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Foliar zinc sulfate application effects on biomass and forage traits of annual ryegrass (*Lolium multiflorum* Lam.) in zinc-deficient soils

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Abstract

Deficiencies of essential micronutrients in forage crops can result in reduced growth, diminished nutrient content, and reduced in forage quality. This can, in turn, affect the nutritional requirements of livestock and the overall productivity of the agricultural sector. The experiment was initiated during the 2023-24 production season and employed four zinc sulfate doses (0, 0.2, 0.4, 0.6 % w/v) and five varieties of annual ryegrass (*Lolium multiflorum* Lam. cv. İlkadım, cv. Master, cv. Baqueno, cv. Caramba, cv. Trinova), with four replications. SPAD measurements were obtained following the foliar application of zinc sulfate, and plant height (cm), flag leaf length (cm), flag leaf width (cm), leaf number per plant, and leaf area (cm^2) parameters were collected through the single mowing of annual ryegrass. After the fresh forage yield (t ha^{-1}) measurements, ADF (%), NDF (%), crude protein ratio (%), crude protein yield (kg da^{-1}) and relative feed value were measured and calculated. As a result of the data obtained, it was determined that foliar zinc sulfate applications can make positive changes in yield and quality, while at the same time increasing the amount of fiber. While İlkadım had the highest average value with 1.48 t da^{-1} in terms of hay yield, the highest value among zinc sulfate doses was obtained from 0.6 % with 1.47 t da^{-1}. High values were obtained at 0.4 % and 0.6 % doses. Among the varieties, İlkadım and Baqueno had higher yield and quality characteristics. However, it is understood that the responses to foliar zinc sulfate applications occurred in different percentages among the others.

Keywords: Foliar fertilizer, Zinc, Ryegrass, Forage quality, SPAD

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INTRODUCTION

Zinc (Zn) is a crucial micronutrient for plant growth and development, playing a significant role in various physiological and biochemical processes. Its importance is underscored by its involvement in enzyme activity, protein synthesis, and regulation of plant hormones, all of which are essential for optimal plant growth and yield. Zinc acts as a structural component and cofactor in numerous enzymes, facilitating critical metabolic pathways including those related to photosynthesis, respiration, and nitrogen metabolism (Broadley et al., 2007; Alloway, 2009).

Zinc deficiency is a widespread issue in agricultural soils, affecting crop yield and quality globally. It is estimated that zinc deficiency impacts a significant portion of the world's arable land, leading to reduced agricultural productivity and nutritional quality of crops (Alloway, 2009; Barman et al., 2018). For instance, in rice cultivation, zinc deficiency has been linked to lower yields, necessitating the application of zinc fertilizers to enhance crop performance (Rasel et al., 2023). Furthermore, zinc plays a critical role in synthesizing chlorophyll, which is essential for photosynthesis, and its deficiency can lead to chlorosis and stunted growth in plants (Khatun et al., 2018).

The mechanisms by which plants acquire and utilize zinc are complex. Zinc transporters, such as the Zn/Fe-regulated transporter family, are crucial for zinc uptake from the soil into plant cells (Khatun et al., 2018; Sinclair et al., 2018). These transporters are upregulated in response to zinc deficiency, highlighting the plant's adaptive mechanisms to cope with low zinc availability (Khatun et al., 2018). Additionally, the interaction between zinc and plant growth-promoting bacteria has been shown to enhance zinc bioavailability and uptake, further supporting plant health and productivity (Jalal, 2024; Upadhayay et al., 2022).

Annual ryegrass (*Lolium multiflorum* Lam.) is increasingly recognized as an important forage crop due to its high yield potential, nutritional quality, and adaptability to various agricultural systems. Its significance is underscored by its role in mixed cropping systems, where it can enhance overall forage production and quality when combined with legumes such as red clover and berseem clover. Studies have shown that while the yield benefits from these mixtures may vary seasonally, annual ryegrass consistently contributes significantly to forage availability, particularly during the mid to late-growing season when clovers are slower to establish (Ryan-Salter & Black, 2012; Iuga et al., 2018).

The application of foliar zinc in annual ryegrass has been shown to significantly enhance both forage yield and quality. Studies have demonstrated that the application of zinc, particularly in the form of zinc sulfate, can lead to improved relative feed value (RFV) in forage crops, including annual ryegrass (Sher et al., 2022). Furthermore, highlighted that biofortification with zinc not only improves forage productivity but also enhances the nutritive value for livestock, making it a dual benefit for agricultural practices (Kumar & Ram, 2021). Additionally, emphasized that agronomic biofortification strategies, including foliar application of zinc, can effectively enhance the micronutrient content of forage crops, thereby improving their overall quality (Dhaliwal et al., 2022).

The increasing importance of annual winter forage crops as cover crops, catch crops and roughage sources has revealed the necessity of research for optimum cultivation of these crops. Annual ryegrass is one of these species. For this purpose, the effect of foliar applications on yield and quality in areas with zinc deficiency in ecologies with Mediterranean climate was investigated.

MATERIALS AND METHODS

Study site description

The experimental site is situated in Aydın Adnan Menderes University Research and Demonstration farms, Aydın within the Mediterranean climate zone (N: 37.762326°, E: 27.758774°). The area has mild and hot temperatures and plenty of sunlight. The long-term climate data for this location indicate that the average total precipitation was 573.7 mm and the average temperature was 13.92 °C during the months of cultivation. It should be noted that the year in which the experiment was conducted differed from the long-term average. In terms of monthly average temperatures, the period in which the experiment was conducted was notably warmer and drier than the long-term average. There were also significant differences between months in terms of precipitation totals. (Figure 1.)

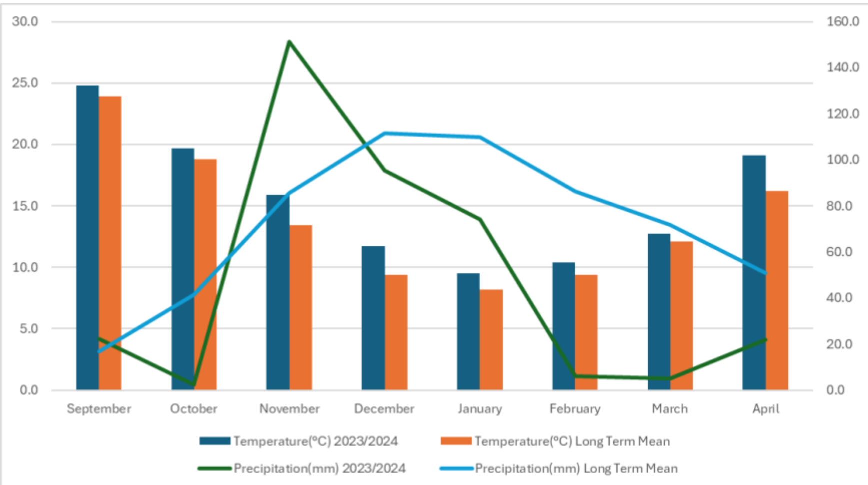

Figure 1. Monthly average temperature and precipitation values in the study area for 2023-2024 cropping season.

The studied soils at the site classified as inceptisols, the basic physical and chemical characteristics of which are as follows: had a pH of 8.09, soil organic matter of 11.4 g kg^{-1}, available phosphorus of 5.11 ppm, exchangeable potassium of 197 ppm, exchangeable calcium (Ca) of 4301 ppm, iron (Fe) of 14.94 ppm, zinc (Zn) of 0.35 ppm (deficient) and texture is sandy loam. Zinc deficiency is observed in soil with concentrations less than 0.5 ppm (Eyüpoğlu et. al., 1998).

Experimental Design

The experiment design was randomized complete block design (RCBD) with factorial arrangements in four replications conducted during the study years 2023/24. The plots were 1.2 meters in width and 5 meters in length, with six rows of plants, separated by 20 centimeters. The seeds were sown with a trial seeder in November 2023 at a depth of 3-4 cm and at a rate of 20 kg per hectare. The experiment utilized five annual ryegrass cultivars (*Lolium multiflorum* Lam. cv. İlkadım, cv. Master, cv. Baqueno, cv. Caramba, cv. Trinova) as the experimental material. Four different zinc sulfate doses (0-Control, 0.2, 0.4, 0.6% w/v) were applied to the plots of each variety. At each foliar application, an aqueous solution 0, 0.2, 0.4, 0.6% (w/v) of $ZnSO_4{\cdot}7H_2O$ ha^{-1} with 800 l ha^{-1} was sprayed in the very late afternoon until most of the leaves were covered. Harvesting was done with a self-propelled meadow mower (BCS-615 SL, Italy) at the flowering stage of the plants.

Measurements of annual ryegrass

Plant height measurements were made on 15 plants in each plot at harvest maturity. Aboveground biomass samples were collected from each plot at a stubble height of 2.5 cm. The samples were weighed and oven dried at 70 °C for 48 hours (Mikrotest Lab., Ankara, Türkiye). Using a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Japan), the relative chlorophyll content of leaves was measured after (BBCH 63 stage) of foliar zinc sulfate treatments by taking measurements in the middle of 15 randomly chosen leaves per plot (Hoel, 1998). The leaf area measurement was carried out with the help of LI-COR 3000C (Lincoln, NE, USA) by measuring the whole leaf area of 15 samples taken homogenously from the plots before and after zinc sulfate application. Total N content was determined by the Kjeldahl method and multiplied by 6.25 to give crude protein content (AOAC, 2003). NDF and ADF contents were measured using the methods described by Van Soest et al. (1991) in an automated fibre analyzer (ANKOM 2000 Fibre Analyser, ANKOM Technology, NY, USA). Briefly, for NDF analysis, a total of 0.5 g of ground sample was placed in the fibre filter bag (F57, ANKOM Technology, NY, USA) and placed in the automatic fibre analyzer to digest the samples with dilute solutions of neutral detergent dry concentrate with triethylene glycol (FND20C, ANKOM Technology, NY, USA). The residues obtained after the determination of NDF can be digested directly with the solution of acid detergent concentrate - dry CTAB powder (FAD20C, ANKOM Technology, NY, USA) for the determination of ADF.

The crude protein yield (t ha^{-1}) and relative feed value were calculated using the obtained data and the procedures of Horrocks and Vallentine (1999).

Data analysis

All data were analyzed for homogeneity and normality using the Kolmogorov-Smirnov test. When significant differences were found via the ANOVA, means were compared using the Fisher's protected least significant difference (LSD) test at $p \leq 0.05$. The 'agricolae' package in R Studio v4 (Mendiburu and Mendiburu, 2019) was used for all one-way analyses, while the 'methane' package (Olivoto and Lucio, 2020) was run for Pearson correlation representations.

RESULTS AND DISCUSSION

The results of the ANOVA demonstrated that the annual ryegrass genotypes and different zinc doses had a significant impact on the plant traits under investigation. Significant differences were observed between the genotypes and the zinc doses in many parameters, including plant height, leaf number, flag leaf length, flag leaf width, fresh forage yield, and hay yield ($p < 0.01$). In particular, the effect of the zinc doses was found to be statistically significant in all of the aforementioned parameters. The interactions between genotypes and zinc doses were, in general, statistically significant. However, for some traits, this interaction was less significant ($p > 0.05$). This was particularly the case for ADF, and RFV parameters, where the genotype × zinc sulfate dose interactions were less significant. This indicates that zinc doses and genotypes alone were effective on these traits, but their interactions were limited. In contrast, in some other parameters, such as fresh forage yield, the genotypes x zinc dose interactions were significant. In these cases, the two factors affected plant performance (Table 1.).

Analysis of the mean values revealed the general tendency of the genotypes towards zinc sulfate doses. In terms of plant height, the highest mean value was observed at 0.4% dose (97.44 cm), followed by 0.6% (93.91 cm) and 0.2% (93.06 cm) doses. The control group had the lowest average plant height (89.85 cm). These results indicate that especially 0.4% dose had the most positive effect on plant height and zinc application significantly increased plant height. Considering the effects of zinc sulfate doses in different genotypes, striking results were observed on plant height. In the Master genotype, plant height increased with a peak (95.69 cm) at 0.4% dose, while it decreased at the higher dose of 0.6%. This indicates that increasing zinc doses do not increase plant height in a linear manner. On the other hand, İlkadım genotype reached the highest height especially at 0.6% dose (101.75 cm), indicating that it was positively affected by the increase in zinc concentration. The Trinova genotype reached the highest height at 0.4% dose (95.95 cm), but decreased at higher doses. Caramba genotype showed a balanced response, reaching the highest height value at 0.4% dose (100.80 cm). Baqueno genotype exhibited a relatively constant response at all doses and showed a limited response to high zinc concentrations.

When the number of leaves per plant was evaluated, the highest average number of leaves was obtained at 0.6% dose (6.29). This was followed by 0.4% (5.98) and 0.2% (5.87) doses, while the control group had the lowest number of leaves (5.32). This indicates that the number of leaves increased in direct proportion with increasing zinc sulfate doses and 0.6% dose promoted leaf growth. Differences were also observed in the number of leaves per plant between genotypes with zinc sulfate doses. Master genotype increased the number of leaves with increasing zinc concentration and reached the maximum value (6.15) at 0.6% dose. Ilkadim genotype reached the maximum number of leaves (7.06) at 0.6% dose, indicating that it was positively affected by high zinc concentrations. In Trinova genotype, the number of leaves increased slightly as the dose increased and reached the highest value at 0.6% dose (6.21). The Caramba genotype showed a more limited response, peaking at 0.6% (5.97). The Baqueno genotype also responded positively to increasing zinc doses, reaching the highest leaf number at 0.6% (6.06).

Table 1. Combined analysis of variance for the effects of foliar zinc sulfate on the traits of annual ryegrass genotypes

SOV	df	Mean Squares						
		Plant Height	Leaf Number	Flag Leaf Lenght	Flag Leaf Width	Fresh Forage Yield	Hay Yield	Leaf Area
Genotypes	4	148.9**	1.35**	35.57**	5.29**	263.8**	150.7**	203.8**
Zinc Doses	3	194.4**	3.27**	108.64**	2.90**	610.4**	318.2**	72.8**
Genotypes × Zinc Doses	12	22.7**	0.14^{ns}	19.75**	0.37**	40.7**	16.8*	21.9**
	df	SPAD	ADF	NDF	CPR	CPY	RFV	
Genotypes	4	47.0**	8.57**	4.91**	0.95**	3443.4**	91.4**	
Zinc Doses	3	56.0**	13.89**	31.53**	22.03**	20493.0**	363.0**	
Genotypes × Zinc Doses	12	14.0**	2.02^{ns}	2.55*	0.55**	658.3**	30.0^{ns}	

** p<0.01, *p<0.05, ns: non-significant

In the flag leaf length averages, the highest value was obtained at 0.6% dose (57.75 cm), followed by 0.4% (56.95 cm) and 0.2% (54.65 cm). The flag leaf length of the control group had the lowest average (52.60 cm). These data show that especially high zinc doses have a positive effect on flag leaf length and the 0.6% dose provides the highest increase in this parameter. When evaluated in terms of flag leaf length, it was observed that as zinc concentration increased in Master genotype, the flag leaf length increased and reached a maximum at 0.6% dose (59.00 cm). İlkadım genotype gave the highest response to zinc application and reached the longest flag leaf length at 0.6% dose (61.00 cm). No consistent response was seen in Trinova genotype and reached the highest value at 0.2% dose (56.75 cm). Caramba genotype showed a slight increase and reached the peak at 0.6% dose (56.00 cm). Baqueno genotype showed a relatively high length at all doses and reached the highest values at 0.4% and 0.6% doses (59.25 cm). In terms of flag leaf width, the highest mean value was observed at 0.6% dose (5.97 cm). This was followed by 0.4% (5.92 cm) and 0.2% (5.74 cm) doses, while the control group had the lowest mean width (5.14 cm). These findings indicate that increasing zinc doses had a positive effect on flag leaf width, especially 0.6% dose, giving the best results in this parameter. According to the genotype × zinc sulfate dose interaction, it was observed that the width of Master genotype increased with zinc concentration and reached the maximum value (6.22 cm) at 0.6% dose. İlkadım genotype reached the highest width (6.87 cm) at 0.4% dose, indicating that it was more sensitive to medium levels of zinc application. A significant increase was observed in Trinova genotype and the highest width was obtained at 0.4% dose (6.02 cm). The Caramba genotype showed a limited response, reaching the highest width at 0.6% dose (5.65 cm). The Baqueno genotype showed a slight increase against doses, peaking at 0.6% (5.77 cm). When the averages were examined in terms of fresh forage yield, 0.4% (4.69 t/da) and 0.6% (4.72 t da^{-1}) doses had the highest yield values, followed by 0.2% (4.55 t da^{-1}). The control group showed the lowest average yield (4.34 t da^{-1}). This situation reveals that zinc application, especially at medium and high doses, increases fresh forage yield and 0.4% and 0.6% doses are ideal for this parameter. Master genotype showed a minimal response among all doses and provided a constant yield of 4.64 t da^{-1}. İlkadım genotype showed that it was positively affected by zinc application by reaching the highest yield at 0.6% dose (4.90 t da^{-1}). Trinova genotype slightly increased as the dose increased and reached the peak at 0.6% dose (4.59 t da^{-1}). The Caramba genotype showed a good response to increasing zinc doses by reaching the highest yield at 0.4% dose (4.80 t da^{-1}). Baqueno genotype also increased with the zinc dose and reached the highest value at 0.6% dose (4.77 t da^{-1}).

Finally, in the evaluation of hay yield, the highest mean value was obtained at 0.6% dose (1.47 t da^{-1}). This dose was followed by 0.4% (1.36 t da^{-1}) and 0.2% (1.32 t da^{-1}) doses, respectively, while the control group had the lowest hay yield (1.16 t da^{-1}). These data show that hay yield increased with increasing zinc doses and especially the 0.6% dose provided the best yield. According to the genotype x zinc sulfate dose interaction, the Master genotype showed limited variation at different doses and reached the highest yield at 0.2% dose (1.39 t/da). The İlkadım genotype was positively affected by zinc doses, especially reaching the highest hay yield at 0.6% dose (1.71 t da^{-1}). The Trinova genotype reached its peak at 0.6% dose (1.37 t da^{-1}). While the Caramba genotype showed the highest hay yield at 0.6% dose (1.44 t/da), the Baqueno genotype also increased as the zinc dose increased and reached the highest yield at 0.6% dose (1.49 t da^{-1}) (Table 2.).

These evaluations detail the responses of each genotype to different zinc sulfate doses and point to potential yield strategies that can be optimized for each genotype according to zinc concentration.

Table 2. Mean comparison of agronomic traits in annual ryegrass genotypes as affected by foliar zinc sulfate

	Plant Height (cm)					**Leaf number per plant**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	91.79dh	93.68be	95.69bc	89.54fh	92.68 b	5.15h	5.93cf	5.93cf	6.15bd	5.79 bc
İlkadım	95.51bc	95.88b	101.16a	101.75a	98.57 a	5.59eh	6.48b	6.27bc	7.06a	6.35 a
Trinova	84.89ı	90.64eh	95.95b	90.54eh	90.50 c	5.18gh	5.83cf	5.96cf	6.21bc	5.80 bc
Caramba	88.23hı	91.98cg	100.80a	94.61bd	93.90 b	5.12h	5.50fh	5.67dg	5.97cf	5.56 c
Baqueno	88.87gh	93.13bf	93.62be	93.13bf	92.18 bc	5.56fh	5.59eh	6.08be	6.06be	5.82 b
Mean	89.85 c	93.06 b	97.44 a	93.91 b		5.32 c	5.87 b	5.98 b	6.29 a	
cv: 2.74 LSD_{var}: 1.82 LSD_{zinc}: 1.62 LSD_{int}: 3.63						cv: 0.71 LSD_{var}: 0.24 LSD_{zinc}: 0.22 LSD_{int}: 0.49				

	Flag Leaf Lenght (cm)					**Flag Leaf Width (cm)**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	50.50gh	55.50cf	56.25be	59.00ac	55.31 a	5.75cf	6.05bc	5.80be	6.22b	5.95 b
İlkadım	55.25df	52.25fh	59.25ab	61.00a	56.93 a	5.82bd	6.70a	6.87a	6.82a	6.55 a
Trinova	55.00ef	56.75be	55.50cf	54.00eg	55.31 a	4.20ı	5.32fh	6.02bc	5.37ch	5.23 d
Caramba	50.25h	52.00fh	54.50ef	56.00be	53.18 b	4.57ı	5.17h	5.27gh	5.65cg	5.16 d
Baqueno	52.00fh	56.75be	59.25ab	58.75ad	56.68 a	5.35eh	5.45dh	5.62ch	5.77bf	5.55 c
Mean	52.60 c	54.65 b	56.95 a	57.75 a		5.14 c	5.74 b	5.92 ab	5.97 a	
cv: 9.50 LSD_{var}: 1.78 LSD_{zinc}: 1.59 LSD_{int}: 3.56						cv: 12.06 LSD_{var}: 0.23 LSD_{zinc}: 0.20 LSD_{int}: 0.46				

	Fresh Forage Yield (t da^{-1})					**Hay Yield (t da^{-1})**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	4.45gı	4.64bf	4.64bf	4.63df	4.59 b	1.14ık	1.39bf	1.28fh	1.34dh	1.29 c
İlkadım	4.64df	4.69be	4.81ab	4.90a	4.76 a	1.28fh	1.44bd	1.48bc	1.71a	1.48 a
Trinova	4.09j	4.41hı	4.54fh	4.59ef	4.41 d	1.04k	1.26gı	1.22hj	1.37cg	1.26 d
Caramba	4.12j	4.43hı	4.80ab	4.72bd	4.53 c	1.11jk	1.22hj	1.40bf	1.44bd	1.29 c
Baqueno	4.39ı	4.58eg	4.69be	4.77ac	4.61 b	1.26gı	1.31eh	1.40be	1.49b	1.36 b
Mean	4.34 c	4.55 b	4.69 a	4.72 a		1.16 c	1.32 b	1.36 b	1.47 a	
cv: 2.06 LSD_{var}: 0.07 LSD_{zinc}: 0.06 LSD_{int} :0.01						cv: 6.39 LSD_{var}: 0.06 LSD_{zinc}: 0.05 LSD_{int} : 0.01				

The effects of foliar zinc sulfate application on the height of annual ryegrass (*Lolium multiflorum* Lam.) have garnered attention in agronomic research due to the significant role of zinc in plant growth and development.

Flag leaf traits, which are critical for crop yield, also benefit from foliar zinc applications. The flag leaf is essential for photosynthesis and grain filling in cereals, and its development can be enhanced by an adequate zinc supply. Research by Razzaq et al. indicates that foliar zinc application influences the mineral status of leaves, which in turn affects vegetative and reproductive growth, ultimately enhancing yield and quality (Razzaq et al., 2013). Moreover, the findings from Mantawy and Elhag emphasize that zinc sulfate application not only improves chlorophyll content but also enhances the overall canopy structure, which is vital for maximizing light interception and photosynthetic efficiency (Mantawy & Elhag, 2018).

Foliar application is recognized as an effective method for enhancing micronutrient uptake, particularly zinc, which is crucial for plant growth and development. Studies indicate that the application of zinc sulfate, both in soil and as a foliar spray, can lead to substantial increases in biomass and quality of forage crops. For instance, a meta-analysis conducted by Iqbal et al. highlights the positive impact of foliar feeding of micronutrients, including zinc, on the productivity of forage crops across diverse climatic conditions. This analysis demonstrated that foliar application can lead to improved forage quality and yield, emphasizing the importance of micronutrient management in sustainable agriculture (Iqbal et al., 2019). Similarly, the research by Dhaliwal et al. supports the notion that biofortification through foliar application of zinc can enhance the nutritive potential of forage crops, thereby improving their overall yield and quality (Dhaliwal et al., 2022). Moreover, specific studies focusing on the effects of zinc sulfate on forage species such as oats and triticale have shown that the application of zinc can significantly improve morphological traits, dry matter yield, and crude protein content. For example, Sher et al. reported that different levels of soil-applied zinc sulfate, combined with appropriate harvesting stages, resulted in enhanced yield and quality of grass forages (Sher et al., 2022). This aligns with findings from a research, who noted that the combination of soil and foliar applications of zinc led to maximum plant height and leaf area index in forage oats, ultimately contributing to increased forage yield (Asif, 2024).

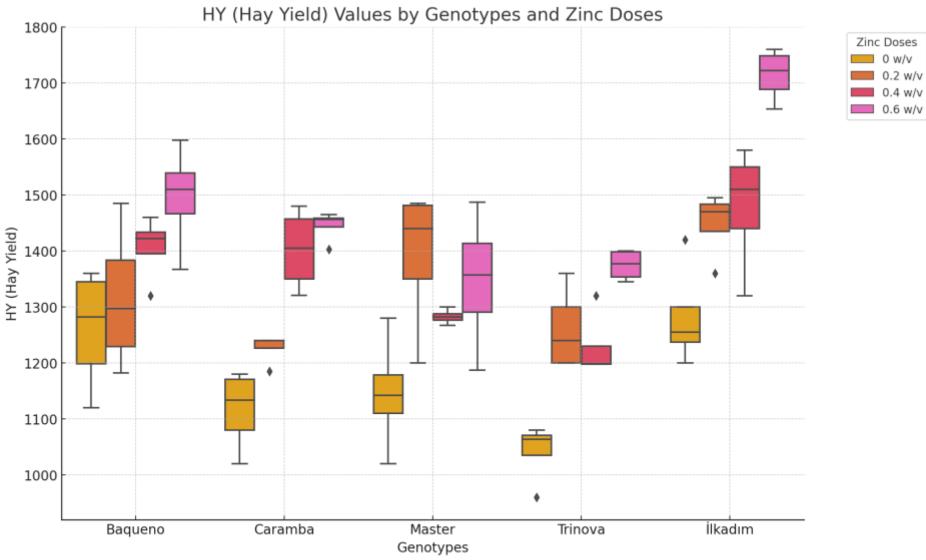

Figure 2. Boxplot of hay yield values by genotypes and zinc doses

The highest value in terms of leaf area was observed in İlkadım genotype at 0.4%, 0.6% and 0.2% doses (87.68 cm², 87.02 cm² and 87.02 cm²). This indicates that İlkadım responded positively to zinc application. On the other hand, the lowest leaf area value was obtained in Baqueno genotype at the control dose (74.60 cm²), indicating that this genotype was limited in leaf area development under zinc-free conditions. Master displayed the highest leaf area at 0.4% (87.06 cm²) and slightly decreased at 0.6% (86.77 cm²), suggesting an optimal response around 0.4% indicating a robust response to zinc. Trinova's leaf area showed minimal variation, with the highest at 0.2% (79.20 cm²), suggesting limited zinc sensitivity. Caramba had a slight increase at 0.6% (80.95 cm²), indicating a moderate response. Baqueno's leaf area also increased, with the highest value at 0.6% (80.73 cm²), suggesting a preference for higher doses. According to the mean values, the highest leaf area was obtained at 0.4% and 0.6% doses (82.41 cm² and 82.57 cm²). This result indicates that zinc sulfate promotes leaf development up to a certain level, but higher doses do not provide any additional gain. The control group had the lowest mean leaf area (78.97 cm²), indicating that zinc deficiency may limit leaf area.

With regard to chlorophyll content, the highest SPAD value was observed in the Caramba genotype at a 0.4% dose (57.42), indicating the potential of moderate zinc doses to enhance chlorophyll content. Conversely, the lowest SPAD value was noted in the Baqueno genotype at both the control and 0.2% doses (48.75), suggesting that zinc is a crucial factor for chlorophyll content increase. Master exhibited the highest SPAD value at 0.6% (56.05), indicating improved chlorophyll content at this dose. İlkadım's chlorophyll content increased gradually, peaking at 0.4% (55.80), reflecting a positive response to mid-range zinc doses. Trinova showed the highest SPAD at 0.6% (55.42), indicating zinc responsiveness. The highest mean SPAD value in terms of chlorophyll content was obtained at 0.4% dose (55.44), indicating that moderate zinc sulfate application provided the greatest increase in chlorophyll content. The lowest SPAD value was observed in the control group (52.26) and 0.2% (52.14), indicating that zinc-free medium had a negative effect on chlorophyll content.

The highest result in ADF values was observed in Baqueno genotype at 0.6% dose (32.17%). This indicates that high zinc concentrations increased the ADF ratio in Baqueno genotype. The lowest ADF value was obtained in Trinova genotype in the control group (28.41%), indicating that ADF content was lower under low zinc conditions. Master responded with moderate increases, peaking at 0.6% (30.84%), suggesting that higher zinc doses may enhance its ADF content. İlkadım exhibited the highest ADF at 0.4% (32.25%), indicating sensitivity to mid-range doses. Caramba showed consistent increases, peaking at 0.6% (31.52%). The highest average ADF content was recorded at 0.6% (31.24%) and 0.4% (30.79%) doses. This result indicates that higher zinc doses tend to increase fiber content. The lowest mean ADF value was recorded in the control group (29.31%), suggesting that zinc deficiency may decrease the fiber content.

The highest value in NDF ratio was observed in İlkadım genotype at 0.6% dose (52.21%). This result reflects the positive response of this genotype to high zinc doses, while the lowest NDF value was obtained in İlkadım genotype at the control dose (47.26%). Zinc seems to be effective in increasing the NDF ratio. Master had a gradual increase in NDF, reaching a maximum at 0.6% (51.12%), suggesting sensitivity to higher doses. Trinova's NDF peaked at 0.6% (50.55%), similar to Master's, indicating responsiveness at high doses. Caramba's NDF was highest at 0.4% (52.66%), suggesting an optimal response at moderate doses. Baqueno's NDF increased steadily, peaking at 0.6% (51.69%). A similar trend was observed in NDF ratio; the highest average value was recorded at 0.4% dose (51.41%). In the control group, the NDF value remained at the lowest level (48.81%). This highlights the positive effect of zinc on plant fiber content and especially the role of medium zinc doses in increasing fiber content.

The genotype with the highest crude protein content was Baqueno, with a dose of 0.6%, which resulted in an impressive 16.28% protein yield. This finding suggests that zinc levels play a significant role in increasing protein content. Conversely, the lowest protein value was observed in the Ilkadim genotype at the control dose, indicating that protein content remained low in the presence of zinc deficiency. Master had the highest crude protein content at 0.6% (16.23%), suggesting a positive response to higher zinc doses. Trinova reached the highest protein content at 0.4% (16.00%), indicating mid-range dose sensitivity. Caramba's protein content was also highest at 0.4% (15.82%), showing an optimal response to moderate zinc The highest mean values in terms of crude protein content were observed at 0.4% (15.72%) and 0.6% (15.92%) doses. In zinc deficiency, crude protein content remained low and was recorded as 13.60% in the control group. These findings indicate that zinc application is effective in increasing protein synthesis and especially high doses can increase protein content.

The highest value in protein yield was obtained in İlkadım genotype at 0.6% dose (271.6 kg $da^{-1)}$, indicating the positive effect of high zinc doses on protein yield. The lowest protein yield was recorded in Trinova genotype at the control dose (143.1 kg da^{-1}), indicating that protein yield was limited under zinc-free conditions. Master's protein yield increased significantly with zinc, peaking at 0.2% (223.9 kg da^{-1}) and then decreasing, indicating an optimal response at this lower dose. Caramba's protein yield was highest at 0.4% (222.1 kg da^{-1}), indicating sensitivity to moderate zinc. Baqueno's yield peaked at 0.6% (243.7 kg da^{-1}), showing a positive response to higher doses. Finally, the highest average value in terms of crude protein yield was obtained at 0.6% dose (234.9 kg da-^{1}). This result reveals that protein yield increased significantly with zinc application. The lowest protein yield was observed in the control group (159.0 kg da^{-1}), indicating that protein yield decreased in zinc-free environment.

The analyzed data reveal the relative feed value interactions between zinc sulfate doses and genotypes. Moreover, when the overall effect of each dose is evaluated over the mean values, the effect of zinc applications on RFV values in annual grass genotypes is clearly seen. The genotype İlkadım reached the highest RFV value at the control dose (131.1) and showed the highest value among all genotypes. However, this genotype decreased with zinc application and obtained one of the lowest values (113.8) at 0.4% dose. This suggests that İlkadım genotype may be sensitive to high zinc doses. Similarly, the Trinova genotype also responded positively to zinc treatments, and obtained a high RFV value (129.2), especially at the control dose. However, the RFV value in Trinova also decreased gradually with increasing zinc doses and the lowest value was observed at 0.6% dose (119.3). While Caramba genotype stood out with its high RFV value (125.3) especially at the control dose, the RFV value showed a downward trend with the effect of zinc applications. Caramba, with the lowest value observed at 0.4% dose (114.1), may have a less tolerant structure to zinc application. In Baqueno genotype, while the highest RFV value was observed at the control dose (122.0), this value decreased with zinc applications and the lowest value was observed at 0.6% dose (114.8). This indicates that Baqueno shows sensitivity to increasing zinc application.

When the mean RFV values were analyzed, the control group had the highest mean value (126.0), followed by 0.2% (122.1), 0.4% (117.5), and 0.6% (117.0) doses, respectively. This indicates that zinc sulfate applications caused a gradual decrease in RFV. In particular, 0.4% and 0.6% doses had the lowest mean RFV values, suggesting that high zinc doses may have a negative effect on RFV.

In the light of these data, it is seen that annual grass genotypes exhibited different sensitivities to zinc sulfate applications and RFV values generally decreased as zinc doses increased. While İlkadım and Trinova genotypes stood out with high RFV values at the control dose, zinc application caused a decrease in these values. In general, it can be said that low zinc doses had less negative effect on RFV, while high doses caused a decrease especially at 0.4% and 0.6% levels.

The impact of foliar zinc sulfate applications on the relative feed value (RFV) of forage crops has been extensively investigated in the scientific literature. The findings of these studies indicate that zinc applications have a significant effect on the nutritive value and digestibility of plants. In this study, zinc doses were observed to result in a general decrease in RFV, particularly at higher doses (0.4 % and 0.6 %). Similarly, the literature discusses the effects of zinc on plant metabolism. Zinc is a vital microelement that plays a crucial role in supporting chlorophyll synthesis and enzyme activities in plants (Cakmak, 2008). However, excessive zinc applications can disrupt the biochemical balance of the plant, negatively affecting cell structure and consequently reducing plant digestibility (Alloway, 2008).

It has been demonstrated that zinc deficiency has a detrimental impact on plant yield and quality parameters. However, high doses of zinc applications can also result in toxicity, leading to adverse alterations in the nutrient composition of the plant (Broadley et al., 2012). The findings of this study indicate that the application of zinc can enhance plant productivity when utilized at optimal doses. However, excessive doses have been shown to reduce plant digestibility and nutritional value. Furthermore, some studies have indicated that zinc may enhance the synthesis of cell wall components and lignin, resulting in the hardening of plant tissue and a subsequent reduction in the digestibility of plant feeds (Marschner, 1995).

Table 3. Mean comparison of forage quality traits in annual ryegrass varieities as affected by foliar zinc sulfate

	Leaf area (cm²)					**SPAD**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	79.46be	79.74be	87.06a	86.77a	83.26 b	51.92ef	50.35fg	52.70e	56.05ab	52.75 b
İlkadım	81.77b	87.02a	87.68a	87.02a	85.87 a	50.32fg	50.77f	55.80ab	55.00bc	52.97 b
Trinova	77.78cg	76.45eg	79.20bf	77.40dg	77.71 c	54.90bd	55.42b	56.05ab	55.42b	55.45 a
Caramba	81.23bc	75.54fg	79.77be	80.95bd	79.37 c	55.42b	55.42b	57.42a	53.22de	55.37 a
Baqueno	74.60g	78.47bf	78.36bf	80.73bd	78.04 c	48.75g	48.75g	55.25b	53.55bd	51.57 c
Mean	78.97 b	79.44 b	82.41 a	82.57 a		52.26 c	52.14 c	55.44 a	54.65 b	
cv: 3.22 LSD$_{var}$: 1.84 LSD$_{zinc}$: 1.65 LSD$_{int}$: 3.69						cv: 2.20 LSD$_{var}$: 0.84 LSD$_{zinc}$: 0.75 LSD$_{int}$: 1.67				
	ADF (%)					**NDF(%)**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	29.95bf	30.58ad	30.12bf	30.84ad	30.37 b	49.78fh	50.05eh	50.55dg	51.12bf	50.37 a
İlkadım	28.61ef	29.48cf	32.25a	30.81ad	30.29 b	47.26j	49.25gı	52.13ac	52.21ab	50.21 a
Trinova	28.41f	28.65ef	29.26df	30.88ad	29.30 c	48.06ıj	48.99hı	50.33dh	50.55dg	49.48 b
Caramba	29.29df	30.75ad	31.13ac	31.52ab	30.67 ab	49.15gı	50.16eh	52.66a	51.08cf	50.76 a
Baqueno	30.30be	31.61ab	31.21ac	32.17a	31.32 a	49.78fh	50.71cf	51.36ae	51.69ad	50.88 a
Mean	29.31 c	30.21 b	30.79 ab	31.24 a		48.81 c	49.83 b	51.41 a	51.33 a	
cv: 4.11 LSD$_{var}$: 0.88 LSD$_{zinc}$: 0.79 LSD$_{int}$: 1.77						cv: 2.03 LSD$_{var}$: 0.72 LSD$_{zinc}$: 0.64 LSD$_{int}$: 1.45				
	Crude Protein Content (%)					**Crude Protein Yield (kg da^{-1})**				
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**	Control	0.2%	0.4%	0.6%	**Mean**
Master	13.85hı	16.08ac	15.59bd	16.23ab	15.44 a	159.1jk	223.9bc	200.0dg	218.8de	200.4 bc
İlkadım	13.45ı	14.91eg	15.51ce	15.84ac	14.93 b	172.2ıj	215.9df	229.4bc	271.6a	222.3 a
Trinova	13.72ı	14.37gh	16.00ac	15.50ce	14.90 b	143.1k	181.3gı	196.6fh	213.0df	183.5 d
Caramba	13.39ı	14.45fh	15.82ac	15.73ac	14.85 b	149.3k	177.2hj	222.1c	227.4bc	194.0 c
Baqueno	13.59ı	15.08df	15.67ad	16.28a	15.16 ab	171.2ıj	198.7eg	220.2cd	243.7b	208.5 b
Mean	13.60 c	14.98 b	15.72 a	15.92 a		159.0 d	199.4 c	213.7 b	234.9 a	
cv: 2.74 LSD$_{var}$: 1.82 LSD$_{zinc}$: 1.62 LSD$_{int}$: 0.64						cv: 2.74 LSD$_{var}$: 18.2 LSD$_{zinc}$: 16.2 LSD$_{int}$: 20.84				

Table 4. Mean comparison of relative feed value in annual ryegrass genotypes as affected by foliar zinc sulfate

Relative Feed Value					
Genotypes	Control	0.2%	0.4%	0.6%	**Mean**
Master	122.5cf	120.9cg	120.5dh	118.1fı	120.5 bc
İlkadım	131.1a	124.7be	113.8ı	115.6gı	121.3 b
Trinova	129.2a	126.4ac	122.1cf	119.3eı	124.3 a
Caramba	125.3bd	120.4dh	114.1ı	117.1fı	119.2 bc
Baqueno	122.0cf	117.8fı	117.0fı	114.8hı	117.9 c
Mean	126.0 a	122.1 b	117.5 c	117.0 c	

cv: 2.74 LSD_{var}: 1.82 LSD_{zinc}: 1.62 LSD_{int} : 5.67

Regression tables were constructed to elucidate the degree and direction of the relationship between the dependent and independent variables utilized in the study. These tables serve as a valuable tool for elucidating the impact of genotypes and zinc doses on plant growth parameters. In this study, regression coefficients demonstrate the positive or negative influence of specific zinc doses on plant productivity The variation of the values does not reveal a linear increase. This is due to the low R^2 values (SPAD: 0.18; leaf area: 0.11; hay yield: 0.41; crude protein yield: 0.61). The inclusion of many values may be difficult for the description of the modeling. However, these tables are also provided to explain the interactions.

These findings offer crucial insights into the effects of zinc applications on crop yield and quality (Figure 3.).

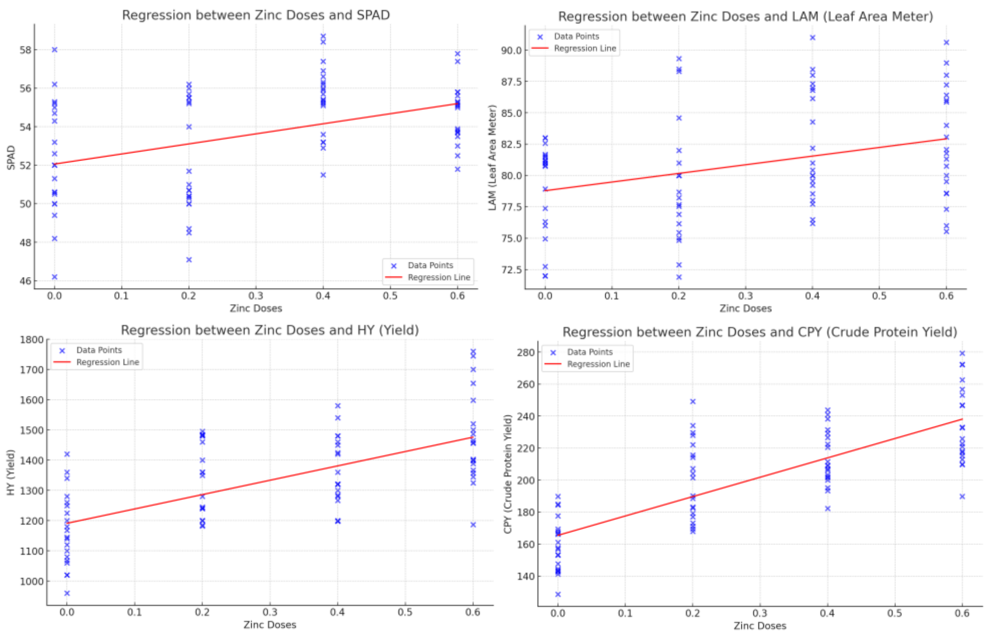

Figure 3. Regression analysis of the relationship between zinc doses and SPAD, leaf area, hay yield and crude protein yield values

Correlation analysis examines the linear relationship between the variables used in the study and reveals the interactions of these variables with each other. Correlation coefficients indicate the direction and strength of the relationship between variables. Positive correlation coefficients indicate that two variables increase or decrease together, while negative correlation coefficients indicate that one variable increases while the other decreases. Parameters with high positive correlation values in the analysis indicate that these variables have a strong linear relationship with each other. For example, high positive correlations between plant height and fresh forage yield or crude protein ratio and crude protein yield indicate that these parameters support each other. In contrast, negative correlations may indicate an inverse relationship between certain parameters The strongest positive interaction was observed between hay yield and crude protein yield with 0.95. The strongest negative interaction was found between NDF and RFV with 0.97 (Figure 4.).

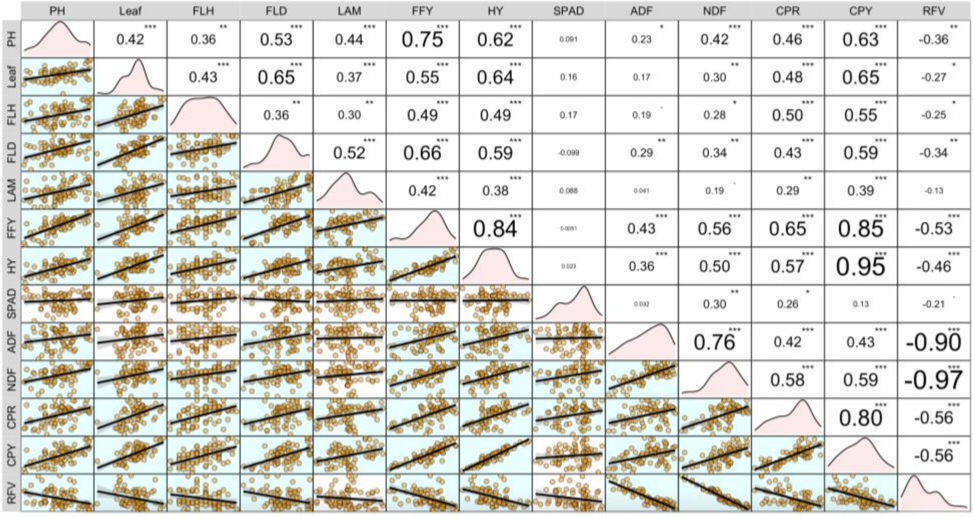

Figure 4. Pearson’s correlation analysis of variables in the experiment (PH: Plant height; FLH: Flag Leaf Height; FLD: Flag leaf width; LAM: Leaf area per plant; FFY: Fresh forage yield; HY: Hay yield; CPR: Crude protein content; CPY: Crude protein yield; RFV: Relative feed value)

CONCLUSION

The cultivation of annual ryegrass is increasing due to its superior characteristics in terms of yield and quality, as well as the positive impact it has on livestock. While there are no specific requirements for cultivating this species, soils lacking sufficient macro- and micronutrients can impede the attainment of optimal yields and quality. Zinc is of particular significance in this context. The objective of this study was to investigate the impact of zinc sulfate application on the agronomic and quality characteristics of zinc-deficient soils. In the study in which different genotypes were tested, it was observed that the genotypes exhibited disparate values concerning their genetic characteristics. Nevertheless, in general, there were notable increases in yield, fiber properties, and crude protein yield with the application of zinc. Among the genotypes, İlkadım and Baqueno exhibited the highest yield and quality values in these increases. As the increase in zinc doses was not significantly different between 0.4% and 0.6%, the application of a 0.4% zinc sulfate dose was deemed sufficient for this experiment. However, it was determined that the optimal dose and genotype can be selected on an economic basis by considering cost calculations.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

Thre are not any conflict of interest.

Author contribution

EK design, experiment and writing. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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