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Biofortification of maize fodder with zinc improves forage productivity and nutritive value for livestock

B. Kumar[1,3] and H. Ram[2]

[1] Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India
[2] Punjab Agricultural University, Ludhiana, Punjab, India

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[3] Corresponding author:
e-mail: dr.balwinderkumar@rediffmail.com

ABSTRACT. Zinc (Zn) deficiency in the soil negatively affects production of maize forage, and consequently dietary Zn intake by the livestock. The aim of the study was to investigate the effect of rate and method of $ZnSO_4 \cdot H_2O$ application on forage yield, quality and digestibility of maize fodder grown on a low DTPA-extractable Zn soil. The treatments were: T_1 = control; T_2 = foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 days after sowing (DAS); T_3 = foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 and 40 DAS; T_4 = soil application of 16 kg/ha $ZnSO_4 \cdot H_2O$; T_5 = soil application of 16 kg/ha $ZnSO_4 \cdot H_2O + T_2$; and T_6 = soil application of 16 kg/ha $ZnSO_4 \cdot H_2O + T_3$. The experiment was performed in a randomized complete block design. The optimal rate and method of $ZnSO_4 \cdot H_2O$ application resulting in improved fodder yield and quality of maize was obtained by T_6 treatment. An increase in the fresh fodder yield, dry matter (DM) yield and Zn uptake by 25, 46.9 and 160.7%, respectively were recorded under T_6 treatment. Digestibility parameters as digestible crude protein, total digestible nutrients, digestible DM, DM intake, net energy for lactation, digestible feed energy, relative feed value and relative forage quality of fodder were significantly ($P \leq 0.05$) improved by soil plus double foliar $ZnSO_4 \cdot H_2O$ fertilization (T_6); however, a significant reduction in fibres content in fodder was noted. So, the Zn enrichment method combining soil addition and foliar spraying is the best one to improve the quality of maize fodder, and thus can be a good way to introduce Zn into animal nutrition.

Introduction

Maize (*Zea mays* L.) is an important cereal crop worldwide, used as food for human beings, feed for livestock, and a raw material for industry (Anees et al., 2016). It is more commonly used in lifestock feeding than other cereal fodder crops due to its higher digestibility and palatability (Wadhwa et al., 2010). Farmers also prefer maize for making high-quality maize-cob silage for feeding the livestock during lean fodder production periods (Kumar et al., 2019).

Zinc (Zn) deficiency in the soil is one of the major micronutrient constraints to crop and pasture production throughout the world in all regions with arid to tropical climate (Alloway, 2008; Cakmak and Kutman, 2018). The major reason for the deficiency of micronutrients, such as Zn, is chiefly ascribed to the introduction of high yielding crop varieties in the past, imbalanced fertilizer application to the soil and low soil organic matter content (Gupta et al., 2016; Kumar et al., 2016; Pal et al., 2020). More than 50% of the Indian soils are now deficient in Zn, particularly in highly intensive cultivated

Indo-Gangetic plains of North-West India (Gupta et al., 2016; Cakmak and Kutman, 2018). The deficiency of Zn in Indian soils is expected to increase to 63% by the year 2025 as more areas of marginal land are brought under intensive cultivation without adequate micronutrient fertilization (Tripathi et al., 2009). Moreover, soil pH, redox conditions, cation exchange capacity (CEC), microbial activity, organic matter and water content are important soil properties governing soil mineral availability to the plants. High soil pH is often considered as the major factor limiting the phyto-availability of Zn and other micronutrients in the rhizosphere solution (Gupta et al., 2016). The soils of the region are Zn-deficient as the critical level of Zn deficiency in soils falls in the range from 0.6 to 1.0 mg Zn/kg (diethylenetriaminepentaacetic acid (DTPA)-extractable). The low level of Zn in soils has resulted in a widespread deficiency of this element in both food and forage crops (Alloway, 2008; Gupta et al., 2016); thereby adversely affecting the health of the human and livestock in tropical countries (Tripathi et al., 2009; Kumar and Dhaliwal, 2021). Moreover, forage crops are generally grown in marginal soils which are deficient in micronutrient contents, particularly in Zn in South Asian countries leading to low yield and quality of fodder for the livestock (Alloway, 2008; Kumar et al., 2016). In a study conducted by Yadav and Khirwar (2000) in Haryana, India, it was reported a positive correlation between low levels of Zn in buffalo milk and low level of Zn in soil and fodder grown on it. Zinc deficiency in adult animals can cause lameness, hoof deformation, impaired locomotion, increased risk of infectious diseases, lower or impaired reproductive efficiency, anoestrus and repeat breeding leading to low milk production (Hosnedlova et al., 2007). A significant increase in Zn content in milk can be achieved by biofortification of forage crop which might further meet the Zn requirements of human beings through milk as well (Hosnedlova et al., 2007).

Maize is reported to be highly responsive to the soil as well as the foliar application of Zn (Alloway, 2008; Ahmad et al., 2012; Ryan et al., 2013). The critical level of Zn in crops (maize, wheat, cowpea etc.) falls in the range of 10–20 mg/kg on a dry matter basis (Kumar and Dhaliwal, 2021). Significant reduction in fodder yield and quality of maize has been reported due to Zn deficiency in soil (Ahmad et al., 2012). There is a strong need for enriching the maize forage with Zn through soil or foliar application which is economical and farmer-friendly. Till now, Zn biofortification of cereals and legumes have been reported at many parts of the world (Alloway, 2008); however, a little information is available in the literature regarding the Zn biofortification of forage crops (Capstaff and Miller, 2018) and its effect on digestibility parameters of the forage. The economic importance of livestock production offers the opportunity to biofortify forage crops, thereby, improving the health of animals and, consequently their product consumers (Capstaff and Miller, 2018). The increased Zn uptake by the crop under Zn fertilization will help to meet the Zn requirements of livestock, particularly in South Asian countries where soils and forage crops are generally deficient in Zn (Alloway, 2008; Ryan et al., 2013; Cakmak and Kutman, 2018). Maize fodder enriched with Zn when fed to the livestock will increase milk production, increase Zn content in milk and reduce the risk of infectious diseases such as metritis and mastitis in the cattle (Hosnedlova et al., 2007; Gupta et al., 2016; Kumar et al., 2016). The present field study was, therefore, planned to assess the effect of soil and foliar-applied Zn on productivity and quality of maize fodder in a semi-arid environment of the Indo-Gangetic plains of North-West India. It was hypothesized that Zn biofortification through the soil and/or foliar application will increase maize herbage yield, quality and digestibility of fodder for livestock nutrition.

Material and methods

Study site, weather and soil characteristics

The field experiment was conducted for two consecutive years during the summer seasons of 2013 (July to September) and 2014 (May to July) at the Fodder Production Area, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India (30°56′ N, 75°52′ E, 247 m above mean sea level). The total amount of rainfall during the cropping seasons was 375.6 and 44.0 mm during 2013 and 2014, respectively. Maximum and minimum air temperatures were 33.5 and 25.8 °C during the 2013 growing season, and 38.4 and 25.4 °C during the 2014 growing season. Mean relative air humidity ranged from 65.8 to 86.8% and from 36.4 to 61.2% for the 2013 and 2014 seasons, respectively. Lower sunshine hours were recorded for the 2013 growing season in comparison to that in 2014.

Soil texture, pH and conductivity (soil:water ratio of 1:2), organic carbon, available N, available (Olsen) P, 1N NH_4OAc-extractable K, available micronutrients (DTPA-extractable Zn, Fe, Cu and Mn) contents were measured on the atomic absorption spectrophotometer (Varian AAS FS 240; Varian, Palo Alto, CA, USA) by standard methods as described by Jackson (1973). The top 15 cm of the soil surface was loamy sand (*TypicUstochrept*) in texture, having pH 8.4 and electrical conductivity (EC) 0.21 dSm^{-1}. The soil of the experimental field was low in organic C (0.22%), low in available N (258 kg/ha), available P (11.9 kg/ha) and DTPA-extractable Zn (0.52 mg/kg) as measured by atomic absorption spectroscopy. The soil had moderate content of available K (Jackson, 1973).

Experimental design and treatments

The experiment was conducted with a set of six treatments in a randomized complete block design (RCBD) with three replications. The six treatments of $ZnSO_4 \cdot H_2O$ application were T_1 = control, T_2 = foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, T_3 = two foliar sprays of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing, T_4 = soil application of 16 kg/ha $ZnSO_4 \cdot H_2O$ at sowing, T_5 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing and T_6 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing. Zinc as Zn sulphate monohydrate ($ZnSO_4 \cdot H_2O$) with 33% Zn was used in the study. The soil Zn application treatment consisted of 16 kg $ZnSO_4 \cdot H_2O$ per ha (5 kg Zn per ha), which was dissolved in water (250 l/ha), then sprayed on the soil surface to ensure uniform distribution and was later incorporated into the soil before planting. In foliar application treatments, 0.75 kg $ZnSO_4 \cdot H_2O$ per ha (0.25 kg Zn per ha) and unslaked lime, were dissolved in 250 l water per ha, and the solution was sprayed on maize foliage 30 (V7 stage) and 40 (V10 stage) days after sowing during evening hours when the wind was calm and the temperature was mild. Applications of foliar sprays on the crop at different stages were made with a manually operated knapsack sprayer pump.

Agronomic practices

Maize cultivar J 1006 (Punjab Agricultural University, Ludhiana, India) was used in the study. Sowing was done on 19 July and 9 May during 2013 and 2014, respectively, using a seed rate at 50 kg/ha with the spacing of 30 × 10 cm (333333 plants/ha) in a plot size of 14.4 m^2. The recommended dose 90 kg N/ha was applied as urea (46% N), split into two halves, one applied at sowing and the other applied as top dressing 30 days after sowing. In addition, 30 kg P_2O_5 was applied at sowing as single super phosphate (16.0% P_2O_5). For weed control, herbicide atrazine 50 WP (1.25 kg/ha) was sprayed immediately after sowing. The crop was manually harvested from a net area measuring 9.9 m^2 in the middle of each plot on 23 September 2013 and 14 July 2014 at the age of 60 days for green fodder purpose (Bhatti and Kaur, 2020). Fresh fodder yield was measured by harvesting the crop from an area of 9.9 m^2 which was then converted to Mg/ha.

Laboratory analyses

Whole maize plants collected from each plot at harvest were washed sequentially with tap water, acidulated water containing 0.01N HCl, distilled water and deionized water. 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 (Thomas Scientific, Swedesboro, NJ, USA) fitted with stainless steel blades and passed through a 40-mm mesh sieve and stored in airtight plastic bags for nutrient composition determination. Crude protein (CP) content was (determined by multiplying N% by 6.25 and expressed as a percentage (AOAC International, 2000). Zinc uptake was calculated by multiplying the Zn content (determined by an atomic absorption spectroscopy) by dry matter (DM) yield of fodder (Page et al., 1982).

Estimation of fodder quality and digestibility parameters

Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using the procedure given by Van Soest et al. (1991). Hemicellulose content was calculated by the difference between NDF and ADF. Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), digestible crude protein (DCP), net energy for lactation (NE_L), digestible feed energy (DFE), relative feed value (RVF), relative forage quality (RFQ) were estimated according to the following equations adapted from Lithourgidis et al. (2006) and Kumar et al. (2016) from the measured variables:

$$\text{total digestible nutrients (TDN, \%)} = 87.84 - (0.7 \times \text{ADF});$$

$$\text{dry matter intake (DMI, \% DM basis)} = 120 / \text{NDF};$$

$$\text{dry matter digestibility (DDM, \%)} = 88.9 - (0.779 \times \text{ADF});$$

$$\text{digestible crude protein (DCP, \%)} = (0.929 \times \text{CP}) - 3.77;$$

$$\text{net energy for lactation } (NE_L\text{, Mcal/kg}) = 1.5 - (\text{ADF} \times 0.0267);$$

$$\text{digestible feed energy (DFE, Mcal/kg)} = 4.4 \times (\text{TDN} / 100);$$

$$\text{relative feed value (RFV, \%)} = (\text{DDM} \times \text{DMI}) / 1.29;$$

$$\text{relative feed quality (RFQ, \%)} = (\text{TDN} \times \text{DMI}) / 1.23.$$

Statistical analyses

The data were subjected to analysis of variance (ANOVA) using IRRISTAT version 92 (IRRI, 1992). Means comparisons were done using the least significant difference (LSD) procedure at $P \leq 0.05$. Pearson's correlation coefficient (r) was calculated among the different variables and a correlation matrix was prepared to quantify the relationship among the different variables and fodder yield and other traits.

Results

Fresh fodder yield and dry matter yield

Fresh fodder yield (FFY) of maize was significantly ($P \leq 0.05$) affected by the Zn fertilization treatments (Table 1). In comparison to the control, all treatments of Zn sulphate application improved the FFY (Table 1). The highest FFY of 57 Mg/ha was achieved with the application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing combined with foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing (T_6), registering an increase of 25% over the control; however, T_6 treatment did not differ significantly from the T_5 treatment (soil application plus singular foliar spray). Zinc application to soil alone (T_4) was superior to singular foliar application of $ZnSO_4 \cdot H_2O$ (T_2) in enhancing the FFY; but there was no significant difference between T_3 (double foliar sprays) and T_4 (soil alone) treatments. Furthermore, no statistical difference in FFY between T_2 (singular foliar spray) and T_3 (double foliar spray) treatments was noticed. The improvement in mean FFY amounted 9.8, 14.6 and 16.7% with the T_2, T_3 and T_4 treatments, respectively, over control.

Similar to FFY, the increasing trend in dry matter yield (DMY) was also recorded with Zn fertilization. Combined application of soil plus foliar $ZnSO_4 \cdot H_2O$ sprays 30 and 40 days after sowing (T_6) increased DMY to the highest level which accounted for 46.9% over control (Table 1), but no significant difference between T_6 and T_5 treatments (soil Zn application plus single foliar Zn) was recorded. Sole foliar application of $ZnSO_4 \cdot H_2O$ 30 days after sowing (T_2) increased DMY to the tune of 18.5% and was significantly superior to the control. The T_2 and T_3 treatments were statistically the same in respect to DMY, but both treatments were found better than the control treatment (T_1). Similarly, the increase in DMY of the crop was also statistically similar in the T_3, T_4 and T_5 treatments (Table 1).

Zinc uptake by fodder

Zinc uptake (ZnU) by maize fodder was significantly affected by the different Zn application treatments (Table 1). The soil $ZnSO_4 \cdot H_2O$ along with two foliar sprays of 0.3% $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing (T_6) recorded 423.7 g/ha of Zn uptake in fodder which was significantly higher than the control (160.7%) and other experimental treatments. Among all Zn fertilization treatments, the lowest increase in ZnU in fodder was recorded with the foliar spray of 0.3% $ZnSO_4 \cdot H_2O$ 30 days

Table 1. Effect of foliar and soil-applied zinc (Zn) sulphate ($ZnSO_4 \cdot H_2O$) on fresh fodder yield (FFY), dry matter yield (DMY) and Zn uptake (ZnU) by maize (mean of two years)

Indices	FFY, Mg/ha	Percent increase over control	DMY, Mg/ha	Percent increase over control	ZnU, g/ha	Percent increase over control
Treatment[1]						
T_1	45.6 ± 1.72^d	–	8.1 ± 0.21^d	–	162.5 ± 6.12^e	–
T_2	50.1 ± 0.75^c	9.8	9.6 ± 0.22^c	18.5	236.3 ± 3.91^d	45.4
T_3	52.3 ± 0.30^{bc}	14.6	10.2 ± 0.24^{bc}	25.9	281.3 ± 3.53^c	73.1
T_4	53.2 ± 0.96^b	16.7	10.5 ± 0.10^b	29.6	273.1 ± 9.03^c	68.0
T_5	55.6 ± 1.87^{ab}	21.9	11.3 ± 0.28^{ab}	39.5	364.2 ± 9.04^b	124.1
T_6	57.0 ± 1.05^a	25.0	11.9 ± 0.25^a	46.9	423.7 ± 15.1^a	160.7
SEM	0.77	–	0.30	–	6.3	–
LSD ($P \leq 0.05$)	2.5	–	0.8	–	20.0	–

[1] treatments: T_1 = control, T_2 = foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, T_3 = two foliar sprays of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing, T_4 = soil application of 16 kg/ha $ZnSO_4 \cdot H_2O$ at sowing, T_5 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, and T_6 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing; SEM – standard error of the mean; LSD – least significant difference; $^{a\text{-}e}$ – means within columns with different superscripts are significantly different at $P \leq 0.05$

after sowing (T_2), yet it was significantly superior to the control. Two foliar sprays of 0.3% $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing caused significantly higher ZnU in fodder over T_2 treatment. On the other hand, basal soil application of 16 kg/ha $ZnSO_4 \cdot H_2O$ (T_4) treatment recorded 22.6% more ZnU in fodder biomass than the sole foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 days after sowing (T_2), but did not differ from the foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing (T_3).

Crude protein content

Significant ($P \leq 0.05$) improvement in forage crude protein (CP) content was recorded with the soil, foliar and soil plus foliar application of Zn (Table 2). The CP content ranged from 8.13 to 9.76%. The maximum mean increase in forage CP content was recorded in the T_6 treatment which did not differ from the T_4 and T_5 treatments involving soil application of 16 kg/ha $ZnSO_4 \cdot H_2O$. The singular foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 days after sowing (T_2) caused an increase in CP content in comparison to control treatment (8.0% increase). The CP content in fodder from the maize treated with double foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing (T_3) was higher than in the T_2 treatment, but did not differ from the T_4 and T_5 treatments.

Table 2. Effect of zinc (Zn) sulphate ($ZnSO_4 \cdot H_2O$) application on the crude protein (CP) content, neutral detergent fibre (NDF), acid detergent fibre (ADF), total digestible nutrients (TDN) and hemi-cellulose (HC) content of maize fodder (mean of two years), % dry matter

Indices	CP	NDF	ADF	TDN	HC
Treatments[1]					
T_1	8.13 ± 0.12^{d}	66.8 ± 0.14^{a}	36.6 ± 0.18^{a}	62.2 ± 0.12^{d}	30.3 ± 0.07^{a}
T_2	8.78 ± 0.42^{c}	65.8 ± 0.07^{b}	35.9 ± 0.03^{b}	62.7 ± 0.03^{c}	29.9 ± 0.09^{a}
T_3	9.02 ± 0.30^{b}	65.7 ± 0.16^{b}	35.5 ± 0.12^{bc}	63.1 ± 0.10^{b}	30.2 ± 0.27^{a}
T_4	9.23 ± 0.29^{ab}	64.6 ± 0.12^{c}	35.4 ± 0.25^{c}	63.0 ± 0.17^{bc}	29.2 ± 0.19^{b}
T_5	9.51 ± 0.18^{ab}	63.8 ± 0.17^{d}	35.2 ± 0.26^{c}	63.2 ± 0.17^{b}	28.6 ± 0.21^{c}
T_6	9.76 ± 0.02^{a}	63.2 ± 0.13^{e}	34.3 ± 0.07^{d}	63.8 ± 0.06^{a}	28.8 ± 0.20^{bc}
SEM	0.23	0.30	0.18	0.09	0.17
LSD ($P \leq 0.05$)	0.72	0.50	0.50	0.30	0.50

[1] treatments: T_1 = control, T_2 = foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, T_3 = two foliar sprays of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing, T_4 = soil application of 16 kg/ha $ZnSO_4 \cdot H_2O$ at sowing, T_5 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, and T_6 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing; SEM – standard error of the mean; LSD – least significant difference; $^{a\text{-}e}$ – means within columns with different superscripts are significantly different at $P \leq 0.05$

Fibres estimation

Neutral detergent and acid detergent fibres (NDF and ADF, respectively) in the fodder decreased with Zn fertilization in the crop (Table 2). The highest decrease in NDF (5.4%) and ADF (6.2%) contents was recorded with treatment involving soil and two foliar applications of $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing (T_6) over the control treatment. Foliar $ZnSO_4 \cdot H_2O$ application treatments (T_2 and T_3) were found statistically similar in respect to NDF and ADF, but were significantly superior over control. Soil application of $ZnSO_4 \cdot H_2O$ (T_4) caused significantly lower NDF values than foliar treatments (T_2 and T_3); however, in the case of ADF the T_4 and T_3 treatments did not differ significantly between each other (Table 2).

Estimated digestibility parameters

Means of TDN of the forage were improved by 2.5% with the T_6 treatment over the control (Table 2). Singular foliar application treatment of $ZnSO_4 \cdot H_2O$ (T_2) was significantly better in improving TDN of fodder by 0.8% over the control treatment, but was found statistically on par with the T_4 treatment. Double foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing (T_3) caused a significantly higher TDN value (63.1%) than the singular foliar spray (62.7%). Similarly, the T_5 treatment (soil plus singular foliar spray) also significantly improved the TDN value than the T_2 treatment. However, statistically similar TDN values were recorded among T_2 (62.7%), T_3 (63.1%) and T_4 (63.0%) treatments, but all were significantly higher than the control (T_1). Zinc fertilization significantly reduced the forage hemi-cellulose (HC) content (Table 2). The T_5 and T_6 treatments recorded the lowest forage HC contents (6.0% than the control).

Zinc fertilization significantly improved the forage DMI (Table 3). The soil treatment with $ZnSO_4 \cdot H_2O$ along with a double foliar spray of $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing (T_6) or singular foliar spray of $ZnSO_4 \cdot H_2O$ 30 days after sowing (T_5) caused the highest DMI (1.90 and 1.88% for T_6 and T_5, respectively) and that was 6.1 and

5.0% more than the control treatment, respectively. The foliar application of 0.3% $ZnSO_4 \cdot H_2O$ 30 days after sowing (T_2) and 30 and 40 days after sowing (T_3) caused statistically similar DMI means (1.82 and 1.83%); however, both treatments were significantly higher than the control. Mean DMI under sole soil $ZnSO_4 \cdot H_2O$ (T_4) fertilizer treatment was significantly higher than the foliar treatments (T_2 and T_3). The forage DDM values were significantly improved with $ZnSO_4 \cdot H_2O$ fertilization, where the highest increase was stated with the T_6 treatment which was statistically superior to all other treatments (Table 3). The mean increase in forage DDM of the T_6 treatment over control reached 3.0%.

Table 3. Effect of zinc (Zn) sulphate ($ZnSO_4 \cdot H_2O$) application on dry matter intake (DMI), digestible dry matter (DDM) and digestible crude protein (DCM) of fodder maize (mean of two years)

Indices	DMI, % DM basis	DDM, %	DCP, %
Treatments[1]			
T_1	1.79 ± 0.003^d	60.3 ± 0.15^e	3.79 ± 0.11^c
T_2	1.82 ± 0.003^c	60.9 ± 0.03^d	4.39 ± 0.40^{bc}
T_3	1.83 ± 0.007^c	61.3 ± 0.07^c	4.61 ± 0.28^b
T_4	1.86 ± 0.003^b	61.3 ± 0.20^c	4.81 ± 0.28^{ab}
T_5	1.88 ± 0.006^a	61.5 ± 0.18^b	5.06 ± 0.17^{ab}
T_6	1.90 ± 0.006^a	62.1 ± 0.09^a	5.30 ± 0.02^a
SEM	0.01	0.11	0.21
LSD ($P \leq 0.05$)	0.016	0.35	0.67

[1] treatments: T_1 = control, T_2 = foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, T_3 = two foliar sprays of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing, T_4 = soil application of 16 kg/ha $ZnSO_4 \cdot H_2O$ at sowing, T_5 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, and T_6 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing; SEM – standard error of the mean; LSD – least significant difference; $^{a-e}$ – means within columns with different superscripts are significantly different at $P \leq 0.05$

Zinc fertilization to the soil, foliar or combination of both treatments significantly improved NE_L of maize fodder (Table 4). The highest NE_L value of 0.583 Mcal/kg was recorded for the T_6 treatment which was statistically superior to all other treatments. The T_3, T_4 and T_5 treatments did not differ among each other but values in these treatments were significantly higher than in the T_1 and T_2 treatments. Significantly higher values of DFE of fodder were recorded in the T_6 treatment involving soil and double foliar $ZnSO_4 \cdot H_2O$ fertilization approaches (Table 4). The T_3, T_4 and T_5 treatments recorded statistically similar values of DFE, but all were significantly higher than the control treatment.

Table 4. Effect of zinc (Zn) sulphate ($ZnSO_4 \cdot H_2O$) application on net energy for lactation (NE_L), digestible feed energy (DFE), relative feed value (RFV) and relative forage quality (RFQ) of fodder maize (mean of two years)

Indices	NEL	DFE	RFV	RFQ
	Mcal/kg		%	
Treatments[1]				
T_1	0.521 ± 0.005^d	2.74 ± 0.003^d	84.1 ± 0.34^e	90.8 ± 0.35^f
T_2	0.540 ± 0.001^c	2.76 ± 0.003^c	86.1 ± 0.11^d	92.9 ± 0.12^e
T_3	0.555 ± 0.003^b	2.78 ± 0.003^b	86.9 ± 0.17^d	93.7 ± 0.17^d
T_4	0.553 ± 0.007^b	2.77 ± 0.009^b	88.3 ± 0.42^c	95.2 ± 0.42^c
T_5	0.559 ± 0.007^b	2.78 ± 0.006^b	89.5 ± 0.47^b	96.5 ± 0.47^b
T_6	0.583 ± 0.002^a	2.81 ± 0.003^a	91.5 ± 0.10^a	98.5 ± 0.15^a
SEM	0.004	0.005	0.26	0.28
LSD ($P \leq 0.05$)	0.012	0.02	0.83	0.89

[1] treatments: T_1 = control, T_2 = foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, T_3 = two foliar sprays of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing, T_4 = soil application of 16 kg/ha$ZnSO_4 \cdot H_2O$ at sowing, T_5 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 days after sowing, and T_6 = soil application of $ZnSO_4 \cdot H_2O$ (16 kg/ha) at sowing plus foliar spray of $ZnSO_4 \cdot H_2O$ (0.3%) 30 and 40 days after sowing; SEM – standard error of the mean; LSD – least significant difference; $^{a-f}$ – means within columns with different superscripts are significantly different at $P \leq 0.05$

Relative feed value and relative forage quality

A significant increase in RFV was observed with Zn fertilization of maize fodder (Table 4). Mean increase in RFV over the control was 9% under the T_6 treatment. Foliar $ZnSO_4 \cdot H_2O$ treatments (T_2 and T_3) recorded statistically similar mean RFV, but both treatments were significantly higher than the control one. Soil $ZnSO_4 \cdot H_2O$ application improved the RFV of fodder over foliar $ZnSO_4$ treatments (T_2 and T_3). The obtained results showed a higher RFQ index with various Zn fertilization treatments (Table 4). The highest RFQ value of 98.5 was recorded in the T_6 treatment, which was significantly higher than in other treatments. Significantly lower RFQ value was recorded in foliar $ZnSO_4 \cdot H_2O$ fertilization (T_2 and T_3) than soil $ZnSO_4 \cdot H_2O$ application, but all the treatments were significantly higher than the control treatment. The mean increases in RFQ under the T_4, T_5 and T_6 treatment reached 4.8, 6.3 and 8.5% respectively, over the control treatment. Foliar $ZnSO_4 \cdot H_2O$ treatment led to 2.3 to 3.1% improvement in mean RFQ over the control.

Correlation analysis

The correlation analysis of maize FFY, DMY, NDF, ADF, digestibility parameters and ZnU (Table 5) indicated significant ($P < 0.05$) or highly

Table 5. Pearson's correlation coefficient and significance level among zinc uptake, quality and digestibility parameters of the fresh fodder yield (mean of two years)

	FFY, Mg/ha	DMY, Mg/ha	CP, %	NDF, %	ADF, %	TDN, %	DDM, %	DMI, %	HC, %	DCP, %	NE_L, Mcal/kg	DFE, Mcal/kg	RVF, %	RFQ, %	ZnU, g/ha
FFY, Mg/ha	–														
DMY, Mg/ha	0.818**	–													
CP, %	0.597**	0.770**	–												
NDF, %	−0.341*	−0.535**	−0.505**	–											
ADF, %	−0.511**	−0.622**	−0.588**	0.868**	–										
TDN, %	0.512**	0.622**	0.588**	−0.869**	−1.001**	–									
DDM, %	0.512**	0.623**	0.589**	−0.868**	−1.000**	1.003**	–								
DMI, %	0.360*	0.551**	0.517**	−0.998**	−0.872**	0.873**	0.873**	–							
HC, %	0.079^{NS}	−0.039^{NS}	−0.233^{NS}	0.320^{NS}	0.332*	−0.332*	−0.332*	−0.327^{NS}	–						
DCP, %	0.527**	0.478**	0.542**	0.004^{NS}	−0.078^{NS}	0.077^{NS}	0.078^{NS}	0.010^{NS}	0.135^{NS}	–					
NE_L, Mcal/kg	0.557**	0.448**	0.356*	0.073^{NS}	−0.068^{NS}	0.069^{NS}	0.069^{NS}	−0.060^{NS}	0.515**	0.595**	–				
DFE, Mcal/kg	0.585**	0.469**	0.365*	0.060^{NS}	−0.083^{NS}	0.078^{NS}	0.080^{NS}	−0.049^{NS}	0.523**	0.576**	0.990**	–			
RVF, %	0.404*	0.577**	0.548**	−0.987**	−0.935**	0.935**	0.935**	0.987**	−0.335*	0.026^{NS}	−0.026^{NS}	−0.014^{NS}	–		
RFQ, %	0.399*	0.573**	0.545**	−0.989**	−0.930**	0.930**	0.930**	0.989**	−0.334*	0.024^{NS}	−0.030^{NS}	−0.017^{NS}	1.000**	–	
ZnU, g/ha	0.170*	0.183*	0.326*	−0.164^{NS}	−0.253^{NS}	0.253^{NS}	0.255^{NS}	0.166^{NS}	−0.365*	0.253^{NS}	−0.180^{NS}	−0.194^{NS}	0.199*	0.196*	–

FFY – fresh fodder yield; DMY – dry matter yield; CP – crude protein content; NDF – neutral detergent fibre; ADF – acid detergent fibre; TDN – total digestible nutrients; DDM – digestible dry matter; DMI – dry matter intake; HC – hemi-cellulose; DCP – digestible crude protein; NE_L – net energy for lactation; DFE – digestible feed energy; RVF – relative feed value; RFQ – relative forage quality; ZnU – zinc uptake; * and ** indicate significance at the 0.05 and 0.01 probability levels, respectively; ^{NS} indicates non-significant probability significant correlations ($P < 0.01$). The quality parameters such as CP, TDN, DDM and DMI were significantly and positively correlated with FFY and DMY. Zinc fertilization significantly reduced HC content of fodder as a significant negative correlation (Table 5) existed between ZnU and HC content (−0.365*), however, NDF and ADF content tended to be negatively correlated (not significantly) with ZnU. Positive ($P < 0.01$) correlations were recorded between FFY and DCP (0.527**); FFY and NE_L (0.583**) as well as FFY and DFE (0.585**) of fodder. Finally, Zn fertilization through soil, foliar and combination of both improved quality of fodder, as positive correlations were recorded with respect to ZnU-RFV index (0.199*) and ZnU-RFQ index (0.196*). A positive correlations ($P < 0.01$) also existed between ZnU and FFY (0.170*), and ZnU and DMY (0.183*). However, no correlation was recorded between ZnU and other digestibility parameters such as TDN, DDM, DMI, DCP, NE_L and DFE.

Discussion

In the present study, the increase in FFY and DMY with Zn application was attributed to better plant growth and development under Zn application treatments. The positive effects of Zn fertilization either through soil or foliar application on the yield of cereals and legumes such as wheat, rice, soyabean, chickpea and cowpea grain had also been reported by several other researchers (Alloway, 2008; Cakmak and Kutman, 2018; Pal et al., 2020; Kumar and Dhaliwal, 2021). However, information on the biofortification of maize fodder with Zn and its impact on green fodder yield and its quality attributes are scarce. Zinc is known to activate various enzymatic reactions and improve photosynthesis and improve carbohydrate assimilate portioning from source to sink which led to an increase in FFY and DMY. Zinc application to soil alone was superior to one and two foliar applications in enhancing the FFY and DMY which might be due to more and longer availability of Zn to the crop. During early growth stages, adequate soil available Zn is important to get high fodder yield as a foliar application of Zn at late growth stage recorded less FFY and DMY than soil Zn application method (Kumar and Dhaliwal, 2021). Plants with Zn deficiency in the early stages of development find it difficult to express their maximum genetic potential which might be due to damage both in the maintenance of enzyme activity as well as enzyme synthetase of tryptophan (Castagnara et al., 2012).

Minerals and trace elements derived from forages play an important role in milk production, reproduction and maintaining livestock health (Kumar et al., 2016; Ali et al., 2019). For better growth and development of cattle, proper supply of trace elements such as Zn through good quality fodder is important. Plant research has focused on the goal of biofortifying cereals, mainly rice and wheat, but there is still potential to improve the nutritional quality of forage crops (Capstaff and Miller, 2018). Different Zn fertilization methods significantly improved ZnU in the fodder which might be due to its role in photosynthesis and metabolic processes, which help in augmenting the production of photosynthates and their translocation to different parts, finally increasing ZnU by the forage crop (Pal et al., 2020; Kumar and Dhaliwal, 2021). Higher Zn availability to cattle through Zn enriched fodder will meet the requirement of Zn which is very important for the immune system in the livestock (Capstaff and Miller, 2018). Zinc applications through the soil and a combination of soil and foliar treatments were more effective in increasing ZnU than the foliar treatment alone. This may be due to the early continuous supply of Zn to the crop, in addition, later foliar applications also loaded more Zn in leaves resulting in higher yield and ZnU in plants. Yerokun and Chirwa (2014) reported that soil application of Zn was more effective in raising yield level, whereas, foliar application of 2–4 kg Zn per ha was most effective for increasing the Zn mass concentration in plant tissues of maize. In the foliar application of Zn (T_2 and T_3), an increase in ZnU occurred due to the easy penetration of Zn into the plant through stomatal pores as reported by Gupta et al. (2016), but both soil and foliar applications of Zn fertilizers enhance plant-available Zn pool. Considering the daily dietary requirement of 30 mg/kg DM for adult cattle, the increased ZnU upon Zn fertilization will meet the requirements of livestock, particularly, in South Asian countries where soils and forage crops are generally deficient in Zn (Ryan et al., 2013; Kumar et al., 2016). Hosnedlova et al. (2007) reported that Zn content in cattle milk can be influenced by forage nutrition. Feeding maize fodder enriched with Zn to the livestock will increase milk production, improve reproduction efficiency and will reduce the susceptibility to infectious diseases such as metritis and mastitis which will further boost the dairy industry as a whole (Hosnedlova et al., 2007; Alloway, 2008; Gupta et al., 2016; Kumar et al., 2016).

Zinc fertilization reduced NDF, ADF and HC contents in the fodder, which could be attributed to the fact that higher Zn availability promoted protein synthesis and decreased the soluble carbohydrate content in the fodder resulting in higher palatability and digestibility (Alloway, 2008; Capstaff and Miller, 2018). Castagnara et al. (2012) also reported significantly lower values of fibres (NDF and ADF) at doses of 0.2 and 0.4 mg/dm^3 of Zn, respectively in the white oats (*Avena sativa* L.) grown in soil with moderate Zn content in the glasshouse. Considerable reduction in the fibre values and enhancement of protein content of fodder with soil and foliar Zn fertilization, in this study, indicated more intake and digestibility of the fodder.

For livestock producers, obtaining high forage yield along with high-quality for animals is the prime objective. Improvement in estimated digestibility parameters such as TDN, DMI, DDM and DCP with soil, foliar and soil plus foliar $ZnSO_4$ application reflected that, under Zn deficient soil, Zn fertilization through soil plus foliar application could be one of the most important criteria for increasing the yield and quality of maize fodder. Total digestible nutrients are a measure of forage energy as well as forage digestibility, the values of which were significantly increased with different Zn fertilization methods. Typically, the greater the value of TDN, the more energy-dense feedstuff is considered (Kumar et al., 2016). Good quality fodder must contain TDN equal or greater than 65% (Ali et al., 2019). However, observed TDN in the present study among different $ZnSO_4$ treatments ranged from 62.7 to 63.7 (mean value), although was significantly higher in treatment T_6, which falls within the range of good quality forage as reported above. Digestible dry matter is the percentage of a forage sample that is digestible (Kumar et al., 2016). Zinc application to the crop increased the DDM as it improved the fodder quality by reducing fibres. Dry matter intake is the amount of DM consumed by the animal and intake increases as the digestibility of forage increases (Kumar et al., 2016; Lithourgidis et al., 2006). The reduction in HC percentage in fodder with Zn fertilization improved the DDM and DMI of the fodder. The improved DMI of fodder with Zn fertilization is an indication of better performance of the livestock in terms of milk and meat production. Rana et al. (2013) also recorded significantly higher *in vitro* DMD (IVDMD) and DDM in sorghum with foliar sprays of 0.5% $ZnSO_4$ 35 and 45 days after sowing over control. The increased ZnU due to various Zn fertilization treatments recorded a significant and positive correlation with CP (0.326*) and TDN percent (0.250*) indicating that Zn application improved these parameters which are essential for livestock health and development.

Relative feed value is an index used to rank the forages according to their overall nutritive value. Relative feed value and RFQ are good indicators for the forage digestibility and quality, in addition to CP content (Lithourgidis et al., 2006; Kumar et al., 2016). Zinc application either through the soil, foliar spraying or combination of both improved RFV and RFQ of fodder over the control treatment, ensuring a better quality of the forage for livestock. Soil application of 16 kg $ZnSO_4 \bullet H_2O$ per ha plus foliar sprays with 0.3% $ZnSO_4 \bullet H_2O$ resulted in the higher RVF and RFQ over control, possibly due to sustained and longer availability of Zn through soil and foliar application which might have helped in better digestibility and DMI of fodder. The higher RFQ, the higher will be the quality of fodder and the better will be the performance of cattle. Lower RVF and RFQ values were reported with foliar $ZnSO_4$ treatments than with soil Zn application, indicating that Zn is essential for maize at early growth stages for getting better quality fodder for livestock. This was confirmed in this study as a positive correlations between ZnU-RFV and ZnU-RFQ of fodder were recorded. Similarly, Sajad et al. (2014) observed the highest RFV of maize fodder (79.3%) in treatment involving 100 kg N/ha + 10 kg Zn/ha than in the control (78.1%).

The present investigation confirmed that fodder maize performed well and produced higher FFY, with better quality in terms of better TDN, DDM, DMI, CP, NE_L, DCP, RFV and RFQ under $ZnSO_4 \bullet H_2O$ fertilization treatments, probably because of the better conversion of assimilates in plants. This was confirmed by the correlation study which showed a positive significant association between the FFY, DMY and fodder quality and digestibility parameters. Nonetheless, Zn fertilization significantly reduced the NDF, ADF and HC content of fodder which was clearly indicated with the negative correlations between Zn and fibres (ZnU-HC; ZnU-NDF and ZnU-ADF), thereby indicating higher digestibility and DMI of fodder by the livestock. Good quality forage has less crude fibre, higher digestibility and DMI (Lithourgidis et al., 2006; Kumar et al., 2016).

Conclusions

In the current study, the application of $ZnSO_4 \bullet H_2O$ resulted in a significant increase in fresh fodder yield (FFY), dry matter yield (DMY), quality and estimated digestibility parameters of fodder maize. Zinc application improved crude protein (CP) content, total digestible nutrients (TDN), dry matter digestibility (DDM) and dry matter intake (DMI) of the fodder. Fodder quality improvement, in terms of reduction in fibre contents (neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose (HC) and enhancement in relative feed value (RFV) and relative forage quality (RFQ) was obtained with soil and foliar $ZnSO_4$ fertilizer application. The increase in FFY, CP content and higher Zn accumulation due to Zn application will improve the economic status of livestock producers. Soil application of 16 kg $ZnSO_4 \cdot H_2O$ per ha at sowing plus foliar applications of 0.3% solution of $ZnSO_4 \cdot H_2O$ 30 and 40 days after sowing was the best treatment in terms of improving yield and quality of maize fodder. This treatment can be recommended to grow maize fodder on soils with low-available Zn content and to obtain a high forage productivity with improved quality.

Conflict of interest

The authors declare that there is no conflict of interest.

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