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Research

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Effects of Zinc on the Yield and Quality of Forage Pea [*Pisum sativum* ssp. *arvense* (L.) Poir.]

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A B S T R A C T

Zinc (Zn) is one of the most important micronutrients that can increase the growth, yield attributes, yield, quality and nutritional value of plants. This study aimed to evaluate the effects of zinc sulphate ($ZnSO_4 \cdot 7H_2O$) application at different concentrations (0, 5, 10, 15, and 20 kg ha^{-1}) on forage yield and quality and mineral content of the plant in forage pea [*Pisum sativum* ssp. *arvense* (L.) Poir.] (cv. Özkaynak) under semi-arid climate conditions. The response variables included stem diameter, plant height, green forage yield, hay yield, crude protein (CP), acid detergent fiber, neutral detergent fiber, total phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg). As a result of the research, it was determined that the Zn doses applied from the soil had meaningful effects on the green forage yield ($p<0.05$) and CP ($p<0.01$), total P ($p<0.05$) and Ca ($p<0.01$) contents of forage pea. The highest green forage yield of 43.60 t ha^{-1} was obtained at Zn dose of 10 kg ha^{-1}. Although it did not show statistically significant changes, improvements were also achieved in hay yield compared to the control at the same dose. In the study, Zn fertilization increased forage CP ratio significantly. In addition, soil Zn application also provided sufficient macronutrient accumulation in forage pea hay for ruminants. According to the research results, it was concluded that in the presence of low level extractable Zn in the soil, 10 kg Zn ha^{-1} application to forage pea would provide prominent increases in forage production, forage quality and nutritional value.

1. Introduction

Pea is one of the oldest crops grown for human food and animal feed, and in some places for green manure purposes (Delchev and Delchev, 2019; Özyazıcı and Açıkbaş, 2021). Pea which is the second most important legume crop in the world (Pawar et al., 2017) has a subspecies that has significant value as a forage and grain feed, *Pisum sativum* ssp. *arvense*, known as forage pea (Açıkgöz, 2001; Manga et al., 2003). With the help of its high biological nitrogen (N) fixation capacity, forage pea [*Pisum sativum* ssp. *arvense* (L.) Poir.] (Özyazıcı and Açıkbaş, 2021), which is an important component of sustainable agricultural systems that include both short-term crop rotation systems and green fertilization, is one of the most important roughage source of livestock enterprises with its high protein content and forage yield (Fraser et al., 2001).

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In recent years, focus has been placed on increasing the production of forage crops in field agriculture to meet the demand for roughage in animal production. This focus has led to an increase in the use of high-yielding forage crop varieties, intensive cultivation methods and micronutrient-free fertilization practices. This contributes to the reduction of essential micronutrients such as iron, copper, zinc (Zn) and manganese in agricultural soils. In this sense, Zn deficiency in agricultural soils has been particularly emphasized (Özyazıcı et al., 2015). Studies have shown that there is a geographical overlap between soils that is characterized by Zn deficient and plant populations experiencing Zn deficiency (Tarakçıoğlu et al., 2003; Özkutlu et al., 2015; Söylemez et al., 2017; Oya and Çimrin, 2023). Deficiency of micronutrients in soil can lead to nutrient imbalances in many plants, reducing the quality of crops and potentially affecting human and animal health (Barrett and Bevis, 2015).

Zinc has important functions in protein and carbohydrate metabolism and the activation of many enzymes (Sharma et al., 2010; Hassan et al., 2020). For this reason, Zn is one of the most important micronutrient elements required for optimum crop growth and development (Hanifuzzaman et al., 2022; Rion et al., 2022). Additionally, Zn directly affects yield and quality due to its remarkable effects on root and shoot growth throughout the growing season (Rengel, 2001; Priyanka et al., 2019).

Many of the minerals necessary for animals are easily met by plants due to their abundance in the soil (Reynolds-Marzal et al., 2021). However, some minerals, such as Zn, are generally found in soil in relatively low concentrations relative to other soil fractions (Reynolds-Marzal et al., 2021). This situation affects the uptake of some other nutrients, especially Zn and some other nutrients that interact with Zn, by plants and plays a role in the different concentrations of mineral contents of forages. When considering Zn application in agriculture, both soil and foliar applications play important roles in increasing plant growth and nutrient uptake (Özyazıcı, 2023; García-Latorre et al., 2024; Özyazıcı and Özyazıcı, 2024). Studies have shown that soil application can lead to increased Zn levels in the soil, which can positively affect plant growth and development (Toğay and Anlarsal, 2008; Özyazıcı, 2020; Boaretto et al., 2024; Devi et al., 2024).

This research aimed to evaluate the effects of soil Zn application at different concentrations on forage yield, quality and nutrient accumulation in forage pea (*P. sativum* ssp. *arvense*).

2. Materials and Methods

2.1. Study site

The study was carried out in the 2019-2020 growing season in the Siirt province (37°58'13.20" N - 41°50'43.80" E, 887 m) in the southeastern Türkiye (Figure 1), in the under rainfed semi-arid climate zone. Some physical and chemical properties of the research soil are given in Table 1. The soil of the research area is clayey in texture and slightly alkaline in character. Soil without salinity problems have a calcareous, low organic matter, high available phosphorus (P) and excess available potassium (K) content. The soil has a good level of available calcium (Ca) and magnesium (Mg) content, while the extractable Zn content is low (Table 1).

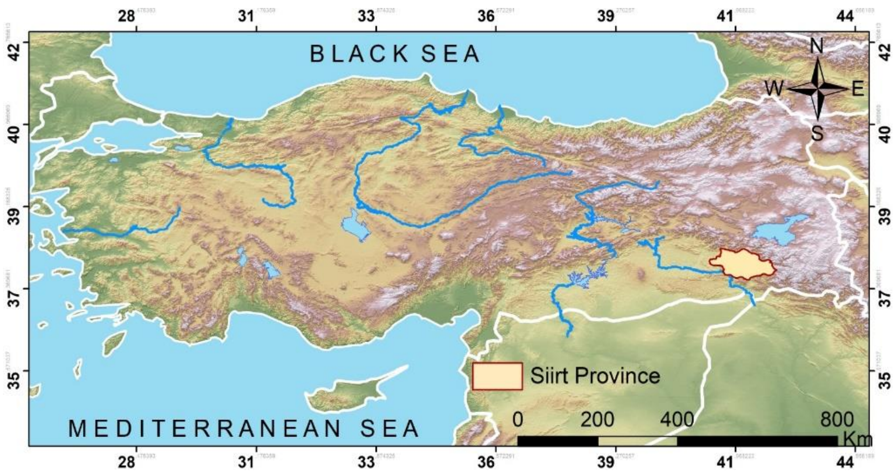

Figure 1. Research area location map

Table 1. Some physical and chemical properties of the soils where forage pea is grown (0-20 cm)*

Parameters	Unit	Value
Clay	%	38.9
Sand	%	43.1
Silt	%	18.0
pH		7.70
Electrical conductivity	dS m^{-1}	0.18
Calcareous ($CaCO_3$)	%	2.8
Organic matter	%	1.64
Available P	kg ha^{-1}	112
Available K	kg ha^{-1}	1882
Available Ca	kg ha^{-1}	11958
Available Mg	kg ha^{-1}	1114
Extractable Zn (DTPA)	ppm	0.79

*: The analyzes were carried out in the laboratory of Science and Technology Application and Research Center-Siirt University, $CaCO_3$: Calcium carbonate, DTPA: Diethylene triamine penta acetic acid

Monthly temperature averages during the period when the forage pea was grown were generally above the long-term average values. During the 7-month vegetation period, the total rainfall amount was 756.8 mm, while the long-term average of total rainfall in the same period was recorded as 652.0 mm. Especially in March and April very high amounts of precipitation has occurred which has supported crop development (Figure 2).

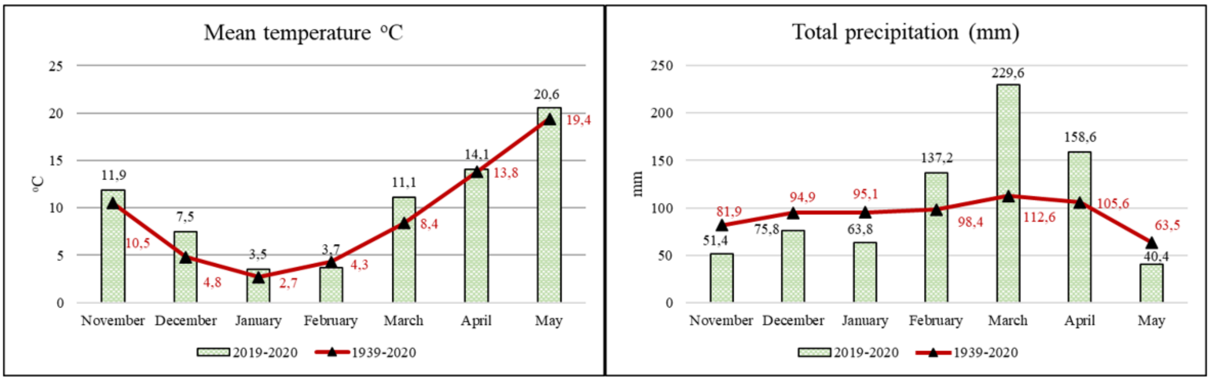

Figure 2. The long-term (1939-2020) and research year (2019-2020) average temperature and total precipitation data for the province of Siirt

2.2. Experimental design and crop management

Özkaynak forage pea (*P. sativum* ssp. *arvense*) variety was used as plant material in the research and 5 different doses of zinc (0, 5, 10, 15, and 20 kg ha^{-1}) were applied as zinc sulphate ($ZnSO_4 \cdot 7H_2O$). The field experiment was set up according to randomized complete block design with 3 replications. Plants were sown in each plot in 4 rows with 30 cm row spacing, and the plot size was 3.6 m^2 (1.2 m × 3 m).

The preliminary crop of the trial area was wheat, and after the wheat harvest, the field was deeply ploughed with a plough, and then the field was made ready for sowing by using a disc harrow, harrow and roller in the autumn of 2019. Before sowing, nitrogenous fertilizer (ammonium sulphate, 21% N) was applied homogeneously to all plots with the calculation of 40 kg ha^{-1} pure N, according to the soil analysis results (Table 1). Since the available P and K contents of the soil were sufficient, fertilization was not done for these elements. Zinc sulphate applied to the plots in different doses and mixed into the soil before sowing. The sowing process was carried out on 07 November 2019 with 100 live seeds per m^2 (Anonymous, 2019). The cutting process for the forage was carried out on May 13, 2020, when the plants were in full bloom in the research. During the cutting process, one row from the parcel edges and 50 cm sections from the parcel heads were removed to avoid edge effects.

2.3. Plant measurements and forage analyses

In the research, stem diameter, plant height, green forage yield, hay yield, crude protein (CP) ratio, acid detergent fiber (ADF) ratio, neutral detergent fiber (NDF) ratio, total P, K, Ca and Mg parameters were examined. Stem diameter and plant height measurements were made on 10 randomly selected plants before cutting in each plot and mean values were calculated for each plot. In each plot, the remaining part was harvested and weighed after removing the edge effects, and green forage yields were determined by taking the harvest area into account. 500 g fresh sample of herbage was taken from each plot for hay yield, forage quality and mineral analyses. These samples, which were withered for a while in laboratory conditions, were then dried in a drying cabinet at 65 $^{\circ}$C for 48 hours. Dried samples were weighed on a precision scale and the hay ratios (%) of the samples were established by calculation. Hay yields were determined by multiplying the hay ratios with the green forage yields of the plots. Dried samples were ground separately and prepared for analysis. Crude protein, ADF, NDF, P, K, Ca and Mg ratios of the ground samples were determined using the #IC-0904FE calibration set (Anonymous, 2020) with a NIRS (Near Infrared Reflectance Spectroscopy) device (Brogna et al., 2009).

2.4. Statistical analysis

The data were subjected to variance analysis according to the randomized complete blocks design, and according to the F test results, the differences between the groups were determined with the LSD (Least Significant Difference) multiple comparison test (Yurtsever, 1984).

3. Results and Discussion

3.1. Effect of zinc doses on forage yield and some yield components

Data on forage yield obtained from forage pea at different levels of Zn applications, and some parameters affecting the yield are presented in Table 2.

Stem diameter and plant height are one of the important morphological growth parameters that affect yield in forage crops and are affected by cultural practices such as fertilization applications. As a result of the research, the effect of Zn doses on stem diameter and plant height of forage pea was found to be statistically insignificant. Depending on the zinc doses, stem diameter varied between 4.80-5.67 mm and plant height varied between 73.00-78.33 cm (Table 2). Öncan Sümer and Yaraşır (2022) stated that the effect of Zn doses on stem diamater in *P. sativum* was insignificant. This result is consistent with the current research findings in terms of stem diamater that is reported by the researchers. Similar to the current research findings, the effect of different doses of Zn applications on plant height was found to be statistically insignificant in some studies conducted on different plant species such as rapeseed (*Brassica napus* ssp. *oleifera* L.) (Aytaç et al., 2016) and oat (*Avena sativa* L.) (Yılmaz and Sonkaya, 2020), as well as legume species such as chickpea (Kurt and Önder, 2024), pea (*Pisum sativum* L.) (Öncan Sümer and Yaraşır, 2022) and alfalfa (*Medicago sativa* L.) (Ersöz Çelik, 2022). On the other hand, in their studies conducted by Yashona et al. (2018) on pigeon pea (*Cajanus cajan*), Erdoğan (2022) on forage cowpea (*Vigna unguiculata* L.), Roy et al. (2022) on faba bean (*Vicia faba* L.), and Sayed et al. (2024) on grass pea (*Lathyrus sativus* L.), reported that the effects of Zn doses were significant and that Zn fertilization increased plant height. Differences between current research results and literature in terms of herbage yield components in forage pea can be explained by genotypic and ecological differences and different reactions of plant species depending on these differences.

In forage pea, 10 kg ha^{-1} Zn fertilization applied from soil significantly increased green forage yield compared to the control; in the following doses, a statistically insignificant decrease in green forage yield was observed at the 15 kg ha^{-1} dose and a significant decrease was observed at the 20 kg ha^{-1} Zn dose. According to this, the highest green forage yield was determined as 43.60 t ha^{-1} at 10 kg ha^{-1} Zn dose. In the study, the lowest green forage yields were determined at 0 (37.36 t ha^{-1}), 5 (37.45 t ha^{-1}) and 20 (37.50 t ha^{-1}) kg ha^{-1} Zn doses, which were statistically in the same group. This difference between Zn doses in terms of green forage yield was found to be statistically significant at $p<0.05$ level. A similar trend was observed in the hay yield in parallel with the increase in zinc doses, however, the variation in Zn doses in terms of hay yield was statistically insignificant (Table 2).

Table 2. Average values of forage yield and some yield components in forage pea applied with different Zn doses*

Zn doses (kg ha^{-1})	Stem diameter (mm)	Plant height (cm)	Green forage yield (t ha^{-1})	Hay yield (t ha^{-1})
0	5.15	73.00	37.36 b	6.09
5	4.80	73.67	37.45 b	6.26
10	5.64	78.33	43.60 a	7.12
15	5.67	75.33	40.37 ab	6.53
20	5.57	75.00	37.50 b	6.30
Mean	5.36	75.07	39.26	6.46
P	0.1426	0.0976	0.0117	0.2424

*: The difference between the means indicated by the same letter in the same group is not statistically significant, P: Significance level

The remarkable effects of zinc on forage pea yield, especially on green forage yield, may be due to the availability of more Zn in the soil during the growth phase and its uptake by plants, depending on the applied Zn levels. The beneficial effects on forage pea biomass can be attributed to soil-applied zinc when considering the roles of zinc in plant chlorophyll formation, biosynthesis of plant growth regulator (Indole-3-acetic acid, IAA), carbohydrate and N metabolism leading to high yield and yield components (Taliee and Sayadian, 2000; Sharma et al., 2010), as well as increasing N-fixation through photosynthesis (Hegazy et al., 1990) and nodule formation (Hegazy et al., 1990; Patel et al., 2011). Padma et al. (1989) and Deotale et al. (1998) stated that zinc also plays a role in the hormone synthesis and contributes to additional growth in the plant compared to the control due to these positive roles in plant metabolism. Moreover, Zn application is thought to be effective in increasing the availability of other nutrients and accelerating the translocation of photo assimilates (Guhey, 1999), which in turn increases the yield of forage in the plant. Enrichment of plant nutrition increases photosynthesis efficiency, assimilation and production (Ali, 2004).

In a study conducted with forage pea (*P. sativum* L.) in Mediterranean conditions in soil with low DTPA extractable Zn content, soil Zn application affected forage yield significantly ($p<0.01$); forage yield increased by 30% when 50 kg ha^{-1} zinc sulphate was applied to the soil before sowing (Reynolds-Marzal et al., 2021). It was established that Zn application in field pea (*P. sativum*) grown in calcareous soils significantly increased seed yield, stover yield and number of nodules per plant compared to the control (Quddus et al., 2018). It was reported that foliar Zn fertilization improves grain and straw yield and quality parameters (Dhaliwal et al., 2022), and Zn element should be applied together with the recommended fertilizer dose to maximize production and net profit (Reddy et al., 2023), in some other studies conducted with field pea (*P. sativum*).

In studies conducted with different species of forage legumes, results consistent with the current research findings were obtained. In fodder cowpea [*Vigna unguiculata* (L.) Walp.], application of 20 kg Zn ha^{-1} in the form of $ZnSO_4$ markedly ($p<0.05$) increased the green fodder yield (Rathore et al., 2015; Kumar et al., 2016). Grain and straw yields of *C. cajan*, a perennial legume forage plant from the Fabaceae family, were found to be 7-25% and 6-18% higher, respectively, under different mode of Zinc application, including Zn doses, compared to the control (no zinc application) (Yashona et al., 2018). In studies conducted with alfalfa (M. sativa) plant, Zn ($ZnSO_4.7H_2O$) application at a dose of 80 kg ha^{-1} applied from soil (Ceylan et al., 2009) and 120 mg L^{-1} applied from leaves (Ersöz Çelik, 2022) increased green forage and hay yield significantly compared to the control. It has been determined that grain yield and yield-effective parameters in grass pea (*L. sativus*) show a positive correlation with Zn application, in this sense the optimum Zn dose was determined as 1.95 kg ha^{-1} and there were decreases in all yield parameters in applications above this recommended Zn dose (Sayed et al., 2024). Similarly, Zn application has been reported to increase the yield of legumes such as chickpea (Valenciano et al., 2010), soybean (*Glycine max* L.) (Chauhan et al., 2013; Choudhary et al., 2014), mungbean (*Vigna radiate* L.) (Islam et al., 2017), garden pea (*Pisum sativum* L.) (Alam et al., 2020; Öncan Sümer and Yaraşır, 2022), faba bean (*Vicia faba* L.) (Roy et al., 2022).

On the other hand, micronutrient deficiencies, especially Zn, have been reported by many researchers as the main reason for the suboptimal yield of crops, including legume forage crop species (Das and Parida, 2020; Islam et al., 2021; Ghosh et al., 2021; Sridhar et al., 2021). For this reason, it is important to fertilize Zn in addition to other nutrients when its deficiency is observed in order to achieve optimum yield in forage peas.

3.2. Effect of zinc doses on forage quality

3.2.1. Crude protein, acid detergent fiber and neutral detergent fiber

Improving productivity in livestock is possible by providing farmers with sufficient amounts of better-quality feed. Forage quality varies depending on cultural practices such as fertilization applied in forage crop cultivation. In this sense, Zn deficiency in soil may be the main reason for the poor quality of legume forages. CP, ADF and NDF ratios of hay are important quality parameters in forage crops.

In the research, the CP content of forage pea hay was significantly affected by Zn doses ($p<0.01$). The CP ratio increased in parallel with the increase in Zn doses, but this increase was insignificant after the 5 kg ha^{-1} Zn dose. Therefore, this statistically significant difference at $p<0.01$ level occurred between the control and other Zn doses. According to this, while the CP rate was 20.17% in the control process, this rate varied between 21.60-25.97% depending on the Zn doses (Table 3). Soil Zn fertilization improved the CP content of forage compared to the control (0 kg ha^{-1} Zn) application in the study. This can be explained by the fact that Zn, which has an important function in nodule formation and N fixation, increases N fixation in the soil and therefore available nitrogen, and also increases N uptake by the plant. Furthermore, Zn plays an important role in various enzyme systems and protein metabolism, which is an important factor in improving the protein content in dry matter of forage pea. In the studies conducted with peas in the literature, results compatible with the current research findings were obtained; it was reported that Zn fertilization increased the CP rate in the plant. For example, in the study conducted with forage pea (*P. sativum*) under Mediterranean conditions, it was emphasized that foliar Zn application significantly affected the CP content of the feed ($p\leq0.01$), the CP level increased by approximately 8% compared to the control, and Zn application was the most effective application to increase the CP rate (Reynolds-Marzal et al., 2021). It was reported that the protein content in grains was significantly affected by Zn application in studies conducted with field pea (*P. sativum*) (Quddus et al., 2018; Dhaliwal et al., 2022). Besides, previous studies with different forage crop species reported that Zn application is an effective method to improve the CP ratio of forage in various crops such as forage sorghum (Sutaria et al., 2013), fodder cowpea (*Vigna unguiculata*) (Rathore et al., 2015), fodder maize (Kumar and Ram, 2021), triticale (x*Triticosecale*) (García-Latorre et al., 2024).

In the research, although the ADF and NDF ratios of forage pea increased according to Zn doses, this increase was found to be statistically insignificant. Accordingly, the ADF ratio of forage pea varied between 33.24-36.85% and the NDF ratio between 38.60-46.93% (Table 3). The effect of Zn applications on ADF and NDF rates was found to be statistically insignificant, in line with the current research findings in the study conducted by Reynolds-Marzal et al. (2021) on forage pea. It has also been reported in the research results conducted on fodder cowpea (*Vigna unguiculata*) (Rathore et al., 2015) and fodder maize (*Zea mays* L.) (Kumar et al., 2017) plants that zinc fertilization does not affect the ADF and NDF rates of feeds.

Table 3. Average values of forage quality parameters in forage pea applied with different Zn doses*

Zn doses (kg ha^{-1})	CP (%)	ADF (%)	NDF (%)
0	20.17 b	33.24	38.60
5	21.60 ab	34.35	42.21
10	25.70 a	34.52	42.24
15	25.74 a	35.52	43.38
20	25.97 a	36.85	46.93
Mean	23.84	34.89	42.67
P	0.0080	0.4616	0.2028

*: The difference between the means indicated by the same letter in the same group is not statistically significant, P: Significance level

3.2.2. Mineral element contents

When considering the forage quality of a forage plant, its nutritional value comes to mind. In determining the nutritional value of forage plants, chemical compositions such as CP, crude fiber, vitamins and minerals are one of the main criteria.

In forage pea, pre-sowing soil Zn fertilizer application significantly affected P ($p<0.05$) and Ca ($p<0.01$), which are mineral components of the hay. The P content in forage pea showed a descending trend with the increase in Zn application over control which gave the highest value of 0.52%. Whereas the Ca content exhibited ascending trend with the application of Zn and the highest value was observed for treatment 20 kg ha^{-1} Zn (1.32%) (Figure 3). The decreasing trend observed for total P content can be attributed to the antagonistic relationship between Zn and P. When elemental soil zinc is high in the soil, the availability of elemental soil phosphorus is in general always low (Ladumor et al., 2019). Due to this interaction between Zn and P, Zn applied to the soil reduced P uptake in the plant. The decrease in the amount of inorganic P with Zn application was also reported by Ghoneim (2016); similar to the current research findings, it has been documented in many studies that the total P amounts in the plant decrease in parallel with the increase in Zn dose (Li et al., 2003; Petković et al., 2019; Dhaliwal et al., 2022). It has also been reported in some studies that the amount of Ca in the plant improves compared to the control depending on the applied Zn fertilization (Petković et al., 2019; Reynolds-Marzal et al., 2021; García-Latorre et al., 2024).

Zn fertilization did not significantly affect the content of K and Mg elements in forage pea. In the study, the total K content of the hay varied between 3.32-3.47% and the total Mg content varied between 0.24-0.27%. At the same time, it was observed that the amount of K increased in parallel with the increase in Zn doses (Figure 3). It can be said that in soils with zinc deficiency, zinc fertilization may contribute to the improvement of the K content of the forage. Petković et al. (2019) reported that plant K and Mg ratios did not show significant changes according to Zn doses in alfalfa, Reynolds-Marzal et al. (2021) reported that soil Zn application did not affect the Mg content of the plant in forage pea, whereas foliar Zn application increased the Mg content significantly ($p<0.01$). It can be indicated that these results in the literature are generally compatible with the current research findings. Contrary to current research findings, García-Latorre et al. (2024) observed that total Mg content in the plant was affected by Zn fertilization, and that Zn application from soil in triticale plants gave better results than other application methods.

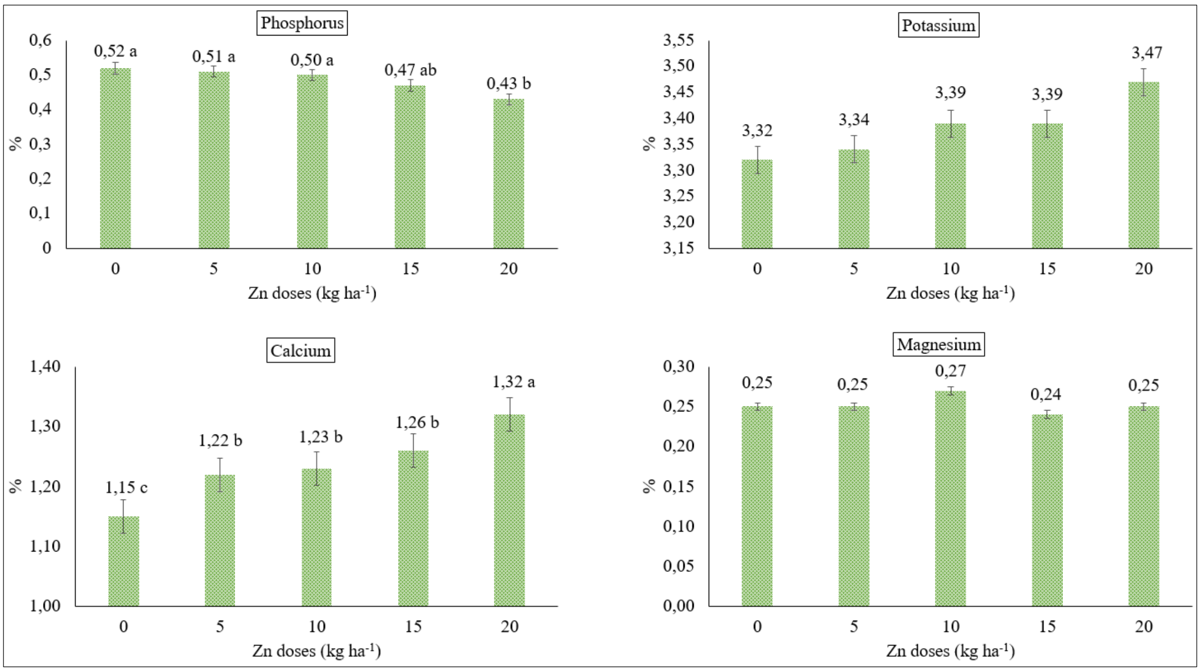

Figure 3. Average values (%) of some macro elements determined in forage pea hay applied with different Zn doses* *: The difference between the means indicated by the same letter in the same group is not statistically significant, Significance level: P_P= 0.0143, P_K= 0.6387, P_{Ca}= 0.0001, P_{Mg}= 0.5944

It is important to compare these macronutrients, which vary according to zinc doses, in terms of the amounts required in rations for animal nutrition. It is reported that in order to meet the macro element needs of animals at a minimum level in feed rations, feed should contain 0.40% P, 1.00% K, 0.90% Ca according to Muller (2009) and 0.25% Mg according to Anonymous (2001). According to these criteria in the literature, it was understood that the total P, K, Ca and Mg values determined in forage pea hay at different Zn doses were at a level to meet the needs of ruminants.

The research result showed that Zn fertilization of forage pea through soil can not only increase the fodder efficiency and quality, but also improve the nutrient uptake of the plant.

4. Conclusion

Research results showed that zinc application may be beneficial in increasing the growth, forage yield and quality of forage legumes such as forage pea in Zn deficient soils in the Southeastern Anatolia Region of Türkiye. In the research, it was concluded that 10 kg Zn ha^{-1} application to forage pea in the presence of low-level extractable Zn in the soil would provide significant increases in forage production. The same Zn dose provided significant improvements in both forage quality and mineral content of the forage.

Consequently, optimum application of micronutrients such as Zn is required for the forage yield and quality of legume forage crops. When considering the complex relationship between soil properties, Zn application and plant uptake, longer-term and more research on the effects of micronutrients is needed.

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