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Agronomic Biofortification of Fodder Maize (*Zea mays* L.) with Zn for Improving Herbage Productivity and Its Quality

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Abstract: Zinc (Zn) deficiency in soils not only reduces the productivity of forage crops, but also results in inadequate dietary zinc intake for livestock. The objective of this study was to evaluate the impact of different rates and methods of applying $ZnSO_4$ to both soil and foliage on the yield and quality of fodder maize grown in a sandy loam soil testing low in DTPA-extractable Zn. A 2-year field experiment was conducted with six treatments including control, foliar application of 0.3% $ZnSO_4$ at 30 days after sowing (DAS) (F_1), foliar application of 0.3% $ZnSO_4$ at 30 and 40 DAS (F_2), soil application of 16 kg ha^{-1} $ZnSO_4$ (S_{16}) and a combination of both soil and foliar $ZnSO_4$ application (S_{16} + F_1 and S_{16} + F_2). Increase in green herbage yield by 25%, dry matter yield by 47% and Zn content by 79% was observed under S_{16} + F_2 treatment over the control. Zinc application improved N, K, Cu and crude protein content of herbage significantly over the control. Thus, the study shows that significant improvement in growth parameters, herbage yield and quality of maize can be achieved with soil Zn application + two foliar sprays of $ZnSO_4$ at 30 and 40 DAS, thereby ensuring availability of improved fodder Zn to the livestock.

Keywords: maize; herbage yield; nutrient composition; quality; Zn biofortification; crude protein

1. Introduction

Zinc (Zn) serves as a vital trace element crucial for the growth and development of plants, human and animals, and is involved in more than 300 enzymes [1,2]. Zn deficiency in soils stands as a significant micronutrient limitation impacting crops and pasture production throughout the world ranging from arid to tropical climates [3,4]. The introduction of high-yielding crop varieties in the past, imbalanced fertilization and low soil organic matter content has contributed towards Zn deficiency in soils in most parts of the world [5,6]. A majority, exceeding 50%, of Indian soils are presently experiencing zinc deficiency, particularly in highly intensively cultivated Indo-Gangetic plains of North-West India. The prevalence of Zn deficiency in Indian soils is anticipated to escalate to over 65.0% by the year 2030 and this projection is attributed to the expansion of intensive cultivation onto marginal lands without adequate micronutrient fertilization [7]. Moreover, soil pH, redox conditions, cation exchange capacity (CEC), microbial activity, soil organic matter and water content are crucial soil properties that influence the availability of soil mineral Zn to plants. Elevated soil pH is frequently recognized as the primary factor constraining the phyto-availability of Zn and other micronutrients within the rhizosphere solution [8]. The low level of Zn content in soils has led to a pervasive deficiency of this element in various food and forage crops [7,8], consequently impacting the well-being of humans and livestock in tropical nations [9].

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Citation: Kumar, B.; Ram, H.; Schoenau, J. Agronomic Biofortification of Fodder Maize (*Zea mays* L.) with Zn for Improving Herbage Productivity and Its Quality. *Agronomy* **2024**, *14*, 912. https://doi.org/10.3390/agronomy14050912

Academic Editor: Leo Sabatino

Received: 21 March 2024
Revised: 12 April 2024
Accepted: 22 April 2024
Published: 26 April 2024

Forage crops are commonly cultivated in marginal soils with limited fertility especially in South Asian regions, where micronutrient deficiencies, notably Zn, are prevalent, which often results in reduced yield and substandard fodder quality for livestock [5,9]. Maize (*Zea mays* L.) holds significant importance as a cereal crop globally, serving as a staple food for human consumption, feed for livestock and a fundamental raw material for various industrial applications [10]. Livestock preference for maize over other cereal fodder crops is attributed to its superior digestibility and palatability, making it a favored choice for feeding purposes [11]. Zn deficiency in the soil reduces herbage yield, as well as adversely impacting the quality of fodder [9,12]. Zn deficiency in soil has been reported to cause a substantial decrease in both the yield and quality of maize fodder [12]. A requirement of 500 mg of Zn day^{-1} for a cow (500 kg body weight) cannot be met via fodder low in Zn content [9,13]. In adult animals, Zn deficiency can lead to various health issues such as lameness, hoof deformities, compromised locomotion, heightened susceptibility to infectious diseases, reduced reproductive efficiency, anestrus and repeat breeding ultimately resulting in low milk production [14]. Therefore, a notable enhancement in Zn content in milk can be achieved by bio-fortification of the forage crop, which not only fulfils the Zn requirement of cattle but also contributes to meeting the Zn needs of humans through the consumption of cattle milk [14].

Maize is reported to be highly responsive to soil additions of Zn fertilizers as well as the foliar application of Zn [12]. There is a strong need for enriching maize forage with Zn through a soil or foliar application approach, which is a farmer-friendly and economical technique. Physiologically accumulated Zn in plants provides a constant source of the Zn element with lesser risk of deficiency and can help to increase the dietary intake of Zn in livestock and humans [4,14]. Although much work on Zn biofortification of cereals and legumes has been reported from many parts of the world [7], meager information is available in the literature regarding the Zn biofortification of forage crops [15]. Elevated Zn concentration in maize fodder resulting from Zn fertilization can play a crucial role in meeting the Zn needs of livestock, especially in regions like South Asia where both soils and forage crops generally exhibit zinc deficiencies [2,4]. The economic importance of livestock production offers the opportunity to biofortify animal feed crops, thereby improving the health of animals and both directly and indirectly their consumers [15]. Feeding maize fodder enriched with Zn to livestock can have several benefits, including increased milk production, elevated Zn content in milk and reduced risk of infectious diseases like metritis and mastitis in cattle [5,14].

The present field study aims to explore methods for enriching fodder through agronomic biofortification techniques and evaluate the impact of soil and foliar application of Zn on the productivity and quality of maize fodder in a semi-arid region of the Indo-Gangetic plains of North-West India. The premise of our study was that bio-fortification of Zn through soil and foliar application would result in increased maize herbage yield, Zn enrichment and improved fodder quality for livestock.

2. Materials and Methods

2.1. Experimental Site, Weather and Soil Characteristics

The field experiment was conducted for two successive years at Ludhiana in Punjab, India (30°56′ N, 75°52′ E, 247 m altitude). The climate in this region exhibits a semi-arid pattern, featuring hot and dry conditions during the summer months from April to June, transitioning to hot and humid weather from July to September, and finally to cold winters from November to January. The annual average rainfall in the area is approximately 705 mm, with the majority occurring during the monsoon season from July to September, and occasional showers in December and January. Throughout the year, the average maximum and minimum temperatures vary between 29 and 32 °C in summer and 15 to 17 °C during winter. The mean maximum temperature registers at 25 °C during winter and 38 °C during summer. Extreme temperatures can reach over 44 °C in May and June and drop as low as 1 °C during December and January. The total amounts of precipitation during the cropping seasons were 375.6 and 44.0 mm during the first and second year, respectively. The maximum and minimum air temperatures recorded were 33.5 °C and 25.8 °C during the year 2013 and 38.4 °C and 25.4 °C during the year 2014. Lower sunshine hours (5.5 h) were recorded during the growing season of the crop during the first year than the second year of the cropping season (8.6 h). Prior to the commencing of the experiment, soil samples were gathered from eight randomly selected spots from the experimental field and a composite sample was created for the initial physico-chemical analysis of the soil. The surface (0–15 cm) soil of the experimental field was loamy sand (*Typic Ustochrept*) in texture having pH 8.4, EC 0.21 dSm^{-1} and a bulk density of 1.43 g cm^{-3}. The soil was low in organic carbon (2.2 g kg^{-1}), low in available nitrogen (258 kg ha^{-1}), available P (11.9 kg ha^{-1}) and DTPA- extractable Zn (0.52 mg kg^{-1}) (AAS-Varian AAS FS 240). The soil was medium in available K (136 kg ha^{-1}) [16]. The $CaCO_3$ content in the soil of experimental field was 141.1 kg ha^{-1}.

2.2. Experimental Setup and Treatment Detail

The experiment comprised six distinct treatments arranged in a randomized complete block design with three replications. The treatments of $ZnSO_4$ application were (1) control (no application of $ZnSO_4$), (2) foliar spray of $ZnSO_4$•H_2O (0.3%) at 30 days after sowing (F_1), (3) two foliar sprays of $ZnSO_4$•H_2O (0.3%) at 30 and 40 days after sowing (F_2), (4) soil application of $ZnSO_4$•H_2O (16 kg ha^{-1}) at sowing (S_{16}), (5) soil application of $ZnSO_4$•H_2O(16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4$•H_2O (0.3%) at 30 days after sowing (S_{16} + F_1) and (6) soil application of $ZnSO_4$•H_2O (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4$•H_2O (0.3%) at 30 and 40 days after sowing (S_{16} + F_2). Zn as zinc sulfate monohydrate ($ZnSO_4$•H_2O) having Zn content of 33% was used in the study. The soil Zn application treatment consisted of $ZnSO_4$•H_2O at 16 kg ha^{-1} (5 kg Zn ha^{-1}), which was dissolved in water (250 L ha^{-1}), then sprayed on the soil surface for ensuring uniform application and was later incorporated into the soil before planting [7]. For foliar application treatments, a specified quantity of $ZnSO_4$•H_2O and unslaked lime was dissolved in 250 L of water (ha^{-1}) and the solution was sprayed onto the maize foliage at two key stages; first 30 days after planting (V7 stage) and then 40 (V10 stage) days after planting during the evening hours when the wind conditions were calm and temperatures were moderate. A foliar spray of $ZnSO_4$•H_2O (0.3%) supplied 0.75 kg ha^{-1} of $ZnSO_4$ or 0.25 kg ha^{-1} of Zn to the crop. Foliar sprays were applied on the crop at various stages using a manually operated knapsack sprayer pump. To prevent spray drift, appropriate measures were taken during the application.

2.3. Crop Husbandry

Irrigation was applied to the field before sowing to maintain sufficient suitable moisture in the root zone near field capacity. Maize fodder cv. J-1006 was used as the test crop. The experiments were conducted during the summer season from July to September during the year 2013 and from May to July during the year 2014 cropping season. The crop was planted in a plot size of 14.4 m^2 at a seeding density of 50 kg ha^{-1} with the spacing of 30 cm × 10 cm resulting in a population density of 333,333 plants ha^{-1}. Before sowing, the seeds were treated with bavistin (Carbendazim 50% WP) at 3 g kg^{-1} of seed for protecting the crop from fungal diseases. The recommended doses of fertilizers were N at 90 kg and P_2O_5 at 30 kg ha^{-1}. Urea (46.0% N) and single superphosphate (16.0% P_2O_5) were used as a source of fertilizer of which a full dose of P and half dose of N were applied at sowing and the remaining half N dose was applied at 30 days after sowing by top dressing. Immediately after the sowing of the crop, the herbicide atrazine 50 WP (1.25 kg ha^{-1}) was sprayed for controlling weeds. The crop was hand-harvested manually from a net plot area spanning 9.9 m^2 in each treatment at the age of 60 days for green fodder purposes.

2.4. Crop Traits Measured

Maize growth parameters such as plant height, leaves plant^{-1}, stem girth, leaf area index, fresh and dry weight plant^{-1} and leaf to stem ratio (LSR) were measured before harvest. The height of ten randomly chosen plants from each experimental plot was measured using a meter ruler, from the base of the plant to the base of the fully opened youngest leaf on a stem. The leaves were counted and averaged from ten randomly selected plants within each experimental plot. The stem diameter was measured using a vernier calliper to measure the circumference of plant stems at about 15 cm above ground level from the same ten plants and averaged. The leaf area was recorded at harvest by sampling the plants from a half-meter row length at two randomly selected places in the middle rows in each plot. Then leaf area was recorded with leaf area meter (Delta T image analyzer, Delta T Devices Limited., Burwell Cambridge, UK). Leaf area was divided with the ground area to compute the leaf area index (LAI) [17]. Five plants per plot were randomly selected to calculate fresh weight and the plants were then partially air-dried at room temperature followed by drying in an electric oven at 60 °C for 48 h for determining the dry weight per plant. Leaf to stem ratio (LSR), the ratio between the weights of fresh leaves per plant to their fresh stem weight was measured from five randomly selected plants from each experimental plot at harvest. The green fodder yield was determined by harvesting the crop from the designated net area plot and then converted to Mg ha^{-1}. A sub-sample of fresh fodder weighing one kg was collected, chopped, sun-dried and then dried to a constant weight in an electric oven for 24 h at 60 °C and the dry weight was recorded. The fresh fodder yield was converted to dry matter yield using the moisture content determined from a sub-sample.

2.5. Plant Analysis for Nutrient Composition and Quality

Following harvest, maize plants gathered from each plot were washed sequentially with tap water, acidulated water containing 0.01 N HCl, distilled water and deionized water. The sub-samples were then air-dried followed by oven drying at 60 °C to a constant weight. The dried samples were ground in a Wiley mill fitted with stainless steel blades and passed through a 40 mesh sieve and stored in airtight plastic bags for nutrient composition determination. The N content in the fodder was determined by the micro-Kjeldahl distillation method [18]. The crude protein (CP) content was determined by multiplying %N by 6.25 and expressed in percentages. The total micronutrient contents viz. Zn, Cu, Fe and Mn in fodder were determined by an Atomic Absorption Spectrophotometer (Varian AAS FS 240 Model) after digesting 0.5 g of grounded maize sample using diacid mixture (HNO_3:$HCLO_4$) as described by [19]. The total phosphorus (P) and potassium (K) contents in maize fodder were estimated by the methods described by [16].

2.6. Statistical Analyses

The data were subjected to an analysis of variance (ANOVA) and analyzed using IRRISTAT version 92 [20]. The difference in two treatment means was compared by the least significant difference (LSD) at $p \leq 0.05$. The polynomial regressions were analyzed using SPSS ver. 16 statistical software. Pearson's correlation coefficient (r) was calculated among different variables and a correlation matrix was prepared to determine the relationship among variables to green fodder yield and other traits. The non-significant treatment differences are denoted as NS.

3. Results

3.1. Growth Parameters

The growth parameters of maize fodder were significantly influenced by foliar and soil application of $ZnSO_4$ (Tables 1 and 2). The plant height, number of leaves plant^{-1}, stem diameter, LAI, fresh weightplant^{-1}, dry weight plant^{-1} and leaf to stem ratio (LSR) of maize fodder were significantly higher under soil application of $ZnSO_4$ (16 kg ha^{-1}) plus a foliar spray of $ZnSO_4$ (0.3%) at 30 and 40 days after sowing (DAS) (S_{16} + F_2) treatment compared to no Zn control. Statistically similar plant heights were recorded under S_{16}, S_{16} + F_1 and S_{16} + F_2 treatments. Foliar (F_1 and F_2), soil (S_{16}) and soil + foliar application of Zn (S_{16} +F_1 and S_{16} + F_2) increased mean plant height by 6.7 to 8.5%, 9.5% and 10 to 11.8%, respectively, over the control. The highest increase (8.7%) in the mean number of leaves plant^{-1} over control was recorded in the treatment that received 16 kg ha^{-1} $ZnSO_4$ as the soil application plus two foliar sprays of 0.3% $ZnSO_4$ solution (S_{16} + F_2) at 30 and 40 DAS. The highest number of leaves plant^{-1} under S_{16} + F_2 treatment was on par with F_2, S_{16} and S_{16} + F_1 treatments (Table 1).

Table 1. Effect of different treatments on growth parameters of maize fodder at harvest.

Treatment	Plant Height (cm)			Leaves Plant^{-1}			Stem Diameter (cm)		
	Year-I	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**
Control	177.5 ^{b}	186.3 ^{b}	181.9 ^{c}	11.6 ^{c}	12.6 ^{b}	12.1 ^{c}	1.69 ^{c}	2.05 ^{c}	1.87 ^{c}
F_1	188.9 ^{a}	199.1 ^{a}	194.0 ^{b}	12.5 ^{b}	13.2 ^{b}	12.8 ^{b}	1.80 ^{b}	2.22 ^{b}	2.01 ^{b}
F_2	192.3 ^{a}	202.5 ^{a}	197.4 ^{ab}	12.9 ^{ab}	13.6 ^{ab}	13.2 ^{b}	1.85 ^{b}	2.29 ^{b}	2.07 ^{b}
S_{16}	192.0 ^{a}	206.3 ^{a}	199.1 ^{ab}	12.8 ^{ab}	13.6 ^{ab}	13.3 ^{ab}	1.86 ^{b}	2.30 ^{b}	2.08 ^{b}
S_{16} + F_1	193.6 ^{a}	208.3 ^{a}	200.1 ^{ab}	13.1 ^{ab}	13.8 ^{ab}	13.5 ^{ab}	1.93 ^{ab}	2.35 ^{b}	2.14 ^{ab}
S_{16} + F_2	195.8 ^{a}	211.0 ^{a}	203.4 ^{a}	13.4 ^{ab}	14.0 ^{a}	13.7 ^{a}	1.98 ^{a}	2.37 ^{a}	2.17 ^{a}
SEm±	1.70	2.38	1.88	0.17 ^{a}	0.12	0.12	0.02	0.03	0.03
LSD ($p \leq 0.05$)	8.2	11.6	6.7	0.80	0.60	0.5	0.10	0.14	0.08

Control: no application of $ZnSO_4$, F_1: foliar spray of $ZnSO_4{\bullet}H_2O$ (0.3%) at 30 days after sowing, F_2: two foliar sprays of $ZnSO_4{\bullet}H_2O$ (0.3%) at 30 and 40 days after sowing, S_{16}: soil application of $ZnSO_4{\bullet}H_2O$ (16 kg ha^{-1}) at sowing, S_{16} + F_1: soil application of $ZnSO_4{\bullet}H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4{\bullet}H_2O$ (0.3%) at 30 days after sowing, and S_{16} + F_2: Soil application of $ZnSO_4{\bullet}H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4{\bullet}H_2O$ (0.3%) at 30 and 40 days after sowing; Means sharing the same case letter do not differ significantly at $p \leq 0.05$.

Table 2. Effect of foliar and soil-applied $ZnSO_4$ on leaf area index (LAI), fresh weight (FW), dry weight (DW) and leaf to stem ratio (LSR) of maize plants at harvest.

Treatment	**LAI**			**FW Plant^{-1} (g)**			**DW Plant^{-1} (g)**			**LSR**		
	Year-I	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**
Control	5.54 ^{c}	6.27 ^{b}	5.91 ^{c}	351.6 ^{b}	386.7 ^{b}	369.2 ^{c}	91.5 ^{d}	102.0 ^{c}	96.8 ^{d}	0.578 ^{b}	0.674 ^{c}	0.626 ^{d}
F_1	6.03 ^{bc}	6.91 ^{b}	6.47 ^{b}	386.7 ^{ab}	421.7 ^{ab}	404.2 ^{b}	106.3 ^{c}	127.7 ^{b}	117.0 ^{c}	0.635 ^{b}	0.747 ^{b}	0.691 ^{c}
F_2	6.29 ^{b}	7.21 ^{ab}	6.75 ^{b}	403.3 ^{a}	436.6 ^{a}	420.0 ^{b}	114.5 ^{c}	136.3 ^{b}	125.4 ^{c}	0.661 ^{b}	0.789 ^{b}	0.725 ^{c}
S_{16}	6.40 ^{b}	7.31 ^{ab}	6.85 ^{b}	411.7 ^{a}	438.3 ^{a}	425.0 ^{a}	115.3 ^{b}	138.7 ^{b}	127.0 ^{c}	0.665 ^{b}	0.790 ^{b}	0.728 ^{bc}
S_{16} + F_1	6.94 ^{a}	7.65 ^{ab}	7.30 ^{a}	420.0 ^{a}	447.3 ^{a}	433.7 ^{a}	127.6 ^{a}	149.4 ^{ab}	138.5 ^{b}	0.709 ^{ab}	0.852 ^{ab}	0.780 ^{b}
S_{16} + F_2	7.35 ^{a}	7.98 ^{a}	7.66 ^{a}	430.0 ^{a}	441.6 ^{a}	445.8 ^{a}	135.0 ^{a}	159.8 ^{a}	147.4 ^{a}	0.775 ^{a}	0.889 ^{a}	0.832 ^{a}
SEm±	0.15	0.15	0.14	7.24	7.18	6.74	3.64	4.60	4.00	0.02	0.01	0.02
LSD ($p \leq 0.05$)	0.50	0.77	0.42	36.4	35.6	23.8	12.4	13.0	8.4	0.091	0.067	0.053

Control: no application of $ZnSO_4$, F_1: foliar spray of $ZnSO_4{\bullet}H_2O$ (0.3%) at 30 days after sowing, F_2: two foliar sprays of $ZnSO_4{\bullet}H_2O$ (0.3%) at 30 and 40 days after sowing, S_{16}: soil application of $ZnSO_4{\bullet}H_2O$ (16 kg ha^{-1}) at sowing, S_{16} + F_1: soil application of $ZnSO_4{\bullet}H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4{\bullet}H_2O$ (0.3%) at 30 days after sowing, and S_{16} + F_2: soil application of $ZnSO_4{\bullet}H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4{\bullet}H_2O$ (0.3%) at 30 and 40 days after sowing; Means sharing the same case letter do not differ significantly at $p \leq 0.05$.

During the first year, S_{16} + F_1 and S_{16} + F_2 treatments produced similar but significantly greater stem diameter compared to the control (Table 1). Foliar $ZnSO_4$ application twice at 30 and 40 DAS (F_2) resulted in superior stem girth over sole foliar $ZnSO_4$ application (F_1) although the differences were non-significant. Treatment S_{16} + F_2 significantly increased the mean stem girth by 16% over the control.

Zn application significantly increased the leaf area index (LAI) of maize over the control (Table 2). The highest increase (30%) in mean LAI was recorded in S_{16} + F_2 treatment over the control, which was statistically similar to S_{16} + F_1. Soil Zn application (S_{16}) resulted in higher mean LAI than the foliar sprays (either at 30 (F_1) or 30 and 40 DAS (F_2)); however, differences were not significant. The other growth attributes of fresh and dry weight of maize with zinc application (soil and foliar) also significantly ($p \leq 0.05$) increased as compared to no Zn treatment (control) (Table 2). However, treatment S_{16} + F_2 resulted in maximum weight (fresh and dry) plant^{-1} closely followed by S_{16} + F_1. Similarly, the leaf to stem ratio (LSR) of maize was significantly increased with Zn application over control (Table 2). Treatment S_{16} + F_2 recorded 33% higher LSR closely followed by S_{16} + F_1 (25%) compared to control. Soil application of $ZnSO_4$ (S_{16}) produced higher values of LSR than the foliar $ZnSO_4$ (F_1 and F_2) treatments.

3.2. Green Herbage Yield (GHY) and Dry Matter Yield (DMY)

Compared to the control, all the treatments of zinc sulfate application (except F_1 0.3% foliar spray at 30 DAS) improved the green herbage yield (Table 3). The highest green herbage yield of 54.8 and 59.1 Mg ha^{-1} in the first and second year of study was recorded in the treatment of application of $ZnSO_4$ (16 kg ha^{-1}) at sowing combined with foliar application of 0.3% $ZnSO_4$ twice at 30 and 40 DAS of the crop, resulting in increases of 23.6% and 24.4%, respectively, over control during the first and second year of study. The sole zinc application to soil (S_{16}) outperformed both single and double foliar applications in enhancing the green herbage yield of the crop. The treatments of foliar sprays of 0.3% $ZnSO_4$ at 30 DAS (48.6 and 51.6 Mg ha^{-1}) and at 30 and 40 DAS (50.5 and 54.1 Mg ha^{-1}) were statistically similar to each other in respect to green herbage yield. The mean green herbage yield (mGHY) was 22 and 25% higher in S_{16} + F_1 and S_{16} + F_2 treatments, respectively, compared to the control. The improvement in mean GHY was to the extent of 9.9, 14.7 and 16.7% with the treatments of F_1, F_2, and S_{16}, respectively, over control.

Table 3. Effect of foliar and soil-applied zinc sulfate on green herbage yield (GHY) and dry matter yield (DMY) of fodder maize.

Treatment	GHY (Mg ha^{-1})			DMY (Mg ha^{-1})		
	Year-I	Year-II	Mean	Year-I	Year-II	Mean
Control	43.7 ^{b}	47.5 ^{b}	45.6 ^{c}	8.0 ^{c}	8.2 ^{c}	8.1 ^{c}
F_1	48.6 ^{b}	51.6 ^{b}	50.1 ^{b}	9.4 ^{b}	9.8 ^{b}	9.6 ^{c}
F_2	50.5 ^{ab}	54.1 ^{ab}	52.3 ^{b}	9.9 ^{b}	10.5 ^{b}	10.2 ^{bc}
S_{16}	51.6 ^{ab}	54.9 ^{ab}	53.2 ^{ab}	10.3 ^{ab}	10.7 ^{b}	10.5 ^{b}
S_{16} + F_1	53.6 ^{ab}	57.7 ^{ab}	55.6 ^{ab}	10.8 ^{ab}	11.9 ^{a}	11.3 ^{a}
S_{16} + F_2	54.8 ^{a}	59.1 ^{a}	57.0 ^{a}	11.4 ^{a}	12.3 ^{a}	11.9 ^{a}
SEm±	1.08	1.25	0.99	0.30	0.34	0.30
LSD ($p \leq 0.05$)	5.9	6.5	4.2	1.3	0.90	0.8

Control: no application of $ZnSO_4$, F_1: foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 days after sowing, F_2: two foliar sprays of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 and 40 days after sowing, S_{16}: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing, S_{16} + F_1: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 days after sowing, and S_{16} + F_2: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 and 40 days after sowing; Means sharing the same case letter do not differ significantly at $p \leq 0.05$.

Among different treatments of Zn application, the combined application of soil + foliar $ZnSO_4$ at 30 and 40 DAS (S_{16} + F_2) increased DMY to the maximum levels, which were 42.5 and 50.0% higher over control during the first and second year of study (Table 3). Sole foliar application of $ZnSO_4$ at 30 DAS (F_1) increased DMY by 20% in the first and second year and was significantly superior to the control. The dry matter yield of the crop was statistically similar in the F_1, F_2 and S_{16} treatments of $ZnSO_4$ application. Maximum mean increase in dry matter yield under different $ZnSO_4$ fertilization treatments accounted for 47% in S_{16} + F_2 treatment over the control. The increases in both GFY and DMY of maize were significantly and positively correlated (r = 0.703 ** and r = 0.805 **, respectively) with the Zn content in maize plant due to soil and/or foliar application of $ZnSO_4$ (Figure 1).

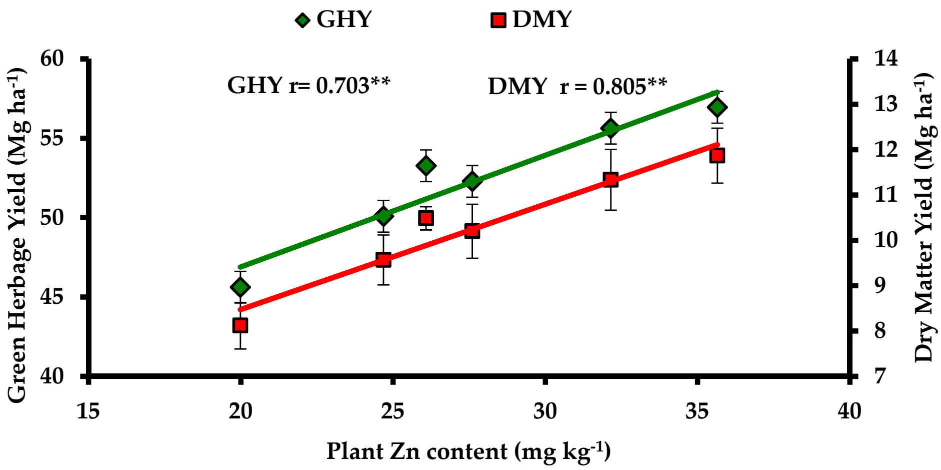

Figure 1. Regression of green herbage yield (GHY) and dry matter yield (DMY) of maize with plant Zn content. **: significant at $p < 0.01$ according to the F test. Vertical bars indicate standard error of the mean ($n = 3$).

3.3. Zn Fertilization and Fodder Micronutrient Composition

Contents of zinc (Zn), manganese (Mn) and copper (Cu) in maize fodder were significantly affected by different methods of Zn application (Table 4). However, iron content in the fodder did not differ significantly due to Zn fertilization. The soil $ZnSO_4$ along with two foliar sprays of 0.3% $ZnSO_4$ at 30 and 40 DAS resulted in fodder Zn concentrations of 34.0 mg Zn kg^{-1} and 37.3 mg Zn kg^{-1} in the two respective years, which were significantly higher than the control. The increase in Zn concentration (on DM basis) was 15.4 mg Zn kg^{-1} during the first year, while during the second year, the increase was 16.0 mg Zn kg^{-1} as compared to the control treatment. Statistically similar Zn concentrations in the plant at harvest were found in the $S_{16} + F_2$ and $S_{16} + F_1$ treatments; however, both these treatments were superior to other Zn application treatments and the control. Out of all the treatments involving Zn fertilization, the smallest rise in Zn concentration in fodder (4.9 mg kg^{-1} in first year and 4.5 mg kg^{-1} in second year) occurred with a foliar spray of 0.3% of $ZnSO_4$ at 30 DAS, but was still significantly higher than the control. The 16 kg ha^{-1} $ZnSO_4$ (S_{16}) soil applied treatment resulted in 7.2 and 4.7% more zinc concentration in fodder biomass than the exclusive foliar application of 0.3% $ZnSO_4$ at 30 DAS (F_1), but 4.0 and 7.4% less than the foliar application of 0.3% $ZnSO_4$ at 30 and 40 DAS treatment (F_2) during the first and second year, respectively. The treatments S_{16}, F_1 and F_2 were found to be statistically similar in respect to zinc content in biomass at harvest.

During the first year, Mn concentration of fodder did not differ significantly among treatments due to zinc fertilization, but significant differences were noticed in Mn in fodder during the second year (Table 4). The Mn concentration of fodder at harvest decreased significantly with Zn application except for one foliar Zn application (F_1) treatment during the second year and there was a non-significant negative correlation (r = −0.252) between Zn and Mn content in fodder (Table 4). The decrease in Mn content was greatest in $S_{16} + F_2$ treatment as compared to the control during the second year. Two foliar spray applications of $ZnSO_4$ at 30 and 40 DAS (F_2) recorded a greater decrease (5.8%) in Mn concentration compared to $ZnSO_4$ treatment of the single foliar application of 0.3% at 30 DAS (F_1).

The Cu concentration in maize fodder at harvest increased significantly with Zn fertilization in all the treatments compared to control (Table 4). A significant positive correlation (r = 0.632 **, $p < 0.1$) was observed between Zn and Cu concentrations in maize fodder. The maximum increase (78.2%) in mean Cu content of the plant was recorded in the $S_{16} + F_2$ treatment, which, similarly to the $S_{16} + F_1$, S_{16} and F_2 treatments, was significantly better than control.

Table 4. Effect of foliar and soil-applied zinc sulfate on Zn, Fe, Mn and Cu concentrations (mg kg^{-1}) of maize fodder (on DM basis).

Treatment	**Zn**			**Fe**			**Mn**			**Cu**		
	(mg kg^{-1} on DM Basis)											
	Year-I	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**
Control	18.6 ^{c}	21.3 c	19.9 ^{e}	105	111	107.8	25.0	27.2 ^{a}	26.1 ^{a}	2.1 ^{b}	2.5 ^{b}	2.3 ^{c}
F_1	23.5 ^{b}	25.8 b	24.7 ^{d}	106	114	109.7	24.8	26.8 ^{ab}	25.7 ^{a}	2.5 ^{b}	3.0 ^{b}	2.8 ^{bc}
F_2	26.2 ^{b}	29.0 b	27.6 ^{c}	107	114	110.7	23.9	25.7 ^{b}	24.7 ^{ab}	3.3 ^{a}	3.5 ^{ab}	3.4 ^{b}
S_{16}	25.2 ^{b}	27.0 b	26.1 ^{c}	101	110	105.1	24.7	26.3 ^{b}	25.5 ^{b}	3.3 ^{a}	3.5 ^{ab}	3.4 ^{b}
S_{16} + F_1	30.6 ^{a}	33.7 ^{a}	32.2 ^{b}	106	115	110.5	24.1	25.6 ^{bc}	24.8 ^{b}	3.5 ^{a}	4.0 ^{ab}	3.8 ^{ab}
S_{16} + F_2	34.0 ^{a}	37.3 ^{a}	35.6 ^{a}	106	115	110.7	23.7	25.3 ^{c}	24.5 ^{b}	3.8 ^{a}	4.5 ^{a}	4.1 ^{a}
SEm±	1.26	1.32	1.26	1.17	0.96	0.18	0.18	0.18	0.17	0.15	0.17	0.15
LSD ($p \leq 0.05$)	4.0	3.7	2.5	NS	NS	NS	NS	0.8	0.7	0.7	1.0	0.6

Control: no application of $ZnSO_4$, F_1: foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 days after sowing, F_2: two foliar sprays of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 and 40 days after sowing, S_{16}: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing, S_{16} + F_1: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 days after sowing, and S_{16} + F_2: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 and 40 days after sowing; Means sharing the same case letter do not differ significantly at $p \leq 0.05$, NS = Non-significant.

3.4. Zn Fertilization and Fodder Macronutrient Composition

A significant increase in the N concentration of the fodder was recorded with $ZnSO_4$ fertilization during both years of study except for F_1 in the second year (Table 5). The highest N concentration in fodder was recorded in the treatment receiving soil $ZnSO_4$ application of 16 kg ha^{-1} plus two 0.3%.

$ZnSO_4$ foliar sprays at 30 and 40 DAS (S_{16} + F_2) treatment were closely followed by S_{16} + F_1 and S_{16} treatments. Foliar application of Zn treatments (F_1 and F_2) also improved mean N content of fodder by 7.4 and 10.8%, respectively, over control. A significant and positive correlation was recorded between Zn and N concentration in fodder (0.668 **). The phosphorus content of fodder decreased significantly with Zn fertilization in the first year; however, the differences were not significant in the second year. Reduced P content in fodder was recorded with Zn application except for F_1 in the first year. Treatments involving soil $ZnSO_4$ application resulted in more reduction in P content of fodder than the treatments involving foliar $ZnSO_4$ fertilization. However, a non-significant negative correlation was observed between Zn and P (-0.053 ^{NS}) in the fodder (Table 6). Zn fertilization improved the K content in herbage significantly during the first year only (Table 6). The maximum increase in the mean K content of herbage was recorded with S_{16} + F_2 (11.9%) followed by the S_{16} + F_1 (10.4%) and S_{16} treatments (8.4%).

Table 5. Effect of foliar and soil-applied $ZnSO_4$ on nitrogen (N), phosphorus (P), potassium (K) concentration (%) of fodder during two growing seasons.

Treatment	**N**			**P**			**K**			**CP**		
	%											
	Year-I	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**	**Year-I**	**Year-II**	**Mean**
Control	1.28 ^{c}	1.32 ^{b}	1.30 ^{c}	0.93 ^{a}	1.03	0.98	1.96 ^{b}	2.09	2.02 ^{b}	8.02 ^{c}	8.25 ^{b}	8.13 ^{c}
F_1	1.40 ^{b}	1.41 ^{b}	1.40 ^{b}	0.91 ^{ab}	0.99	0.96	2.06 ^{b}	2.14	2.10 ^{b}	8.75 ^{b}	8.81 ^{b}	8.78 ^{b}
F_2	1.42 ^{b}	1.46 ^{ab}	1.44 ^{b}	0.89 ^{b}	0.99	0.94	2.09 ^{b}	2.22	2.15 ^{ab}	8.91 ^{b}	9.13 ^{ab}	9.02 ^{b}
S_{16}	1.46 ^{ab}	1.49 ^{ab}	1.47 ^{b}	0.86 ^{bc}	0.97	0.91	2.16 ^{ab}	2.23	2.19 ^{ab}	9.14 ^{ab}	9.31 ^{ab}	9.23 ^{b}
S_{16} + F_1	1.51 ^{ab}	1.53 ^{ab}	1.52 ^{ab}	0.88 ^{b}	0.97	0.92	2.20 ^{ab}	2.26	2.23 ^{a}	9.43 ^{ab}	9.58 ^{ab}	9.51 ^{ab}
S_{16} + F_2	1.55 ^{a}	1.57 ^{a}	1.56 ^{a}	0.84 ^{c}	0.97	0.90	2.26 ^{a}	2.27	2.26 ^{a}	9.72 ^{a}	9.79 ^{a}	9.76 ^{a}
SEm±	0.02	0.03	0.02	0.01	0.01	0.01	0.03	0.04	0.03	0.15	0.16	0.15
LSD ($p \leq 0.05$)	0.11	0.12	0.07	0.03	NS	NS	0.16	NS	0.11	0.70	0.80	0.5

Control: no application of $ZnSO_4$, F_1: foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 days after sowing, F_2: two foliar sprays of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 and 40 days after sowing, S_{16}: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing, S_{16} + F_1: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 days after sowing, and S_{16} + F_2: soil application of $ZnSO_4 \bullet H_2O$ (16 kg ha^{-1}) at sowing plus foliar spray of $ZnSO_4 \bullet H_2O$ (0.3%) at 30 and 40 days after sowing; Means sharing the same case letter do not differ significantly at $p \leq 0.05$, NS = Non-significant.

Table 6. Pearson correlation coefficient and significance level among growth, micro-nutrient contents, dry matter and green herbage yield of maize (pooled data of two years).

	PH	LN	SD	LAI	FW	DW	LSR	Zn	Fe	Mn	Cu	DMY	GHY
PH	-												
LN	0.684 **	-											
SD	0.776 **	0.731 **	-										
LAI	0.769 **	0.786 **	0.769 **	-									
FW	0.740 **	0.747 **	0.688 **	0.771 **	-								
DW	0.832 **	0.769 **	0.795 **	0.846 **	0.796 **	-							
LSR	0.849 **	0.723 **	0.823 **	0.830 **	0.741 **	0.874 **	-						
Zn	0.706 **	0.711 **	0.556 **	0.819 **	0.729 **	0.845 **	0.798 **	-					
Fe	0.470 **	0.482 **	0.687 **	0.304 NS	0.460 **	0.487 **	0.622 **	0.289 NS	-				
Mn	0.165 NS	0.116 NS	0.498 **	0.070 NS	0.001 NS	0.017 NS	0.197 NS	−0.252 NS	0.496 **	-			
Cu	0.772 **	0.772 **	0.830 **	0.758 **	0.696 **	0.791 **	0.799 **	0.632 **	0.536 **	0.325 NS	-		
DMY	0.734 **	0.745 **	0.545 **	0.784 **	0.702 **	0.838 **	0.735 **	0.805 **	0.276 NS	−0.227 NS	0.649 **	-	
GHY	0.645 **	0.686 **	0.572 **	0.809 **	0.741 **	0.776 **	0.721 **	0.703 **	0.268 NS	−0.046 NS	0.534 **	0.818 **	-

PH: plant height; LN: leaf number; SD: stem diameter; LAI: leaf area index; FW: fresh weight; DW: dry weight; LSR: leaf to stem ratio; Zn: zinc content; Fe: iron content; Mn: manganese content; Cu: copper content; DMY: dry matter yield; GHY: green herbage yield; **: significant at the 0.01 probability level; NS: Non-significant.

3.5. CP Content of Fodder

A notable enhancement ($p \leq 0.05$) in the crude protein content of fodder was evident with the application of Zn to the soil, foliage and combination of both (Table 5). During the first year, the foliar spray of 0.3% $ZnSO_4$ at 30 DAS resulted in significantly higher crude protein content over the control, whereas differences were found to be non-significant during the second year. The highest mean increase in crude protein content of fodder was recorded in soil + foliar $ZnSO_4$ application followed by solo soil $ZnSO_4$ and foliar application treatments over the control. The crude protein content ranged from 8.02 to 9.72% and 8.25 to 9.79% during the first and second, respectively (Table 5). Foliar, soil and soil + foliar Zn application resulted in 8–11%, 13.5% and 17–20.0% increases in mean crude protein content of fodder over control. Treatment S_{16} + F_2 was similar to the S_{16} + F_1 treatment for mean crude protein content but fared significantly better than the other treatment.

3.6. Correlation Studies

A correlation analysis of maize fodder growth parameters, nutrient contents, dry matter yield and green herbage yield (Table 6) revealed significant ($p < 0.05$) or highly significant correlations ($p < 0.01$). Green herbage and dry matter yield were highly significantly correlated with plant height (0.645 ** and 0.734 **), number of leaves plant^{-1} (0.686 ** and 0.745 **), stem girth (0.572 ** and 0.545 **), leaf area index (0.809 ** and 0.784 **), fresh weight plant^{-1} (0.741 ** and 0.702 **), dry weight plant^{-1} (0.776 ** and 0.838 **) and leaf to stem ratio (0.721 ** and 0.735 **). Also, green herbage yield and dry matter yield were highly significantly correlated (0.703 ** and 0.805 **) with Zn concentration in the plant (Table 6). On the other hand, both green fodder and dry matter yield were negatively correlated with Mn concentrations in fodder (−0.046 and −0.227), but differences were non-significant (Table 6). A significant positive correlation was found between green fodder yield and dry matter yield with Cu (0.534 ** and 0.649 **). The zinc (Zn) content of fodder showed significant ($p < 0.01$) and positive correlation with different growth parameters such as plant height (0.706 **), number of leaves plant^{-1} (0.711 **), stem girth (0.556 **), leaf area index (0.819 **), fresh weight plant^{-1} (0.729 **), dry weight plant^{-1} (0.845 **) and leaf to stem ratio (0.798 **). In regard to the correlation of the zinc concentration in fodder with other micronutrients, a non-significant negative correlation with Zn-Mn concentration in fodder was found (r = −0.252), whereas a significant positive correlation (0.632 **, $p < 0.01$) with Zn-Cu concentration was observed (Table 6).

4. Discussion

4.1. Effect of Zn on Growth Parameters

The results obtained from this investigation indicate that Zn fertilization has a significant effect on the growth, yield, nutritional content and quality of maize. Increases in various growth parameters viz. plant height, number of leaves plant^{-1}, stem girth, LAI, fresh weight/dry weight plant^{-1} and leaf to stem ratio (LSR) of maize fodder (Tables 1 and 2) were significantly higher under soil Zn application (16 kg ha^{-1}) plus the foliar spray of $ZnSO_4$ (0.3%) at 30 and 40 DAS after sowing (S_{16} + F_2) treatment, which can be attributed to the involvement of Zn in synthesizing plant growth hormones such as indole acetic acid (IAA) and auxins that take an active role in the elongation and enlargement of plant cells [7,21]. Soil and foliar Zn application increased the plant height over control treatment possibly due to increases in cell division and chlorophyll content as described by [10].

The girth, i.e., stem diameter of the plant is an important criterion that determines its strength and ability to resist lodging and contributes significantly to green fodder and the dry matter yield of the crop. Leaf area index (LAI) is the main physiological determinant of the yield in crops. Zn application was found to exhibit a significant effect on stem diameter (Table 1) and leaf area index (Table 2). Zinc plays an important role in nitrogen metabolism and chlorophyll synthesis in plants, which might have led to increase in stem diameter and LAI in our study [1] due to the activation of different physiological processes like stomata regulation, chlorophyll synthesis and cell division [8]. The leaf to stem ratio (LSR) of the crop has a direct relationship with the herbage yield and quality of fodder [5]. Zinc has a positive effect on the chlorophyll content of the crop and helps in water and nutrient absorption through enhanced root depth, which in turn improves plant growth parameters such as biomass (fresh and dry) and leaf stem ratio and nutrient uptake [7,9].

4.2. Effect of Zn on Green Herbage and Dry Matter Yield

In the present study, the increase in green herbage yield (GHY) and dry matter yield (DMY) with Zn application (Table 3) was attributed to expansion in yield-related traits such as plant height, leaves plant^{-1}, stem girth, LAI, FW plant^{-1}, DW plant^{-1} and LSR in maize fodder (Tables 1 and 2). Several other researchers have also reported the beneficial effects of Zn fertilization, whether applied through soil or foliage, on the growth and yield of cereals and pulses such as wheat, rice, soybean and chickpeas [2,4]. Zinc is recognized for its role in activating various enzymatic reactions and improving photosynthesis, which results in the generation of more food reserves in the plants and thus increase in green herbage yield and dry matter yield (Table 3). Zinc also enhances carbohydrate assimilate partitioning from source to sink in the plant, which led to the increase in fodder yield [21].

The application of Zn solely to the soil proved superior to one or two foliar applications in enhancing the yield, potentially indicating a higher amount of Zn applied and an extended-duration Zn availability to the crop under this method [22]. Foliar application at the late growth stage attributed to less vigorous vegetative growth than basal application indicates that during the early growth stage, adequate soil-available Zn is important to receive a high herbage yield. Secondly, the amount of nutrient applied through one foliar application might not be sufficient to meet the requirement of plants, as two foliar applications of $ZnSO_4$ resulted in higher GHY and DMY. Plants experiencing Zn deficiency during early development stages may struggle to reach their maximum genetic potential possibly due to impairment in both enzyme activity maintenance and the synthesis of tryptophan enzyme [22,23].

4.3. Zn Biofortification and Effect on Other Micronutrients

Minerals and trace elements obtained from forage play a crucial role in milk production, reproductive health, and overall livestock well-being [5]. To ensure the optimal growth and development of cattle, it is very essential to provide adequate trace minerals such as Zn through high-quality fodder. While much of plant research has focused on biofortifying cereals like rice and wheat, there remains untapped potential to enhance the nutritional quality of forage crops through biofortification approaches [15]. Different Zn fertilization methods significantly improved Zn content in the fodder in this study (Table 4), reflecting Zn's pivotal role in photosynthesis and metabolic processes, which contribute to boosting the production of photosynthates and their translocation to various plant parts [15]. Increased Zn availability to cattle through zinc-enriched fodder can fulfill their Zn requirement, which is very crucial for bolstering the immune system of the livestock [15]. Zinc applications through soil and a combination of soil and foliar treatments proved more effective in augmenting zinc content in fodder compared to foliar treatment alone (Table 4). This is attributed to the continuous supply of zinc to the crop, especially from the sequential foliar applications that loaded more zinc into the leaves [22]. In the foliar application of Zn treatments (F_1 and F_2), an increase in Zn concentration in fodder occurred due to the entry of Zn into the plant through stomatal pores as reported by Gupta et al. [6]; however, both soil and foliar application of Zn fertilizers enhance the plant-available Zn pool. With the daily dietary requirement for adult cattle estimated at 40 mg kg^{-1} DM, the increased zinc content resulting from Zn fertilization in fodder can adequately meet the livestock's needs, particularly in regions like South Asia where soils and forage crops typically lack sufficient zinc [9]. Hosnedlova et al. [14] reported that Zn content in cattle milk can be influenced by forage nutrition. Feeding livestock maize fodder enriched with Zn can increase milk production, enhance reproductive efficiency and reduce the risk of infectious diseases such as metritis and mastitis, which could potentially boost the dairy industry [14].

Plant Fe required for normal plant growth ranges from 100 to 200 mg kg^{-1}, but higher Fe levels from 250 to 500 mg kg^{-1} in the green herbage may be toxic and can produce Cu deficiency in animals [5]. In this study, Fe content in maize fodder ranged between 105 and 115 mg kg^{-1} during the two-year study, which was sufficiently high for meeting normal functioning of the plant. Considering the daily dietary requirement of 50 mg kg^{-1} of dry matter of Fe by an adult animal, the Fe content in fodder was in the sufficient range to meet the requirement of the animal after Zn fertilization. Furthermore, a non-significant Zn-Fe correlation was noted in this study (Table 6), indicating that Zn fertilization did not affect Fe concentration in fodder. On the contrary, Adiloglu [24] from Turkey showed 32 and 15% decreases in the Fe concentration of maize plants in a pot study in sandy and clayey calcareous soils deficient in Zn due to antagonism between Zn and Fe.

Mn content of fodder decreased significantly with foliar, soil-alone and soil + foliar Zn application in maize, yet its content was in sufficient range in the crop (Table 4); however, a non-significant negative correlation (-0.252^{NS}) was observed in this study (Table 6). The presence of Mn in moderate availability range in the soil of the study site might have contributed to the uptake of Mn by the plants, although high soil pH generally restricts its uptake [24]. In soils deficient in Zn, Adiloglu [24] also observed a decrease in Mn content of the maize plant with either soil or foliar Zn fertilization. For maize, a plant tissue analysis showing a value of 15 mg Mn kg^{-1} dry matter would indicate the critical range of nutrient status, with 15–20 mg kg^{-1} as low and 20–150 mg kg^{-1} as sufficient [25]. In our study, the Mn content of the plant at harvest was in the sufficient range (23.7 to 27.2 mg kg^{-1}) for the vital functioning of the plant (Table 4). According to Weiss et al. [26], it has been reported that for cattle, a diet containing approximately 14 mg of Mn kg^{-1} of DM will meet the total Mn requirement for a 600 kg cow producing 30 L day^{-1} of milk. However, Hansen et al. [27] suggested that feeding gestating heifers a diet containing 16.6 mg kg^{-1} of Mn was not adequate for proper fetal growth and development.

The copper content in the leaf was sufficiently high (2.5 to 4.5 mg kg^{-1}) as Cu levels in plant tissue below 2.0 mg kg^{-1} are considered inadequate for plant growth [28]. There are conflicting reports on the effect of Zn application on Cu content in plant tissue. While Aref [25] has recorded enhancement in Cu content in the maize plant with the foliar application of Zn, other researchers have recorded antagonistic effects between Zn application and Cu content in the plant [29]. There was a significant and positive correlation between Zn and Cu (0.632 **, $p < 0.01$) indicating that either Zn application through soil or foliage or a combination of both promoted Cu uptake in the herbage. The possible reason for an increase in Cu concentration in the plant tissue is due to positive Zn interaction with N, which further improves the absorption of minerals such as Cu and Mn, etc., in the plants [6,8]. Since the soil application of $ZnSO_4$ plus a foliar spray of 0.3% $ZnSO_4$ at 30 and 40 DAS improved plant Zn content and had no adverse effect on fodder Fe, Mn or Cu content, it can therefore be considered as an appropriate dose and method for enriching maize fodder with zinc.

4.4. Zn Biofortification and Effect on Macronutrients

Zinc fertilization improved nitrogen concentration of the plant significantly at harvest as Zn is involved in N metabolism in plants, which helped to increase the N content in maize fodder [1]. There has been widespread documentation of the crosstalk between Zn and N, and recent studies have demonstrated a positive effect of Zn nutrition in improving grain and foliage N content in various crops [6]. This study also strongly confirmed this fact as a significantly strong positive correlation was recorded between Zn and N (0.668 **), signifying that zinc application either to the soil or on plant improves the N content of the plant (Tables 5 and 6).

Soil Zn application alone (S_{16}) and soil + foliar Zn application (S_{16} + F_1 and S_{16} + F_2) treatments caused a greater reduction in the P content of maize than foliar Zn application treatments. Studies by other researchers [28] also corroborate that phosphorus uptake in the shoot and its content in the leaves decreased due to Zn sufficiency in plants. Zinc can interact with inorganic phosphate to form insoluble $Zn_3(PO_4)_2$ in the soil, rendering it unavailable for root uptake, and demonstrates a negative relationship regarding zinc–phosphorus crosstalk [6]. However, P content in maize fodder in this study was much higher (>0.90 mg kg^{-1}) than the sufficient range (0.25–0.50 mg kg^{-1}) of P according to Olsen's Agricultural Laboratory, Plant Tissue Interpretative Guidelines [30]. Also, a non-significant and a very weak negative correlation between Zn and P (-0.052^{NS}) existed in this study (Table 6), indicating that Zn fertilization did not greatly influence the P content of maize.

The possible reason for the increase in K content in maize fodder due to Zn application is not known. The experimental soil had a moderate level of available K, and improved root growth with Zn fertilization might have led to the better absorption and transport of K from the soil to plant. In Pakistan, Anees et al. [10] a positive relationship between Zn and K contents in rainfed maize grown conditions was also observed.

4.5. Zn Effect on Herbage Crude Protein

For dairy farmers, the primary goal is to achieve high fodder yield while maintaining high-quality fodder. The crude protein (CP) content of forage is one of the most important criteria for forage quality evaluation [5]. Since crude protein is directly related to total N content in the plant, the increase in crude protein content is similar to that reported for total N content (Table 5). Zinc plays an important role in protein and carbohydrate synthesis and is involved in regulating metabolism, including saccharides, nucleic acid and lipid metabolism in plants [7,22]. Zinc controls the activity of RNAse, the enzyme that hydrolyzes RNA, leading to a decrease in protein synthesis [22,31] if Zn is deficient in the plant. Anees et al. [10] also reported significant improvement in N and crude protein content of maize grain with soil and foliar application of Zn in semi-arid conditions.

5. Conclusions

In this study, the application of $ZnSO_4 \bullet H_2O$ resulted in a significant increase in growth parameters, green herbage and dry matter yields and Zn content of maize fodder. Zinc application also improved the crude protein content of the fodder. The increase in fresh herbage yield, dry matter yield and Zn content due to Zn application is beneficial to the livestock farmer. Soil application of $ZnSO_4 \bullet H_2O$ at 16 kg ha^{-1} during seeding, combined with foliar applications of 0.3% solution of $ZnSO_4$ at 30 and 40 days after sowing, was the optimal treatment in terms of improving fodder maize productivity as well as quality of the fodder. This treatment almost doubled the Zn content of fodder and can be recommended to farmers for obtaining high forage maize productivity with improved quality.

Author Contributions: Conceptualization: B.K.; methodology: B.K.; formal analysis and investigation: B.K. and H.R.; writing, original draft preparation: B.K.; writing, review and editing: H.R. and J.S.; resources: B.K.; supervision: B.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no funding.

Data Availability Statement: The data presented in this study are available upon fair request to the corresponding author.

Conflicts of Interest: The author declares no conflict of interest.

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