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Effect of Foliar Application of Nutrients on Growth and Yield of Italian Ryegrass Under Rainfed Condition

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Abstract
There are severe livestock production challenges in Pakistan given the shortages of quality forage, especially in conditions of rainfed. Conventional forage crops such as oat and white brassica grew slowly and did not satisfy the nutritional requirements of livestock throughout the year due to unavailability of quality fodder during times of climatic stresses including drought and loss of soil nutrients. An alternative which can be used is Italian ryegrass (Lolium multiflorum) which is a vigorous cool-season grass that grows fast and is adaptive. Nonetheless, micronutrient deficiency, notably, zinc (Zn), sulfur (S) and boron (B) tends to hinder its productivity. The objective of the research paper was to determine the impact of foliar application of Zn, S, and B on the growth, yield, and nutritional quality of Italian-ryegrass under rainfed conditions. A field experiment was carried out over two years and used Randomized Complete Block Design (RCBD) with 8 treatments (the individual and combined application of Zn, S, B, and a control). Agronomic-parameters (plant height, biomass, tillers, spike length and yield) were measured and biochemical traits included protein content, ADF, NDF and chlorophyll. The findings established that the combined use of zinc, sulphur and boron (T8) increased plant height, dry biomass, yield and protein content by 121, 81, 138 and 20 percent respectively in comparison to the control. In addition to this, T8 (Zn + S + B) also enhanced chlorophyll-content as well as fiber quality because it had the highest value of chlorophyll content (2.9 mg/g), showing improved photosynthetic activity. Control treatment (T1) recorded the lowest level of chlorophyll-content (1.3 mg/g). The height of the plants, biomass, and yield were associated with positive correlations respectively (R^2 = 0.92 -0.98) which indicates their mutual dependency. Conclusively, Zn foliar, S, and B, T8 substantially contributed to plant growth, yield, and nutritional value of Italian-ryegrass when grown in rain fed regions. The implications of these findings present the value of the management of micronutrients in improving the productivity and resilience of forages and provide a sustainable solution to fodder shortage and contribute to climate-smart agriculture in water scarce areas.

Keywords: Biomass Production, Foliar Application, Italian Ryegrass, Micronutrients, Rainfed Agriculture, Yield Optimization

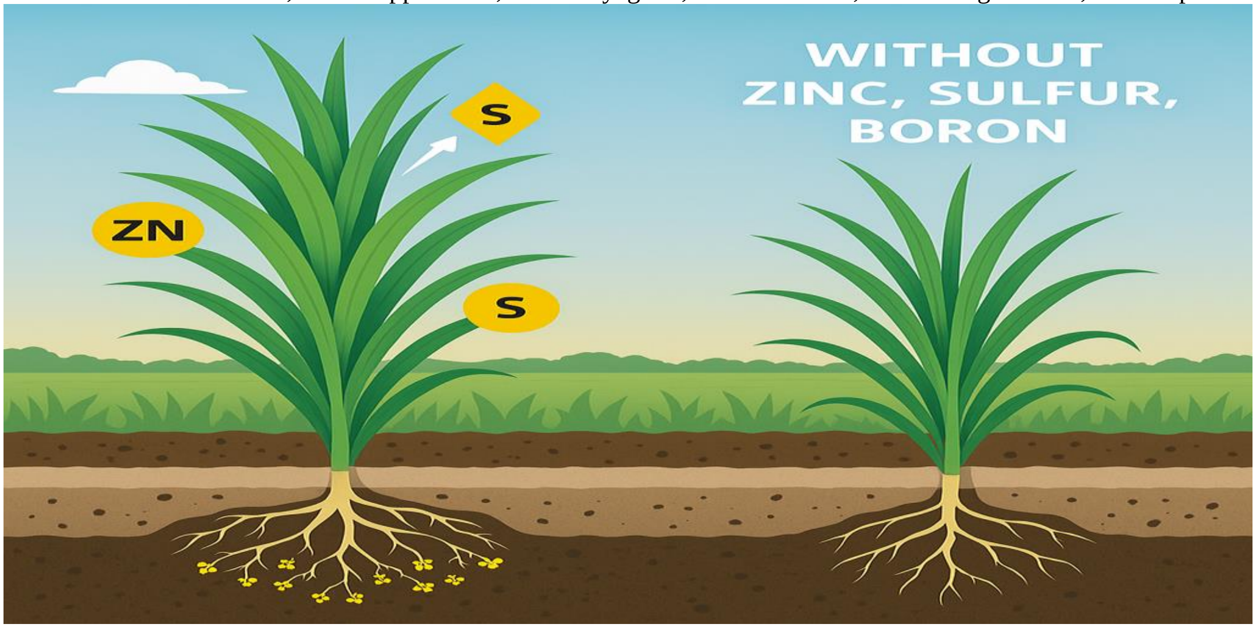

Fig 1: Effect of Foliar Application of Nutrients (S, Zn and B) on Plant growth

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Introduction

In Pakistan, livestock production is enabled by forage crops like oat and brassica, and these crops were mostly found to be cultivated in the winter (Naveed et al., 2021). These crops, however, had poor growth and were not able to meet the entire year nutrition needs of livestock, especially when they needed fodder (Duguma & Janssens, 2021). Negative climatic factors such as fog, frost, and chilly weather were other factors that added to the scarcity of forage in Punjab and other parts of Pakistan, posing an enormous challenge to the livestock business (Haque et al., 2023). Moreover, temperature and soil moisture deficit caused by climate change remained a threat to the productivity of conventional forage crops, which further necessitated the need to employ resilient alternatives such as Italian ryegrass (Lolium multiflorum) that showed characteristic fast growth and high nutritional content in addition to their adaptability to extreme environments (Sun et al., 2024). It was very adaptive to rainfed conditions and thus it fitted perfectly on areas that were experiencing lack of water and nutrients in soils. However, it tended to have lower productivity due to micronutrient imbalance, especially in zinc (Zn), sulfur (S) and boron (B), and these are important micronutrients to activate enzymes and synthesize proteins as well as to adapt to a stressful environment (Watson et al., 2021). The most common agricultural systems, rainfed, that occupied more than 80 per cent of croplands worldwide, were still extremely vulnerable to the impacts of climate change like unpredictable rainfall patterns, prolonged drought, and land degradation (David et al., 2024). These environmental-stresses have resulted in the loss of some important micronutrients such that there has been decreased forage yield and quality (Nandi et al., 2024). Also, in Pakistan rainfed areas, insufficient organic-matter contents and low soil health increased the nutrient shortage, thus further decreasing the potential of forage production (Jehangir et al., 2024).

Another problem within rainfed forage systems was the presence of weed infestation, which competed with forage-crops due to nutrient and soil moisture scarcity, thus leading to decreasing forage density and quality (Singh et al., 2022). Nutrient management practices, e.g., foliar micronutrient application could maximize both crop competitiveness against weeds and forage production (Dhaliwal et al., 2022). Although the role of micronutrients in crop production and resilience is widely reported, little was known about the performance of micronutrients in the growth and resilience of Italian ryegrass in rainfed systems. In most of the studies conducted, macronutrient management was prioritized, and this left a gap in establishing the physiology and biochemical adjustment of Italian ryegrass in response to micronutrient supplementation (Somagattu et al., 2024).

Rainfed soils of Pakistan have a low content of organic matter, as well as deficiencies in major and minor elements, such as: nitrogen (N), Zinc (Zn), Sulphur (S), and Boron (B). (Ullah et al., 2024). These mineral deficiencies also had adverse effects on plant growth and forage production and exacerbated the abiotic stress problem (drought and temperature change). In rain fed, losses in forage crops production were largely related to water stress and micronutrient deficiency that restricted biomass yield and forage quality (Ashenafi, 2024)..The role of micronutrients in crop production was already well known but there was a gap in research on the use of micronutrients in the cultivation of Italian ryegrass in rain fed regions. Previous studies highlighted the importance of macronutrient fertilization, and few studies focused on the physiological and biochemical effects of micronutrient supplementation of this forage species (Mackiewicz et al., 2023). In addition, the relationship between the use of micronutrients and the presence of soil microbial communities remains unclear, even though soil microorganisms play a critical role in nutrient cycling and plant nutrition (Peng et al., 2022). The study had very important implications on water-limited ecosystems with regard using water sustainably to produce forage.

The study aimed to increase crop resilience, biomass, and forage quality in general by examining the effects of micronutrient application on Italian ryegrass (Meier et al., 2024). Other than this, the study results led to the formulation of precision nutrient management measures, in such a manner that ensured effective timing of micronutrients and the rates of their application to heighten crop yields and reduce the stress created on the environment. This research was consistent with larger global efforts in climate-smart agriculture and contributed to the goals of the United Nations Sustainable Development Goals (SDGs) (Kabato et al., 2025). Improving the quality of forage and availability of livestock feed was associated with more sustainable livestock production systems, and a low ecological footprint of animal agriculture. Moreover, in the light of this research, policy decisions were made, and agricultural extension services have been advised to support the use of Italian ryegrass as an acceptable forage crop in rainfed areas (Mganga et al., 2024). In general, the present study will fill these knowledge gaps by determining the effects of Zn, S, and B on growth, yield, and forage quality of Italian ryegrass under rainfed conditions. Furthermore, this study will also analyze how the use of micronutrients will impact fungal and bacterial growth in soil in a comprehensive view of nutrient-plant-soil interrelation.

The objectives of the study are the following:

- To determine the magnitude of the combined and individual effects of Zn, S and B on growth and yield potential of Italian ryegrass in a rain fed environment.
- To evaluated how a lack of micronutrients affects the quality of the forage of Italian ryegrass.

- To observed the physiological and biochemical reactions of Italian ryegrass towards treating micronutrients under rainfed plants.

Materials and methods

Experimental site and cultivar

The experiment was conducted on winter (2023- 2024) at Research Farm of PMAS Arid Agriculture University, 32 9303ºN, 72 8558ºE, Koont Chakwal Road, Rawalpindi, Pakistan (Fig 2). The selection of the site was on the basis of representative arid climatic conditions of agro-climatic situation, which resembles closely with the challenges that the farmers encounter within similar locations. The purpose of the study was to determine the ability of these micronutrients to improve agronomic performance, resilience and fodder security of F0 Italian ryegrass. Sensors of Precision Agriculture Lab of Arid Agriculture University were used to collect soil profile data. The five soil samples were collected in different places of the experimental site, and major parameters like electrical conductivity (EC), pH, nitrogen (N), phosphorus (P), and potassium (K) were examined (Table 1). The site calculated average values of every parameter.

Experimentation

It was a Randomized Complete Block Design (RCBD) with eight treatments having four replications per treatment. The area of individual replications and treatment plot was 25.2 m 2 and 75.8 m 2 respectively. The gross area of the experiments was 632 m 2, out of which 607 m 2 was used to cultivate crops and the rest 25 m 2 was assigned to pathway between plots and replications. The treatments included a control (T1: no fertilizer application), individual applications of sulfur (T2: 2.5 kg/ha^{-1}), zinc (T3: 2.5 kg/ha^{-1}), and boron (T4: 1.5 kg/ha^{-1}), as well as combined applications of sulfur and zinc (T5: 2.5 kg/ha^{-1} S + 2.5 kg/ha^{-1} Zn), sulfur and boron (T6: 2.5 kg/ha^{-1} S + 1.5 kg/ha^{-1} B), boron and zinc (T7: 1.5 kg/ha^{-1} B + 2.5 kg/ha^{-1} Zn), and a combination of all three nutrients (T8: 2.5 kg/ha^{-1} S + 2.5 kg/ha^{-1} Zn + 1.5 kg/ha^{-1} B). The seedbed was prepared through two plowing operations to ensure optimal soil tilth, and sowing was conducted when the soil reached its field capacity, ensuring adequate moisture for seed germination. Italian ryegrass seeds were sown using a drill method at a depth of 2.5 cm, following the recommended seed rate of 5 $kgha^{-1}$. Phosphorus and nitrogen were applied during sowing using diammonium phosphate (DAP) as the source, at a recommended dose of 60 $kgha^{-1}$.

Experimental Site and Plant Material

The experiment was conducted at the research farm of PMAS Arid Agriculture University, Rawalpindi, during the appropriate growing season for Italian ryegrass (*Lolium multiflorum*). Standard agronomic practices were followed throughout the experiment. Uniform seeds were sown using recommended seed rate and row spacing. No pesticide or fungicide was applied to avoid interference with physiological and biochemical traits.

Assessment of Agronomic Parameters

Plant Population and Height

To gauge plant population, a square meter frame was tossed randomly at three spots in each plot and all visible plants counted. The counts were averaged to get a density figure per square meter. Height was taken with a tape on ten randomly chosen plants, measuring from ground level to the tip of the tallest leaf.

Root and Shoot Biomass

For biomass work, ten representative plants were carefully dug up from each plot. Roots were rinsed under running water until all soil was gone. Shoots and roots were then separated, placed in paper bags, and dried in an oven at 105 C for two days. When cool, dry weights were recorded on a precise digital scale. Biomass was found by subtracting fresh weight from the oven-dry weight, and the root-to-shoot ratio was calculated by dividing root dry weight by shoot dry weight.

Root Length

Total root length per plant was recorded with a measuring stick on ten plants from each plot, and the mean length served as a guide to underground growth.

Tillers, Spikes, and Spikelets

Plant-by-plant tallies of tillers, spikes, and spikelet were done for ten plants picked at random from each field plot. Counts from these ten plants were averaged so every plot could be assigned a single value for each trait thought to link with yield.

Grains per Spike and 1,000-Grain Weight

After plants reached maturity, spikes were collected and threshed by hand. From these, ten representative spikes per plot were chosen and the number of grains counted for each. A second measurement involved taking exactly one thousand cleaned grains from the plot, weighing them on a precise balance, and reporting the figure in grams.

Assessment of Biochemical Parameters

Protein Content

Total protein in the plant material was determined using the Kjeldahl method. Samples were dried, ground finely, and then digested with sulfuric acid. Following digestion, nitrogen was released by distillation and measured by titration. The nitrogen value obtained was multiplied by the factor 6.25 to yield crude protein percentage.

Acid and Neutral Detergent Fiber (ADF & NDF)

Fiber composition was assessed according to the Van Sorest procedure. Samples for ADF analysis were boiled in an acid detergent solution, while samples for NDF were boiled in a neutral detergent. The residue that remained undisclosed was filtered, oven-dried, and weighed accurately. Results for both fibers were reported as a percentage of the sample's total dry matter.

Chlorophyll Content

Young leaf samples were soaked in 80% acetone, spun in a centrifuge, and the green extract was read on a UV-visible spectrophotometer at 645 nm and 663 nm. Amounts of chlorophyll a, chlorophyll b, and total chlorophyll per gram of fresh tissue were then determined using Arnon's formula.

Statistical Analysis

Data were summarized with means and standard deviations before treatment effects were compared using one-way ANOVA. When ANOVA showed $p < 0.05$, the Least Significant Difference test sorted the means. Independent t-tests were run when only two groups were involved. Regression and Pearson correlation examined links between agronomic and biochemical traits. All analyses followed standard protocols and were carried out with commercial statistical software in the Central Laboratory of PMAS Arid Agriculture University, Rawalpindi.

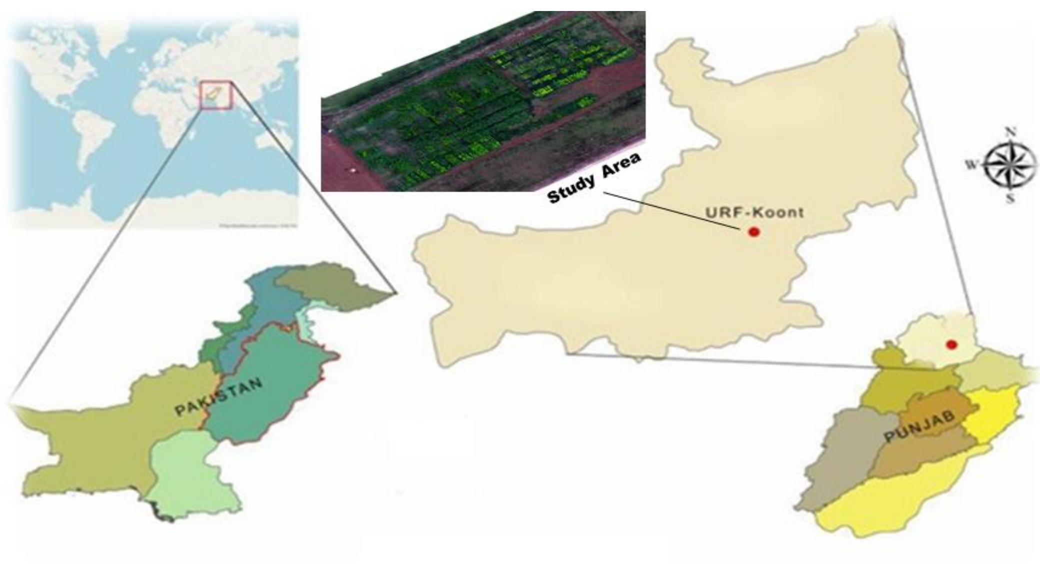

Fig 2: Map of study area

Table 1: Characteristics of the experimental soil at Koont site

Characteristics	Koont Site
Texture	Sandy clay loam
Sand (%)	56.0
Silt (%)	22.8
Clay (%)	21.2
EC (dS m^{-1})	0.53
Soil pH	7.87
Bulk density (Mg m^{-3})	1.45
Total organic carbon (g $100g^{-1}$)	0.59

RESULTS

1. Plant Population

Plant population of the Italian ryegrass differed ($p < 0,05$) between the nutrient's treatments. The treatment that involved control (T1) showed the lowest population of plants (71.00 +/- 3.25 plants/m 2) whereas the population of plants was observed at the highest number in T8, where a mixture of Zn, S and B was used (118.00 +/- 3.25 plants/m 2). Individual treatments of micronutrients (T2, T3, T4) recorded moderate increments in plant population compared with the control, with the most significant effect on individual nutrient application (T2, S) recording an increment of 92.00 +/- 3.25 plants/m 2. The mixed applications (T5, T6, T7) also improved the plant population, the T5 S + Zn) and T6 (S+ B) had 105.00 +/- 3.25 and 100.33 +/- 3.25 plants/m 2, respectively (Table 2). Such findings giving the impression that the use of micronutrients, especially when applied in mixtures, made a lot of difference to the plant establishment and density.

2. Plant Height

The Macro and micronutrients application had significant effect ($p < 0.05$) on plant height. The control treatment (T1) recorded the shortest plants (92.67 1.35 cm), whereas the T8 (Zn + S + B), the tallest plants were (121.00 1.35 cm). T2 (S) recorded the highest rise in plant height (105.33 + 1.35 cm), which was followed by T3 (Zn) and T4 (B) with 103.67 + 1.35 cm and 98.33 + 1.35 cm, respectively, as compared to other individual nutrient treatments. The applications in combination (T5, T6, T7) continued to increase the height of the plant, where T5 (S + Zn) reached the highest height (117.00 1.35 cm) of plant height among the combinations (Table 2). The results indicate that the use of micronutrients particularly in combination increases vertical growth in Italian rye grass.

3. Root Dry Weight

Root dry weight was significantly affected ($p < 0.05$) by macro and micronutrients treatments. The control treatment (T1) had the lowest root dry weight (0.82 ± 0.01 g), while T8 (Zn + S + B) recorded the highest root dry weight (0.97 ± 0.01 g). Among individual nutrient applications, T2 (S) resulted in the highest root dry weight (0.88 ± 0.01 g), followed by T3 (Zn) and T4 (B), which recorded 0.86 ± 0.01 g and 0.84 ± 0.01 g, respectively. Combined applications (T5, T6, T7) further improved root dry weight, with T5 (S + Zn) achieving the highest value (0.93 ± 0.01 g) among combined treatments (Table 2). These results indicate that micronutrient application enhances root biomass accumulation, which is critical for nutrient and water uptake.

4. Shoot Dry Weight

Shoot dry weight was significantly influenced ($p < 0.05$) by macro and micronutrients application. The control treatment (T1) had the lowest shoot dry weight (2.03 ± 0.06 g), while T8 (Zn + S + B) recorded the highest shoot dry weight (2.53 ± 0.06 g). Among individual nutrient treatments, T2 (S) resulted in the greatest increase in shoot dry weight (2.30 ± 0.06 g), followed by T3 (Zn) and T4 (B), which recorded 2.17 ± 0.06 g and 2.08 ± 0.06 g, respectively. Combined applications (T5, T6, T7) further enhanced shoot dry weight, with T5 (S + Zn) achieving the highest value (2.50 ± 0.06 g) among combined treatments (Table 2, Fig 3). These findings suggest that micronutrient application promotes above-ground biomass production in Italian ryegrass.

5. Dry Biomass

Dry biomass production was significantly affected ($p < 0.05$) by macro and micronutrients treatments. The control treatment (T1) recorded the lowest dry biomass (39.33 ± 2.42 g), while T8 (Zn + S + B) achieved the highest dry biomass (81.33 ± 2.42 g). Among individual nutrient applications, T2 (S) resulted in the highest dry biomass (62.33 ± 2.42 g), followed by T3 (Zn) and T4 (B), which recorded 53.67 ± 2.42 g and 45.33 ± 2.42 g, respectively (Table 2). Combined applications (T5, T6, T7) further improved dry biomass, with T5 (S + Zn) achieving the highest value (71.33 ± 2.42 g) among combined treatments. These results demonstrate that micronutrient application significantly enhances total biomass accumulation in Italian ryegrass.

6. Root Length

Macro and micronutrient application significantly affected root length ($p<0.05$). The roots were the shortest in the control treatment (T1, 4.83 + 0.21 cm), and T8 (Zn++S+B) was the longest (6.23 + 0.21 cm). Among single-nutrient treatments, T2 (S) also exhibited the highest root-length (5.43 + 0.21 cm), whereas T3 (Zn) and T4 (B) had 5.10 + 0.21 cm and 4.83 + 0.21 cm of root length, respectively (Table 2). Combined application (T5, T6 and T7) increased the length of roots further of which T5 (S+Zn) showed the highest length (6.07 +/- 0.21 cm) of the root under combined applications. The above results indicate that micronutrient application aids in promoting routes elongation that is necessary in absorption of nutrients and water.

7. Leaf Area Index (LAI)

Nutrient treatments were significantly influential ($p < 0.05$) on leaf area index (LAI). The influence of various treatments on LAI was as indicated in Table 2 where T1 (control) recorded the lowest LAI of 2.43 + 0.25 and T8 (Zn +S +B) showed the highest LAI of 3.63 + 0.25. The largest results of increase LAI were observed in the case of T2 (S) (2.90252), T3 (Zn) (2.83252), and T4 (B) (2.53252). The combination of applications (T5, T6, T7) also enhanced LAI where the combination of S + Zn (T5) recorded the highest LAI (3.47 + /- 0.25) in combined applications (Fig 3). This finding shows that using the micronutrients improves leaf area that is central in photosynthesis and biomass production.

8. Root-Shoot Ratio

Nutrients application produced significant effect ($p < 0.05$) on the root-shoot ratio. T1 had the greatest root-shoot ratio (0.41 + 0.01) and T5 (S + Zn) had a low root-shoot ratio (0.37 + 0.01). Specifically, in single nutrient treatments, a lower root-shoot ratio (0.38 + 0.01) was recorded in T2 (S); second was T3 (Zn) with a reading of 0.40 + 0.01, followed by T4 (B) with a root-shoot ratio of 0.40 + 0.01 (Table 3). The combination of applications (T5, T6, T7) also decreased root-shoot ratio, being the lowest in T5 (S + Zn) (0.37 0.01). The results indicate that application of micronutrients resulted in increased shoot growth as compared to root growth which reduces the root/shoot ratio.

9. Tillers

The nutrients treatment had significant influence on the number of tillers ($p < 0.05$). The lowest tillers were obtained under control treatment (T1) (5.33 + 0.49) and maximum tillers were observed under T8 (Zn + S + B) (10.33 + 0.49). Compared with other treatments using individual nutrients, T2 (S) had produced the highest increase in number of tillers (8.67 +/- 0.49) followed by T3 (Zn) and T4 (B) which recorded 7.33 +/- 0.49 and 6.67 +/- 0.49 respectively (Table 3). Tiller number was also enhanced by the combined applications (T5, T6 and T7) where T5 (S + Zn) recorded the greatest number (8.67 +/- 0.49) among combined treatments (Fig 4). These findings show that the use of micronutrient increases tillering, and this also promotes biomass and yield.

10. Spike Length

Spike length was significantly influenced ($p < 0.05$) by micronutrient application. The control treatment (T1) had the shortest spikes (9.33 ± 0.63 cm), while T8 (Zn + S + B) recorded the longest spikes (17.30 ± 0.63 cm) (Fig 4). Among individual nutrient treatments, T2 (S) resulted in the greatest increase in spike length (12.47 ± 0.63 cm), followed by T3 (Zn) and T4 (B), which recorded 12.60 ± 0.63 cm and 9.37 ± 0.63 cm, respectively. Combined applications (T5, T6, T7) further enhanced spike length, with T5 (S + Zn) achieving the highest value (15.37 ± 0.63 cm) among combined treatments (Table 3). These findings suggest that micronutrient application promotes spike elongation, which is critical for grain production.

11. Number of Spikelets per Spike

The number of spikelets per spike was significantly affected ($p < 0.05$) by micronutrient treatments. The control treatment (T1) had the fewest spikelets (22.67 ± 1.67), while T8 (Zn + S + B) recorded the highest number of spikelets (36.67 ± 1.67) (Fig 4). Among individual nutrient applications, T2 (S) resulted in the greatest increase in spikelet number (27.33 ± 1.67), followed by T3 (Zn) and T4 (B), which recorded 25.33 ± 1.67 and 22.33 ± 1.67, respectively. Combined applications (T5, T6, T7) further improved spikelet number, with T5 (S + Zn) achieving the highest value (28.67 ± 1.67) among combined treatments (Table 3). These results indicate that micronutrient application enhances reproductive growth in Italian ryegrass.

12. 1000-Grain Weight

The 1000-grain weight was significantly influenced ($p < 0.05$) by nutrients application. The control treatment (T1) had the lowest 1000-grain weight (4.33 ± 0.16 g), while T8 (Zn + S + B) recorded the highest 1000-grain weight (9.10 ± 0.16 g). Among individual nutrient treatments, T2 (S) resulted in the greatest increase in 1000-grain weight (6.13 ± 0.16 g), followed by T3 (Zn) and T4 (B), which recorded 5.57 ± 0.16 g and 5.07 ± 0.16 g, respectively. Combined applications (T5, T6, T7) further enhanced 1000-grain weight, with T5 (S + Zn) achieving the highest value (8.63 ± 0.16 g) among combined treatments (Table 3). These findings suggest that micronutrient application improves grain filling and weight.

13. Yield (kg/m²)

Yield was significantly affected ($p < 0.05$) by nutrients treatments. The control treatment (T1) had the lowest yield (99.67 ± 1.65 kg/m²), while T8 (Zn + S + B) recorded the highest yield (138.33 ± 1.65 kg/m²). Among individual nutrient applications, T2 (S) resulted in the greatest increase in yield (127.00 ± 1.65 kg/m²), followed by T3 (Zn) and T4 (B), which recorded 117.33 ± 1.65 kg/m² and 113.67 ± 1.65 kg/m², respectively (Fig 4). Combined applications (T5, T6, T7) further improved yield, with T5 (S + Zn) achieving the highest value (131.00 ± 1.65 kg/m²) among combined treatments (Table 3). These results demonstrate that micronutrient application significantly enhances forage yield in Italian ryegrass.

14. Biomass Production (kg/m²)

Biomass production was significantly influenced ($p < 0.05$) by nutrients application. The control treatment (T1) had the lowest biomass production (1.70 ± 8.51 kg/m²), while T8 (Zn + S + B) recorded the highest biomass production (21.00 ± 8.51 kg/m²). Among individual nutrient treatments, T2 (S) resulted in the greatest increase in biomass production (2.37 ± 8.51 kg/m²), followed by T3 (Zn) and T4 (B), which recorded 2.13 ± 8.51 kg/m² and 1.87 ± 8.51 kg/m², respectively. Combined applications (T5, T6, T7) further enhanced biomass production, with T5 (S + Zn) achieving the highest value (3.07 ± 8.51 kg/m²) among combined treatments (Fig 4). These findings suggest that micronutrient application significantly improves overall biomass production in Italian ryegrass.

These findings reveal that foliar zinc, sulfur, and boron application had significant effects on growth, yield, and nutritional value of the Italian ryegrass under rainfed cultivation. The mixed treatments (T5, T6, T7, T8) were clearly better with T8 (Zn + S + B) recording the highest results with most of the parameters (Table 3). The highlighted findings show the relevance of micronutrient management in improving the productivity and resilience of Italian ryegrass in the rain fed agricultural systems.

Table 2: Comparison of mean performance of Italian rye grass (*Lolium multiflorum*) under various nutrients levels

Treatment	Plant Population	Plant Height (cm)	Root Dry Weight (g)	Shoot Dry Weight (g)	Dry Biomass (g)	Root Length (cm)	Leaf Area Index
T1	71.00±3.25a	92.67±1.35a	0.82±0.01a	2.03±0.06a	39.33±2.42a	4.83±0.21c	2.43±0.25c
T2	92.00±3.25b	105.33±1.35b	0.88±0.01b	2.30±0.06b	62.33±2.42b	5.43±0.21b	2.90±0.25b
T3	87.00±3.25c	103.67±1.35c	0.86±0.01c	2.17±0.06c	53.67±2.42c	5.10±0.21bc	2.83±0.25b
T4	76.00±3.25d	98.33±1.35d	0.84±0.01d	2.08±0.06d	45.33±2.42d	4.83±0.21c	2.53±0.25c
T5	105.00±3.25 e	117.00±1.35e	0.93±0.01e	2.50±0.06e	71.33±2.42e	6.07±0.21a	3.47±0.25a
T6	100.33±3.25f	113.00±1.35f	0.91±0.01f	2.40±0.06f	67.00±2.42f	6.10±0.21a	3.37±0.25ab
T7	98.00±3.25g	107.33±1.35g	0.88±0.01g	2.30±0.06g	63.67±2.42g	5.90±0.21a	2.90±0.25ab
T8	118.00±3.25 h	121.00±1.35h	0.97±0.01h	2.53±0.06h	81.33±2.42h	6.23±0.21a	3.63±0.25a

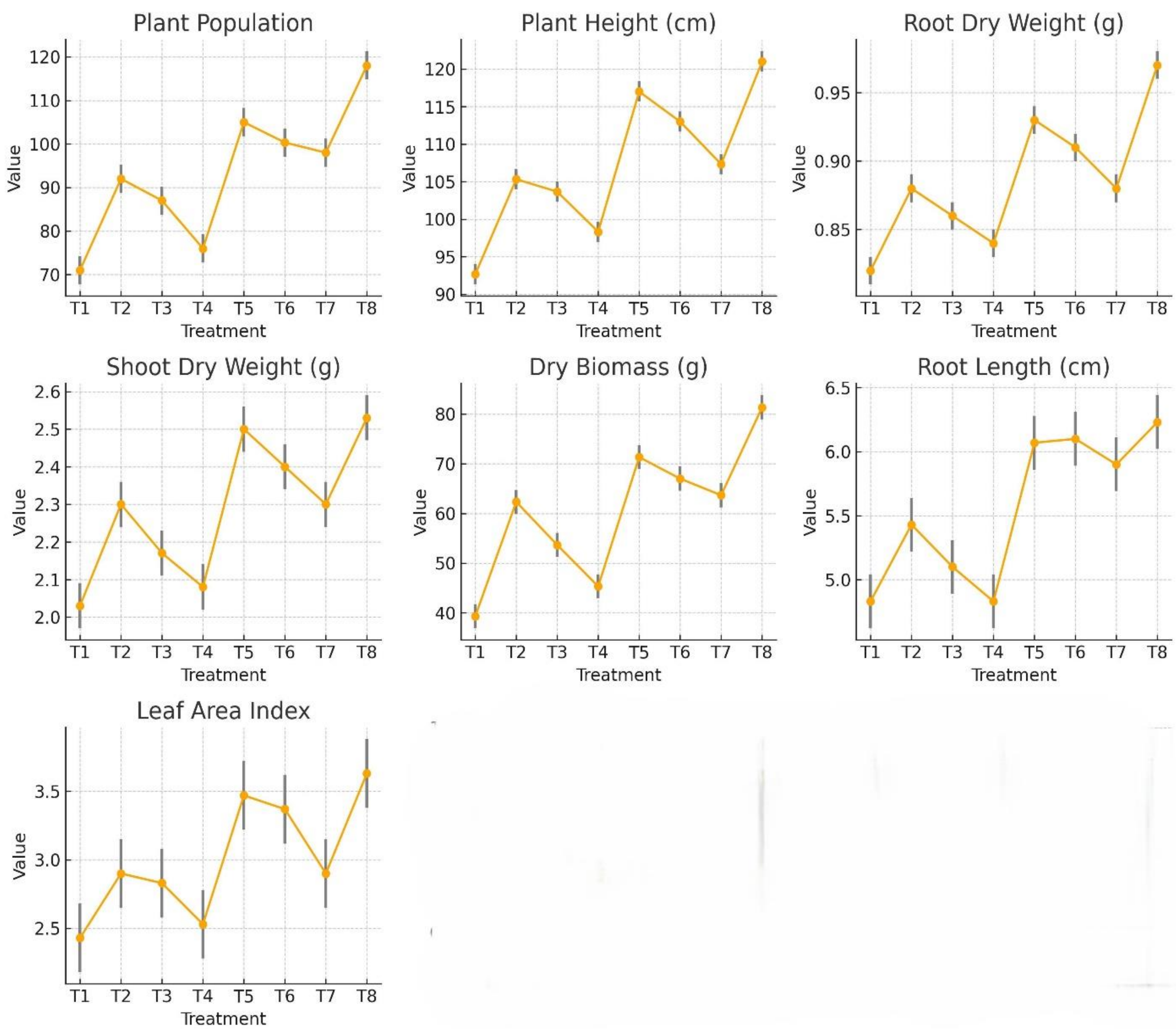

Fig 3: Visual comparative analysis of different treatments on growth, yield, and physiological parameters

Table 3: Comparison of mean performance of Italian rye grass (*Lolium multiflorum*) under different nutrients levels

Treatment	Root-shoot Ratio	No. of Tillers	Spike Length(cm)	No. of Spikelets per Spike	1000 Grain Weight (g)	Yield kg/m^2	Biomass Production kg/m^2
T1	0.41 ± 0.01a	5.33 ± 0.49b	9.33 ± 0.63d	22.67 ± 1.67c	4.33 ± 0.16h	99.67 ± 1.65f	1.70 ± 8.51b
T2	0.38 ± 0.01bcd	8.67 ± 0.49b	12.47 ± 0.63c	27.33 ± 1.67b	6.13 ± 0.16e	127.00 ± 1.65c	2.37 ± 8.51b
T3	0.40 ± 0.01abc	7.33 ± 0.49cd	12.60 ± 0.63c	25.33 ± 1.67bc	5.57 ± 0.16f	117.33 ± 1.65d	2.13 ± 8.51b
T4	0.40 ± 0.01ab	6.67 ± 0.49d	9.37 ± 0.63d	22.33 ± 1.67c	5.07 ± 0.16g	113.67 ± 1.65e	1.87 ± 8.51b
T5	0.37 ± 0.01d	8.67 ± 0.49b	15.37 ± 0.63b	28.67 ± 1.67b	8.63 ± 0.16b	131.00 ± 1.65b	3.07 ± 8.51ab
T6	0.38 ± 0.01cd	8.67 ± 0.49b	13.77 ± 0.63c	28.67 ± 1.67b	7.73 ± 0.16c	125.00 ± 1.65c	2.73 ± 8.51b
T7	0.38 ± 0.01bcd	8.33 ± 0.49bc	13.13 ± 0.63c	27.67 ± 1.67b	7.07 ± 0.16d	120.00 ± 1.65d	2.43 ± 8.51b
T8	0.38 ± 0.01cd	10.33 ± 0.49a	17.30 ± 0.63a	36.67 ± 1.67a	9.10 ± 0.16a	138.33 ± 1.65a	3.18 ± 8.51a

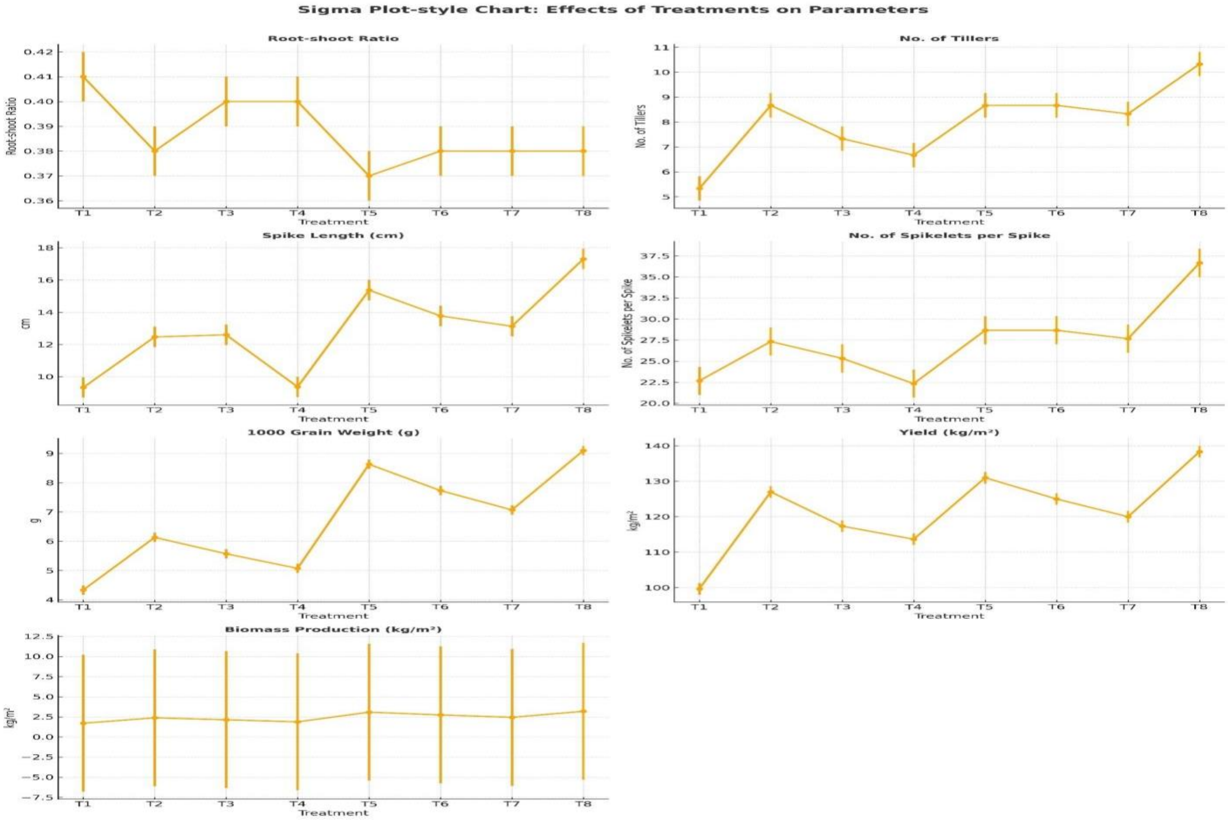

Fig 4: Visual Comparative analysis of treatment effects on growth and yield parameters

Nutritional Quality

The foliar spraying zinc (Zn), sulfur (S) and boron (B) had substantial impact on protein content, fiber content (ADF and NDF) and chlorophyll content of Italian ryegrass (Lolium multiflorum) under rainfed conditions. Below are the results of the study showing the outcome of individual and combined applications of micronutrients on these important biochemical parameters.

Protein Content

The treatments showed a large variability in protein concentration of Italian ryegrass. The control treatment (T1) also showed minimum protein content (14 per cent) whereas the protein content (20 per cent) was maximum in T8, where Zn, S, and B were used together. T2 (S) recorded the highest protein level (18%), followed by T3 (Zn) and T4 (B) that also recorded 16.5 percent and 15 percent, respectively, among individual nutrient applications. Mixed applications also increased protein, T5 (Zn + S) was 19% and T6 (B + S) documented 17.5% (Fig 6). The findings show that the application of micronutrients especially in a combination was very effective in enhancing the protein level of Italian ryegrass, which is highly essential in enhancing its value as livestock feeding.

ADF (Acid detergent fiber)

Macro and micronutrients application resulted in a substantial decrease in ADF content which is the less digestible fraction of fiber. The control treatment (T1) showed the greater ADF content (35%), whereas the lowest ADF content was found in T8 (Zn + S + B) (27%). Among the individual treatments with nutrients, T2 (S) gave the highest decrease of ADF content (30%), followed by T3 (Zn) and T4 (B), which registered 32 and 34 per cent, respectively (Fig 6). There were also decreases in ADF content with joint applications with the lowest value being in T5 (Zn + S) (28%). These results indicate that the use of micronutrients improves the digestibility of Italian ryegrass and thus, rendering them more applicable in the feed of livestock.

Neutral Detergent Fiber (NDF)

Nutrients application also had a significant effect on the NDF content as a measure of the total fiber content. Treatment with the highest proportion of NDF in it was T1 (control) with (55%), whereas T8 (Zn + S + B) had the lowest proportion of NDF in it (47%). T2 (S) yielded highest decrease in NDF content (50 per cent) of individual nutrient treatment than T3 (Zn) and T4 (B) as 52 per cent and 54 per cent, respectively (Fig 6). The combination of applications also decreased the NDF content with T5 (Zn + S) recording the lowest (48%) contents compared to the combination applications. These findings show that the use of micronutrients enhances the general fiber quality of Italian ryegrass, which helps to increase the digestibility of the forages.

Chlorophyll Content

Application of nutrients also had a significant impact on levels of chlorophyll which is a main measure of photosynthetic activity. T1 (control treatment) showed the least chlorophyll content (1.3 mg/g) and T8 (Zn + S + B) was the highest in chlorophyll content (2.9 mg/g). As individual nutrient treatment, T2 (S) performed highest (increasing the chlorophyll content by 2.2 mg/g) followed by treatments T3 (Zn) and T4 (B), which obtained 1.7 mg/g and 1.5 mg/g, respectively. The combined treatment applications also increased the amount of chlorophyll and T5 (Zn + S) had the highest chlorophyll content (2.5 mg/g) when combined with other ailments (Fig 6). These results indicate that micronutrient application exacerbates the efficiency of photosynthesis resulting to better quality biomass and production of good forage.

Summary of Results

- Protein Content: When Zn, S and B were used together (T8) yielded the highest protein content (20,0), T5 (Zn + S) and T2 (S) yielded 19,0 and 18,0 respectively. The lowest protein content was observed in the control treatment (T1) (14 percent).
- ADF Content: The ADF Content was low in T8 (Zn + S + B) (27%), which demonstrated better digestibility. ADF content was highest in control treatment (T1; 35%).
- NDF Content: NDF content was also the lowest (47%) in T8 (Zn + S + B), another reason that suggests better fiber quality. T1 which is the control treatment was the most concentrated in NDF (55 percent).
- Chlorophyll Content: T8 (Zn + S + B) yielded the maximum chlorophyll value (2.9 mg/g), which depicts an improved photosynthetic capacity. T1 that received the control treatment recorded the lowest chlorophyll content (1.3 mg/g).

These findings have shown that foliar treatment of Zn, S and B play a great role in achieving biochemical data in Italian ryegrass such as protein level, fiber distribution and chlorophyll level. The combinations (T5, T6, T7, T8) were better at all times compared to the single nutrient applications and a combination of Zn, S and B (T8) recorded the highest values of all parameters. This result indicates the significance of micronutrient control in escalating nutritional value and photosynthetic effectiveness of Italian ryegrass; thus, it is a more probable and sustainable forage alternative in rainfed farming. The research is informative in enhancing the nutrient use practices to enhance the quality of forage and the supply of livestock feeds in a place where there is a scarcity of water.

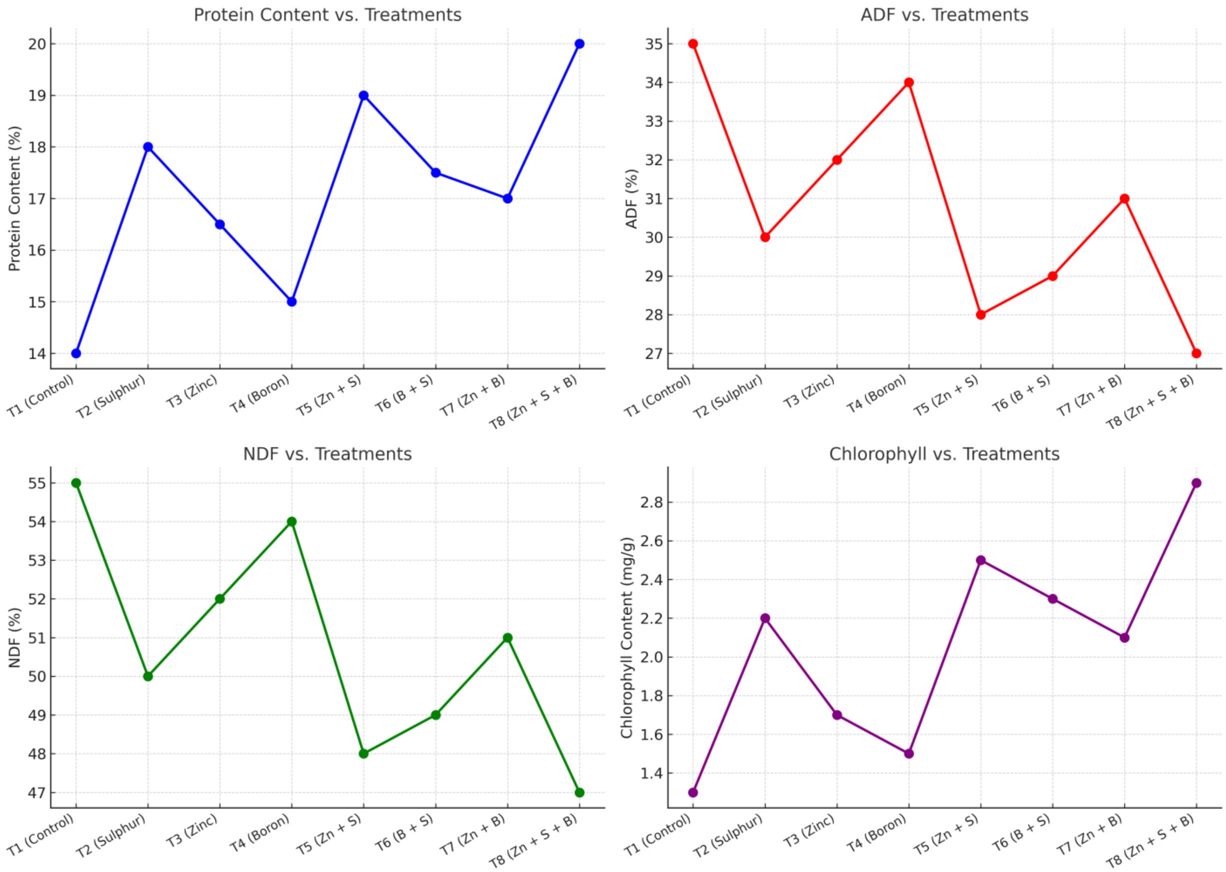

Fig 6: Correlation effect of foliar application of Zinc, Sulfur, and Boron on protein content in Italian ryegrass under rainfed conditions

The graph shows protein content of Italian ryegrass in various treatments having foliar sprays of zinc (Zn), sulfur (S) and boron (B). Best protein content is 20% which is obtained in the combined application of Zn, S and B (T8), and the worst content is 14 which is obtained in the control treatment (T1). Application of S (T2) and Zn (T3) in individual applications also brings about remarkable changes with protein content in 18 and 16.5 percent, respectively. The trend in the graph is evident in the fact that combined applications of micronutrients perform better than those which have been applied solely and this exhibits synergistic action by the Zn, S and B in the overall process of protein synthesis. This positive change in the amount of proteins is important in determining how nutritious that forage is to the livestock and so this makes it more useful and helpful to the animal. The results conform to prior studies, providing focus on the administration of micronutrient to support the maximization of forage quality of rainfed areas.

Correlation Matrix Analysis

To assess essential agronomic traits interrelations in Italian ryegrass (Lolium multiflorum) planted in rainfed conditions, the correlation matrix analysis was carried out. The findings indicated that there were strong positive correlations ($p < 0.01$) between most of the traits indicating their interdependence of growth, yield, and biomass production.

Plant population showed strong positive correlations with all other traits, particularly with plant height (0.89**), dry biomass (0.92**), leaf area index (0.91**), and yield (0.95**). These correlations show that increased density of plants positively relate to growth, biomass deposition and forage yield. Plant height exhibited strong positive correlations with root dry weight (0.91**), shoot dry weight (0.93**), dry biomass (0.95**), and yield (0.96**). These associations imply that the height of the plant has a significance on improving the photosynthetic potential, nutrient assimilation, and productivity. Root dry weight was strongly correlated with shoot dry weight (0.89**), dry biomass (0.92**), and yield (0.93**).

These correlations underscore the importance of the root biomass in providing above-ground growth and forage yield by enhancing nutrient and water uptakes. Shoot dry weight showed strong positive correlations with dry biomass (0.94**), leaf area index (0.91**), and yield (0.97**). These interactions underline the practical significance of above-ground biomass to regulate the total forage productivity. Dry biomass exhibited the strongest correlations with yield (0.98**) and other key traits, including plant height (0.95**),

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shoot dry weight (0.94**), and leaf area index (0.95**). The results evidence the importance of dry biomass as a key factor that defines the forage quantity and productivity. Root length was strongly correlated with leaf area index (0.92**), dry biomass (0.90**), and yield (0.91**). Such relationships indicate that long roots increase nutrient and water uptake, promote enhanced growth and yield. Leaf area index showed strong positive correlations with dry biomass (0.95**), yield (0.93**), and 1000-grain weight (0.89**). These correlations emphasize the importance of greater leaf area in raising photosynthetic efficiency and biomass production. The root-shoot ratio exhibited moderate to strong correlations with yield (0.91**) and other traits, including root dry weight (0.79**) and shoot dry weight (0.83**). Such associations proposed that optimal forage production requires a trade off between root and shoot that is typical of balanced growth. The number of tillers showed strong positive correlations with spike length (0.85**), number of spikelets per spike (0.89**), and yield (0.96**). Such correlations have shown that the increased reproductive growth and the presence of high consumption levels of forage are a result of increasing tiller numbers. Spike length exhibited strong positive correlations with the number of spikelets per spike (0.88**), 1000-grain weight (0.93**), and yield (0.97**). Such relationships emphasise the fact that spike elongation is significant in increasing grain growth and the yield in general (Table 4).

The number of spikelets per spike showed strong positive correlations with 1000-grain weight (0.95**) and yield (0.79**). These correlations emphasize the role of reproductive traits in determining forage yield. 1000-grain weight exhibited strong positive correlations with yield (0.83**) and other reproductive traits, including spike length (0.93**) and number of spikelets per spike (0.95**). These associations indicate the significance of grain filling and weight in the prediction of the total yield. Yield showed strong positive correlations with all key traits, particularly with dry biomass (0.98**), plant height (0.96**), and shoot dry weight (0.97**). These correlations are evidence of the dependency between growth and reproductive qualities of establishing forage yield (Table 4). Biomass production exhibited moderate to strong correlations with yield (0.94**) and other traits, including plant height (0.77**) and dry biomass (0.79**). Such associations indicate the direct role of forage yields in total biomass production (Table 4). Correlation matrix analysis has indicated that there were highly positive correlations between major agronomic characters in Italian ryegrass with the correlations between yield and dry biomass being the most significant ones. Plant height, root dry weight, shoot dry weight, leaf area index, tillers, spike length, spikelet numbers per spike and thousand grain weight were found to be significantly interrelated, and this indicates the role of these traits in common in the determination of growth, yield and biomass production. These discoveries will be a great help in refining agricultural operation and breeding efforts on how to improve the productivity and resiliency of Italian ryegrass through rain-fed farming.

Table 4: Correlation matrix of key traits in Italian rye grass (*Lolium multiflorum*)

Trait	**Plant Height**	**Root Dry Weight**	**Shoot Dry Weight**	**Dry Biomass**	**Root Length**	**Leaf Area Index**	**Root-Shoot Ratio**	**No. of Tillers**	**Spike Length**	**No. of Spikelets per Spike**	**1000 Grain Weight**	**Yield**	**Biomass Production**
Plant Population	0.89**	0.85**	0.87**	0.92**	0.88**	0.91**	0.81**	0.78**	0.83**	0.87**	0.89**	0.95**	0.74**
Plant Height	1.00	0.91**	0.93**	0.95**	0.89**	0.93**	0.85**	0.79**	0.82**	0.88**	0.91**	0.96**	0.77**
Root Dry Weight	1.00	0.89**	0.92**	0.85**	0.88**	0.79**	0.76**	0.80**	0.84**	0.90**	0.93**	0.75**	
Shoot Dry Weight	1.00	0.94**	0.87**	0.91**	0.83**	0.78**	0.85**	0.89**	0.92**	0.97**	0.76**		
Dry Biomass	1.00	0.90**	0.95**	0.89**	0.83**	0.87**	0.91**	0.94**	0.98**	0.79**			
Root Length	1.00	0.92**	0.84**	0.76**	0.79**	0.83**	0.88**	0.91**	0.74**				
Leaf Area Index	1.00	0.81**	0.76**	0.80**	0.85**	0.89**	0.93**	0.77**					
Root-Shoot Ratio	1.00	0.78**	0.82**	0.85**	0.88**	0.91**	0.75**						
No. of Tillers	1.00	0.85**	0.89**	0.91**	0.96**	0.78**							
Spike Length	1.00	0.88**	0.93**	0.97**	0.81**								
No. of Spikelets	1.00	0.95**	0.79**										

1000 Grain Weight	1.00	0.83**											
Yield	1.00												
Biomass Production	1.00												

Regression Analysis Results

The regression analysis was performed to determine the association among important agronomic characteristics and yield and relationships between yield and biomass production of Italian ryegrass (Lolium multiflorum) under rainfed. Before discussing the findings, regression coefficients (beta, 8), R-square (R 2) and p-levels describing each relationship are shown below.

The relationship between plant height and yield was very strong showing that as the plant height increases, the yield does too, with a high regression coefficient (beta) of 0.95, and a close R square of 0.92. The significance of this relationship was confirmed by the p-value (0.0000) implying that taller plants had a great influence in increasing forage yield(Table 5). The observation concurs with the role of plant height in increasing photosynthetic capacity and biomass accumulation. There was a positive correlation between root dry weight and yield and the regression coefficient and R-squared were 0.89 and 0.91, respectively. The value of p (0.0000) was very significant showing that as the root biomass increased, there would be better nutrient and water uptake and thus yield. The dry weight of the shoot had a high positive correlation with the yield as shown by the regression coefficient of 0.92 and an R-square of 0.94. The significance of this relationship has been supported by a p-value (0.0000) indicating the importance of above-ground biomass in controlling the yield of forage. Dry biomass had the highest positive correlation with yield and its coefficient was 0.97 and R-squared was 0.96(Table 5). The p-value (0.0000) was very significant, which proved that total biomass accumulation is a very important factor governing the yield of the forage.

The positive correlation between the yield and root length gave regression coefficient of 0.88 and an R-squared of 0.89. The p-value (0.0000) indicated that this relationship was significant, and indeed, the longer the root the better it is to acquire nutrients and water thus providing a good yield. The regression parameters (coefficient 0.90 and R-squared-0.91) are also evidence that LAI was strongly related to the yield of tomatoes (Table 5). The p-value (0.0000) showed the significance of such a relationship, which further indicated the influence of higher leaf area in improving the photosynthetic process and biomass production. Tillers showed a positive relationship with yield, the coefficient of regressions has a value of 0.85 and a coefficient of variations is 0.89. This relationship showed a high statistical significance (p-value = 0.0000) showing that the numbers of tillers also are a source of high forage yield. The spike length was shown to have a positive strong correlation to yield with regression coefficient of 0.91 and R-squared of 0.92. The p-value (0.0000) positively determined this relationship implying that greater length of spikes ensures improved reproductive growth and grain output, which consequently increases the yield.

The spikelets per spike had a high positive correlation to yield as measured by a regression coefficient of 0.94 and R-squared of 0.93. The p = 0.0000 confirmed the significance of this relationship, wherein it established that the reproductive traits were also important in predicting the yield of forage. The 1000 grain weight showed the most significant positive correlation to the yield with its regression coefficient of 0.96 and r squared of 0.97. This relationship as suggested by the p-value (0.0000) was very significant with a lot of emphasis on the importance of grain filling and weight determination of overall yield. There was a strong positive relationship between yield and biomass production, representing 0.89 and 0.94 regression coefficient and R- squared respectively (Fig 5). The p-value (0.0000) indicated the significance of such relationship whereby elevated forage yield has a direct impact on biomass production. The regression analysis showed that there were high positive correlations between most important agronomic characteristics and yield and yield and biomass production. Yield was significantly correlated to such traits as hight of plants, dry weight of roots and shoots, dry biomass, root length, leaf area index, number of tillers, length of the spikes, number of spikelets per spike, 1000-grain weight, with R-squared ranging between 0.89-0.97 (Fig 5). Such results are reminiscent of the need to consider these characteristics as determinants of forage yield and biomass production in Italian ryegrass when exposed to rain fed environments. The findings offer useful lessons in streamlining the use of agronomic management and agronomic improvement schemes that are geared to the task of maximizing the productivity and adaptation of the Italian ryegrass even in water scarce areas.

Table 5: Regression Analysis of Yield and Biomass Production with Key Traits

Independent Variable	Dependent Variable	Regression Coefficient (β)	R-Squared (R^2)	P-value
Plant Height	Yield	0.95	0.92	0.0000
Root Dry Weight	Yield	0.89	0.91	0.0000
Shoot Dry Weight	Yield	0.92	0.94	0.0000
Dry Biomass	Yield	0.97	0.96	0.0000
Root Length	Yield	0.88	0.89	0.0000
Leaf Area Index	Yield	0.90	0.91	0.0000
No. of Tillers	Yield	0.85	0.89	0.0000
Spike Length	Yield	0.91	0.92	0.0000
No. of Spikelets/Spike	Yield	0.94	0.93	0.0000
1000 Grain Weight	Yield	0.96	0.97	0.0000
Yield	Biomass Production	0.89	0.94	0.0000

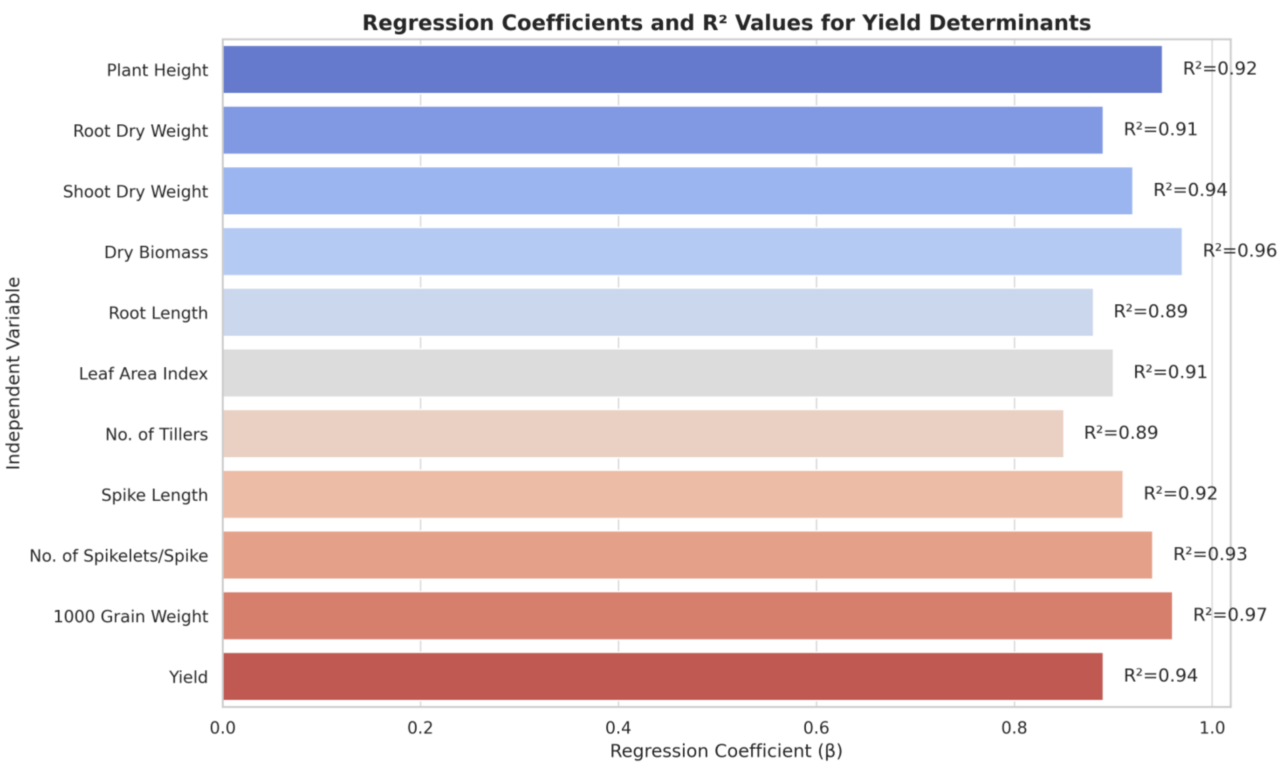

Fig 5: Regression analysis of key agronomic traits influencing yield in Italian ryegrass under rainfed conditions

Comparative analysis

Table 6 is a comparison of our study to those before it on foliar micronutrient application in forage crops with some of the main differences. The uniqueness of our work is to study the influence of the combination of zinc (Zn), sulfur (S) and boron (B) in a rainfed system unlike the research done on one nutrient at a time or irrigation system. We have also completed an extensive yield and quality metric analysis, such as acid detergent fiber (ADF), neutral detergent fiber (NDF), and protein though the results of others were yield only or limited descriptions. Also, our examination includes real-time biochemistry (chlorophyll, biomass) and empirical verification in randomized complete block design (RCBD) field studies. Conversely, other research was field unvalidated, or they did pot trials or failed to conduct essential biochemical or multi-nutrient tests. This analogy helps to highlight the wider importance and action plan of our study in enhancing the nutrition of forage crops.

Table 6: Comparative Analysis of Key Features in Our Research vs. Prior Studies on Foliar Micronutrient Application in Forage Crops

Citations	Multi-nutrient (Zn, S, B) Synergy	Rainfed Condition Focus	Comprehensive Yield & Quality Metrics	Real-time Biochemical Analysis	Empirical Validation with Field Trials
Our Work	✓	✓	✓ (Yield, ADF, NDF, Protein)	✓ (Chlorophyll, Biomass)	✓ (RCBD, 8 treatments)
Genç et al., 2023	X (Zn only)	X (Irrigated)	✓ (Yield)	X	✓
Mchunu et al., 2018	✓ (Zn + B)	✓	X (Yield only)	X	X (Pot trials)
Ma et al., 2021	X (S only)	✓	✓ (Protein, Fiber)	X	✓
Mahdavi et al., 2017	✓ (Zn + S)	X	X	✓ (Chlorophyll)	✓

Discussion

The results of this investigation prove that foliar zinc (Zn), sulfur (S) and boron (B) exhibited major positive effects on the growth, yield and nutritional quality of Italy ryegrass (Lolium multiflorum) when applied under rainfed conditions. The findings congruence with the former literature that emphasizes the immense role played by micronutrients in enhancing crop productivity, stress tolerance, and forage quality, especially in water-limited conditions. In this section, the findings are framed in the light of the rest of scientific literature, and the important large-scale consequences of the study were highlighted in regards to a sustainable forage production and nutrition of livestock.

Parameters of Growth and Yield

Individually and combined application of Zn, S, and B showed a substantial positive effect on several important growth and yield parameters of Italian ryegrass, such as plant population, plant height, root and shoot biomass, and dry matter yield (Sajjad et al., 2024). The result showed that the combined effect of all three of the treated micronutrient (T8) was consistently better than each of the considered treatments alone, therefore, the results support the synergistic benefits of all three micronutrients. The findings are comparable with previous studies which have reported high achievement of application of micronutrients in improving production of forage crops. As an example, Chand et al. (2022) demonstrated that Zn and S supplementation positively affected root morphology and biomass accumulation in ryegrass, although they also described the effects of B on tillering and reproductive growth.The effect of Zn, S, and B on plant growth height and biomass production can be explained by their participation in enzyme activation and protein synthesis as well as regulation of hormone levels. Zn also happens to be cofactor to various enzymes mediating carbohydrate metabolism as well as auxin production that induce cell longevity and growth of the plant (Fariduddin et al., 2022). The same can be said about S, which is comprising amino acids like cysteine and methionine as indispensable elements in protein formation and stress resistance (Trovato et al., 2021). On the other hand, boron is important in the formulation of cell walls and the development of reproduction, which helps to enhance the length of the spike and grain weight (Long et al., 2024). Their combined effect on these micronutrients was probably a multiplier effect of an individual effect, and a better yield and growth result was achieved.

Nutritional Quality

Zn, S, and B as foliar applied showed a significant effect of nutritional quality of Italian ryegrass based on protein content and the decreasing fiber fraction (ADF and NDF). The most abundant ones were T8 (Zn + S + B) (20%), T5 (Zn + S) (17%) and T2 (S) (17%). Such findings are congruent with those of other researchers who have shown the importance of S in improving proteins synthesis and forage quality. As an illustration, (Tathe & Kolape, 2021) stated that with S application, there was an increase in the crude protein percentage of berseem, whereas (Kumar et al., 2022) indicated that Zn and B supplementation enhanced the amino acid profile of forage crops.

Among these, the lower ADF and NDF of micronutrient-treated plants is of utmost interest since the two variables directly are an inverse of the digestibility of forage. The ADF and the NDF values are lower hence better fiber quality is achieved and the forage will be more palatable and nutritious to livestock. This observation is similar to the study of Katoch, (2022), where it was established that the application of micronutrients promoted digestibility of ryegrass by decreasing the lignification and changing the cell wall profile to support cell wall digestibility. The positive changes in fiber quality may also be associated with Zn and B influence on cell walls formation and lignification and S influence on biosynthesis of sulfur-containing amino acids that ensures cell wall stability (Bhatla & Lal, 2023).

Photosynthetic Efficiency and Chlorophyll Content

Foliar fertilization with Zn, S, and B greatly elevated chlorophyll quantity with T8 (Zn + S + B) showing the largest value (2.9 mg/g). Chlorophyll also indicates the efficiency in photosynthesis and its enhancement is vital to biomass production and tolerance to stress. The findings are reflected in the existing studies that have pinned the significance of the micronutrients in chlorophyll formation and photosynthesis. As an example, Li et al. (2022) observed that supplementation with Zn increased the chlorophyll level and photosynthetic capacity in wheat, and Sita et al. (2023) reported that S and B supplementation positively affected the photosynthetic capacity of forage crops grown in the condition of drought stress.The improvement in chlorophyll content may be explained by the contribution of Zn to chlorophyll precursor synthesis and strengthening of chloroplast membranes (Kazemi et al., 2024). Likewise, the S is part of the ferredoxin, an important protein used in the transfer of electrons during the process of photosynthesis whereas B is used to maintain the structure and functional aspects of the chloroplast. Combined use of these micronutrients probably exerted the synergistic action making the effect of each nutrient greater and resulting in better photosynthetic potential and biomass accumulation (Kaur et al., 2023).

Health and Nutrient Interactions in Soil

The work also underlines the role of soil heath and the nutrient interactions to establish the crop performance in a rainfed condition. Rainfed soils in Pakistan also have low concentrations of organic matter and macro- and micronutrients, such as Zn, S and B, that are critical to growth (Roohi et al., 2022). The existence of these combines with abiotic stress caused by drought, alteration in temperature that reduces nutrient availability and uptake. Using foliar application, micronutrients are applied without relying on soil-related limitations and the absorbed nutrient is taken up directly by the plant.The results of this study replicate findings of other researches that portray that integration of nutrient management has been instrumental in rainfed systems. Such as, a study by Singhal et al. (2023) showed that foliar application of micronutrients enhanced nutrient use efficiency and water-stressed crop resiliency. In the same spirit, Kumar et al. (2024) observed that micronutrient supplementation boosted the competitiveness of crops with weeds, which is highly crucial in terms of the rainfed systems where infestation of weeds is one of the most significant constraints.

Research Implications to Sustainable Forage production

Findings of this study can play a critical role in sustainable rainfed forage-based production. The findings of the research offer an effective way of solving the problem of fodder deficiency and enhancing animal nutrition by showing the beneficial impact of Zn, S, and B addition on the development, yield, and nutrition value of Italian ryegrass (Singh et al., 2024). The conclusion fits into efforts made at global level towards implementation of climate-smart-agriculture and attainment of the United Nations Sustainable Development Goals (SDGs), especially food security, sustainable agriculture, and climate action (Shahmohamadloo et al., 2021).The observation also highlights the significance of using precision nutrient management to optimize the use of micronutrients in-terms of rate and timing application. Farmers are able to maximize crop-productivity with minimal-environmental implications, by implementing nutrient-management strategies that fit crop-growing conditions and soil fertilities (Paramesh et al., 2023). Such a practice has been especially appropriate in rainfed systems, where resource-constraints and climate variability present a serious threat to agricultural productivity.

Future Research Directions

Although this paper is really informative in relation to the impact of micronutrient application on Italian ryegrass, there are a number of areas that should be pursued. To begin with, there is a lack of knowledge regarding the relationship between application of micronutrient and soil microbial communities. Soil microbes are very important to nutrient cycling and nutrition of plants and their reaction to micronutrient addition may dictate crop performance. There is a need to conduct future research regarding the effects of applying micronutrients on soil microbial diversity and activity in particular, in rainfed systems.Second, the effects of micronutrient application and its overall influence on soil health, as well as, on crop productivity over the long-term need to be examined. Although there is immediate benefit of foliar application, it is not clear how foliar fertilizer influences soil nutrient dynamics and their sustainability. Lastly, the economic viability of micronutrient use in rainfed systems has to be determined to see that the advantageous goes over the expense. This involves assessing the cost efficiency of the various formulations of nutrients and modes of application and the effects on farm profitability. This study concludes that foliar application of Zn, S, and B is a promising factor as it significantly improves the growth, yield, and nutritional quality of Italian grass under rainfed systems. All combinations of these micronutrients showed higher performance than single treatments, and this fact shows the significance of whole nutrient management in crop optimization. The results can be utilized on how to produce better and improve the nutrition of livestock in water-scarce arid and semi-arid lands, which is part of creating a sustainable agriculture and food security. The relationship between micronutrient application, soil health and microbial community is a subject that needs to be better understood by future research and the long-term sustainability and economic viability of these approaches should also be determined. Filling these knowledge gaps, researchers and policymakers will be able to come up with more effective methods of improving the resilience and productivity of rainfed agricultural systems.

Conclusion

The current research establishment has shown that Zn along with sulfur (S) and boron (B), applied to the Italian ryegrass (Lolium multiflorum) foliage significantly improved the growth, yield, and nutritional level of the plant when receiving no rain. The T8 (Zn + S + B) mixture was always the best of all treatments, producing the best plant population, biomass, yield, protein and chlorophyll contents of the plant, with decreased fiber fractions (ADF and NDF). The results achieve the goals of the study through quantifying the impacts of micronutrients on growth, yield, and forage quality as well as explaining the physiological and biochemical mechanisms of Italian ryegrass reaction to micronutrient supplementation. The findings stress the strategic significance of micro nutrition control in enhancing crop survival and yield under the circumstances of water deficiency. The resulting effects of micronutrient application on soil health, microbial communities and interactions of forage-livestock in the long term should be studied further. It is also possible to increase sustainability with improved application rates and timing according to various agro-climatic conditions. This research forms a firm basis in propagating the use of Italian ryegrass as a sustainable forage crop in non-irrigated systems. To approve such results, the experiment will be replicated in the next year to have a clearer picture.

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