (%)	RFV
P_0	18.1	31.1	43.0	140.0
P_3	18.9	32.2	43.4	136.7
P_6	18.9	33.4	44.5	131.6
P_9	19.4	33.4	43.8	133.8
P_{12}	18.4	33.8	44.7	131.0
P_{15}	18.3	33.4	45.8	127.7
Significance level				
Tukey value	3.01	6.69	3.61	19.04
F probability	ns	ns	ns	ns

P_0=0.0 kg P ha^{-1}, P_3=0.3 kg P ha^{-1}, P_6=0.6 kg P ha^{-1}, P_9=0.9 kg P ha^{-1}, P_{12}=1.2 kg P ha^{-1}, P_{15}=1.5 kg P ha^{-1}, ns – no significant difference the CP ratio ranged from 18.1% to 19.4%, ADF from 31.1% to 33.8%, NDF from 43.0% to 45.8%, and RFV from 127.7 to 140.0, depending on the phosphorus fertilizer doses. The effect of phosphorus fertilization on CP, ADF, NDF, and RFV parameters was found to be statistically insignificant (Table 2). Contrary to the findings of this study, phosphorus fertilization has been reported to show significant effects in different legume plant species (Yılmaz 2008, Albayrak et al. 2009, Sürmen et al. 2011, Yıldız, Türk 2015).

One of the parameters used to determine forage quality is the CP content of roughages (Hu et al. 2021). Therefore, the protein content of roughages in feed rations is important in animal nutrition. Meen (2001) emphasized that, in general, the CP content in feed rations should be at least 7% to meet the nutritional requirements of ruminants. According to the threshold values reported by Rohweder et al. (1978) (<19% – top quality, 17-19% – very good, 14-16% – good, 11-13% – medium, 8-10% – bad, and <8% – unacceptable), the CP values of berseem fell within the "very good" and "top quality" categories. Overall, it was observed that berseem contains a high level of protein sufficient to meet the protein requirements in animal rations, making it a valuable high-protein forage.

The values for P, K, Ca, Mg, Ca/P, and K/(Ca+Mg) ratios, determined according to the effect of phosphorus fertilizers on Berseem in the study, are shown in Figure 2. The effect of phosphorus fertilizers on K ($p<0.01$) and K/(Ca+Mg) ($p<0.05$) ratios was found to be statistically significant. The effect of phosphorus fertilizers on the examined macronutrient parameters such as P, Ca, Mg, and Ca/P was found to be statistically insignificant. When examining the effect of phosphorus fertilizers on K and K/(Ca+Mg) ratios in Berseem, the highest values were obtained at P_0, P_3, P_6, and P_9 doses. However, the lowest values for both parameters were observed at the P_{15} dose.

In general, a Ca/P ratio between 1:1 and 2:1 is recommended in feed rations (National Academy of Sciences 1984); excessive levels have been reported to cause milk fever in animals (Açıkgöz 2001). Considering this threshold, it was observed that the Ca/P ratio of berseem was high. However, since animals are thought to tolerate Ca/P ratios up to 7:1 (Buxton, Fales 1994), it can be stated that the Ca/P ratios obtained in our study were below the tolerable limit.

It has been observed that phosphorus fertilization statistically affected the K and K/(Ca+Mg) ratios in berseem, and similar results were also obtained in a study conducted with hairy vetch (Türk et al. 2009). Increased phosphorus application led to a decrease in K and K/(Ca+Mg) ratios (Figure 2). The reason for this is that the uptake of mineral components by plants depends on various parameters, and meteorological conditions, particularly factors such as soil and air temperature, affect the concentration of mineral substances in plants (Roche et al. 2009). In forage crops, the K/(Ca+Mg) ratio is reported to be ideally below 2.2 (Mayland et al. 1992); some researchers

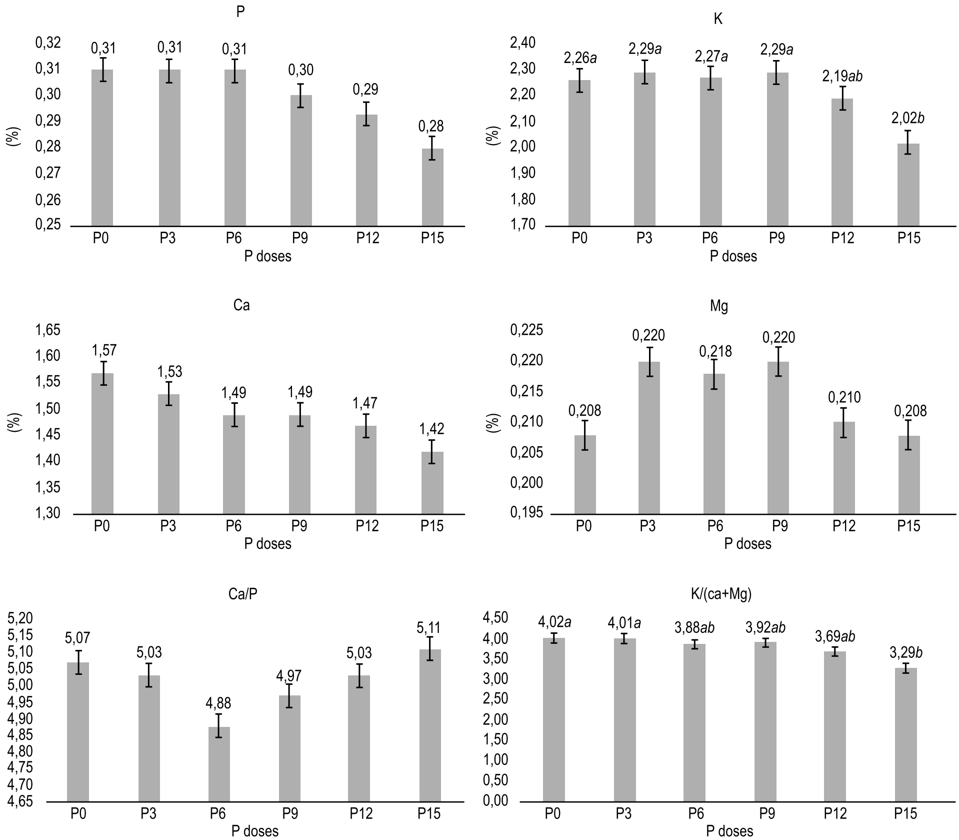

Fig. 2. Mineral matter content of berseem to phosphorus doses: K was found to be statistically significant at the $p<0.01$ level, and K/(Ca+Mg) was found to be statistically significant at the $p<0.05$ level

have reported that when this ratio is 2.2 or higher, the risk of grass tetany increases (Elkins et al. 1977, Crawford et al. 1998). In light of this information from the literature, it can be said that the K/(Ca+Mg) ratio determined in the berseem evaluated in our study poses a risk of grass tetany for animals.

CONCLUSIONS

According to the results of the study, under semi-arid climate conditions and in soils with very low and/or low levels of phosphorus, a dose of 0.9 kg P ha^{-1} is recommended for berseem cultivation aimed at forage production. Phosphorus fertilization was found to have no significant impact on the quality parameters of green forage. Regarding the mineral content of berseem, phosphorus fertilization did not create any differences in nutrient content such as P, Ca, and Mg according to the doses, while phosphorus fertilization negatively affected K starting from a dose of 1.5 kg P ha^{-1}.

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Author contributions

M.A.S. – Material, methodology, investigation writing-original draft original draft preparation; S.A. – conceptualization, material, methodology, investigation, visualization, supervision, writing-original draft original draft preparation, writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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