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Bio-Fortification of Oats Fodder through Zinc Enrichment to Reduce Animal Malnutrition

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Abstract: The availability of poor quality fodders for livestock feeding is a major hurdle for the dairy industry in South Asia. The low content of Zn in fodders, leads to its malnutrition in animals. In fodders, Zn facilitates protein synthesis, gene expressions, energy production and involves in various metabolic activities of plants, animals and humans. To achieve these objectives, multi-location field trials were conducted on enrichment of oats fodder with Zn through bio-fortification. The experiment consists of eight different treatment combinations for enrichment of oats fodder with Zn including control, soil and soil plus foliar application of Zn at different stages of plant growth. The results of the study reported that soil application of Zn at the rate of 25 kg/ha and soil + foliar application of Zn at 0.5% at 60 days after sowing (DAS) (jointing stage), 90 DAS (booting stage) and both 60 DAS and 90 DAS showed improvement in plant growth parameters. The results further reported that foliar application of Zn at the rate of 0.5% at 60 DAS, 90 DAS, both 60 DAS and 90 DAS reported significant increase in biomass yield and fodder quality. Integrated application of Zn at 25 kg/ha in soil along with foliar Zn at the rate of 0.5% at 60 DAS and 90 DAS showed maximum Zn enrichment, green fodder yield, dry fodder yield, plant height, and Zn accumulation in oats fodder as compared to other treatments of Zn application. These treatments of Zn application through integrated mode also reported significant improvement in fodder quality with maximum crude protein (CP) and crude protein yield (CPY) while neutral detergent fiber (NDF), acid detergent fiber (ADF), ash content and organic matter showed a non-significant effect. Thus, the results of experimental study concluded that soil and foliar application of Zn at 25 kg/ha and 0.5% Zn, respectively, at 60 DAS and 90 DAS enhanced the growth, yield and quality of oats fodder which will certainly improve livestock production through bio-fortification.

Key words: Zn, oats fodder, growth parameters, fodder yield, Zn enrichment, fodder bio-fortification.

1. Introduction

Livestock is the backbone of Indian agriculture. India has 15% of world's total cattle population with 482 million heads [1]. It ranks first in cattle population in world followed by Brazil and China. However, in order to meet the ever increasing demands of increasing population, a larger area is used for the production of food and cash crops, and very less arable land is available for fodder production. This is the main reason that fodder production is under focus in present agriculture. To meet such high demands, indiscriminate and continuous use of chemical fertilizers had adverse effects on soil health and led to decline in productivity due to limitation of one or more micronutrients. Fodder requirement of India is 53.19 dry matter in million tonnes but present production of green fodder is 22.74 dry matter in million tonnes [2]. So, there is a huge gap in demand and supply. There is a need for integrated nutrient management not only for securing high yield and productivity but also to arrest the deterioration of soil environment [3].

In oats production, the main constraint in achieving yield potential of fodder crop is imbalanced, and overuses of fertilizers results in micronutrients

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deficiency viz. Zn, Cu, Fe and Mn in crop's grain and straw. This has detrimental effects on animal health and causes several disorders. Application of micronutrients like Zn is also essential in addition to other macronutrients so there is no deficiency in animal fodder. Excessive use of phosphatic fertilizers causes Zn deficiency in the soil which is essential for basic metabolic processes of plant cycle such as tryptophan and chlorophyll synthesis, starch and nitrogen metabolism as well as for transportation of assimilates. It also facilitates protein synthesis and gene expressions, enzymes structure, energy production and Krebs cycle [4]. Therefore, micronutrient bio-fortification has been considered to be one of the new alternative approaches. Research on bio-fortification of oats fodder with Zn has already been started in many developing countries. However, information on extent of Zn enrichment in oats fodder and stage of Zn absorption was lacking. Therefore, the objective of this experiment was to investigate the effects of soil and foliar application of Zn on growth, herbage yield and quality of oats fodder under field conditions.

2. Materials and Methods

2.1 Experiment Location

The field experiment was conducted at two locations viz. Research Farm Area, Department of Soil Science, Punjab Agricultural University, Ludhiana (Location I) and Research Farm, Krishi Vigyan Kendra (KVK), Tarn Taran (Location II) during *rabi*, from November 2017 to March 2018. Ludhiana is situated at the latitude of 30°54′ N and longitude of 75°48′ E at an altitude of 307.42 m above sea level and KVK, Tarn Taran is situated at latitude of 30°45′ N and longitude of 74°92′ E at an altitude of 307.42 m above sea level.

2.2 Experimental Design and Details

The field was prepared with cultivator and disc harrow till the soil is brought a fine tilth. The fields at both locations were cultivated followed by planking. The broad dimensions of two fields were 64×24 m^2 and 20×25 m^2 and were divided into 54 plots each of size 6×3 m^2 and 3.3×2.5 m^2 at Punjab Agricultural University, Ludhiana and KVK, Tarn Taran, respectively. The remaining field was considered as non-experimental area. Oats, cv. OL-10 was sown during November 2017 at Ludhiana (Location I) and at KVK, Tarn Taran (Location II), respectively, using seed rate at 62.5 kg/ha with a row spacing of 20 cm and plant spacing of 6 cm.

2.3 Treatment Details

The field experiments during 2017-2018 were conducted with a set of 10 treatments in randomized block design (RBD) at same experimental sites. The details of treatments are given in Table 1. The recommended half dose N and a full dose of P in the form of diammonium phosphate were also applied before the sowing of the crop. The remaining half dose of N in the form of urea was applied at 35 days after sowing (DAS). The crop was harvested manually using sickle at the age of 110 d for green fodder purposes.

Table 1 Treatments detail of Zn bio-fortification experiment.

Treatments	Treatment details
T1	Control
T2	Soil application of Zn at 25 kg/ha
T3	Soil + foliar application of Zn at 0.5% at 60 days after sowing (DAS)
T4	Soil + foliar application of Zn at 0.5% at 90 DAS
T5	Soil+ foliar application of Zn at 05% at 60 DAS and 90 DAS
T6	Foliar application of Zn at 0.5% at 60 DAS
T7	Foliar application of Zn at 0.5% at 90 DAS
T8	Foliar application of Zn at 0.5% at 60 DAS and 90 DAS

2.4 Observations and Measurements

Before harvesting, various plant parameters as plant height, the number of tillers per plant were also recorded at 60, 90 and 110 DAS. Moreover, random samples of fodder were taken from each plot in order to estimate the Zn concentration at various stages of crop growth. Fresh green herbage yield (GHY) was measured by weighing the harvested fresh green plants on plot basis and then converted to yield per hectare (q/ha). For dry matter (%) estimation, 1,000 g sample of green fodder from harvested plots was collected, chopped, sun dried and kept in oven for 24 h at 60 °C for obtaining constant weight and then reweighed using electronic balance. Then, dry matter (%) from each treatment was multiplied with GHY to obtain dry matter yield (DMY) and converted to q/ha.

2.5 Estimation of Diethylenetriamine Pentaacetic Acid (DTPA)-Extractable Zn in Soil

For soil samples availability Zn was assessed by extracting 10 g portion of soil sample with 20 mL of DTPA extractant (0.005 M DTPA + 0.01 M $CaCl_2·2H_2O$ + 0.1 M triethylamine (TEA) buffer adjusted to pH 7.3) as described by Lindsay and Norvell [5]. The determination of Zn cation was performed on the same extract with an atomic absorption spectrophotometer (Varian AAS-FS 240 model) after appropriate dilutions.

The estimation of Zn from above ground plant samples was carried out with the method given by Isaac and Kerber [6]. The plant samples were washed with 0.2% liquid detergent followed by N/10 HCl solution and then with double distilled water. These washed plant samples were air dried by keeping them in paper bags and then at oven at 65 °C for 3 d and dried samples were ground in Thomas Wiley mill (Model ED-5). These grounded plant materials were digested in diacid mixture of HNO_3 and $HClO_4$ (3:1, *v*/*v*) for analysis of Zn concentration by using atomic absorption spectrophotometer (Varian AAS-FS 240 model).

2.6 Forage Quality Estimation

Fresh fodder samples collected from each plot at harvest were washed sequentially with tap water, acidulated water containing 0.01 N HCl, distilled water and deionized water. Samples were then air-dried followed by oven drying at 60 °C to a constant weight. The dried samples were ground in Wiley mill (Thomas Scientific, India) with stainless steel blades to pass through 40 mesh sieve and then used for chemical composition estimation.

2.7 Crude Protein (CP) Content and Yield (%)

The N content of the oven dried 24 samples was estimated by the Modified-Kjeldahal method [7]. The 0.5 g plant sample was digested with 10 mL H_2SO_4, along with 5-6 g of digestion mixture (K_2SO_4 and $CuSO_4$ in 9:1, *v*/*v*). The final volume of the digested material was made 50 mL and then distillation was done with 40% NaOH. The ammonia gas was liberated and was absorbed in the 25 mL solution of 4% boric acid having mixed indicator (bromocresol green and methyl red in 5:1 (g/g) ratio prepared in ethanol 95%), the complex ammonium borate was formed. This ammonium borate was titrated with standard H_2SO_4. The blank reading was also calculated by taking all reagents except plant sample. The percent nitrogen was determined by subtracting the blank reading.

$$\text{N content (\%)} = (0.00028 \times \text{volume of 0.1 N } H_2SO_4 \text{ used} \times \text{dilution factor})/\text{weight of sample (g)} \quad (1)$$

To calculate the CP content percent nitrogen was multiplied by the factor 6.25 as CP contain 16% N.

$$\text{CP (\%)} = \text{percent nitrogen content} \times 6.25 \quad (2)$$

Crude protein yield (CPY) was worked out by multiplying the CP percentage with DMY.

$$\text{CPY (kg/ha)} = \text{CP (\%)} \times \text{DMY (kg/ha)} \quad (3)$$

2.8 Ash Content and Organic Matter (%)

Ash content was estimated by method given by Association of Official Analytical Chemists (AOAC) [8]. For the determination of ash content 5 g sample was first incinerated on hot plate till no more smoke came out. It was kept in a crucible to place in a muffle furnace at 550 °C for 5 h, the weight of residue is determined, and result was expressed in percent.

$$\text{Ash (\%)} = \frac{\text{Weight of residue (g)}}{\text{Weight of sample (g)}} \times 100 \quad (4)$$

Since the ash (%) represents the inorganic constituent of sample thus the content of organic matter can be calculated as percentage of sample from ash content by using the formula:

$$\text{Organic matter (\%)} = 100 - \text{ash content (\%)} \quad (5)$$

2.9 Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF)

ADF and NDF were determined using the procedure outlined by Van Soest *et al.* [9]. For ADF, firstly acid detergent solution (ADS) was prepared by dissolving acetyltrimethyl ammonium bromide (20 g) in 500 mL of 1 N H_2SO_4 and volume was made to 1 L with 1 N H_2SO_4. A sample of 1 g was taken in the already weighed crucible. Crucibles were fitted to Fibra plus fibre estimation system. About 150 mL ADS was added to this system. The instrument was switched on and initial temperature was set to 500 °C. When boiling has just started, the temperature was reduced to 400 °C and the samples were digested for 1 h. The solution was drained and samples were washed twice with distilled water. The crucibles were removed and dried in hot air oven. The crucibles were cooled in desiccator and weighed. ADF was calculated by using the formula:

$$\text{ADF (\%)} = [(\text{weight of crucible + fiber} - \text{weight of crucible})/\text{weight of sample (g)}] \times 100 \quad (6)$$

For estimation of NDF, neutral detergent solution (NDS) was prepared. Disodium salt of ethylene diamine tetraacetate dihydrate, i.e., EDTA (18.61 g) and sodium borate decahydrate (6.81 g) were placed in 2 L capacity beaker and about 700 mL distilled water was added and heated until dissolved, to this solution sodium lauryl sulphate (30 g) and 2-ethoxyethanol (10 mL) were added. In another beaker, disodium hydrogen phosphate (anhydrous) (4.56 g) was dissolved in 100 mL distilled water. Both the solutions were mixed and transferred to 1 L volumetric flask. The volume was made to 1 L with distilled water. The pH of the solution was adjusted to 6.9-7.1.

To determine the NDF, follow the same procedure of ADF by using 1 g of samples with NDS. Similarly, the NDF was calculated by using the formula:

$$\text{NDF (\%)} = [(\text{weight of crucible + fibre} - \text{weight of crucible})/\text{weight of sample (g)}] \times 100 \quad (7)$$

2.10 Statistical Analysis

The experiment was laid out in RBD with three replications. The statistical analysis was done with the help of method described by Panse and Sukhatme [10] and means were compared by using least significance difference (LSD) test at a 0.05 level of probability.

3. Results

3.1 Plant Height

Plant height was increased with the age of plant and at 60 DAS the highest increase in height of 44.1 cm and 60.2 cm was recorded in T4 (soil + 0.5% foliar Zn at 90 DAS) and T5 (soil + 0.5% foliar Zn at 60 DAS and 90 DAS) at Location I and Location II, respectively (Table 2), which was significantly at par with T5 and T4, respectively. The smaller height (37.1 cm and 50.3 cm, respectively) was recorded with T1 (control). Maximum plant height was reported at 90 DAS, i.e., 117.9 cm and 117.7 cm with treatment receiving soil Zn 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS (T5) at Location I and Location II, respectively. These results were also statistically at par at Location I (117.1 cm) and Location II (117.4 cm) under T3 treatment where Zn at 25 kg/ha (soil) + 0.5% foliar Zn at 60 DAS was applied, respectively. Similar trend for plant height (124.1 cm and 142.5 cm) was recorded with T5 treatment made at 110 DAS respectively at both locations, which was on par with T3. Minimum plant height at 90 DAS (98.8 cm and 107.7 cm) and at 110 DAS (107.8 cm and 130.3 cm) was recorded in control plants at Location I and Location II, respectively.

3.2 The Number of Tillers per Plant

The number of tillers per plant was found significant at different growth stages of oats fodder (Table 3). However, at 60 DAS the highest number of tillers per plant 13.7 with T3 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS) and 15.0 with T5 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) was recorded at Location I and Location II, respectively. The results were also significantly at par with T5 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) and T3 treatments (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS) at Location I and Location II, respectively. On the other hand, the lowest number of tillers per plant at Location I (8.7) and at Location II (10.0) was recorded with treatment T1 (control only).

At 90 DAS the highest number of tillers per plant, i.e., 14.7 and 15.0 was recorded with treatment receiving soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS (T3) at Location I and Location II, respectively, which was statistically at par (14.3) with T5 treatment (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) at both the locations. At 110 DAS maximum number of tillers per plant was recorded with soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS (T5)

Table 2 Effect of foliar application of Zn on plant height (cm) at different growth stages of oats fodder.

Treatments	60 DAS		90 DAS		110 DAS	
	Location I	Location II	Location I	Location II	Location I	Location II
T1	37.1	50.3	98.8	107.7	107.8	130.3
T2	43.0	59.7	113.5	115.3	119.4	138.5
T3	43.3	59.0	117.1	117.4	122.1	141.7
T4	44.2	60.0	113.5	115.2	121.2	140.0
T5	43.7	60.3	117.9	117.7	124.1	142.5
T6	40.0	50.7	109.0	112.3	116.8	136.7
T7	40.3	50.4	99.3	108.7	117.7	136.9
T8	40.7	49.0	109.2	112.7	118.9	137.3
LSD (p = 0.05)	3.24	3.19	2.74	4.13	2.59	5.16

Table 3 Effect of foliar application of Zn on the number of tillers per plant at different growth stages of oats fodder.

Treatments	60 DAS		90 DAS		110 DAS	
	Location I	Location II	Location I	Location II	Location I	Location II
T1	8.7	10.0	9.3	9.0	9.7	9.3
T2	13.0	14.0	13.0	12.3	13.3	12.3
T3	13.7	14.7	14.7	15.0	14.3	13.7
T4	13.0	14.3	13.7	12.7	13.0	13.3
T5	13.3	15.0	14.3	14.3	15.0	14.0
T6	11.0	11.0	12.0	12.0	11.0	11.0
T7	11.3	10.7	11.0	11.0	12.3	11.7
T8	11.2	11.0	12.3	12.3	12.7	12.3
LSD (p = 0.05)	2.43	2.55	2.77	2.66	2.35	2.45

which was 15.0 and 14.0 at Location I and Location II, respectively, which is at par with T3 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS) at both the locations. The lowest number of plants per meter row length at 90 DAS (9.3 and 9.0) and at 110 DAS (9.7 and 9.3) was recorded in control plants (T1) at Location I and Location II, respectively.

3.3 Zn Concentration

Application of Zn had significant effect on Zn concentration in oats at different stages (60, 90 and 110 DAS) of oats fodder (Table 4). The results of study reported that for oats at 60 DAS, the maximum Zn concentration of 68.6 mg/kg and 72.6 mg/kg was recorded in T5 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) at Location I and Location II, respectively, which was significantly on par with T2 (soil Zn at 25 kg/ha) at Location I and with T3 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS) at Location II (Table 2). Similarly at 90 DAS the highest Zn concentration in oats (73.0 mg/kg and 72.2 mg/kg) was recorded with T5 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) at Location I and Location II, respectively. At 110 DAS maximum Zn concentration was observed with T5 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) which was 70.7 mg/kg and 69.2 mg/kg at both locations resulting in increases in Zn concentration by 100% over the control.

3.4 Zn Uptake by Oats Fodder

Zinc uptake was calculated using dry biomass yield of oats fodder in ton/ha multiplied with concentration measured in mg/kg (Table 5). Zn application had significant effect on Zn uptake by oats fodder. The data reported that the highest Zn uptake by oats fodder

Table 4 Effect of foliar application of Zn on concentration of Zn (mg/kg) in oats sample at various growth stages.

Treatments	60 DAS				90 DAS				110 DAS			
	Location I	Location II	Overall mean	Increase (%)	Location I	Location II	Overall mean	Increase (%)	Location I	Location II	Overall mean	Increase (%)
T1	46.5	42.8	44.7	-	40.2	36.5	38.4	-	37.2	32.9	35.0	-
T2	68.4	69.1	68.8	53.9	60.5	62.3	61.4	59.8	58.1	59.6	58.9	68.2
T3	66.5	72.5	69.5	55.4	69.8	71.9	71.0	84.8	64.3	66.4	65.4	86.8
T4	67.6	70.7	69.2	54.8	57.1	61.5	59.3	54.4	62.8	64.0	63.4	81.1
T5	68.6	72.6	70.6	57.9	73.0	72.2	72.5	88.8	70.7	69.2	70.0	100.0
T6	49.4	44.8	47.1	5.3	57.7	59.4	58.6	52.6	53.9	54.7	54.3	55.1
T7	48.4	46.2	47.3	5.8	46.9	45.9	46.4	20.8	52.6	54.4	53.5	52.8
T8	48.6	45.2	46.9	4.9	56.4	57.1	56.7	47.6	56.5	57.9	57.2	63.4
LSD (p = 0.05)	9.03	8.34	5.69	-	9.69	9.72	6.31	-	9.92	11.50	6.99	-

Table 5 Effect of foliar application of Zn on uptake of Zn by oats fodder.

Treatments	Zn uptake (g/ha)			
	Location I	Increase (%)	Location II	Increase (%)
T1	1,095.7	-	1,020.5	-
T2	2,117.7	93.2	2,390.7	134.2
T3	2,440.7	122.7	2,717.5	166.2
T4	2,425.8	121.3	2,673.5	161.9
T5	2,960.5	170.1	2,986.5	192.6
T6	1,772.5	61.7	1,972.3	93.2
T7	1,819.6	66.0	2,018.3	97.7
T8	1,969.7	79.7	2,211.6	116.7
LSD (p = 0.05)	518.36	-	525.69	-

Table 6 Effect of foliar application of Zn on green fodder yield of oats fodder (q/ha).

Treatments	Green fodder yield (q/ha)				
	Location I	Increase (%)	Location II	Increase (%)	Overall mean
T1	295.9	-	309.9	-	303.0
T2	364.2	23.0	399.5	28.9	381.9
T3	378.9	28.0	411.4	32.7	395.2
T4	384.5	29.9	417.9	34.8	401.2
T5	419.5	41.7	431.3	39.1	425.4
T6	330.3	11.6	360.9	16.4	345.6
T7	342.6	15.7	369.5	19.2	356.0
T8	351.7	18.8	386.2	24.6	369.0
LSD (p = 0.05)	62.03	-	67.51	-	42.39

was recorded by treatment receiving soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS (T5) which was 2,960.5 g/ha at Location I and 2,986.5 g/ha at Location II. This treatment closely followed T3 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS) and found significantly superior over the rest of treatments at both the locations. The lowest Zn uptake was recorded in control (T1) which was 1,095.7 g/ha and 1,020.5 g/ha at Location I and Location II, respectively. The data on Zn uptake revealed that increase of 170.1% and 192.6% was recorded over the control with treatment receiving soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS at Location I and Location II, respectively.

3.5 Herbage Biomass Yield

Significant (p = 0.05) improvement in GHY was recorded with application of soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS (Table 6). The data revealed that maximum average green fodder yields of 419.5 q/ha and 431.3 q/ha were recorded with treatment receiving soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS (T5) at Location I and Location II, respectively. Also, the fodder yields were at par with T4 treatment at Location I (384.5 q/ha) and at Location II (417.9 q/ha) where soil Zn at 25 kg/ha + 0.5% foliar Zn at 90 DAS was applied. On the other hand, treatment (T5) receiving soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS recorded 41.7% and 39.1% increase in green fodder yields as compared to control at Location I and Location II, respectively.

Similarly, there was significant increase in DMY with application of Zn (Table 7). Maximum mean DMY was recorded with T5 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS), i.e., 98.8 q/ha and 84.9 q/ha at Location I and Location II, respectively, which was at par with T4 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 90 DAS) 90.6 q/ha at Location I and 82.3 q/ha at Location II and significantly superior than other treatments. In DMY as in green fodder yield treatment (T5) receiving soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS recorded 41.7% and 39.1% increase in DMY as compared to control at Location I and Location II, respectively.

3.6 Effect on Forage Quality

Soil plus foliar sprays of Zn on crop increased CP content and CPY significantly (p = 0.05) over control (Table 8). CP ranged from 6.5% to 9.0% at Location I and from 6.3% to 8.9% at Location II. The higher CP content was recorded with T5 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) which was 9.0% and 8.9% at Location I and Location II, respectively, and was significantly superior to control (T1) at both locations. Treatment T5 was at par with T3 with 8.7% at Location I and 8.5% at Location II. The lowest CP concentration 6.5% and 6.3% was recorded in control plants (T1) at Location I and Location II, respectively. Increase in CP content up to

38.46% and 41.26% was recorded with application of soil Zn applied at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS at Location I and Location II, respectively, over control. CPY also had a similar trend as CP percent at both the locations. The results showed that Zn application failed to produce any significant effect on NDF and ADF percent at both the locations (Table 9). Organic matter and ash content also showed non-significant effect on application of Zn at both the locations.

Table 7 Effect of foliar application of Zn on dry matter yield (DMY) of oats fodder (q/ha).

Treatments	DMY (q/ha)				
	Location I	Increase (%)	Location II	Increase (%)	Overall mean
T1	69.7	-	61.0	-	65.4
T2	85.8	23.0	78.7	28.9	82.3
T3	89.3	28.1	81.0	32.7	85.2
T4	90.6	29.9	82.3	34.8	86.4
T5	98.8	41.7	84.9	39.1	91.9
T6	77.8	11.6	71.0	16.4	74.5
T7	80.7	15.6	72.7	19.2	76.7
T8	82.9	18.8	76.0	24.6	79.5
LSD (p = 0.05)	14.61	-	13.29	-	9.13

Table 8 Effect of foliar application of Zn on crude protein (CP), crude protein yield (CPY) and organic matter of oats fodder.

Treatments	CP (%)		CPY (q/ha)		Organic matter (%)	
	Location I	Location II	Location I	Location II	Location I	Location II
T1	6.5	6.3	4.5	3.8	94.7	94.1
T2	7.6	7.3	6.5	5.8	94.2	93.5
T3	8.7	8.5	7.7	6.9	95.1	93.3
T4	8.6	8.4	7.8	6.9	94.9	93.2
T5	9.0	8.9	8.9	7.5	95.0	93.4
T6	8.0	7.8	6.2	5.6	95.0	92.7
T7	8.0	7.7	6.4	5.6	96.1	92.9
T8	8.2	8.0	6.8	6.1	95.2	93.5
LSD (p = 0.05)	0.63	0.74	1.21	1.07	NS	NS

NS: non significant.

Table 9 Effect of foliar application of Zn on acid detergent fiber (ADF), neutral detergent fiber (NDF) and ash content of oats fodder

Treatments	ADF (%)		NDF (%)		Ash content (%)	
	Location I	Location II	Location I	Location II	Location I	Location II
T1	48.0	44.1	66.5	70.2	5.0	6.0
T2	37.2	48.7	67.3	73.6	5.5	6.2
T3	34.9	48.5	66.9	66.8	5.3	6.2
T4	37.2	43.7	67.8	71.2	5.5	6.3
T5	40.5	42.9	70.4	72.1	5.4	6.1
T6	37.8	41.5	65.9	69.2	5.3	6.4
T7	37.0	44.9	65.6	73.9	4.9	6.5
T8	39.5	42.7	65.6	69.2	5.3	6.5
LSD (p = 0.05)	NS	NS	NS	NS	NS	NS

NS: non significant.

4. Discussion

The results obtained in this investigation indicated that soil plus foliar sprays of Zn had the dominant effect on the plant parameters, growth, herbage yield, better nutrition and quality of oats. Plant height increased with Zn application which may be due to its inter-relationship with auxin production, which is an important plant growth promoter regulating cell enlargement and stem elongation. These results were in agreement with study of Khanda *et al*. [11]. At 60 DAS soil $ZnSO_4$ at 25 kg/ha recorded maximum plant height that may be due to increased availability of Zn near rhizosphere which ultimately increased nutrient content and uptake. These results were in line with study conducted by Chaphale and Badole [12], Chaudhary and Sinha [13] and Rao [14]. At 90 DAS and 110 DAS, T5 (soil Zn at 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) recorded the highest increase in plant height which might be due to increase in Zn content of rhizosphere as well as quick utilization of Zn through foliar spray. These results were in line with findings of Singh *et al*. [15], Ram *et al*. [16], Khan *et al*. [17], Zayed *et al*. [18] and Sandhu *et al*. [19].

The increase in number of tillers per plant at 60 DAS and 90 DAS was recorded with T3 (soil Zn 25 kg/ha + 0.5% foliar Zn at 60 DAS), and this might be because of superiority of $ZnSO_4$ in sustaining high Zn content in the soil rhizosphere. These results were similar to the findings of other scientists like Singh and Sharma [20], Pulla and Shukla [21], Kulandaivel *et al*. [22] and Mustafa *et al*. [23]. At 110 DAS the highest number of tillers was recorded with T5 (soil Zn 25 kg/ha + 0.5% foliar Zn at 60 DAS and 90 DAS) that is both soil and foliar application of Zn, which might be the result of high concentration of Zn in soil rhizosphere and better utilization of Zn through foliar feeding. This resulted in increase in plant height, leaf area index that helped in increasing the photosynthetic rate of plant, which in turn helped in formation of tillers. Above results were in thorough agreement with results of scientists like Sandhu *et al.* [19], Ravikiran and Reddy [24] and Singh and Sharma [20].

Increase in Zn concentration might be due to increased availability of Zn in rhizosphere soil and absorption from foliar spray and rapid translocation to storage organs besides arresting the fixation loses and retention of this fertilizer on leaf surface. These results were in conformity with findings of Stalin *et al.* [25] and Ravikiran and Reddy [24]. Similar results were reported by Yilmaz *et al*. [26] that all the methods receiving Zn increased Zn concentration in shoot and grain of wheat as compared to control. Soil + foliar application resulted in significant increase in Zn concentration in both shoot and grain as compared to other methods of Zn application. Zou *et al*. [27] also found that foliar Zn application alone or in combination with soil application, significantly increased grain Zn concentrations from 27 mg/kg with no Zn to 48 mg/kg and 49 mg/kg across all of 23 site-years, resulting in increases in grain Zn by 84% and 90%, respectively.

Increase in Zn uptake may be due to greater availability of Zn in rhizosphere soil and absorption from foliar spray and rapid translocation to storage organs besides arresting the fixation loses. These results were in conformity with findings of Stalin *et al*. [25], Ravikiran and Reddy [24]. It might also be due to easy availability of Zn with higher concentrations and rapid absorption and mobility when applied as foliar spray. These results were related with findings of scientists like Sandhu *et al.* [19], Das *et al*. [28] and Yadav *et al*. [29].

Zn addition to the crop through soil plus foliar sprays enhanced the GHY and DMY which may be due to high concentration of Zn content in soil, which resulted in higher Zn uptake and in turn contributed to increased green fodder yield as well as DMY. Better utilization of Zn by foliar feeding results in better supply of Zn, which plays specific role in various metabolic activities. These results found support from the work of many scientists like Ram *et al*. [16], Das *et al*. [28] and Reddy *et al*. [30]. Further, Stalin *et al*. [25] observed that foliar spraying of Zn results in better absorption of this nutrient, thereby helping in photosynthetic activity and effective translocation to storage organs, thus contributed to increased green fodder yield as well as dry fodder yield.

High forage yield along with proper nutrition is important for the livestock farmers for better growth and development of cattle. CP content of forage is a vital criterion for fodder quality evaluation, while for livestock producer's CPY is important for determining the supplemental protein feed for the reduction of expenditure on feed costs [31]. The higher the CPY of fodder less will be the expenditure on concentrate feeding to the livestock. The NDF values are important in ration formulation for the livestock because they reflect the amount of forage the animal can consume. The ADF values are important because they relate to the ability of an animal to digest the forage. As NDF and ADF percentage decreases, herbage intake and digestibility will generally increase.

Similar to GHY and DMY, Zn application on crop also improved CP content and CPY of oats. This might be because application of Zn increased N-metabolism, which enhanced accumulation of amino acids and increased the rate of protein synthesis and consequently increased CP and CPY in oats fodder. Zn application in soil plus foliar enhanced the Zn concentration in plant which associated with RNA and ribosome induction the result of which accelerates protein synthesis [32]. The results were in consonance with the findings of Mishra [33], Dhaliwal *et al.* [34] and Keram *et al.* [35].

5. Conclusions

Soil plus foliar application of Zn improved Zn concentration, herbage yield (GHY and DMY) and quality of oats fodder. Soil application of Zn at 25 kg/ha with foliar application at 0.5% of Zn at 60 DAS and 90 DAS recorded maximum plant height, the number of tillers per plant, Zn enrichment, green fodder yield and dry fodder yield in oats fodder as compared to other methods of Zn application. Among the various fodder quality parameters CP and CPY were also recorded maximum with application Zn at 25 kg/ha with foliar at 0.5% Zn at 60 DAS and 90 DAS, but Zn application failed to produce any significant effect on other fodder quality parameters.

Conflict of Interest

The authors declare that they have no conflict of interest.

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