RESEARCH ARTICLE Agricultural Science Digest, Volume 45 Issue 5: 839-844 (October 2025)

Enhancing Crop Yield and Nutritional Values Through Zinc Biofortification in Maize-soybean Strip Intercropping System

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10.18805/ag.D-6028

ABSTRACT

Background: Zinc, an essential micronutrient performing a key function in agricultural system, influencing crop yield, nutritional valueand overall development and growth of plants. Intercropping is referring to the most important practices to improving land utilization rates in India, which has limited arable land resources.

Methods: The study aim to investigate the effect of different zinc levels on yield and qualitative characteristics of maize and soybean in 2:2 strip intercropping. The field study was conducted during *kharif* 2023 at Lovely Professional University, Punjab. The experiment was conducted with a set of nine treatments in randomized block design (RBD) with three replications.

Result: The results revealed that the maximum yield and yield attributes such as cobs per plant (1.44), number of rows per cob (13.81), number of grains per row (25.07), number of grains per cob (372.28) and grain yield (17.47 q ha^{-1}), number of pods per plant (35.31), number of seed per pod (2.82), number of seeds per plant (95.33), seed index (13.02). Maximum maize equivalent yield (48.57 q ha^{-1}), maize production efficiency (18.39 kg ha^{-1} day^{-1}), soybean production efficiency (12.06 kg ha^{-1} day^{-1}) were recorded in T_9 (soil supplementation of 19 kg ha^{-1} of $ZnSO_4$ + foliar spray of 0.2% $ZnSO_4$). In contrast, T_2 (sole maize) showed the highest grain yield (32.33 q ha^{-1}), stover yield (67.57 q ha^{-1}), biological yield (96.80 q ha^{-1}). Similarly, T_1 (sole soybean) showed the highest pod yield (24.97 q ha^{-1}), haulm yield (51.18 q ha^{-1}), biological yield (76.15 q ha^{-1}) but lower values for other parameters compared to T_9. Applying zinc significantly rises the zinc concentration in maize grains, stover (24.92 to 28.05 mg kg^{-1}, 26.90 to 35.25 mg kg^{-1}), soybean pods, haulm (23.92 to 35.03 mg kg^{-1}, 25.95 to 41.38 mg kg^{-1}), with variations across treatments.

Key words: LER, Maize, MEY, Soybean, Strip intercropping.

INTRODUCTION

The Agricultural sector is experiencing severe pressure to fulfil the food demands of an increasing worldwide population, which is projected to exceed 9 billion by 2025 (Alexandratos *et al.,* 2012). Worldwide, almost 50% of agricultural soils suffer from zinc deficiency, which causes challenges for the well-being of both humans and animals (Cakmak *et al.,* 2008) and projections suggest that this percentage might rise to 63% by 2025 (Singh, 2009). Globally, 17.3% of the population are suffering from zinc deficiencyand in South Asian countries, that number climbs to 30% (Gupta *et al.,* 2020). With its inadequate dietary intake posing significant health risks and correlating with various diseases. According to WHO (World Health Organization), Zn deficiency is considered the 11th most prominent issue on a global basisand 5th most significant factor in developing countries causing illness and diseases. It is also responsible for numerous health concerns, such as stunted physical development, weakened immune system, cognitive aptitudeand all together susceptibility to infections, DNA damage, increases the risk of cancer development (Prasad *et al.,* 2007).

Zinc is a vital micronutrient that acts has a significant impact on diverse essential plant processes. It acts as a catalyst for numerous enzymes and is involved in the synthesis of tryptophan, an amino acid that forms the growth hormone IAA (Indole Acetic Acid). Additionally, it is engaged in different physiological processes such as protein and carbohydrate synthesis (Yadavi *et al.,* 2014). Micronutrients actively participate in various plant metabolic activities, including cell development, respiration, photosynthesis, chlorophyll production, enzyme activityand nitrogen fixation.

Maize (*Zea mays* L.) is the 3rd important food crop in the world. It was grown on a worldwide acreage of 197 million ha in 2021 (FAO, 2021). Soybean is a significant provider of plant-based protein in animal feed and is responsible for over 50% of global oil output (Ainsworth *et al.,* 2012). Maize is an exhausting crop that depletes soil nutrients (Baishya *et al.,* 2021). Soybean, a restorative crop, may replace soil nutrients (Fattah Abdul *et al.,* 2023).

Intercropping is getting prominence as the “new green revolution” due to its ability to increases land productivity

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How to cite this article: Jeevana, E.S., Rajeev and Siyon, M.S. (2025). Enhancing Crop Yield and Nutritional Values Through Zinc Biofortification in Maize-soybean Strip Intercropping System. *Agricultural Science Digest.* **45(5):** 839-844. doi: 10.18805/ag.D-6028.

Submitted: 22-04-2024 **Accepted:** 01-07-2024 **Online:** 13-12-2024

through the utilization of species complementarities and facilitate sustainable agricultural intensification (Martin *et al.,* 2018). The practical implementation of fundamental ecological concepts, such as variety, competitionand facilitation, for the benefit of agricultural production (Layek *et al.,* 2018). It promises increased production, optimal use of resourcesand more revenue (Maitra *et al.,* 2021). Intercropping can be classified into different types based on the extent of temporal and spatial mixing. These types include row intercropping (where crops are grown in separate regular rows), mixed cropping (without any specific row or spatial configuration), strip cropping (where several rows of a crop species are grown in separate adjacent rows or strips)and relay cropping system (where a component crop is planted during the middle stage of the major crop's life cycle (Lithourgidis *et al.,* 2011; Talukdar *et al.,* 2022).

Biofortification is a crop-based strategy that aims to increases the levels of minerals and improve the availability of essential nutrients in the edible parts of plants. This is achieved through various methods such as agronomic intervention, breeding practices, genetic modificationand microbiological changes (Sharma *et al.,* 2020). Agronomic biofortification refers to the process of applying fertilizers and using agronomic methods in agricultural production. This approach has tremendous potential in combating hidden hunger on a global basis (Rajan *et al.,* 2020). Mineral micronutrients can be added to granular fertilizers that are spread on the soil or applied directly to the leaves as foliar sprays (Parmar *et al.,* 2023). Implementing agronomic biofortification using fertilizers containing Zn to enhance the nutritional content of food crops should be considered as a public health strategy in countries with high rates of nutrient shortage (Joy *et al.,* 2015). It can enhance to meet micronutrient deficiencies (Birol *et al.,* 2015), by targeting rural populations in areas with low resources and restricted availability of animal-based diets or fortified foods. Thus, the main objective of this study was to determine (i) The effect of soil and foliar-applied different levels of Zn application through $ZnSO_4$. H_2O on growth, to enhance crop yield of maize and soybean, (ii) to increases the Zn concentration in grains/pods, straw and uptake by maize/ soybean strip intercropping eventually to minimize the deficiencies of Zn.

MATERIALS AND METHODS

The field experiments was conducted at research farm of Department of Agronomy, School of Agriculture, Lovely Professional University, Punjab (31°24′39″N latitude, 75°69′54″E longitude and at 245 m altitude above mean sea level). The total amount of rainfall received at the experimental site was 411.3 mm for the *kharif* 2023 cropping seasons. The total precipitation is 346 mm, mean maximum and minimum temperature for the experimental site were in the range of 40.9°C-18.2°C for the 2023 cropping season (from middle June to early October). The soil was a sandy loam, with the following characteristics: 8.21 pH, 0.51% organic matter, 198.22 kg ha^{-1} total nitrogen (N), 20.91 kg ha^{-1} available phosphorous (P), 238.40 kg ha^{-1} available potassium (K). The initial soil diethylenetriamine pentaacetate (DTPA)-extractable Zn concentration was 0.37 mg/kg, which indicated Zn deficiency (low, 0-0.5 mg/ kg; medium, 0.51-0.8 mg/kg: high, >0.8 mg/kg). The experiment was conducted with a set of nine treatments in randomized block design (RBD) with three replications having nine treatments each measuring 49 m^2 (7 m in width and 7 m in length). The strip intercropping pattern used in this study consisted of two rows of maize alternated with two rows of soybean. Additionally, both spices were grown as sole crops (Fig 1). The nine treatments were T_1 =

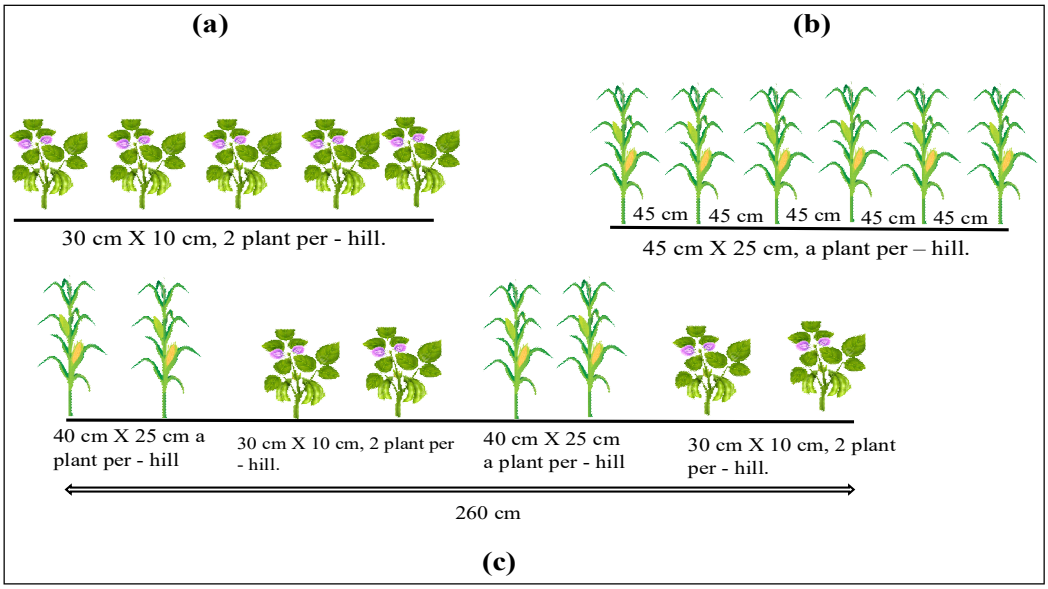


Fig 1: The design comprises of three patterns: (A): sole soybean, (B): sole maize, (C): maize-soybean strip intercropping, where two rows of maize are alternated with two rows of soybean.

Sole soybean (spacing size 30×10 cm), T_2 = Sole maize (spacing size 45 × 25 cm), T_3 = Soybean + maize (2:2) – line sown, T_4 = Strip cropping (2:2) + Soil supplementation of 20 kg ha^{-1} of $ZnSO_4$,T_5 = Strip cropping (2:2) + Soil supplementation of 22.5 kg ha^{-1} of $ZnSO_4$, T_6 = Strip cropping (2:2) + Soil supplementation of 25 kg ha^{-1} of $ZnSO_4$, T_7: Strip cropping (2:2) + Soil supplementation of 15 kg ha^{-1} of $ZnSO_4$ + foliar spray of 0.4% $ZnSO_4$, T_8 = Strip cropping (2:2) + Soil supplementation of 17.5 kg ha^{-1} of $ZnSO_4$ + foliar spray of 0.3% $ZnSO_4$, T_9 = Strip cropping (2:2) + Soil supplementation of 19 kg ha^{-1} of $ZnSO_4$ + foliar spray of 0.2 % $ZnSO_4$. A total of 27 plots were made and the well decomposed farmyard manure, the recommended doses of nitrogen, phosphorus and potassium by agriculture Department in Government of Punjab, for maize were applied @ 80 kg ha^{-1} N: 60 ha^{-1} kg P_2O_5: 20 kg ha^{-1} K_2O and for soybean were applied @ 20 kg ha^{-1} N: 60 kg ha^{-1} P_2O_5: 20 kg ha^{-1}, K_2O, respectively were mixed well and uniformly incorporated to each plot. Macro nutrients (NPK) were applied in the form of urea, single super phosphate (SSP)and muriate of phosphate (MOP) respectively. For maize entire dose of phosphorous and potassium and half of N fertilizer were applied at the time of sowing and remaining half of N was applied at 45 DAS. Zinc as Zn sulphate monohydrate ($ZnSO_4$. H_2O) with 33% Zn was used in the study. All the soil Zn application treatments were applied at the time of sowing. The foliar application treatments of Zn were done at 60 DAS for both crops.

Data analysis

The data was statistically analysed using XLSTAT 2024. One-way ANOVA was conducted to determine the significance of the differences among all the treatments. Mean values of treatment comparison were done using the LSD (least significant difference) evaluated at the P≤0.05. Mean values are presented mean ±SE (standard error), based on the 3 independent replicate per treatment.

RESULTS AND DISCUSSION

Yield and yield attributes of maize

The perusal of data on yield parameters with different methods of zinc application presented in (Table 1). The maximum cobs per plant (1.44), number of rows per cob (13.81), number of grains per row (25.07), number of grains per cob (372.28) and grain yield (17.47 q ha^{-1}) were recorded in T_9 (Soil supplementation of 19 kg ha^{-1} of $ZnSO_4$ + foliar spray of 0.2% $ZnSO_4$) and the minimum cobs per plant (1.13), number of rows per cob (11.92), number of grains per row (17.99), number of grains per cob (300.40) were recorded with T_2 (sole maize). In contrast, T_2 (Sole maize) showed the highest grain yield (32.33 q ha^{-1}), stover yield (67.57 q ha^{-1}), biological yield (96.80 q ha^{-1}) but lower values for other parameters compared to T_9. The harvest index ranged from 23.09% (T_3) to 29.48% (T_2), indicating variability in efficiency of converting biomass to grain among the treatments. Similarly, Arya and Singh, (2000) reported that applying zinc to plants enhanced both grain yield and biological yield. Zinc acts as an activator for several enzymes involved in plants metabolism, which may directly or indirectly impact the production carbohydrates and proteins.

Yield and yield attributes of soybean

Yield attributing characteristics like number of pods per plant, number of seed per pod, number of seed per plant, seed index, pod yield (q ha^{-1}), haulm yield (ha^{-1}), biological yield (q ha^{-1}) harvest index (%) showed positive correlation with yield. The data from the (Table 2) revealed with the soil supplementation of 19 kg ha^{-1} of $ZnSO_4$ + foliar spray of 0.2 % $ZnSO_4$ (T_9) shows the maximum number of pods per plant (35.31), number of seed per pod (2.82), number of seed per plant (95.33), seed index (13.02) and the minimum number of pods per plant (29.96), number of seed per pod (2.27), number of seed per plant (75.87), seed index (10.44) were recorded with sole soybean (T_1). In contrast, T_1 (sole

Table 1: Influence of different zinc fertilization methods on yield of maize.

Treatments	No. of cobs $plant^{-1}$	No. of rows cob^{-1}	No. of grains row^{-1}	No. of grains cob^{-1}	Grain yield (q ha^{-1})	Stover yield (q ha^{-1})	Biological yield (q ha^{-1})	Harvest index (%)
T_1	-	-	-	-	-	-	-	-
T_2	1.13	11.92	17.99	300.40	32.33	64.57	96.80	29.48
T_3	1.20	13.13	19.88	326.07	16.31	48.50	64.81	23.09
T_4	1.27	13.41	23.35	307.53	16.31	50.85	67.16	24.29
T_5	1.26	13.49	24.22	334.09	16.73	51.49	68.22	24.59
T_6	1.28	13.71	24.54	349.47	17.27	52.97	70.23	24.61
T_7	1.39	13.45	23.48	323.00	16.70	52.01	68.71	24.30
T_8	1.43	13.77	24.40	340.61	17.23	53.27	70.50	24.65
T_9	1.44	13.81	25.07	372.38	17.47	54.46	71.93	24.27
LSD (0.05)	0.334	0.513	2.538	87.42	1.489	7.702	7.60	3.335
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

soybean) showed the highest pod yield (24.97 q ha^{-1}), haulm yield (51.18 q ha^{-1}), biological yield (76.15 q ha^{-1}) but lower values for other parameters compared to T_9. The harvest index ranged from 23.69 % (T_3) to 32.79% (T_1). Similarly, (Alam *et al.*, 2016) reported that levels of zinc application significantly improved the translocation of photosynthesis towards storage organs (pods and seeds) and thus enhanced the yield.

Effect of combination between strip intercropping system and zinc biofortification on LER, RCC, CR, ATER, MEY, maize production efficiency, soybean production efficiency

Data in Table 3 show that to evaluate the effects of various treatments (T_1-T_9) on maize and soybean production efficiency under different conditions. The most significant variable studied were maize production efficiency (MPE) and soybean production efficiency (SPE), both measured in kg ha^{-1} day^{-1}. Additionally, measures include the land equivalent ratio (LER), relative crowding coefficient (RCC), competitive ratio (CR), area time equivalent ratio (ATER) and maize equivalent yield (MEY), quantified in q ha^{-1}. The result show that soil supplementation of 19 kg ha^{-1} of $ZnSO_4$ + foliar spray of 0.2% $ZnSO_4$ (1.12 LER, 0.095 RCC, 0.94 CR, 1.01 ATER) was the most efficient treatment for maize and soybean production. T_9 had the highest LER, suggesting that intercropping in this treatment was more productive than monocropping. The comparatively high RCC and ATER values indicate a strong competitive advantage, which might be attributed to optimum utilization of resources and crop interactions. Maize equivalent yield was significantly the highest in maize + soybean strip intercropping system with T_9 (48.57 q ha^{-1}) and the lowest in T_3 (44.19 q ha^{-1}). The maize production efficiency varied from 16.14 to 30.33 kg ha^{-1} day^{-1}, while in soybean production efficiency varied from 11.08 to 19.81 kg ha^{-1} day^{-1}. The maximum maize production efficiency (18.39 kg ha^{-1} day^{-1}) was obtained in T_9 followed by T_8 (18.14 kg ha^{-1} day^{-1}). The minimum production efficiency (16.14 kg ha^{-1} day^{-1}) was obtained in T_3. Similarly, the maximum production efficiency (12.06 kg ha^{-1} day^{-1}) was recorded in T_9 followed by T_8 (11.97 kg ha^{-1} day^{-1}). The minimum production efficiency (11.08 kg ha^{-1} day^{-1}) was recorded in T_3. The results obtained in this study are consistent with the findings of (Aasim *et al.,* 2008)

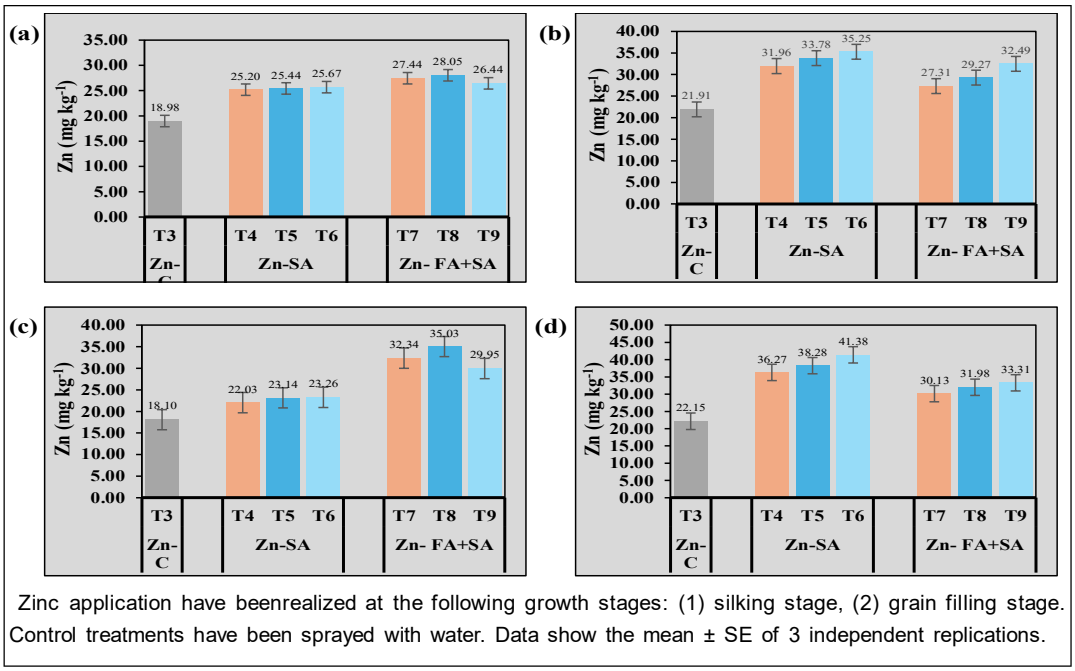
Table 2: Influence of different zinc fertilization methods on yield of soybean.

Treatments	No. of pods $plant^{-1}$	No. of seed pod^{-1}	No. of seed $plant^{-1}$	Seed index (g)	Pod yield (q ha^{-1})	Haulm yield (q ha^{-1})	Biological yield (q ha^{-1})	Harvest index (%)
T_1	29.96	2.27	75.87	10.44	24.97	51.18	76.15	32.79
T_2	-	-	-	-	-	-	-	-
T_3	31.13	2.46	82.00	10.56	13.42	39.53	52.95	23.69
T_4	32.05	2.34	81.73	12.97	13.63	43.33	56.97	24.30
T_5	32.80	2.67	85.80	13.11	13.90	43.75	57.65	24.41
T_6	33.86	2.74	91.07	13.28	14.36	44.70	59.06	24.43
T_7	32.71	2.56	82.33	12.45	14.30	42.48	56.78	24.63
T_8	33.44	2.77	89.07	12.69	14.37	43.61	57.97	24.79
T_9	35.31	2.82	95.33	13.02	14.47	45.11	59.58	24.89
LSD (0.05)	3.95	0.713	9.06	0.682	1.456	4.456	5.56	1.44
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Table 3: Influence of treatments on LER, RCC, CR, ATER, MEY, maize production efficiency, soybean production efficiency.

Treatments	LER	RCC	CR	ATER	MEY (q ha^{-1})	Maize production efficiency (kg ha^{-1} day^{-1})	Soybean production efficiency (kg ha^{-1} day^{-1})
T_1	1	-	-	-	-	-	19.81
T_2	1	-	-	-	-	30.33	-
T_3	1.02	0.071	0.91	0.25	44.19	16.14	11.08
T_4	1.05	0.076	0.93	0.95	45.62	17.17	11.36
T_5	1.08	0.082	0.93	0.97	46.62	17.61	11.58
T_6	1.11	0.092	0.93	1.00	48.14	18.18	11.97
T_7	1.09	0.085	0.90	0.98	47.45	17.58	11.92
T_8	1.11	0.091	0.93	1.00	48.12	18.14	11.97
T_9	1.12	0.095	0.94	1.01	48.57	18.39	12.06

LER: Land equivalent ratio, RCC: Relative crowding coefficient, CR: Competition ratio, MEY: Maize equivalent yield, ATER: Area time equivalent ratio.



Zinc application have beenrealized at the following growth stages: (1) silking stage, (2) grain filling stage. Control treatments have been sprayed with water. Data show the mean ± SE of 3 independent replications.

Fig 2: Influence of Zn applications on (a) maize grain Zn concentration (mg kg $^{-1}$) (b) maize stover Zn concentration (mg kg $^{-1}$) (c) soybean pods Zn concentration (mg kg $^{-1}$) (d) soybean straw Zn concentration (mg kg $^{-1}$).

about cotton intercropped with cowpea, (Nurbakhsh *et al.,* 2013) regarding sesame intercropped with bean.

Quantifying the zinc concentration in maize grains and stover, as well as soybean pods and straw, across different zinc application treatment

The zinc concentration in maize grains varied from 18.98 to 28.05 mg kg^{1}, while in maize stover it varied from 21.91 to 35.25 mg kg^{-1}. while in soybean, the zinc concentration in pods varied from 18.10 to 28.05 mg kg^{-1} and in haulm it ranged from 22.15 to 41.38 mg kg^{-1}. These variations were seen under various Zn applications treatments, as shown in Fig 2. All treatments showed a substantial rise in the concentration of zinc in both maize grains and straw compared to no- Zn control. The average zinc concentration in maize grains and soybean pods was found to be 25.53 mg kg^{-1} (SD = 2.51, SE= 0.79), 25.65 mg kg^{-1} (SD = 5.16), with significant difference seen among different treatments. Applying a rate of 17.5 kg ha^{-1} of $ZnSO_4$ soil supplementation with 0.3% Zn foliar spray led to the highest zinc concentration of 28.05 mg ka^{-1}, 35.03 mg ha^{-1} compared with Zinc-C treatment had the lowest zinc concentration at 18.98 mg kg^{-1}, 18.10 mg kg^{-1}. Similarly, the average zinc concentration in maize stover and soybean haulm showed an average zinc concentration of 29.41 mg kg^{-1} (SD=3.98, SE= 1.26), 31.36 mg/kg^{-1} (SD = 6.06, SE = 1.92) was showing significant heterogeneity among all treatments. Addition of soil supplementation of 25 kg ha^{-1} of $ZnSO_4$ led to the highest zinc concentration of 35.25 mg ka^{-1}, 41.38 mg ha^{-1} compared with Zinc-C treatment had the lowest zinc concentration at 21.91 mg kg^{-1}, 22.15 mg kg^{-1}. Zhang *et al.* (2010) found that applying Zn by foliar spraying during the grain development stage may increase the Zn concentration levels in seeds. This is because zinc initiates development by enhancing seedling vigor, root growthand chlorophyll content, leading to increased nutrient absorption and crop productivity.

CONCLUSION

In conclusion, based on the findings zinc fertilization improves maize and soybean performance by enhancing yield attributes parameters and productivity. The application of 17.5 kg ha^{-1} of $ZnSO_4$ to the soil, along with a foliar spray of 0.3% $ZnSO_4$, led to the highest concentration of zinc in the grains and pods. On the other hand, applying 25 kg ha^{-1} of $ZnSO_4$ to the soil resulted in the highest concentration of zinc in the straw of maize and soybean crops. Biofortification is proposed as the most favourable crop-based approach to increase the quality and bioavailability of vital nutrients in the edible part of crops. These systems achieved a balance between maximizing crop production and ensuring long-term environmental and ecological viability in agriculture. Therefore, it may be concluded that intercropping has an ability to contribute as a profitable and sustainable methods of reducing poverty and decreasing susceptibility to drought, especially among small-scale farmers in developing nations.

ACKNOWLEDGEMENT

We extend our sincere appreciation to Dr. Rajeev, Department of Agronomy, M. Siyon Kumari, Department of Agronomy for their invaluable contribution to this research endeavour. Their expertise, guidance and support have greatly enriched the quality of our work.

Conflict of interest

The author declare that the research was carried without any commercial or financial references that might be considered as a feasible conflict of interest.

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